Université catholique de Louvain

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OPIOID-INDUCED HYPERALGESIA:

A PATHOLOGICAL OR PHYSIOLOGICAL PHENOMENON?

Study development of an animal model of acute hyperalgesia to sufentanil under general anesthesia

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“One answer simply leads to another question”

Ben-David and Chelly.

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ACTH:	*AdrenoCorticoTropin Hormone*
Ad.C:	*Adenylyl Cyclase*
α-MSH:	*alpha-Melanocyte Stimulating Hormone*
AMPA:	*2-Amino-3-(5-Methyl-3-oxo-1,2-oxazol-4-yl) Propanoic Acid*
AR:	*Adrenoceptor, AdrenoReceptor*
CCK:	*CholeCystoKinin*
CGRP:	*Calcitonin Gene-Related Peptides*
CNS:	*Central Nervous System*
COX:	*Cyclo-Oxygenase Enzyme*
CRF:	*Corticotropin-Releasing Factor*
DLF:	*Dorsal Lateral Funiculus*
DNIC:	*Diffuse Noxious Inhibitory Control*
DH:	*Dorsal Horn*
DRG:	*Dorsal Root Ganglia*
DYN:	*DYNorphin*
EAAs:	*Excitatory Amino Acids*
GABA:	*Gamma- AminoButyric Acid*
Glut:	*Glutamate*
GLUT:	*GLUtamate Transporters*
GPCR:	*G-Protein Coupled Receptor*
GPRK:	*G-Protein-Regulated Kinases*
5-HT:	*Serotonine*
IL:	*InterLeukin*
i.p.:	*intraperitoneal*
i.t.:	*intrathecal*
i.v.:	*intravenous*
MAC:	*Minimal Alveolar Concentration*
MAPK:	*Mitogen-Activated Protein Kinase*
M6g:	*Morphine-6-Glucuronide*
MH:	*Mechanical Hyperalgesia*
MLPC:	*Multi-Lineage Progenitor Cells*
NMDA:	*N-Methyl-D-Aspartate*
NE:	*NorEpinephrine*
NK-1	*NeuroKine-1*
NO:	*Nitric Oxide*
N_2O:	*Nitrous Oxide*
N/OFQ:	*Nociceptin/Orphanin peptide*
NPY:	*Neuropeptide Y*

***NSAID**: NonSteroidal Anti-Inflammatory Drug*

***OIA:** Opioid-Induced Analgesia*
***OIH**: Opioid-Induced Hyperalgesia*

***PACU**: Post-Anesthesia Care Unit*
***PAF**: Primary Afferent Nerve Fiber*
***PAG**: Peri-Acqueductal Gray matter of the midbrain*
***PKA**: Protein Kinase A*
***PKC**: Protein Kinase C*
***PENK**: Pro-ENKephalin*
***PGs**: ProtaGlandines*
***PNS**: Peripheral Nervous System*
***POMC**: ProOpioMelanoCortin*
***PSNL**: Partial Sciatic Nerve Ligation*

***QST**: Quantitative Sensory Testing*

***s.c.**: subcutaneous*
***SG**: Substantia Gelatinosa (lamina II in the spinal cord)*
***SP**: Substance P*
***SMIR**: Skin Muscle Incision Retraction*

***TENS**: Transcutaneous Electrical Nerve Stimulation*
***TH**: Thermal Hyperalgesia*
***TNF**: Tumor Necrosis Factor*
***TMH**: Trans-Membrane Helical*
***TRPV**: Transient Receptor Potential Vanilloid*

***RVM**: RostroVentromedial Medulla*

***VIP**: Vasoactive Intestinal Peptide*

***WHO**: World Health Organization*

Glossary

***Allodynia**:* *pain perception evoked by a stimulus that under normal conditions evokes non-painful sensations*

Antinociception: *inhibition of behavioural responses to nociceptive stimuli in animals*

***Anesthesia**:* *derived from the Greek ἀν-,* an-, *"without"; and αἴσθησις,* aisthēsis*, "sensation. Pharmacological-induced reversible state of amnesia, analgesia, loss of responsiveness, loss of motor reflexes and decreased stress response*

***Balanced anesthesia**:* *technique of general anesthesia based on the concept that administration of a mixture of several drugs i.e. combination of analgesic and anesthetic drug summates the advantages but not the disadvantages of the individual components of the mixture*

***Central sensitization**:* *phenotypic changes in CNS pathways that leads to increase of the processing of nociceptive stimuli*

***Hyperalgesia**:* *enhanced pain perception evoked by a stimulus that under normal conditions evokes painful sensations*

- ***Primary**:* *within the injured area*
- ***Secondary**:* *in the surrounding uninjured tissue*

***Hypnosis**:* *a subjective state in which alterations of perception or memory can be elicited by suggestion*

MAC: *Minimal Alveolar Concentration: partial pressure of an inhaled anesthetic in the lung at which 50% of non-relaxed patients remain immobile during a skin incision (concentration of anesthetic required to block movement)*

MAC-awake: *MAC that results in 50% of patients, being on the verge of unconsciousness, a loss of the response to verbal command*

MAC-BAR: *MAC that Blocked Adrenergic Responses (cardiovascular and neuroendocrine responses) to skin incision in 50% of all patients. A positive response was arbitrarily defined as an increase of 10% or more from mean pre-incision value to mean*

	post-incision value in heart rate, blood pressure or norepinephrine levels (Roizen, Horrigan et al. 1981)
MAC-sparing effect:	*decrease in MAC after an analgesic drug*
Nociception:	*behaviors evoked by the application of a brief nociceptive stimulus*
***Nociceptors**:*	*primary nerves fibers that evoke and transport nociceptive information in respond to tissue injury or nociceptive stimuli*
***Plasticity**:*	*dynamic functional and/or structural changes occurring in the nervous system as a result of an injury or disease*
***Sparing-effect**:*	*synergistic interaction between two drugs allowing to reduce their effective amount*
***Narcotic**:*	*derived from the Greek* ναρκωσις *(narcosis), the term used by Hippocrates for the process of numbing or the numbed state, and is defined as any drug that would induce sleep*
***Opium**:*	*extract of plant (poppy Papaver Somniferum), opium is the source of many opiates, including morphine, thebaine, codeine, papaverine, and noscapine*
***Opiates**:*	*oldest term, related to substances of synthetic morphine derivates which have no peptide structure*
***Opioids**:*	*all endogenous or exogenous (natural or synthetic) substances which produce similar effect to morphine and which are blocked by antagonist as naloxone*
***Wind-up**:*	*frequency-dependent increased response of second order neurons in the dorsal horn to repeated electrical activation of afferent C-fibers.*

Introduction

Opioid analgesics are the most frequently used drugs to relieve moderate to severe pain in both cancerous and non-cancerous chronic conditions as recognized by the World Health Organization (WHO; Cancer pain relief, 2nd ed. Geneva, 1996). Moreover, morphine and its derivates are widely-used in acute pain conditions; particularly in anesthetic practice as a component of anesthesia and in postoperative pain management.

In addition to their analgesic effects, opioids induce dependence and tolerance. These phenomena are well-known in the setting of chronic pain therapy (Compton 1994; Compton, Miotto et al. 2004). Moreover, opioids can unexpectedly enhance pain and prolong pain states even after a single administration (Richebe, Rivat et al. 2005; Angst and Clark 2006). This condition, termed hyperalgesia, is broadly defined as an increased sensitivity to nociceptive stimuli.

Because, in daily clinical practice, opioids are frequently used for balanced anesthesia and postoperative pain management, the experimentally proved hyperalgesic effect of opioids leads clinicians and researchers to question how drugs recognized to alleviate pain, may have the opposite effect. In other words, in clinical setting, **do opioids decrease pain sensitivity, enhance pain, or promote postsurgical persistent pain?**

In the two last decades, major improvements have been made in the understanding of the mechanisms underlying acute postoperative pain thanks to the development of incisional pain models in animals (Brennan 2005). New analgesic and anesthetic drugs as well as minimally invasive surgical procedures were expected to reduce postoperative pain. Nevertheless, post-surgical pain continues to be a major challenge in daily anesthetic practice (Aubrun, Langeron et al. 2003; Jensen, Kehlet et al. 2009).

Postoperative pain is a major source of suffering and disability, which negatively impacts on patient's rehabilitation. Further, severe acute postoperative pain is currently pointed out as a striking factor involved

in the risk to develop chronic postsurgical pain (Perkins and Kehlet 2000; Macrae 2001; Poleshuck, Katz et al. 2006; Woolf 2007).

By consequence, additional research is mandatory to optimize perioperative pain management (Dolin, Cashman et al. 2002; Apfelbaum, Chen et al. 2003). Postoperative pain involves central nervous system sensitization which is the result of surgical injury but might also be enhanced by a paradoxical effect of the opioid analgesics administered perioperatively (Angst and Clark 2006; Koppert and Schmelz 2007).

The present work will focus on the intriguing concept of opioid-induced hyperalgesia (OIH). Its originality is the development of a new animal model where the paradoxical effect of opioids is expressed under general anesthesia, mimicking clinical practice.

Throughout the manuscript, the terms opioids and opiates will be used. As described in the glossary, opioids represent endogenous or exogenous substances which produce similar effect to morphine whereas opiates are related to synthetic morphine derivates. To facilitate the reading, the term of opioids will be implied most of the time. The term of opiates will be used when specifically we will refer to synthetic substances.

Aim of the thesis

The present work will explore the mechanisms of paradoxical OIH in a surgical context. For this purpose, an experimental model mimicking perioperative conditions has been developed and validated to investigate whether the perioperative use of opioid analgesics may increase pain sensitivity. We will explore:

1) the dose-dependent analgesic and hyperalgesic effects of μ-opioid administration under general anesthesia,

2) the interaction between analgesic and anesthetic drugs (volatile and intravenous agents),

3) the modulation of OIH by analgesic adjuvant drugs used in perioperative pain management,

4) the impact of various preexisting nociceptive conditions.

The **first section** of the thesis presents, in a first part, a brief review of the history and pharmacology of opioids, the physiopathology of pain and the role of opioids in modulating nociceptive processing. In a second part, the clinical, experimental expression and the relevance of the paradoxical opioid-induced hyperalgesia, as well as its physiopathology will be explored.

The **second section** points out the objectives of the thesis.

The **third section** is devoted to the experiments investigating the development of paradoxical OIH in perioperative conditions, specifically under general anesthesia.

Section 1: State of the art

1.1. Basic concepts

1.1.*1*. Pharmacology of opioids

1.1.*1.1*. A brief history of opioids from opium to recent forms of opiates

Opium, an extract derived from the poppy *Papaver somniferum*, is composed of a number of alkaloids and is probably the oldest known medically-useful substance, as it was used by the ancient Sumerians (4000 B.C.) and Egyptians (2000 B.C.). At first, opium was employed as a euphoriant in religious rituals or was co-administered with hemlock to induce a quick, painless death. It then came to be used medically to relieve pain and diarrhea, a remedy that was initially considered dangerous because it varied in potency and absorbance rate. In 1600s, manuscripts appeared describing opium abuse and tolerance.

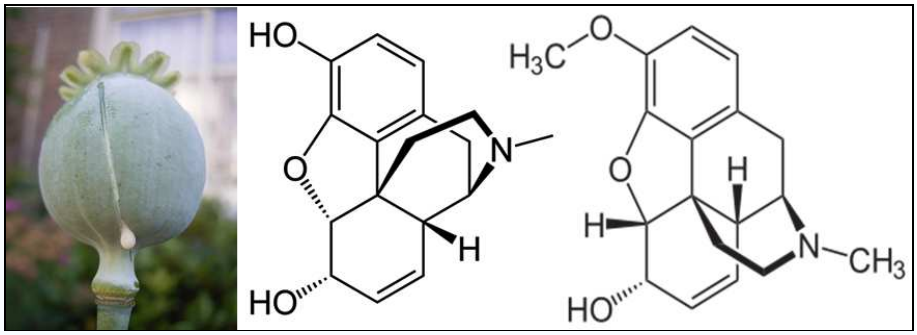


Figure 1: morphine and codeine (alkaloids) are the biologically active chemical constituents of opium

The main active ingredient in opium is the alkaloid morphine, which was first isolated in crystalline form in the 1800s by Sertürner, who named it after Morpheus, the god of dreams. A few years later, another active ingredient, codeine, was isolated.

Identification of the structures of morphine and codeine soon led to the synthesis of many analogs in attempts to avoid the problems encountered with morphine, such as respiratory depression, constipation and sedation, as well as addiction. Pharmaceutical

companies in particular mounted a large synthesis program, and in the late 1800s, the Bayer Company developed pure morphine and a method of administering it parenterally via the hypodermic needle. Morphine began to be used for surgical procedures in postoperative pain and as an adjunct to general anesthetics. Claude Bernard first investigated the use of morphine for premedicating experimental animals to reduce the amount of chloroform needed to produce anesthesia.

With the goal of minimizing its addictive properties, morphine was acetylated. In 1898, diacetylmorphine was released as a potent analgesic, which was initially marketed as a non-addictive alternative to morphine. However, it soon became apparent that diacetylmorphine, also known as diamorphine or heroin, was far more addictive than morphine. Meperidine was discovered in 1939, followed by the synthesis of methadone in 1946. Hundreds of opioid derivates have been generated to date in an effort to avoid side effects and addictive potential. Most have properties similar to morphine and act through mu (μ) opioid receptors, but structurally, these agents are quite various. Many retain the rigid chemical structure of morphine and codeine, while others such as methadone and fentanyl have completely unrelated structures.

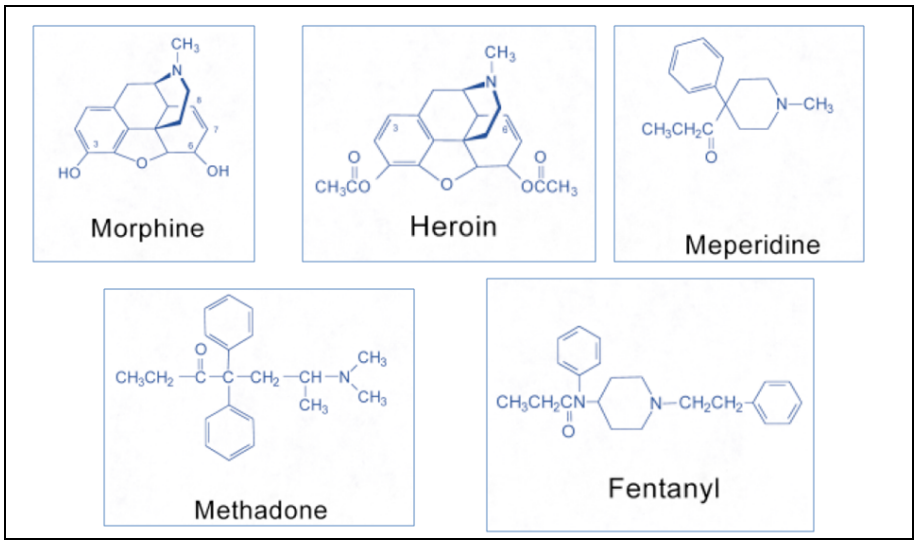


Figure 2 : structures of μ-opioid analgesics
Adapted from Pasternak, 2001 (Pasternak 2001)

Among the most recent morphine derivatives, remifentanil is probably the most original. It is a potent μ-agonist that retains all of the pharmacodynamic characteristics of its class (i.e., analgesia, respiratory depression, muscle rigidity, nausea and vomiting, pruritus) but with a unique pharmacokinetic profile due to rapid metabolism by non-specific tissue esterases. Remifentanil can be used as the sole agent for sedation during painful procedures in patients breathing spontaneously, or as the analgesic component in intensive care sedation and during surgical anesthesia. A precise titration and safe administration requires continuous infusion or the use of sophisticated drug delivery systems such as target-controlled infusion (TCI) (Egan and Shafer 2003). Remifentanil has permitted important experimental and clinical research in the study of interactions between opioid and glutamatergic systems, leading to a better understanding of postoperative OIH and acute tolerance to the analgesic action of opioids.

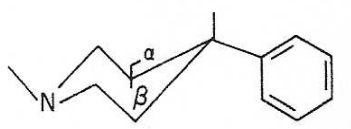
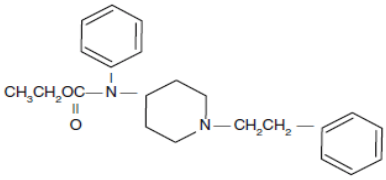
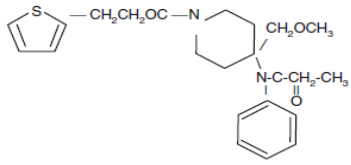
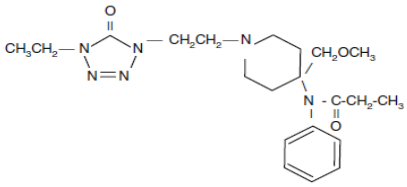
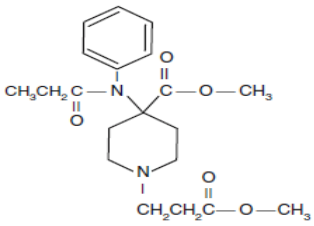
	*Phenylpiperidine family*
	***Fentanyl*** *(Fentanyl®)* *Synthesized in 1960 by Paul Janssen. Many other fentanyl analogs were developed and introduced in medical practice*
	***Sufentanil*** *(Sufenta®)* *Synthesized in 1976*
	***Alfentanil*** *(Rapifen®)* *Synthesized in 1984*
	***Remifentanil*** *(Ultiva®)* *Marketed by GlaxoSmihkline and Abbott as Ultiva*

Figure 3: chemical structure of opioids used in anesthesia
Anilinopiperidine family includes fentanyl, alfentanil, sufentanil and remifentanil. While all share similar pharmacological properties (metabolized hepatically), remifentanil, the newest one, has a unique pharmacokinetic profile due to a rapid metabolism by non-specific tissue esterases

1.1.*1.2.* Endogenous opioid peptides

After the discovery of opioid antagonists nalorphine (mixed agonist-antagonist) and naloxone (first pure antagonist), it became clear by the mid-1960s that the effects of opioid agonists, antagonists and mixed agonist-antagonists could be explained by action at multiple opioid receptors (Goldstein, Lowney et al. 1971). In 1973, it was shown that these receptors are stereospecific opioid binding sites in the CNS and that they have non-uniform distributions. When Akil et al. found that stress-induced analgesia was partially reversed by naloxone, the existence of endogenous opioids seemed obvious.

Nervous tissue was observed to contain endogenous opioid peptides, including: met-enkephalin and leu-enkephalin (1975), β-endorphin (1976), dynorphins (1981) and deltorphins (1989). In mammals, each of these opioid peptides was described as part of a larger precursor protein: proenkephalin, prodynorphin, or proopiomelanocortin (POMC). These three precursors gave rise to more than 20 opioid ligand candidates.

Neurophysiological and behavioral observations of several selective opioid compounds (lacking some side effects) and incomplete cross-tolerance among the opioids provided strong evidence for the existence of different types of receptors, named after the drugs used to delineate them: μ (mu for morphine), κ (kappa for ketocyclazocine) and σ (sigma for SKF 10,047) (Martin, Eades et al. 1976). Later, a fourth type of opioid receptor was proposed, the δ (delta) receptor, so named because it was present in the mouse vas deferens. The σ-receptor was then removed from the opioid receptor family, as it displayed neither the stereo-selectivity of other opioid receptors nor antagonism by opioid antagonists.

At that time, the three major families of opioid receptors (μ, κ and δ) were proposed based on pharmacology, as opioid binding was the only way to define receptor sites. However, gene cloning confirmed this classification with the clones MOR 1 (μ) (Chen, Mestek et al. 1993), KOR 1 (κ) (Li, Zhu et al. 1993) and DOR 1 (δ) (Evans, Keith et al. 1992; Kieffer, Befort et al. 1992). The three receptors were closely related structurally and demonstrated the ligand selectivities predicted

from binding studies in the brain (Reisine and Bell 1993). Because of strong sequence homology across receptors, the entire opioid receptor gene family was readily cloned in the following years. By the mid-1990s, the entire endogenous opioid system, including peptides and receptors, was characterized at the molecular level (Akil, Watson et al. 1984; Kieffer 1995).

Several groups identified a fourth opioid receptor, originally named Opioid Receptor-Like (ORL1), but then OP4 (Mollereau, Parmentier et al. 1994; Pan, Cheng et al. 1995). Originally, this was an orphan receptor, but its endogenous ligand was later identified and termed orphanin (FQ) (Reinscheid, Nothacker et al. 1995) or nociceptin (OFQ/N) (Meunier, Mollereau et al. 1995). The receptor was thus renamed the nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor (Chiou, Fan et al. 2004; Hu, Calo et al. 2010). Despite structural similarities with other members of the opioid receptor family, this receptor is unlike a "traditional" opioid receptor: it lacks the high affinity of classical opioid receptors and is naloxone-insensitive. Furthermore, heterogeneity of NOP receptors has been reported. N/OFQ has been implicated in various physiological functions, including nociception, the stress response, feeding, learning and memory, pituitary functions, and even cardiovascular regulation. It reverses morphine analgesia and induces spinal analgesia and supraspinal hyperalgesia (Mogil and Pasternak 2001; Chiou, Fan et al. 2004).

Based on pharmacological data, additional opioid receptor types have been proposed, including epsilon (ε), iota (ι), lambda (λ) and zeta (ζ). These receptors are poorly characterized and are not currently considered "classical" opioid receptors (Kieffer and Gaveriaux-Ruff 2002).

Based on gene sequencing, the primary amino acid structure of the seven trans-membrane G-protein opioid receptor family placed these receptors into a large family of rhodopsin-like G-protein coupled receptors (GPCRs). This family includes receptors for dopamine, epinephrine, serotonin (5-HT), acetylcholine, and many peptide neurotransmitters and hormones (Lagerstrom and Schioth 2008). Approximately 670 genes representing 2-3% of the transcribed

human genome are dedicated to this receptor family. Despite the diversity of their ligands, GPCRs share many characteristics: they all interact with heterotrimeric G proteins that serve as a transduction system for conveying the binding signal into the cell.

1.1.*1.3.* Structure and function of opioid receptors

Overall, μ, κ and δ receptors show 60% amino acid sequence homology. Extracellular domains, including three extracellular loops and the N-terminal domain, determine their selectivity; these domains differ strongly among receptors and likely form a protein gate that allows particular agonists or antagonists to enter the binding pocket, thereby contributing to μ, κ and δ selectivity. Like other GPCRs, opioid receptors convey extracellular signals within the cell by modulating cytoplasmic receptor domains that interact with the G-protein. Agonist binding modifies helical positioning of the receptor to switch it from an inactive to an active conformation. The μ, κ and δ receptors have identical intracellular domains, and their stimulation produces intracellular signaling events consistent with activation of a Gi/o linked GPCR. G-protein subunits dissociate from the activated receptors and then modulate intracellular effectors and pathways. Intracellular signaling, in turn, leads to short-term inhibition of neuronal activity or long-term genomic effects (Kieffer and Evans 2009). Opioid receptor activation produces an inward K^+ conductance to cause hyperpolarization, closes voltage-gated Ca^{++} channels (N- and P-type) and inhibits adenylyl cyclase to reduce the formation of cyclic adenosine monophosphate (cAMP). It may also modulate other signal transduction systems. Additional opioid-modulated pathways involve N-methyl-D-aspartate (NMDA) receptors, mitogen-activated protein kinase (MAPK) and phospholipase C (PLC) (Stein and Zollner 2009).

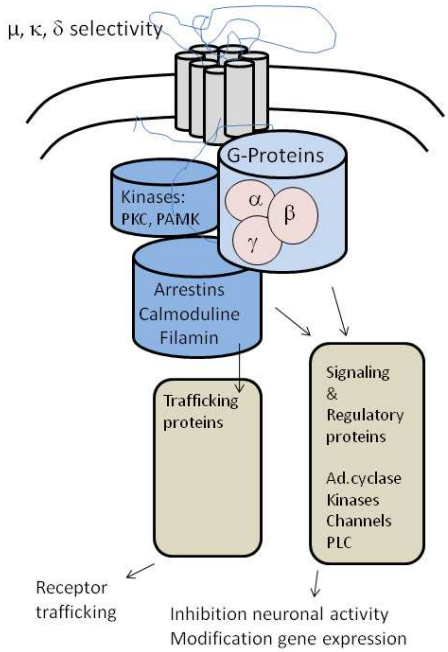


Figure 4: opioid receptor structure
Opioid receptors are coupled to inhibitory G-proteins and form signaling complexes with many proteins. The signaling is highly regulated by receptor phosphorylation and trafficking via intracellular pathway. Opioid receptor activation modifies ion channel activities (decreased neuronal excitability or neurotransmitter release), decreases cAMP levels via inhibition of adenylyl cyclase (Ad.C) and activates phosphorylation pathways that lead to transcriptional regulation
Adapted from Kieffer, 2009

After activation, various enzymes such as phosphokinase C (PKC) and G-protein-regulated receptor kinases (GPRK) can phosphorylate opioid receptors, leading to separate it from the G-protein. The receptor increases then its affinity for intracellular arrestin molecules. The formation of arrestin-receptor complexes prevents G-protein coupling and promotes internalization (endocytosis), leading to opioid receptor desensitization. The receptor is either recycled and will re-express at the cell surface or it will be degraded. Recycling of opioid receptors to the plasma membrane promotes rapid re-sensitization to signal transduction. Interestingly, both μ and δ receptors internalize after exposure to agonists, whereas κ receptors do not. Morphine

does not possess a significant capacity for receptor internalisation but exhibits a high potential for tolerance development.

The relative contribution of these signaling events to function depends on the site of receptor expression (Kieffer and Evans 2009; Stein and Zollner 2009). The receptor types are dynamic multi-component units rather than single protein entities, and different agonist ligands confer different patterns of signaling and receptor trafficking based on the anatomical distribution of their receptors. This leads to different functions for each receptor subtype, such as euphoria (μ) versus dysphoria (κ) or supraspinal analgesia (μ) versus supraspinal antagonism of opioid analgesia (NOP). The diversity of opioid receptor physiology at the behavioral, cellular, and molecular levels may therefore account for the wide diversity of opioid receptors reported by pharmacological studies. Studies with mutant mice confirm a role of opioid receptors in pain, as mice lacking μ, κ, or δ receptors exhibit enhanced pain sensitivity (Martin, Matifas et al. 2003; Kieffer and Evans 2009). It is interesting to note that each receptor creates a distinct pattern of activity: μ-receptors modulate mechanical, chemical and supraspinally thermal nociception; κ-receptors modulate spinally mediated thermal nociception (Martin, Matifas et al. 2003) and visceral and inflammatory pain (Gallantine and Meert 2008); while δ-receptors do not regulate acute pain, but are implicated in inflammatory and neuropathic pain (Nadal, Banos et al. 2006; Gaveriaux-Ruff, Karchewski et al. 2008). Moreover, phenotypes of mutant mice have suggested a low endogenous opioid tone in the regulation of physiological pain and a specific role for each receptor in regulating the diversity of pain modalities.

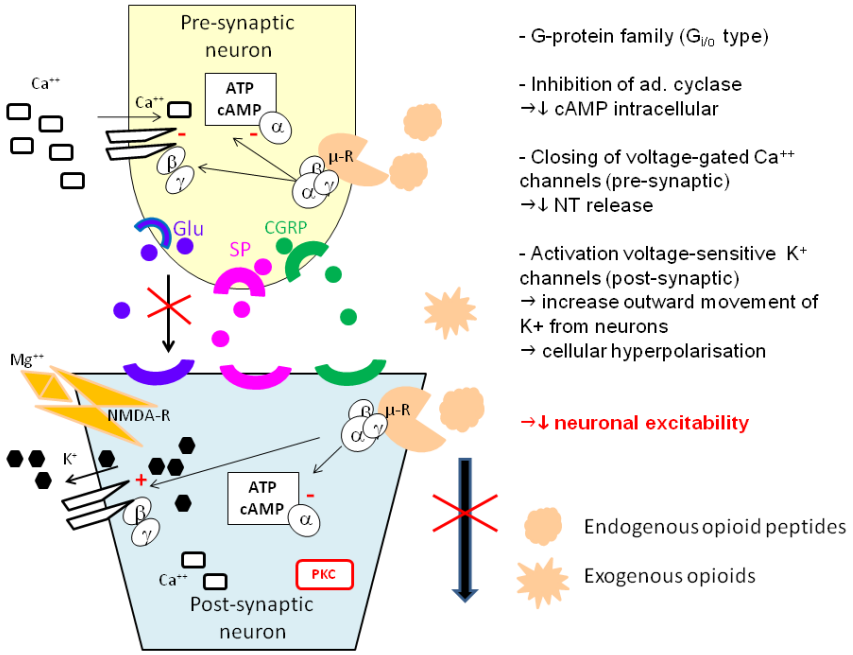


Figure 5: schematic summary of antinociceptive mechanisms mediated by μ-agonists
The opioid receptors are largely present in dorsal horn (substantia gelatinosa) of spinal cord. Activation of opioid receptor produces a decrease in release of neurotransmitters (Glut, SP, acethylcholine,…) by inhibition of voltage-gated Ca^{++} channels (pre-synaptic) and a cellular hyperpolarisation by opening of K^{+} channels (post-synaptic) reducing neuronal excitability.
μ-R: μ-receptor; Glu: glutamate; SP: substance P, CGRP: calcitonine gene related peptide; PKC: protein phosphokinase C; NMDA: N-methyl-D-aspartate receptor

Today, the μ-opioid analgesics remain the clinical mainstay for control of pain. Morphine and most clinical opioid analgesics act through μ-opioid receptors. However, clinical observation and animal studies have suggested several μ- receptor subtypes. A tolerant patient regains analgesic sensitivity when switched from one μ-analgesic to another, implying that the mechanisms of action of these analgesics are probably only partially overlapping (Rossi, Brown et al. 1996). Based on affinity and selectivity for a wide range of opiates and opioid peptides, two subtypes of μ-receptors were distinguished, with the higher affinity subtype designed μ_1 and the lower affinity, morphine-selective subtype designed μ_2 (Wolozin and Pasternak 1981). Both μ

subtypes were blocked by the μ-selective antagonist β-funaltrexamine, consistent with their classification as μ-receptors; however, naloxonazine and naloxazone selectively blocked μ_1 but not μ_2 (Pasternak 1993; Corbett, Henderson et al. 2006).

Kappa-opioid receptors, defined by their high affinity for the endogenous opioid peptide dynorphin, are effective analgesics in both animals and humans, but their use is limited by side effects, including psychotomimetic and dysphoric effects.

Delta-opioid receptors have anti-nociceptive and anti-depressant-like properties, as well as pro-convulsant effects in animals. While κ- and δ-receptor agonists may prove to be important in the future, their present clinical impact is negligible (Peng, Zhang et al. 2009).

Summary

Since the isolation of morphine from opium and its synthetic production, hundreds of opioid derivates have been generated in an effort to avoid side effects and addictive potential, a goal that is far to be reached. The existence of endogenous opioid peptides and of different types of opioid receptors was first demonstrated pharmacologically and later confirmed by gene cloning. The "traditional" opioid receptors (μ, κ and δ) are GPCRs that trigger intracellular signaling upon activation. The end result of receptor activation depends upon the neuroanatomical site of receptor expression. The μ-opioid analgesics remain the mainstay clinical approach to control pain.

	μ - MOP	***δ - DOR***	***κ - KOP***	***NOP - ORL***
Receptor type	*G protein-GRCP*			
Transduction mechanism	*Inhibition of adenylyl cyclase, inhibition Ca^{++} channels, outward K^{+} conductance*			
Subtypes	*μ_1 μ_2 μ_3*	*δ_1 δ_2*	*κ_1 κ_2 κ_3*	
Location	*Brain: cortex, thalamus, PAG* *Spinal cord (SG)*	*Brain: amygdala, pontine nuclei, olfactory bulbs, deep cortex*	*Brain: PAG, hypothalamus, claustrum* *Spinal cord (SG)*	*Brain: amygdala, locus coeruleus* *Spinal cord*
Endogenous peptide	*Endomorphin*	*Enkephalin*	*Dynorphin*	*Nociceptin*
Nociception	*Analgesia*	*Analgesia*	*Analgesia*	*Analgesia (spinally) Hyperalgesia (supraspinal)*
Other actions	*Euphoria, respiratory depression, miosis, constipation, immune function, physical dependence, …*	*Euphoria, physical dependence*	*Dysphoria, dieresis, feeding, neuroendocrine secretions*	*Anxiolysis, modulation of feeding*
Clinical agonist	*Morphine Fentanyl Sufentanil Alfentanil, Remifentanil*	*None*	*None*	*None*
Clinical antagonist	*Naloxone*	*Naloxone*	*Naloxone*	*None*

Table 1: summary of major properties and actions of opioid receptors
SG: Substantia Gelatinosa
PAG: Peri-Acqueductal Gray

1.1.*1.4.* Endogenous opioid signaling

For a long time, studies focused on the pharmacological properties of "exogenous" opioids (i.e., morphine and morphine-like substances), based on the historical belief that morphine was not present endogenously. However, in 1973, three researchers independently described an opioid receptor in nervous tissue and hypothesized the existence of an endogenous opioid (Lord, Waterfield et al. 1977). Isolation of sufficient material for analysis from the pig brain required about two years, but finally resulted in the identification of two closely-related endogenous opioids termed enkephalins, a name derived from the Greek *enkephalos*, meaning "in the head" (Kosterlitz 1979). Soon after, β-endorphin was discovered and shown to be a potent opioid agonist. Within 5 years, three families of opioid peptides were described. They derive from different precursors: proenkephalin (PENK), POMC and prodynorphin. All of the opioid peptide cleavage products contain the sequence of either Met-enkephalin or Leu-enkephalin as the first five amino acids. These peptides vary in their affinity for μ- , δ- and κ- receptors and have negligible affinity for the NOP receptor, but none binds exclusively to one opioid receptor type.

Later, the endogenous opioids morphine and codeine were demonstrated in various vertebrate tissues, including the nervous and immune systems (Stefano, Digenis et al. 1993). In addition to the important discovery of morphine presence and signaling in plant, invertebrate and vertebrate cells, animal cells and complex organ systems were demonstrated to be able to synthesize morphine via enzymatic pathways that were strikingly similar to that originally described in the opium poppy (Poeaknapo, Schmidt et al. 2004; Zhu, Cadet et al. 2005; Zhu, Mantione et al. 2005; Stefano, Kream et al. 2008)

Precursor	***Opioid peptides***	***Specificity***
Pro-opiomelanocortin (POMC)	*β-endorphins (YGGFM-)*	*μ- and δ- receptor (κ- receptor)*
Pro-enkephalin (PENK)	*(Met)-enkephalin (**YGGFM**)* *(Leu)-enkephalin (**YGGFL**)* *Peptide E (YGGFM-)* *Metorphamide (YGGFM-)*	*δ- receptor (μ- receptor)*
Pro-dynorphin	*Dynorphin A (YGGFL-)* *Dynorphin B (YGGFL-)* *α-Neoendorphin (YGGFL-)* *β-Neoendorphin (YGGFL-)*	*κ- receptor (μ- and δ- receptor)*
Pro-nociceptin/Orphanin-FQ	*Nociceptin/Orphanin-FQ*	*ORL_1*
	Endomorphin-1 *Endomorphin-2*	*μ- receptor*
Pro-dermorphin and pro-deltorphin	*Atypical opioid peptides (physiological significance unclear)* *Dermorphin* *Deltorphin* *Deltorphin I* *Deltorphin II*	*μ- receptor* *δ- receptor*

Table 2: endogenous opioid peptides
The penta-peptide sequences corresponding to (Met)-enkephalin and (Leu)-enkephalin are contained in other opioid peptides. β-endorphin and most of the opioid peptides derived from PENK contain (met)-enkephalin at their N-termini whereas the sequence of (leu)-enkephalin is present in those peptides from pro-dynorphin
Adapted from Pasternak, 2001 and Corbett, 2006 (Pasternak 2001; Corbett, Henderson et al. 2006)

At this turning point, a quest began to identify the possible roles played by this group of endogenous messenger molecules under both physiological and pathological conditions. Opioid signaling is not as simple as originally described. New molecular insights into endogenous opioids signaling pathways should illustrate the complexity of opioid pharmacology. These events are physiological processes in humans that were probably derived from phylogenetic evolution to preserve cellular functionality and integrity. For example of this complex and physiological process, endogenous opioid peptides in peripheral nervous system and endogenous morphine signaling will be exposed.

1.1.1.4.1. Endogenous opioids in peripheral nervous system

Pain is an essential warning mechanism necessary to minimize tissue injury damage and hence to prolong survival. At spinal and supraspinal sites, the integration of signals from proalgesic neurotransmitters and cognitive factors results in the perception of pain. Endogenous mechanisms that counteract pain sensation in the brain and spinal cord are well-described and consist of descending pain inhibitory pathways, which contain mostly opioid peptides, noradrenaline and 5-HT and their associated receptors (Bodnar 2008). These pathways will be discussed in section 1.2. Less well-known is that similar counter-regulatory mechanisms also exist in the peripheral nervous system (PNS), producing effects via interactions between leucocyte-derived opioid peptides, anti-inflammatory cytokines and peripheral nociceptor terminals expressing opioid receptors (Rittner, Brack et al. 2008).

Opioid receptors were demonstrated in dorsal root ganglia (DRG) neurons, where they are co-expressed with sensory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP). These receptors are synthesized in DRG, then transported to peripheral nerve terminals. Coupled to inhibitory G-protein, they inhibit adenylyl cyclase and modulate ion channels (Mousa, Zhang et al. 2001; Wang and Wessendorf 2001; Zollner, Mousa et al. 2008). Opioid peptides are found in human subcutaneous and synovial cells, mast cells, lymphocytes and macrophages (Mousa, Machelska et al. 2002; Bergstrom, Ahmed et al. 2006; Mousa, Straub et al. 2007). As a result, opioid agonists can attenuate the excitability of nociceptors, the propagation of action potentials and the release of proinflammatory neuropeptides (SP, CGRP) from nociceptor endings, resulting in anti-nociception. The prevailing peptides in these regions are β-endorphin and met-enkephalin, whereas only minor amounts of dynorphins are detectable. Met-enkephalin and dynorphin have been detected in T-lymphocytes, granulocytes and macrophages. Immune cells in the blood and in inflamed tissue co-express the machinery required for processing POMC into functional β-endorphin. POMC-related opioid peptides have been found in leucocytes of many vertebrates and invertebrates.

Inflammatory response to tissue injury triggers various proinflammatory and pro-algesic mediators, thus activating specialized peripheral sensory neurons that signal pain. It is important to note that nociceptive but not sympathetic neurons are involved in the attraction of opioid-containing leukocytes during inflammation (Heurich, Mousa et al. 2007).

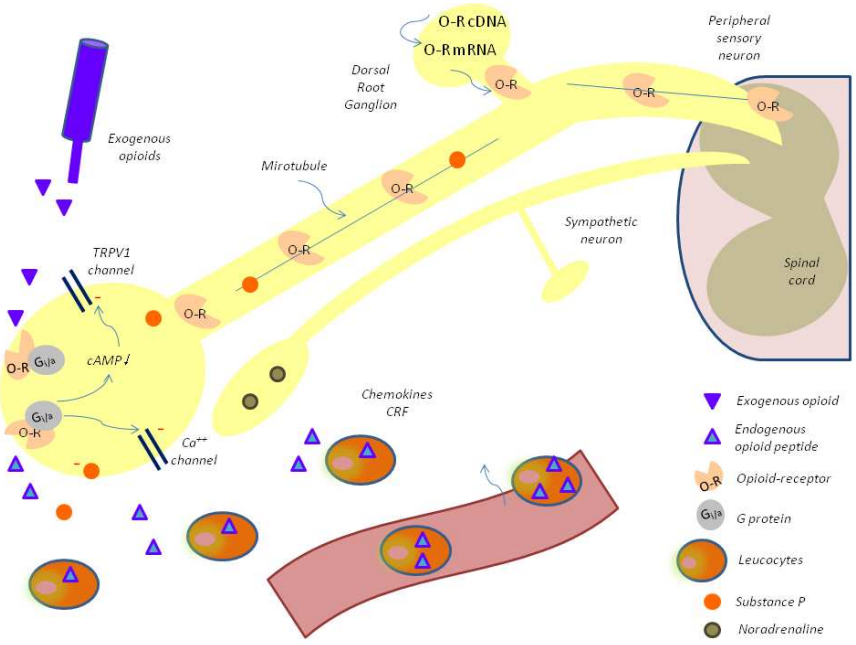


Figure 6: peripheral nociceptor terminals
Endogenous opioid peptides and exogenous opioids bind to peripheral opioid receptors. Opioids receptors are synthesized in dorsal root ganglia and transported along intra-axonal microtubules to peripheral terminals. The subsequent inhibition of ion channels (e.g.TRPV-1, ca^{++}) and SP release results in antinociceptive effects. Circulating leukocytes containing opioid peptides extravasate upon activation of adhesion molecules and chemotaxis
Adapted from Stein, 2009

Several mechanisms contribute to improve the anti-nociceptive opioids efficacy in inflamed tissue. First, increased synthesis of opioid receptors in DRG neurons, coupled with enhanced axonal transport, results in their up-regulation and enhanced G-protein coupling at peripheral nerve terminals. This is dependent on neuronal activity, production of pro-inflammatory cytokines and availability of endogenous nerve growth factor within the inflamed tissue. Second,

the perineural barrier is disrupted, which facilitates access of opioid agonists to their receptors. Finally, leukocytes are activated by chemokines released from endothelial and inflammatory cells and presented on the endothelium. Leukocytes then transmigrate through the endothelium into injured tissues, apparently under modulation by central mechanisms.

Effective central inhibition of pain reduces the need for recruitment of opioid-containing cells to injured tissues: morphine administrated i.t. at analgesic doses (Schmitt, Mousa et al. 2003) and epidural analgesia significantly reduced the number of β-endorphin-containing leucocytes in inflamed paw tissue (Heurich, Mousa et al. 2007). Blocking intra-articular opioid receptors by local naloxone administration in patients undergoing knee surgery significantly increased postoperative pain (Stein, Hassan et al. 1993). This suggests that in a stressful situation (such as post-surgery), opioids are tonically released in inflamed tissue and activate peripheral opioid receptors to attenuate clinical pain (Stein and Zollner 2009).

Some of the previous observations provide interesting insights into the intrinsic mechanisms of endogenous opioids in pain control. It is intriguing that in the presence of painful paw inflammation, chronic morphine treatment does not induce tolerance at peripheral μ-opioid receptors, suggesting that immune cell-derived opioids do not readily produce cross-tolerance to morphine at peripheral opioid receptors (Zollner, Mousa et al. 2008). Intra-articular morphine is an equally potent analgesic in patients with or without opioid-producing inflammatory synovial cells (Stein, Pfluger et al. 1996). Furthermore, opioid analgesia resulting from this neuroimmune interactions and occurring in peripheral tissue is devoid of central effects such as respiratory depression, nausea, dysphoria, addiction and tolerance. The continuous availability of endogenous opioids in inflamed tissue increases recycling and preserves signaling of μ-receptors in sensory neurons and thereby counteracts the development of peripheral opioid tolerance. Opioids peptides not only modulate pain transmission and sensitization but exert also an auto-regulation of sensory nerve function.

1.1.1.4.2. Endogenous morphine

Although the capacity to synthesize morphine was initially thought to be restricted to plants, animal cells have been demonstrated to carry out de novo synthesis of morphine via highly regulated enzyme-linked catalytic steps. The synthesis of morphine begins with a small molecule derived from L-tyrosine and proceeds via a strikingly similar biochemical pathway to that described in the opium poppy.

In addition to the two main μ-opiate receptor subtypes, μ_1 and μ_2, functional studies have provided biochemical, molecular and pharmacological characterization of two unique six-transmembrane helical domain (TMH) opiate receptors derived from the μ-opioid receptor gene (Stefano, Digenis et al. 1993; Stefano, Hartman et al. 1995; Cadet, Mantione et al. 2007). Designated μ_3- and μ_4-receptors, comparative phylogenetic analysis suggests that these six-TMH domain μ_3- and μ_4-receptors may be prototypic evolutionary models that have given rise to seven TMH domain μ-, δ-, κ- receptors. The μ_3- receptor is expressed on immunocytes and neural tissues of invertebrates, as well as on human monocytes, granulocytes and vascular endothelial cells. It has also been identified in human multi-lineage progenitor cells (MLPCs) derived from post-partum umbilical cord blood (Cadet, Mantione et al. 2007). The μ_3- receptor is selective for opioid alkaloids (morphine) but insensitive to opioid peptides; and maintains naloxone sensitivity, thus demonstrating its opioid receptor properties. A tissue that expresses μ3- does not express the μ1-opioid receptor subtype, and in each of these tissue types, the μ_3-receptor appears to be coupled to constitutive NO release (Cadet, Mantione et al. 2003). Cells transfected with μ3-receptor cDNA exhibit dose-dependent release of NO following administration of morphine but not opioid peptides, such as met-enkephalin and endomorphin. Opioid peptides endomorphin-1 and -2 and orphanin FQ do not bind to μ_3, nor does the synthetic phenylpiperidine μ_1-opiate agonist fentanyl. 6-glucuronide, but not the 3-glucuronide metabolite of morphine, binds to μ_3 (Stefano, Kream et al. 2008). This selectivity of the μ_3-receptor subtype supports therefore the hypothesis of morphine as an endogenous signaling molecule (Cadet, Mantione et al. 2003). Morphine stimulate constitutive NO release in

macrophages, granulocytes and various type of human endothelial cells by mechanisms that are antagonized by naloxone and NO synthase inhibition. NO has anti-inflammatory and anti-proliferative effects. Production of NO causes cytostasis and may reduce apoptosis resistance in cancer cells, thus leading to down-regulation of tumor growth. NO has been associated with anti-nociception, as well as tolerance and dependence. It has been suggested that basal NO serves to limit micro-environmental noise and maintain cells in a state of inhibition, thus sub-serving essential processes of cellular preservation during states of biological readiness (Stefano, Kream et al. 2008).

1.1.1.4.3. Endogenous regulation of cellular physiology

The observations that both invertebrates and mammals, organisms that diverged evolutionarily 500 million years apart, express opioid receptors in their nervous systems and can synthesize morphine de novo led scientists to pose **the questions of why these receptors are so widespread and why they have persisted throughout evolution.**

Recent observations may provide possible answers to the questions.

1. The anti-proliferative effects of morphinergic signaling. Morphine has been demonstrated to have an inhibitory effect on tumor growth and dissemination. Repeated morphine administration not only has clear anti-nociceptive properties, but also reduces cancer cell-induced bone lesions (El Mouedden and Meert 2007): both pre- and post-operative administrations of analgesic morphine doses reduce the spread of tumor cells (Page, McDonald et al. 1998; Payabvash, Beheshtian et al. 2006) and enhance human cytotoxic T-lymphocyte activity (Page, Ben-Eliyahu et al. 1994; Fuggetta, Di Francesco et al. 2005). Furthermore, only some human cancerous tissues contain morphine and express μ-receptors which modulate cell proliferation (Olsen, Rasmussen et al. 2005; Kream and Stefano 2006; Zhu and Stefano 2009). Interestingly, the synthetic phenylpiperidine μ_1-receptor opiate agonist fentanyl suppresses NK cell activity, increasing the risk of tumor metastasis (Stefano, Scharrer et al. 1996; Forget, Collet et al. 2010). The opposite pharmacological

effects of the naturally opioid alkaloid morphine and the synthetic opioid analgesic on NK cell activity linked to *in vitro* tumor progression may be functionally linked to their differential effects on μ_3- receptor activation. Opioid peptides appear to have proinflammatory properties, whereas opioid alkaloids like morphine may represent a class of immune and vascular anti-inflammatory factors that inhibit or down-regulate key factors. Therefore, morphine appears to be protective in many tissues in response to stress and cancer expanding (Stefano, Fricchione et al. 2005; El Mouedden and Meert 2007).

2. The neuroimmune protective response to combat an immediate non-cognitive threat. It has been demonstrated that the pro-enkephalin from invertebrates contain the antibacterial peptide enkelytin. The sequence of enkelytin exhibits a 98% sequence identical with mammalian enkelytin. The opioid peptides stimulate immunocyte chemotaxis and phagocytosis, while the secreted cytokines and simultaneously-liberated enkelytin attack bacteria immediately (Stefano, Scharrer et al. 1996; Stefano, Salzet et al. 1998). The co-processing and liberation of enkelytin and met-enkephalin may represent a unified neuroimmune protective response. Bacteria and viruses are persistent environmental factors that are threats to any organism. Such a unified response might therefore represent a successful survival strategy across diverse species (Stefano, Digenis et al. 1993; Cadet, Mantione et al. 2003). In fact, a similar phenomenon is found in humans: endogenous morphine plasma levels increase in patients undergoing coronary artery bypass grafting, arguing that morphine is part of the anti-inflammatory response to cardiac surgery (Brix-Christensen, Tonnesen et al. 1997).

3. The physiological excitatory rebound effect. The ambiguity of morphine's action (inhibitory versus facilitatory) could also be interpreted differently by considering the μ3-receptor subtype that is coupled to NO release, even in human stem cells (Cadet, Mantione et al. 2007). In tissues examined, including immune, basal morphine levels are low (Weitz, Lowney et al. 1986; Guarna, Neri et al. 1998). In stress studies, the levels of naturally morphine rise dramatically to insure their operation (Stefano 1998). Over the short term, morphine-

induced NMDA activation enhances NO levels and induces greater inhibition. Morphine appears down-regulating the immunocyte responsiveness. Following this down-regulating effect, immunocytes both *in vitro* and *in vivo* are more sensitive toward excitatory signal stimulation. Stefano poses an interesting hypothesis (Stefano, Esch et al. 2009; Stefano, Kream et al. 2009). This enhanced activation following the inhibition results as a rebound effect, which could be the mechanism underlying hyperalgesia observed following exogenous opioids administration. Once morphine's influence is over, the immune system cannot remain in an inhibited state. Enhanced sensitivity allows an immunocyte "resetting" in alert state. This enhanced immunocytes response serves a vital immune function that has survival value as noted by the diverse cells which exhibit this phenomenon, e.g. immune, vascular, and neural (Cadet, Mantione et al. 2003; Pryor, Zhu et al. 2005; Stefano, Cadet et al. 2008). Therefore, hyperalgesia could represent a physiologically-relevant phenomenon that allows organisms to have enhanced pain sensitivities following a depression of pain sensitivity and to return at basal levels.

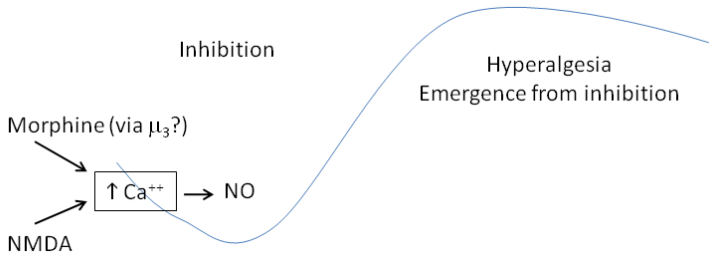


Figure 7: a model of cellular morphine expression
The inhibition is followed by enhanced sensitivity. Both morphine and NMDA receptor activation may increase intracellular Ca^{++} transients, promoting the stimulation of NO synthase activity and subsequent NO release. NO induces cellular inhibition (cell down-regulatory activity). Following this inhibition, there is a rebound period that may represent hyperalgesia
Data adapted from Stefano, 2009

From this point of view, cellular and physiological effects associated with the pharmacological administration of morphine and related alkaloids may be re-evaluated. If morphine acts to continually down-regulate activity, morphine tolerance may be a mechanism to ensure

that the continued presence of morphine does not prevent an excitatory state, which may be required to overcome a traumatic event (Stefano, Kream et al. 2009). This view is important to further understand the role of endogenous opioids and opioid receptors in tolerance to pain and in drug addiction.

Summary

Endogenous opioids and morphine participate in physiological processes, making their important chemical messengers. Several functional and evolutionary linkages between cellular inhibition and facilitation, nociception and anti-nociception can been observed.

The association of opioid peptides and enkelytin in proenkephalin suggest that, evolutionarily, the opioid pentapeptides may have originated as immune-signaling molecules. A functional relationship between endogenous opioids and the immune system is apparent.

Opioid receptors have been demonstrated on peripheral endings of sensory nerves. In injured tissue, opioid peptides (e.g., β-endorphin, met-enkephalin) mediate anti-nociceptive and anti-inflammatory effects via a well-orchestrated series of events. Opioids peptides are continuously released and counteract hyperalgesia elicited by many known pro-inflammatory agents present in inflammation.

In addition to expressing the well-known endogenous opioid peptides, animal cells have the ability to carry out *de novo* synthesis of morphine. Endogenous morphine is thus considered to be a hormone or neurotransmitter that down-regulates the activity of immune, vascular, neural and gut tissues under normal circumstances and following traumatic injury situations. Morphine in some cancerous tissues can modulate cell proliferation. Furthermore, the cellular "morphinergic"/NO-coupled regulation of intracellular Ca^{++} signaling mediated by μ_3- receptors involves cellular regulatory effects via highly selective binding of morphine-related opioid alkaloids with strict exclusion of all classes of endogenous opioid peptides.

Endogenous opioid peptides and alkaloid morphine are important because, to survive and reproduce, organisms had to evolve processes to combat non-cognitive threat and maintain cells in states

of biological readiness. Neuroimmune activation may interact with opioid peptide analgesia in peripheral inflamed tissues. Endogenous morphine interacting with the protective NOS-dependant cellular pathway may initiate a regulatory signaling cascade with anti-proliferative, apoptotic and anti-neoplastic properties. **The question remains of what happens when high concentrations of exogenous morphine or opioid agonist are administrated. Does this exclusively infer potent analgesia?**

1.1.*1.5.* Opioids in perioperative medicine: concept of balanced anesthesia

The concept of balanced anesthesia was first introduced by a surgeon (Crile 1913) through his theory of anoci-association as a technique to reduce surgical stress and improve postoperative states. He hypothesized that psychological stimuli associated with surgery could be prevented by association of light general anesthesia and infiltration of local anesthetics to block noxious impulses arising from the surgical wound. The term "balanced anesthesia" was introduced in 1926 by Lundy, who used a combination of premedication, regional and general anesthesia to produce pain relief while maintaining unconsciousness via a balance of agents. With the introduction of curare in 1942, anesthesia with controllable muscle relaxation was obtained without the need for very deep levels of anesthesia. Muscle relaxation became one of the essential components of the anesthetic state, defined as narcosis, analgesia and muscle relaxation by Gray and Rees (Gray and Rees 1952).

In 1957, Woodbridge expanded the definition of anesthesia to include abolition of autonomic reflexes. Four modalities (motor, sensory, mental and reflex) are affected by the different components of anesthesia via muscular relaxation, analgesia, amnesia, and autonomic reflex abolition, respectively, while maintaining homeostasis (Woodbridge 1957). Woodbridge proposed different patterns of depth and signs of anesthesia. Moreover, he instructs anesthetists to choose the best drug according to their particular aim: "Before each operation and before each phase of each operation the anesthetist needs to ask himself: how much sensory blocking is needed? How much relaxation is needed? How much blocking of

reflexes is needed? How much mental blocking is needed? And with what drug will I produce each of these actions? He may decide on a single drug, which combined with suitable preliminary medication, will cover the needs of the operation, with a wide margin; or, on the other hand, he may fit the effect more closely to the need in each component by using drugs having more specific actions".

Combination of halogenated vapor or thiopental and nitrous oxide often allowed non-efficacious analgesia to prevent a rising pulse rate or blood pressure or other similar reactions to painful stimuli. A great improvement was made in 1947 by introduction of the opioid analgesic *pethidine* into the combination (Mushin and Rendell-Baker 1949). This technique rapidly became popular, and other opioids were introduced. The inclusion of an opioid as a component of balanced anesthesia offered several advantages: hemodynamic stability (Grell, Koons et al. 1970), decreased requirement of inhaled anesthetics (Hecker, Lake et al. 1983) and improved postoperative analgesia.

The use of opioids during anesthesia was largely generalized around 1960 when Paul Janssen and other researchers discovered fentanyl, a piperidine derivative and pure μ-agonist. Fentanyl has now been in use for over 30 years and remains the most widely-used opioid in surgery. The pharmacodynamics and pharmacokinetics of its molecular design define fentanyl as one of the most powerful analgesic drugs, combined with high selectivity and low toxicity. In addition, the ability of large doses of fentanyl to inhibit hyperglycemia, the cortisol and growth hormone response to surgery, serves to ameliorate the catabolic response to trauma (Hall, Young et al. 1978).

Because of their hemodynamic stability properties (Bennett and Stanley 1979) and ability to inhibit the stress response to surgical trauma (Hall, Young et al. 1978), opioid analgesic drugs were largely recommended for use in a surgical context, particularly in cardiovascular surgery, where they decrease sympathetic and somatic responses to noxious stimulation without negative inotropic effects, even at high doses and in patients with impaired cardiac function. Fentanyl and its analogue sufentanil (Rosow 1984; Flacke, Bloor et al. 1985), which has a more rapid onset and shorter duration

of action, are the most potent μ-agonists known and are still widely-used as adjuncts for surgical anesthesia.

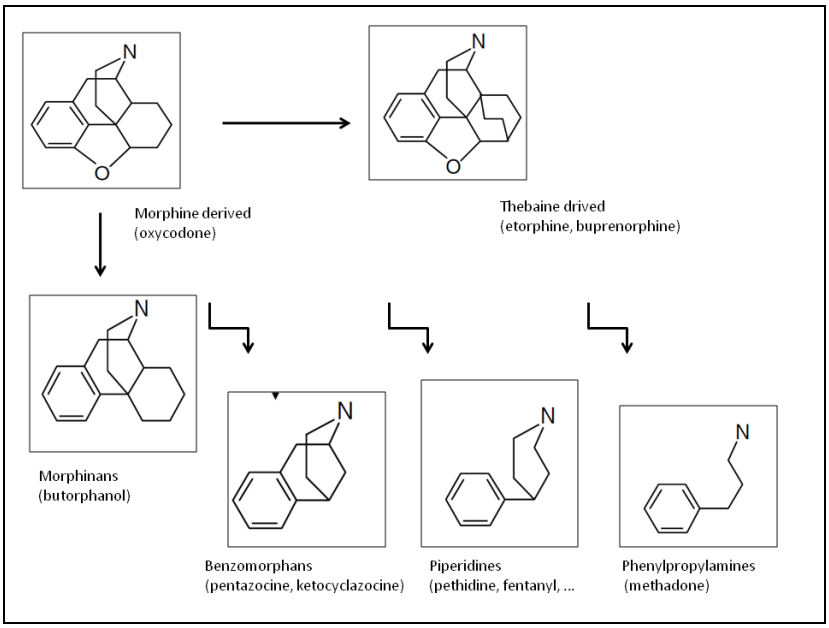


Figure 8: progressive simplification of opiates
Most clinically available opioid analgesics are μ-agonists derived from chemical templates and are related to natural opium alkaloids. There is a progressive simplification through the morphinans to the benzomorphans and the piperidines, and to the phenylpropylamines such as methadone
Adapted from Corbett, 2006

A shorter-acting analogue of fentanyl, *alfentanil*, has been achieved by substitution of an ester group (Feldman, James et al. 1991). The newest and probably the most original analogue, however, is *remifentanil* (Egan 1995). Remifentanil is rapidly metabolized by blood and tissue esterases, has an elimination half-life of less than 10 min and does not accumulate in tissues. Consequently, it requires continuous infusion in a perioperative setting.

Unfortunately, the attractive concept of “stress-free anesthesia” was not maintained over the long-term, and the clinical benefit of high-dose opioids became uncertain (Bovill, Sebel et al. 1984). Moreover, introduction of the ultra-short-acting opioid remifentanil in anesthesia strengthened the notion of acute tolerance to opioid effects and confirmed that hyperalgesia can be induced by opioid agonists (Cox,

Ginsburg et al. 1968; Michelsen and Hug 1996; Vinik and Kissin 1998; Guignard, Bossard et al. 2000).

Opioids	μ	δ	κ
Morphine, Codeine *Oxymorphone* *Dextropropoxyphene*	+++	+	+
Methadone	+++	-	-
Pethidine (Meperidine)	++	+	+
Fentanil *Sufentanil* *Alfentanil* *Remifentanil*	+++	+	-
Buprenorphine	*(+++)*	-	*[++]*
Pentazocine	*[+]*	+	++
Nalbuphine	*[+]*	+	*(++)*
Nalorphine	*[++]*	-	*(++)*
Naloxone	*[+++]*	*[+]*	*[++]*

Table 3: selectivity of opioids for receptors
Advances in opioid chemistry introduced molecules interacting with different receptors and so producing a wide spectrum of effects
+ agonist; [+] antagonist; (+) agonist partial
Adapted from Beaulieu, 2005

Summary

Opiates were largely used in anesthesia for their attractive properties in the surgical context: powerful and selective analgesia, decreased sympathetic and somatic responses to nociceptive stimulation and hemodynamic stability even at high doses. However, these attractive properties always co-occur with bothersome side effects (respiratory depression, urinary retention, pruritus, sedation, ileus…) including the paradoxical effect of pain hypersensitivity (hyperalgesia).

1.1.*2.* Physiopathology of pain and opioid modulation

1.1.*2.1.* A protective system

Pain, “an early warning system signaling the production of damage”, plays an essential role in human behavior and is defined by the International Association for the Study of Pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”. This subtle definition implies a complex system and recognizes that both perception and experience of pain are multi-factorial.

Acute pain has a perceptual pattern consisting of a trigger that evokes the expected *physiological reactions* following the activation of the nociceptive pathway by an appropriate stimulus, such as an increase in heart rate and blood pressure, perspiration, spinal reflexes and heightened vigilance. Moreover, this immediate sensorial reaction of the nociceptive system following the exposure of the skin or other organs to potentially-damaging injury is considered to be an *alarm signal to protect the organism* via activation of spinal reflex withdrawal mechanisms.

Pain is the result of *complex interactions* among physiological, biochemical and psychological mechanisms that involve most parts of the PNS and CNS. The afferent transmission of pain from the periphery to higher areas, where the conscious phenomenon of pain arises, depends on the integration of *three levels of the nervous system* (Millan 1999; Woolf and Salter 2000; Zeilhofer 2005; DeLeo 2006). It is possible to identify *first-order neurons* localized at the level of the DRG and trigeminal ganglia; *second-order neurons* located in the posterior horns of the spinal medulla, which converge at the level of the anterior commissure to ascend through the spinothalamic tracts; and *third-order thalamic neurons* that project into the primary somatosensory and cingulate cortices. The painful stimulus is transmitted from the periphery to the spinal medulla and brainstem by myelinated Aδ and small-diameter unmyelinated C-fibers. These axons transduce and propagate noxious stimuli from peripheral tissues (such as skin, muscles, joints and viscera) to the dorsal horn

of the spinal cord. The third type of sensory fibers is myelinated and fast conduction Aβ-fibers. They transmit low-intensity signals such as innocuous, mechanical stimulation of the skin. Under normal circumstances, only C and Aδ-fibers transmit nociceptive information.

A large range of mediators are involve in the excitatory and sensitization of primary afferent nerve fibers (PAFs). The process of peripheral sensitization may involve direct activation of the peripheral nociceptor through ligand-gated ion channel interaction (ATP, 5-HT, SP, bradykinin, histamine, VIP, CGRP, NO, glycine) or through indirect activation by inflammatory mediators such as cytokines, growth factors and the phospholipid metabolites, prostaglandins (PGs). Many peptides present at the peripheral terminals of PAFs are also present in their central terminals: adenosine, NO, glutamate, aspartate, cholecystokinin (CCK), neuropeptide Y, SP. The activity of the dorsal horn neurons is modulated by inhibitory inputs, mediated by GABA (released by interneurones) and glycine. Descending pathways originated from the brainstem induce the release of inhibitory noradrenergic and serotoninergic transmitters and influence also the pain process (Zeilhofer 2005).

Synaptic transmission between primary afferent nerve fibers (PAFs) and dorsal horn projection neurons is not a fixed process but is subject to dynamic control by local interneurons, descending pro- or anti-nociceptive pathways and chemical mediators released from neurons, as well as inflammatory mediators such as cytokines and glial cells (Suzuki and Dickenson 2005). Maladaptive neuroplastic events within the three levels of central neurons, as a consequence of pathological damage to their afferents, may be becoming a key factor in the genesis and maintenance of pathologic pain (Woolf and Salter 2000).

1.1.*2.2.* Physiological and pathological pain

Neuronal changes that lead to increased peripheral responses, including broadening of receptor fields and increased excitability, create a state defined as "central sensitization". This increased responsivity can be observed at the site of the lesion (primary hyperalgesia) and in surrounding areas (secondary hyperalgesia) and

is sustained by functional changes in both the PNS and CNS. Conditions in which hypersensitivity is known to occur include spontaneous pain; allodynia, defined as pain due to a stimulus that does not normally provoke pain; and hyperalgesia, defined as an increased response to a stimulus that is normally perceived as painful. The role of the sensory nervous system is to interpret stimuli from the outside world, and simultaneous presentation of multiple sensory inputs would create chaos without filtering to focus resources on the most important stimuli; physiological pain serves to focus attention on stimuli that may cause injury or even mortality.

The term “central hypersensitivity” describes a functional alteration in the processing of afferent impulses from the periphery, whereas the concept of “central structural neuroplasticity” relates to the development of new synaptic connections. In both cases, the result is an increase in the sensitivity of central neurons to stimuli from the periphery. Central hypersensitivity can develop under physiological conditions; in this case, it is considered a normal and reversible *physiological* response of the undamaged nervous system. Conversely, central hypersensitivity is considered *pathological* only if it persists after removal of the painful stimulus that induced it.

Glutamate plays a major role in the process of central sensitization and can produce hyperexicitability throughout the neuraxis. This excitatory neurotransmitter can act at both N-methyl-D-aspartate (NMDA) and non-NMDA ionotropic receptors, as well as at metabotropic receptors. Under conditions of persistent injury, C-fibers fire repeatedly, and the dorsal horn (DH) response increases. This phenomenon, referred to as *wind-up,* is dependent upon glutamate release (Sandkuhler 2009) and represents an acute form of pain amplification that occurs during rapidly repeated stimulation of the skin by a noxious stimulus (i.e., temporal summation).

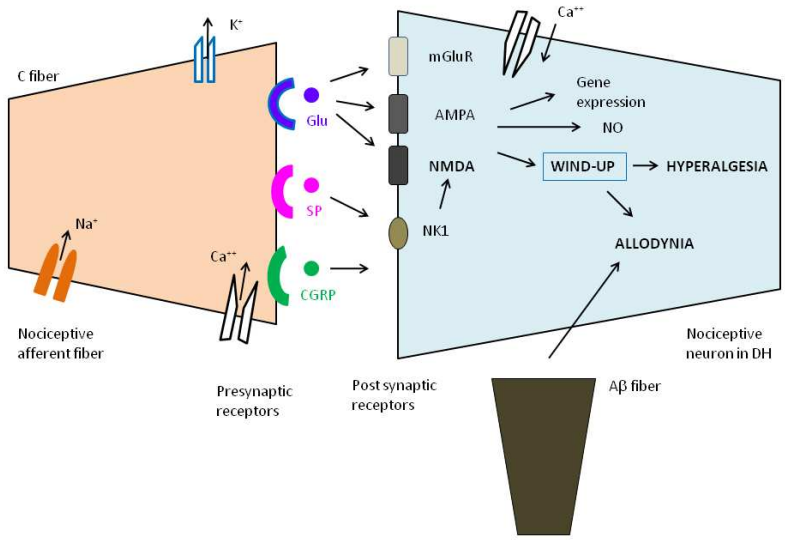


Figure 9: central sensitization
Central sensitization is initiated when prolonged activation of nociceptors in DH produces the release of Glu, as well of SP and CGRP. Sustained activation of NMDA and NK-1 (neurokin) receptors induces intracellular cascades, which decrease the pain threshold and causes hyperalgesia and allodynia
Adapted from Beaulieu, 2005 and Woolf 2004

Brief but intense nociceptor activity is required to initiate central sensitization. The process may be produced by sensitized nociceptors during inflammation, by spontaneous ectopic activity generated in sensory neurons after nerve injury or by a surgeon creating an incision through the skin. Central and peripheral sensitization are the major causes of hypersensitivity to pain after surgery. Central sensitization begins in the DH of the spinal cord. Release of transmitters from nociceptor terminals triggers a cascade of events that induces alterations in synaptic receptor density, threshold, kinetics and activation and thus dramatically increases transmission of pain. One key receptor involved in these changes is the glutamate-activated NMDA receptor. During sensitization, the NMDA receptor is phosphorylated, which increases its sensitivity to glutamate. Moreover, changes occur in gene transcription, induction of new proteins, and in expression levels of existing proteins (Woolf 2004).

Furthermore, strong evidence indicates that these cellular mechanisms are involved in opioid-induced pain sensitivity (Mao 2002).

1.1.*2.3.* Modulation of nociceptive transmission

Nociceptive messages impinging upon the DH of the spinal cord from skin, viscera and other tissues are not automatically transferred to higher centers, but are profoundly modified prior to being dispatched to supra-spinal centers. Many diverse mechanisms are recognized to be involved in filtering and modifying nociceptive transmissions in the DH and elsewhere, but they are far from being completely understood.

Many classes of DH neurons, efferent fibers and PAFs themselves exert powerful modulating influences upon the transfer of nociceptive information from the spinal cord to the brain.

In the **periphery**, the voltage-gated transient receptor potential (TRP) channels constitute a large family with a wide range of physiological functions, including nociceptive transmission. One member of this family, the transient receptor potential vanilloid-1 (TRPV1), plays an important role in development of the inflammation-induced hyperalgesia (Caterina and Julius 2001) and in the expression of morphine-induced hyperalgesia (Vardanyan, Wang et al. 2009). In inflamed tissue, opioid peptides (e.g., β-endorphin and met-enkephalin) mediate anti-nociceptive and anti-inflammatory effects via a well-orchestrated series of events. Opioid peptides are continuously released and counteract hyperalgesia elicited by many known pro-inflammatory agents (Stein and Zollner 2009).

The **DH in the spinal cord** is the main site of synaptic integration in the pain pathway. The spinal cord is critical in pain modulation and is also implicated in pathologically exaggerated pain sensations. Within the DH itself, three types of neurons have been classified based on their responses to nociceptive input. The first class comprises the *non-nociceptive neurons.* The second class are typically *silent, nociceptive-specific neurons*, which have limited stimulus-encoding capability and are activated exclusively by noxious stimuli via C- and

Aδ-fibers. The third are *multi-receptive neurons* that can produce a dynamic response over a broad stimulus range from innocuous to noxious and are therefore called *wide dynamic range (WDR) neurons. WDR* neurons possess complex receptive fields and react to both innocuous and noxious stimuli with excitation, whereas large fibers from the surrounding regions react to non-noxious stimuli with inhibition. This phenomenon is known as the “gate control theory,” and it accounts for the pain-relieving effect of additional external stimuli (as in trans-cutaneous electrical nerve stimulation, *TENS*). If the large myelinated Aβ fibers are stimulated, they activate an inhibitory interneuron, which in turn inhibits the activity in the C-fiber and projection neuron, cutting of the pain signal. The mechanisms of this modulation are complex and probably involve several classes of neurotransmitters. Evidence suggests that spinal components may be activated or augmented after either inflammation or peripheral nerve injury to contribute to a general increase in sensory transmission.

The **descending pathway** is a combination of descending inhibition (DI) and descending facilitation (DF). Intriguingly, the substrates controlling these processes are separated anatomically, and the stimulation of a single supraspinal structure may simultaneously trigger both DI and DF. Furthermore, a single neurotransmitter may, via divergent actions at different receptor types, concomitantly promote and suppress nociceptive transmission in the DH; this serves as a supplemental example of the complexity and sophistication of the pain control pathway. Modulation of the descending pathway originates from the mesencephalic periaqueductal gray (PAG), rostral ventromedial medulla (RVM), hypothalamus, amygdala, parabrachial nucleus, nucleus tractus solitarius, locus coeruleus and raphe nucleus. The PAG is closely associated with areas of the brainstem, including the RVM, and these regions are critical in descending modulation of spinal activity through serotonergic and noradrenergic pathways, respectively. PAG and RVM can exert both DI and DF influences on the spinal cord (Suzuki and Dickenson 2005; D'Mello and Dickenson 2008). Diffuse noxious inhibitory controls (*DNIC*) provide a neural substrate to explain the observation that pain can inhibit further pain (Le Bars, Dickenson et al. 1979; Le Bars 2002). For example, a noxious stimulus applied to one part of the body inhibits the activity of nociceptive neurons in the spinal cord with

receptive fields outside of the stimulated area (Figure 10). In some cases, RVM may enhance spinal mechanisms of pain independently and have an excitatory influence on development and maintenance of persistent pain states (Porreca, Ossipov et al. 2002). RVM has been implicated in fentanyl-induced hyperalgesia (Rivat, abstract Neuroscience Oct 2006). Moreover, ascending and descending pathways may form a loop that ultimately underlies hypersensitivity in injury-induced and opioid-induced hyperalgesia (Hunt and Mantyh 2001).

At the **supraspinal level**, complex interactions and functional projections among the thalamus and cortical and subcortical areas underlie evaluation of stimulus characteristics that are critical for deciding how to respond. Variability in pain expression among healthy subjects depends on a complex neuroanatomical circuit that integrates different motivational, cognitive and affective components of the pain experience (Almeida, Roizenblatt et al. 2004). Placebo analgesia recruits a descending opioidergic pain control system. The brain regions associated with this pain modulation include the dorsolateral prefrontal cortex, rostral anterior cingulate cortex and PAG (Eippert, Bingel et al. 2009). Hypnotic suggestions activate cortical and subcortical regions. It is proposed that both hypnosis and DNICs influence the same descending pathways to modulate pain perception (Sandrini, Milanov et al. 2000; Wobst 2007).

At all levels of the pain transmission, both inhibitory and facilitatory influences may modulate the integration of nociceptive information.

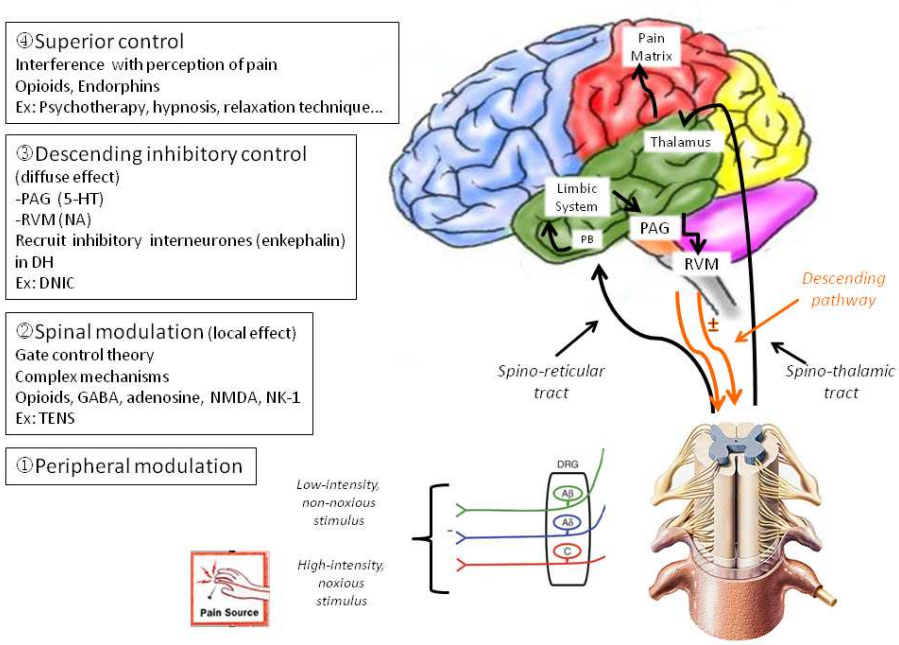


Figure 10: modulation of nociceptive transmission
Physiological pain is an essential warning mechanism to minimize tissue damage. Peripheral terminals of Aδ and C-fibers transduce and propagate noxious stimuli from peripheral tissues to the DH of the spinal cord and then the brain. At spinal and supraspinal sites, integration of signals from pro-algesic neurotransmitters, as well as environmental and cognitive factors, eventually results in the sensation of pain. Endogenous mechanisms that counteract pain development exist in the brain and spinal cord and consist of descending pain inhibitory pathways, which contain mostly opioid peptides, NA (noradrenaline) and 5-HT (serotonin). Similar counter-regulatory mechanisms are also induced in inflamed peripheral tissues, e.g. anti-inflammatory cytokines or interactions between leukocyte-derived opioid peptides and peripheral nociceptor endings expressing opioid receptors
Adapted from D'Mello, 2008, DeLeo, 2006, Suzuki 2005, Millan, 2002

1.1.*2.4.* Three areas of pain modulation

Peripheral, spinal and supraspinal sites can be targeted to modulate pain. Specifically, pain can be attenuated or suppressed by:

1. intervention at the periphery with the use of non-steroidal anti-inflammatory drugs (NSAIDs), steroids, opioids, regional analgesia or neural ablative procedures;

2. activation of inhibitory processes in the spinal cord and brain by opioids, α_2-adrenergic agonists (e.g., clonidine) or tricyclic antidepressants; and
3. interference with the perception of pain. Perception varies between individuals, but equally importantly, pain varies widely within an individual depending on the situation in which the nociceptive insult occurs. For example, despite greater severity of the wounds, analgesic requirements of victims during catastrophes or soldiers during World War II were far less than those of civilians undergoing elective surgery, demonstrating that the stress of life-threatening situations markedly impacts pain perception and tolerance (Beecher 1946).

For better targeted pain management, identification of the origin of a particular pain is important (e.g., peripheral *versus* central; inflammatory neuropathic *versus* functional neuropathic).

1.1.*2.5.* Opioid receptors

The opioid receptors are localized to all strategic sites of nociceptive signaling system. The mechanisms of action of opioid-induced analgesia are mediated by effects at spinal and supraspinal sites, as well as peripheral sites.

Opioid receptors are widely distributed throughout the brain and spinal cord. Receptor binding studies have found high densities in regions such as the caudate-putamen (in striatal patches and in the subcallosal streak), thalamus, interpeduncular nuclei, locus coeruleus, nucleus of the solitary tract and DH. Receptors are greatly enriched in the PAG, where electrical stimulation produces analgesia that is blocked by naloxone. The medial thalamus, which conveys information about emotional components of pain to the cerebral cortex, has a higher receptor density than the lateral thalamus. Limbic regions of the brain that regulate emotional behavior, such as the amygdala, also possess high opioid receptor density. Recently, the anterior cingulate cortex has been identified as a brain region with a major impact on opioidergic pain modulation (Snyder and Pasternak 2003; Baumgartner, Buchholz et al. 2006). Autoradiography has

revealed extremely dense concentrations of opioid receptors in brain nuclei, including: the locus coeruleus, the source of the major noradrenaline-containing cell bodies in the brain; the substantia gelatinosa of the spinal cord and brain stem; and vagal nuclei, such as the nucleus ambiguus and nucleus solitarius. Differences in receptor distribution are reported according to gender and painful disease states (Henriksen and Willoch 2008). Opioid receptors are implicated in ascending and descending pain pathways, where they may have an indirect effect by stimulating GABAergic inhibitory neurons, which then inhibit release of SP and glutamate. In the PNS, opioid receptors are found on PAFs, in the dorsal root ganglion (DRG) and on immune cells.

The powerful analgesic effects of opioids are due to complementary pre- and post-synaptic mechanisms that inhibit nociceptive transmission. Pre-synaptically, opioids block Ca^{++} channel-mediated release of excitatory neurotransmitters. Post-synaptically, opioids induce a cellular hyperpolarization, which is mediated by increased K^+ conductance via enhanced Gi protein coupling. Moreover, opioids act both at spinal and supraspinal sites and that synergistic interaction also account for the high effectiveness of opioid-mediated analgesia. Opioid receptors localized in the DH fulfill a crucial role in the mediation of anti-nociception. These segmental receptors operate in synergy with their supraspinal counterparts. Furthermore, descending noradrenergic pathways mediate supraspinal opioidergic anti-nociception, and spinal α_2-adrenoceptors (ARs) contribute to the "multiplicative" interaction between cerebral and spinal populations of opioid receptors. Opioids and α_2-ARs share common intracellular mechanisms, notably suppression of adenylyl cyclase activity and positive and negative influences upon K^+ and Ca^{++} currents, respectively (Eisenach, De Kock et al. 1996; Millan 2002). While minimal cross-tolerance between anti-nociceptive properties of α_2-AR agonists and opioids in the DH has been reported, the synergism between clonidine and morphine is maintained following induction of tolerance to morphine (Fairbanks and Wilcox 1999). This may suggest fundamental differences in cellular function during development of tolerance (Smith and Elliott 2001).

Summary

Physiological pain is an essential warning system necessary to minimize tissue damage and to promote survival. The nociceptive message is encoded as an extraordinarily complex and interactive series of mechanisms, which progressively transmit the message from peripheral to higher nervous centers. At each level of transmission, the nociceptive information is modulated. This modulation may be inhibitory or facilitatory. Changes within the spinal and descending modulatory networks (i.e., reduction of inhibitory pathway and/or enhancement of facilitatory pathway) have been implicated in pathological pain dysfunction. Thus, prevention of such facilitation may reduce hypersensitivity to pain. Moreover, strong evidence suggests that some cellular changes and neural circuit impairments underlying the development of pathological pain may also be implicated in the development of opioid-induced pain sensitivity (Mao 2002).

Opioids produce analgesic effects through their modulatory role in nociceptive transmission. Opioid receptors are expressed throughout that transmission system and are localized at all strategic sites of nociceptive signaling.

If OIH requires activation of opioid receptors, which are located at all pain transmission levels, the question should be whether OIH mechanisms exist at all these levels?

1.1.*3.* Summary of chapter 1.1.

Since the isolation of morphine from opium, researchers have undertaken efforts to synthesize opioids, and pure μ-agonists are now available. Opioids are largely used in so-called balanced anesthesia for their attractive properties, such as powerful analgesia, decreased sympathetic and somatic responses to noxious stimulation and hemodynamic stability even at high doses. Specifically, opioids can induce a potent anti-nociceptive effect by activating their G-protein-coupled receptors (GPCRs), thus triggering intracellular signaling and modulating pain transmission. Opioid receptors are expressed throughout the pain transmission system and are localized at all strategic sites of pain signaling

The existence of endogenous opioid peptides and of different types of opioid receptors has been demonstrated and confirmed by gene cloning. Endogenous opioids and the natural opioid alkaloid morphine serve as important chemical messengers in normal physiological processes. Several functional and evolutionary links between opioid-induced inhibition and activation have been observed at the cellular level. For example, opioid peptides can auto-regulate sensory nerve function and modulate pain transmission.

Pain is the result of complex interactions among physiological, biochemical and psychological mechanisms that involves most parts of the PNS and CNS. Pain may be considered to be a protective system. At each level of pain transmission, modulation of the nociceptive signal may occur. From the periphery to the cortex, mechanisms exist to facilitate or inhibit nociceptive signaling, and it is this inherent plasticity that is thought to be perturbed in chronic pain states. Strong evidence suggests common cellular changes and neural circuit impairments underlying pathological pain-induced hypersensitivity and opioid-induced pain.

Therefore, we should question what happens when exogenous opioid agonists are administrated in pathological pain. Does this provoke potent analgesia and/or exacerbate an underlying increased excitability?

1.2. Opioids and hyperalgesia

1.2.*1.* Tolerance and opioid-induced hyperalgesia (OIH)

1.2.*1.1.* Definition

Besides their analgesic effects, opioids can induce dependence, tolerance and hyperalgesia. Dependence and tolerance are well-known phenomena after chronic pain therapy or in individuals who become addicted to opioids (Compton 1994; Compton, Charuvastra et al. 2001). However, opioid administration can also produce hyperalgesia, termed OIH (Fallon and Colvin 2008; Silverman 2009).

Tolerance refers to a phenomenon in which exposure to a drug results in diminution of the effect or need for a higher dose to maintain the effect. Tolerance is a pharmacological concept related to desensitization of the anti-nociceptive opioid pathway. Tolerance may develop not only to the analgesic effects but also to undesirable effects that are observed with opioid administration, such as pruritis, nausea, sedation and respiratory depression.

In contrast, **OIH** is defined as a paradoxical response to an opioid agonist, whereby instead of an analgesic effect, a pro-nociceptive effect occurs. Thus, there is an increase in perceived pain (sensitization). Hyperalgesia is defined as an exacerbated painful response to noxious stimuli. Allodynia is a related phenomenon, defined as pain elicited by innocuous stimuli (IASP Pain Terminology).

The development of OIH is closely linked to the development of pharmacological opioid tolerance. Both are initiated by opioid administration and could contribute to the manifestation of apparent opioid tolerance.

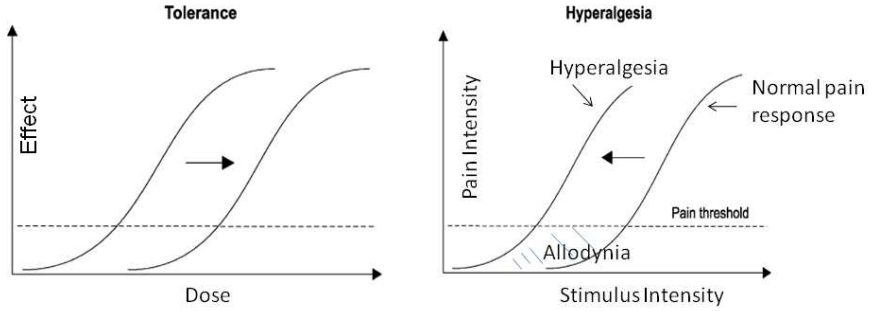


Figure 11: tolerance and hyperalgesia to opioids
Tolerance is characterized by decreasing analgesic effect and requires increasing the dose of opioid to get the same analgesic effect. Hyperalgesia is characterized by a left-ward shift of the stimulus. A normally non painful stimulus becomes noxious (allodynia) and normally painful stimulus increases in intensity (hyperalgesia)
Adapted from Koppert, 2007

1.2.*1.2.* Clinical implications

While the definition of OIH as a paradoxical increase in pain secondary to opioid administration seems relatively clear, the situation is much more complex in clinical practice. Distinguishing between tolerance, OIH and enhanced pain caused by malignancy recurrence or infection remains a clinical challenge. When opioid tolerance is apparent, dose escalation has been a logical approach to restoring effectiveness of opioid analgesics. Of course, this approach is effective only if tolerance has in fact developed; if apparent opioid tolerance instead is due to OIH, dose escalation could enhance the pro-nociceptive process and will exacerbate pain. Observations that may suggest the presence of OIH include: failure of previous opioid dose escalation to provide the expected analgesic effect and inexplicable exacerbation of pain after an initial period of effective analgesia (Mao, 2008).

The differentiation between OIH and tolerance in clinical setting will require clinical trials to directly assess pain sensitivity (Wilder-Smith and Arendt-Nielsen 2006). OIH can be differentiated from pre-existing pain because it is less defined and more diffuse, extending to areas beyond the region that was initially affected.

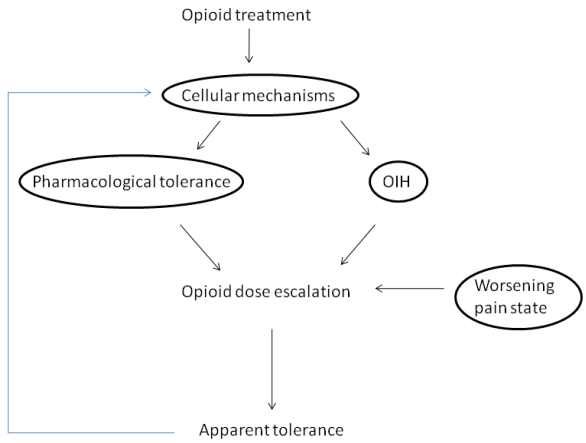


Figure 12: opioid dose escalation aggravates pain state
Apparent clinical opioid tolerance results from pharmacological tolerance, a worsening pain and/or opioid-induced pain sensitivity
Adapted from Mao, 2008

The neurobiology of OIH is complex and involves more than one mechanism. Interestingly, some neural processes of opioid tolerance and OIH may interact with mechanisms of pathological pain, such that opioid administration can exacerbate pathological pain, at least in animal models (Mao, Price et al. 1995; Mao 2002; Angst and Clark 2006).

OIH is detrimental in the postoperative period for the following reasons:

1. Induced hyperalgesia may amplify postoperative pain (Aubrun, Langeron et al. 2003). Increased pain sensitivity is detrimental in the postoperative period because it has a negative impact on patient's rehabilitation (Breivik 1998; Apfelbaum, Chen et al. 2003).

2. OIH and CNS sensitization are interrelated phenomenons. Increased excitability may induce permanent modifications in the CNS, leading to the development of persistent pain. Abnormal persistence of nervous system sensitization is now considered to be a risk factor for the development of chronic pain (Woolf and Salter 2000; Lavand'homme, De Kock et al.

2005). Moreover, it is worth noting that both preoperative pain increase the vulnerability to post-surgical chronic pain development due to central facilitation. Preoperative pain is associated with differences in central sensory processing and is currently one of the best predictors of severe pain in the early postoperative period (Caumo, Schmidt et al. 2002; Wilder-Smith, Tassonyi et al. 2002; Kalkman, Visser et al. 2003).

By consequences, does OIH increase perioperative pain?

The following sections will review: the evidences supporting the existence of OIH in humans, especially after surgery (Chapter 1.2.2.1), the evidence of OIH in animal studies (Chapter 1.2.2.2) and the results from electrophysiological recordings (Chapter 1.2.2.3). Finally, the underlying mechanisms suspected to be involved in OIH will be reviewed (Chapter 1.2.3).

1.2.*2.* Clinical observations and experimental settings

1.2.*2.1.* Human evidence

1.2.2.1.1. Measurement of pain thresholds

A majority of clinical trials assess the subjective experience of pain either directly with the evaluation of pain intensity using a visual analog scale (VAS) or indirectly with analgesic consumption. The reliable diagnosis of hyperalgesia is difficult and can't rely on clinical symptoms only. Its detection should be based on comparison of stimulus-response curves before and after nociceptive stimulus or drug application. Therefore, the quantification of hyperalgesia requires the formal determination of stimulus-response curves under standardized conditions, a process termed quantitative sensory testing (QST). The use of QST is a more accurate method of measuring pain, as it allows for detection and quantification of nociceptive neuroplasticity (i.e., hyperalgesia). It is worth noting that the association between QST-demonstrated hyperalgesia and clinical pain measures is consistently weak (Wilder-Smith 2000; Wilder-Smith, Tassonyi et al. 2003). The weakness of this relation is not surprising according to the subjective complexity and wide interindividual variability of pain. Moreover, association of both measures may provide different but complementary information (Wilder-Smith, Tassonyi et al. 2003).

QST may be based on different stimulations: electrical (Wilder-Smith, Tassonyi et al. 2002; Wilder-Smith, Tassonyi et al. 2003), thermal (Chen, Malarick et al. 2009; Aasvang, Brandsborg et al. 2010), or mechanical stimulations. The latter are made by using either a pressure algometer (Luginbuhl, Gerber et al. 2003; Joly, Richebe et al. 2005) or by application of von Frey filaments (Joly, Richebe et al. 2005; Lavand'homme, De Kock et al. 2005; Lavand'homme, Roelants et al. 2008). The use of mechanical stimuli for QST seems more accurate in perioperative setting because postoperative pain is mostly "mechanical by nature" i.e. mostly caused by mechanical stimulations such as cough, movements. Mechanical QST measures are realized at different sites which include the wound (primary hyperalgesia),

close to the wound (secondary hyperalgesia) and distant from the wound (generalized effects, supraspinal inhibitory pathway). Mapping of the area of punctuate mechanical hyperalgesia surrounding the wound correlates with the level of central sensitization (De Kock, Lavand'homme et al. 2001). Mechanisms for central sensitization which underlies the development of secondary hyperalgesia may be separated from those involved in the ongoing pain and primary hyperalgesia, explaining that clinical drugs that modulate punctuate secondary hyperalgesia do not necessary affect pain scores and postoperative analgesic requirements in patients.

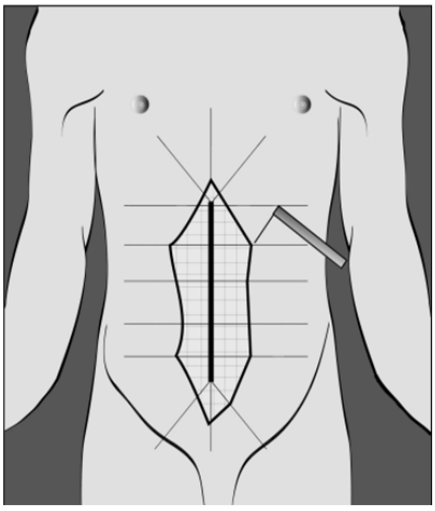


Figure 13: mechanical hyperalgesia
Mapping of the area of punctuate mechanical hyperalgesia surrounding the surgical incision. Stimulation with von Frey filament starts from the periphery toward the surgical incision

QST should provide new insights into nociceptive mechanisms and allow the diagnosis necessary for mechanism-based approaches to perioperative nociception and pain management (Wilder-Smith and Arendt-Nielsen 2006). Studies of pain perception present ethical challenges, especially in humans (Kawamata, Watanabe et al. 2002), and extrapolation from animal studies to the clinical context is difficult, making collection of human data critical.

1.2.2.1.2. Context of pain management

Aggravated postoperative pain, i.e. higher VAS pain scores and/or increased analgesics use have been found in patients receiving high-doses of opiates during surgery by comparison to those receiving lower doses. Various opioid μ-agonists have been associated with that "OIH" phenomenon as well as different doses and routes of administration of the drugs.

Co-administration of intrathecal opioids with bupivacaine for spinal anesthesia during caesarean section was once a common practice in obstetric surgery. Postoperative morphine requirements and pain scores were significantly higher 6 h after delivery with *intrathecal fentanyl* compared to saline (Cooper, Lindsay et al. 1997). In contrast, following intrathecal diamorphine, visual analogue pain scores were reduced for at least 12 h (Cowan, Kendall et al. 2002).

Chia et al. observed increased pain intensity and fentanyl consumption in the postoperative period after female patients underwent total abdominal hysterectomy with higher *fentanyl* doses during surgery (Chia, Liu et al. 1999).

Guignard et al. reported similar findings in individuals who underwent major abdominal surgery with *remifentanil* (Guignard, Bossard et al. 2000). However, Cortinez failed to observe this phenomenon in a study similar to Guignard et al. The main differences in Cortinez's study were: total intraoperative opioid doses were lower, and nitrous oxide, which is known to be an NMDA receptor antagonist, was also administered (Cortinez, Brandes et al. 2001; Billard, Servin et al. 2004). Moreover, using intraoperative low-dose ketamine, Guignard et al. decreased both intraoperative remifentanil and postoperative morphine consumption (Guignard, Coste et al. 2002).

In a study conducted to identify perioperative variables associated with severe immediate postoperative pain, Aubrun et al. demonstrated that a high dose of intraoperative *sufentanil* induced severe postoperative pain (defined as a dose of morphine in the post-anesthesia care unit higher than 0.15 mg/kg to obtain pain relief and/or a lack of pain relief at the end of morphine titration (Aubrun, Langeron et al. 2003).

1.2.2.1.3. Remifentanil

Remifentanil is used during general anesthesia to attenuate hemodynamic, autonomic and somatic intraoperative responses which promote intraoperative hemodynamic stability, to rapid and predictable emergence and reduces the incidence of respiratory depression during recovery (Scott and Perry 2005; Servin and Billard 2008). Clinical reports and experimental pain studies with remifentanil are interesting because its unique pharmacokinetic profile offers a new approach to the OIH phenomenon. Remifentanil is a potent μ-agonist that is rapidly metabolized by non-specific tissue esterases. As acute OIH associated with remifentanil occurs very rapidly, it offers a unique opportunity for the real-time study of cellular changes underlying its development. A pharmacological study of remifentanil distribution in dogs during intravenous administration revealed a high penetration into cerebrospinal fluid, equal to 74% of venous levels (Kabbaj, Vachon et al. 2005). Therefore, pharmacodynamic effects of remifentanil in the spinal cord are relevant for the study of mechanisms underlying the development of OIH (Luginbuhl, Gerber et al. 2003).

In *human volunteers*, remifentanil administrated at a constant rate (0.1 μg/kg/min) for 4 h induced an analgesic effect, which decreased after 60-90 min (despite constant infusion), indicating an acute 'tolerance' (Vinik and Kissin 1998). Mechanical hyperalgesia developed within 30 min after stopping a 90-min infusion of remifentanil, and the skin area with pre-existing mechanical hyperalgesia was significantly enlarged even after the remifentanil infusion ended (Angst, Koppert et al. 2003; Hood, Curry et al. 2003; Koppert, Angst et al. 2003). Moreover, hyperalgesia after remifentanil was more pronounced than after naloxone (Koppert, Angst et al.

2003). Co-administration of the NMDA-receptor antagonist ketamine or the α_2- agonist clonidine prevented post-infusion hyperalgesia. In contrast, clonidine, but not ketamine, reduced elevated pain ratings post-infusion, suggesting that different mechanisms may underlie post-infusion anti-analgesia and secondary hyperalgesia (Angst, Koppert et al. 2003; Koppert, Sittl et al. 2003).

In the *surgical context*, the use of relatively large doses of intraoperative remifentanil in patients undergoing major abdominal surgery facilitates the expression of postoperative secondary hyperalgesia (increase by 50% the area of secondary hyperalgesia) measured by application of von Frey hair adjacent to the surgical wound. Larger morphine requirements were also observed. This remifentanil-induced hyperalgesia was prevented by low-dose ketamine (Joly, Richebe et al. 2005) (Figure 14). In scoliosis surgery, adolescents who received intraoperative infusion of remifentanil rather than intermittent morphine had higher postoperative morphine consumption (Crawford, Hickey et al. 2006).

Differentiation between OIH and tolerance remains difficult and frequently authors say about acute tolerance. Increased postoperative pain and augmented opioid consumption can be speculated to be a result of OIH.

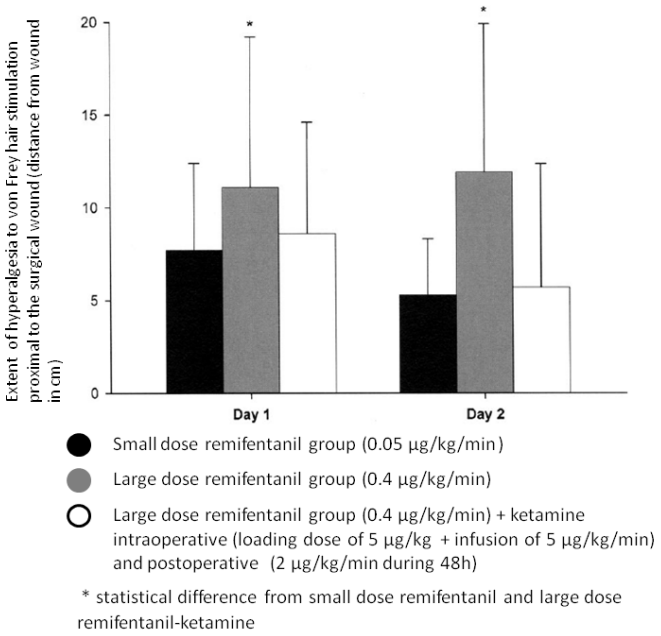


Figure 14: periincisional mechanical hyperalgesia
Extent of hyperalgesia in patients undergoing major abdominal surgery and receiving intraoperative small-dose, large dose of remifentanil, or large dose with ketamine
Result are expressed as mean±SD
Adapted from Joly, 2005

1.2.2.1.4. Is there enough evidence for existence of OIH in humans?

Although numerous reports seem to support the occurrence of OIH in humans, one evidence-based structured review (Fishbain, Cole et al. 2009) concludes that there is insufficient evidence to support or refute the existence of OIH in humans, except in the case of normal volunteers receiving opioid infusions. It is often difficult to make a distinction between acute tolerance and OIH, and most human studies provide only indirect evidence for OIH (Wilder-Smith and Arendt-Nielsen 2006). Prospective studies to assess nociceptive thresholds before and after opioid administration are needed. The introduction and adaptation of QST to clinical practice may constitute a valid method for reliably diagnosing hyperalgesia after surgical

intervention and highlighting the role of intraoperative opioid administration.

Nevertheless, aggressive treatment of pain with opioids may predispose patients to enhanced pain sensitivity later. Patients who use opioids chronically experience increased levels of postoperative pain despite higher doses of postoperative pain medication (Carroll, Angst et al. 2004). Patients who currently use or formerly used opioids also display enhanced pain responses to minor procedures like venipuncture (Compton, Charuvastra et al. 2000). Conceivably, long-term use of opioids may also exacerbate, rather than ameliorate, chronic pain.

The question remains open whether intraoperatively administered opioids can exacerbate postoperative pain

1.2.*2.2.* Animal studies

When clinical questions are formulated, animal studies can help us to understand the underlying mechanisms and to explore clinically-relevant pharmacological approaches.

Hyperalgesia has been extensively documented in animals during withdrawal after cessation of opioid administration, after precipitating withdrawal with opioid antagonists (Larcher, Laulin et al. 1998; Celerier, Laulin et al. 1999) and on occasion while animals were still experiencing opioid stimulation (Christensen and Kayser 2000; Li and Clark 2002). However, OIH has been demonstrated after acute administration: multiple bolus doses (Celerier, Rivat et al. 2000) (Laulin, Maurette et al. 2002), a single high dose (Van Elstraete, Sitbon et al. 2005), a single low dose (Crain and Shen 2001; Esmaeili-Mahani, Shimokawa et al. 2008) or a subanalgesic dose (Wu, Thompson et al. 2004; Holtman and Wala 2005; Galeotti, Stefano et al. 2006).

The first animal studies in this area investigated the effects of opioid tolerance and OIH on pain management and treatment of chronic pain, while more recent studies examined the structure of the opioid system and the signaling pathways involved.

We will focus especially on acute opioid administrations which are more relevant for perioperative pain management.

1.2.2.2.1. Opiates and animal models

Nociceptive testing At first, opioid effects were explored using different end points of anesthesia: loss of righting reflex, abolition of purposeful movement responses to painful stimuli (usually regarded as an index for opioids analgesic action) and abolition of heart rate responses to painful stimuli (which is one of the goals of anesthesia). Comparison between opioids and their interactions with anesthetic drugs were therefore the primary variables investigated (Kissin, Kerr et al. 1983; Kissin and Jebeles 1984). Later, the OIH phenomenon has been explored in many different behavioral assays (e.g., motor response or vocalization tests), using thermal (Crain and Shen 2001; Sweitzer, Wong et al. 2004; Holtman and Wala 2005), mechanical (Celerier, Rivat et al. 2000; Vanderah, Gardell et al. 2000), electrical (Wilcox, Mikula et al. 1979) and chemical (Rivat, Laulin et al. 2002) noxious stimuli. The susceptibility of different pain signaling pathways to express OIH is extremely variable.

Various opiates have been tested, including morphine (Galeotti, Stefano et al. 2006), heroin (Laulin, Larcher et al. 1998; Celerier, Laulin et al. 2001), methadone (Holtman and Wala 2007), fentanyl (Celerier, Rivat et al. 2000), remifentanil (Cabanero, Campillo et al. 2009) and sufentanil (Minville, Fourcade et al. 2010).

Various routes of administration have been evaluated, including subcutaneous (s.c.) (Celerier, Rivat et al. 2000; Shen and Crain 2001; Sweitzer, Wong et al. 2004; Galeotti, Stefano et al. 2006), intrathecal (i.t.) (Vanderah, Gardell et al. 2000; Mao, Sung et al. 2002; Wu, Thompson et al. 2004; Van Elstraete, Sitbon et al. 2005) and intravenous (i.v.) (Cabanero, Campillo et al. 2009).

1.2.2.2.2. In normal animals

Galeotti et al. (2006) observed a bimodal response in mice receiving different s.c. doses of *morphine*. Using thermal testing, an OIH effect appeared 15 min after administration of moderate doses of morphine (1-10 µg/kg), persisting almost unchanged for up to 45 min and then

diminishing. Lower doses (0.01-0.3 µg/kg) and higher doses (30-300 µg/kg) were devoid of any effect, whereas very high doses (1-7 mg/kg) induced analgesia. Naloxone administration completely reversed the OIH effect observed after moderate doses of morphine.

A single dose of intrathecal ***morphine***, on the other hand, induced a biphasic effect on mechanical noxious stimulus: early analgesia lasting 3-5 h followed by delayed OIH lasting 1-2 days (Van Elstraete, Sitbon et al. 2005).

After using different design with ***heroin*** (Larcher, Laulin et al. 1998; Laulin, Larcher et al. 1998; Laulin, Celerier et al. 1999), Simonnet and collaborators explored the effects of various doses of ***fentanyl*** on nociceptive threshold using a paradigm designed to partially mimic its use in human surgery. As previously described, fentanyl is a potent µ-opiate analgesic widely used for human surgery. Four fentanyl boluses (every 15 min) elicited a dose-dependent biphasic effect on nociceptive threshold (in the paw pressure vocalization test): a short-lasting increase followed by a long-lasting decrease (OIH) (Celerier, Rivat et al. 2000) (Figure 15). A subsequent morphine administration showed a reduction of its analgesic effect (Laulin, Maurette et al. 2002). Ketamine pretreatment suppressed OIH and restored the full effect of a subsequent morphine injection. OIH development was prevented by gabapentin (i.p. or i.t.; probably by binding to the $\alpha_2\delta$ subunits of voltage-gated Ca^{++} channels) (Van Elstraete, Sitbon et al. 2008) and by magnesium sulphate (i.p.; physiological block of the ion channel on the NMDA receptor) (Van Elstraete, Sitbon et al. 2006).

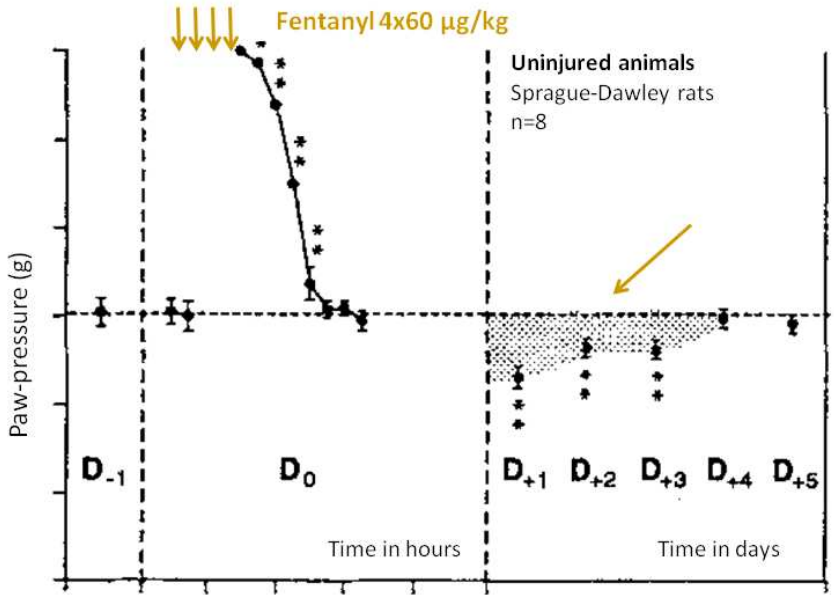


Figure 15: effect of high dose fentanyl in uninjured and awake rats
Nociceptive thresholds are determined by paw pressure vocalization after fentanyl administration. There is a biphasic time-dependent effect on nociception. The nociceptive threshold first increases in the first hours (analgesia) and then decreases in the following days (hyperalgesia).
Results are expressed as mean nociceptive threshold ± SEM (g)
*** p<0.01 compared with the basal nociceptive threshold value*
Adapted from Celerier, 2000

In **summary**, the paradoxical hyperalgesic effect of opioid administration may be apparent immediately after low-dose administration or later after analgesic doses when the opioid concentration is expected to be low due to metabolism. A delayed hyperalgesic effect observed after an analgesic effect doesn't come from a decrease in opioid effectiveness, but rather from nociceptive facilitation, which may no longer be masked by the anti-nociceptive inhibitory system at these later time points.

1.2.2.2.3. Acute and chronic pain animal models: surgical conditions

As persistent postoperative pain is a significant clinical problem (Macrae 2001), laboratory investigations have modeled several

clinical postoperative pain scenarios. These models appear to be useful for quantifying the efficacy of treatments to reduce the frequency and severity of long-term pain and to understand its underlying mechanisms.

Two models of *acute incision pain* have been used. Brennan et al. (Brennan, Vandermeulen et al. 1996; Zahn and Brennan 1999) used a 1 cm incision through the skin, fascia and muscle of the rat plantar hindpaw, while Duarte et al. (Duarte, Pospisilova et al. 2005) employed a 1 cm incision in the hairy back skin of the rat (under halogened anesthesia). These models evoke 3-5 days of mechanical hypersensitivity. Using in vivo microdialysis, the EAAs aspartate and glutamate have been demonstrated to be increased from 10 to 30 min after paw incision. Although concentrations returned to baseline by 1h post-incision, mechanical hyperalgesia persisted, implicating EAA-induced dorsal horn sensitization (Zahn, Sluka et al. 2002). A review of the rat plantar hindpaw model of postoperative pain is available, which proposes mechanisms for enhanced excitability of sensory neurons following the incision (Brennan, Zahn et al. 2005).

Li and colleagues (2001) demonstrated that hyperalgesia and allodynia resulting from the incision were additive to OIH induced by *morphine* (administered over 6 days via s.c. osmotic minipumps). Moreover, naloxone administered chronically for 6 days before the incision and then discontinued markedly reduced incision-induced hyperalgesia and allodynia. In contrast, naloxone (1 mg/kg) administered acutely after hind paw incision increased hyperalgesia and allodynia. This study suggests that chronic preoperative opioid use can lead to excessive postoperative pain. Furthermore, Richebe et al. (Richebe, Rivat et al. 2005) demonstrated that animals receiving high doses of *fentanyl* during hind paw plantar incision (under halothane anesthesia) show early anti-nociception, as well as exaggerated postoperative pain (Figure 16).

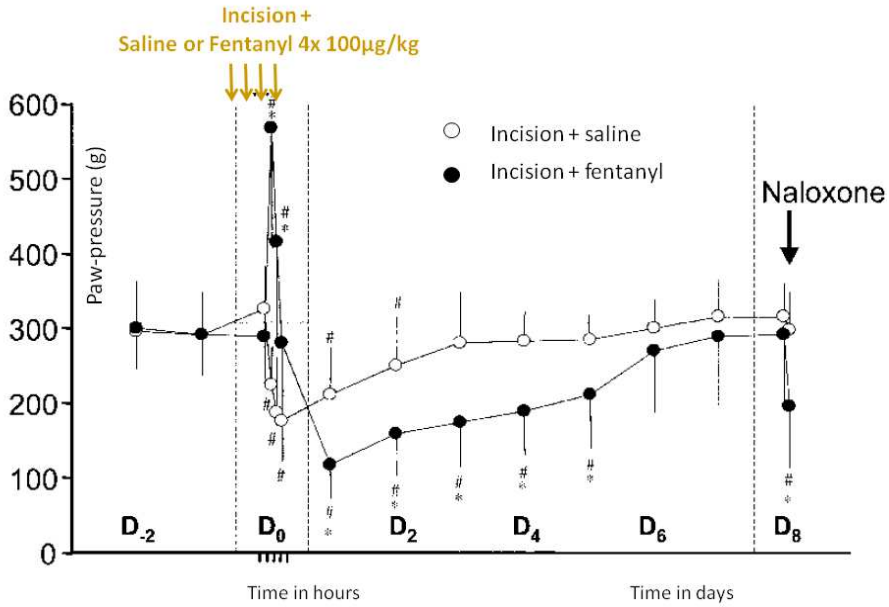


Figure 16: incisional pain and fentanyl
Mechanical hyperalgesia induced by paw incision ± s.c. fentanyl administration (400 µg/kg). The pain threshold (paw pressure vocalization) was evaluated before surgery, on D_0 and once daily for 8 days. Hind paw plantar incision produced a significant decrease of the nociceptive threshold for 2 days. Fentanyl administration initially induced an analgesic effect for 6h and worsened mechanical hyperalgesia for 5 days. Naloxone administration on D8 (after rats had returned to normal) induced hyperalgesia only in fentanyl-treated rats
#: significantly different compared with the D_0 basal value
**: significant difference between groups*
Adapted from Richebe, Anesthesiology 2005

The carrageenan model is a well-established animal model for localized ***inflammatory pain*** (Hargreaves, Dubner et al. 1988). Carrageenan elicits an early edema with long-lasting hyperalgesia (24-96 h) that peaks after 1-4 days (acute pain) (Vinegar, Schreiber et al. 1969). Rivat et al. (Rivat, Laulin et al. 2002) demonstrated that hyperalgesia induced by carrageenan injection into the hind paw was dose-dependently enhanced effects by ***fentanyl*** in duration and magnitude. Hyperalgesia was also observed in the hind paw contralateral to carrageenan injection in fentanyl-treated animals, suggesting that central sensitization in inflammatory pain states is reinforced by opiate use. A second carrageenan injection

exaggerated hyperalgesia, particularly in fentanyl-treated rats. Pretreatment with ketamine (NMDA antagonist) prevented the long-lasting hyperalgesia induced by carrageenan and fentanyl. These observations indicate that central sensitization in inflammatory pain states is reinforced by an opioid treatment, which could be prevented by NMDA receptors blockade.

SMIR surgery (skin/muscle incision and retraction) has led to a new model of persistent postoperative pain (Flatters 2008). The incision and retraction of skin and superficial muscle of the medial thigh evoked at least 3 weeks of hypersensitivity to mechanical stimulation of the plantar ipsilateral paw without affecting the contralateral paw. Tissue retraction for 1 h may cause stretching of the saphenous nerve and neurodegeneration. In contrast to the thoracotomy model (rib-retraction for 60 min produced allodynia lasting more than one month) (Buvanendran, Kroin et al. 2004), no significant peripheral neuronal damage has been observed after this procedure, suggesting that nerve damage is not a causal factor in persistent postoperative pain evoked by SMIR. Instead, it may involve a combination of nociceptive and inflammatory processes.

Using the *SMIR surgery* model, we have demonstrated that the intrasurgical administration of high-dose *fentanyl* (4x60 μg/kg, s.c. at 15-min intervals, according to Celerier (Celerier, Rivat et al. 2000) induces an early hypersensitivity in the contralateral paw, demonstrating that intraoperative opioid use may enhance hyperalgesia and worsen postoperative pain. SMIR evoked significant mechanical hyperalgesia in the ipsilateral paw immediately and in the contralateral paw after a delay (Docquier et al. 2008) (Figure 17).

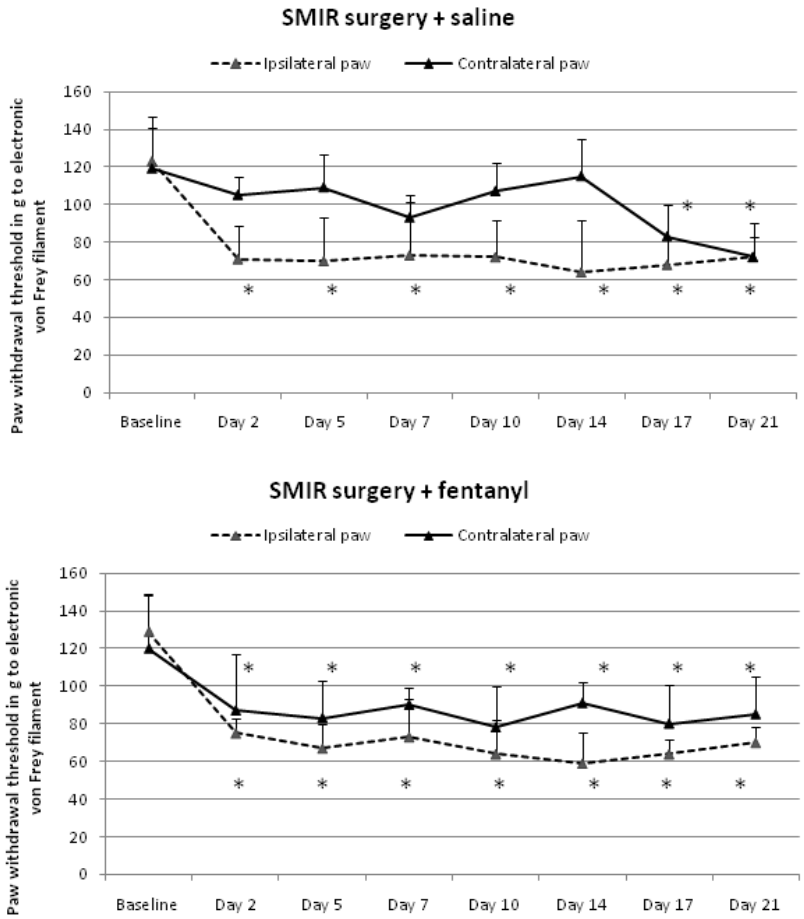


Figure 17: SMIR surgery and fentanyl
Under sevoflurane anesthesia, adult male Wistar rats underwent SMIR surgery, and received either saline (n=6) or fentanyl (n=6) (4x60 µg/kg s.c. at 15-min intervals). Postoperative development of mechanical hyperalgesia was evaluated by paw withdrawal threshold (in g) (electronic filament). Statistical analysis used repeated measure ANOVA and t-test, p< 0.05 was considered as significant
**: significant difference compared to baseline value*
From Docquier et al. Eur J Anaesth, 2008

To mimic the conditions in which opioids are used in humans, Célerier et al. (2006) studied the effects of *fentanyl*, *alfentanil* and *remifentanil* (sevoflurane anesthesia ± s.c. opioid infusion over 30 min) on nociceptive sensitivity and on incisional pain in mice. Nociception was evaluated over 7 days with thermal and mechanical stimuli and with punctuate stimuli (allodynia). Intraoperative infusion of opioids significantly enhanced incision pain and duration of postoperative pain on all tests. The most prominent effects were observed with remifentanil. In inducible nitric oxide synthase (NOS) knockout mice, both remifentanil- and incision-induced pronociceptive effects were attenuated. In these mice, remifentanil still enhanced incision pain, but the pronociceptive effect was significantly attenuated. A role of the NO system is therefore suggested in the cause of acute postoperative pain and opioid-induced pronociception.

More recently, a mouse model of *orthopedic surgery* was developed, which is of particular interest because it combines anesthetic management with postoperative effects (Minville, Laffosse et al. 2008; Minville, Fourcade et al. 2010). Bone fracture in mice was performed under general anesthesia and during *sufentanil* administration (4x10 μg/kg per injection, s.c., at 15 min intervals). Sufentanil produced a short anti-nociceptive effect and led to development of mechanical hyperalgesia for 3 days and thermal hyperalgesia for 4 days. Ketamine prevented sufentanil hyperalgesia and improved postoperative morphine effectiveness.

One interesting study mimicked the *stress analgesia* induced by endogenous opioid release (Rivat, Laboureyras et al. 2007). In naïve rats, a first non-nociceptive environmental stress (NNES; induced by exposing animals for 1 h to a new environment) induced a moderate and limited analgesia. In contrast, in pain- and opioid-experienced rats, NNES induced hyperalgesia for several hours. Repetition of NNES induced a 20-fold enhancement of stress hyperalgesia (3-4 days), which was still observed 4 months later. Moreover, ultra-low dose fentanyl administration, mimicking stress analgesia in naive rats, induced hyperalgesia in pain- and opioid-experienced rats. These observations demonstrate that opioids may induce opposite effects

(analgesia versus hyperalgesia), depending on prior life events. There exists a pain vulnerability associated with prior life events.

1.2.2.2.4. Conclusion of observations

Based on these different experimental models, it appears that:

1) Opiates simultaneously activate a pain-inhibitory system (associated with short-lasting analgesia) and a pain-facilitatory system (associated with longer-lasting hyperalgesia). Pronociceptive effects may be induced after the first exposure to an opiate but only manifest on termination of antinociceptive effect, leading to an excitatory effect that lasts longer than opiate-receptor stimulation. High-dose μ-opioid agonists (e.g., fentanyl, heroin) show time- and dose-dependent, biphasic analgesic-hyperalgesic responses (Celerier, Rivat et al. 2000; Celerier, Laulin et al. 2001). Low-dose μ-opioid agonists show an early and shortly hyperalgesic response (Crain and Shen 2001; Galeotti, Stefano et al. 2006).

2) Induction of OIH reduces the analgesic efficacy of subsequent opioid applications (Laulin, Maurette et al. 2002), and once generated, may remain latent for long time periods (i.e., latent pain sensitization) (Celerier, Laulin et al. 2001; Rivat, Laboureyras et al. 2007).

3) Exaggerated postoperative pain may result not only from the nociceptive inputs related to tissue damage, but also from pain sensitization induced by intra- and post-operative opioids. Opioid administration in incision and inflammatory pain models exacerbate injury-induced hypersensitivity (Rivat, Laulin et al. 2002; Richebe, Rivat et al. 2005).

4) NMDA antagonists prevent the development of opioid-induced hyperalgesia (Celerier, Rivat et al. 2000; Galeotti, Stefano et al. 2006; Van Elstraete, Sitbon et al. 2006; Minville, Fourcade et al. 2010).

All of these studies offer insights into the underlying mechanisms of OIH. Although, some animal models have been used in attempts to reproduce perioperative nociceptive conditions, **none have evaluated the occurrence of OIH under general anesthesia.**

1.2.*2.3.* Electrophysiological recordings

Electrophysiological studies may provide quantifiable measures of synaptic activity useful in the functional analysis as well as new insight into the molecular mechanisms regulating neurotransmission. Therefore *in vitro* studies may reinforce a role for some pathways suspected in behavioral observations.

In decerebrate, spinalized, unanesthetized rats with intact or sectioned sciatic nerves, low doses of i.t. morphine (10 ng in rats with intact nerves; 10 or 100 ng in rats with sectioned nerves) facilitated the flexor reflex. Higher doses of *morphine* caused reflex facilitation followed by depression. Facilitation of the flexor reflex is prevented by i.t. naloxone and suppressed by the tachykinin antagonist, indicating that the reflex facilitation evoked by low doses of morphine may be due to the release of SP and perhaps other neuropeptides (Wiesenfeld-Hallin, Xu et al. 1991).

Zhao et al. (2008) investigated the effect of *remifentanil* in spinal neurons that displays an augmentation in NMDA receptor currents after chronic morphine treatment. They demonstrated that exposure to 4, 6, or 8 nM remifentanil, but not higher or lower concentrations, significantly increased NMDA-evoked peak currents during the 36-min remifentanil perfusion and for 40 min after washout. This enhancement of NMDA responses was attenuated by μ- or δ-opioid antagonists, suggesting that the concurrent activation of μ- or δ-opioid receptors by remifentanil is required to increase NMDA-evoked current.

1.2.*3.* Molecular mechanisms that may underlie OIH

A range of molecular mechanisms is likely involved at all levels of the nociceptive system. A great deal of experimental evidence strongly supports a role of the **spinal cord** as the neuroplastic site OIH genesis, including acute receptor desensitization and up-regulation of the cAMP pathway, spinal PGs, protein kinase C (PKC) and the NO pathway. Activation of spinal NMDA receptors seems to play a key role (Dunbar, Karamov et al. 2000; Vanderah, Gardell et al. 2000; Li, Angst et al. 2001; Mao, Sung et al. 2002; Raghavendra, Rutkowski et al. 2002). Furthermore, changes in the **peripheral nerves** involved in pain processing, including intracellular messengers and TRPV1 (Vardanyan, Wang et al. 2009), as well as alteration in **supraspinal systems** responsible for the descending modulation (inhibitory or facilitatory pathway) of perceived pain, are also implicated (Mercadante and Arcuri 2005; Colpaert, Deseure et al. 2006; Gardell, King et al. 2006; Grecksch, Bartzsch et al. 2006; Chang, Chen et al. 2007).

OIH is postulated to be related to activation of excitatory pathways by opioids. The mechanisms are numerous, interrelated and physiological to activation of μ-receptors. To simplify, each hypothetic mechanism will be discussed individually, though most are interrelated. Figures 18, 19 and 20 summarize the mechanisms suspected to be implicated by μ-receptor activation.

1.2.*3.1.* Receptor trafficking and "desensitization"

Earlier, neuroadaptive processes, such as acute receptor desensitization and downregulation, were suggested as underlying mechanisms for opioid tolerance after long-term morphine treatment. After activation by as little as a few minutes of agonist exposure, the opioid receptor becomes phosphorylized by G-protein-regulated receptor kinases (GPRK), which causes separation from the G-protein. The receptor increases its affinity for the cellular protein arrestin, and the subsequently activated receptor-arrestin complex can initiate endocytosis, thus causing desensitization. Once internalized, the receptor is either degraded or re-expressed at the

cell surface (“recycling”). Via internalization and re-cycling, the opioid receptor is intermittently detached from the cell membrane and can initiate other adaptive intracellular processes (Borgland 2001).

Desensitization has been demonstrated for many opioids in clinical use and is attributed to protein kinase C (Bot, Blake et al. 1998; Whistler, Chuang et al. 1999). At least a dozen PKC isoforms have been described, of which PKC-γ has the greatest impact on the regulation of spinal nociceptive processes (PKC-γ localization in excitatory interneurons in laminae II of the spinal cord) (Polgar, Fowler et al. 1999). Moreover, activation of PKC causes phosphorylation in many receptors and ion channels, including μ-opioid and NMDA receptors (Mayer, Mao et al. 1995; Velazquez, Mohammad et al. 2007).

Some data indicate that agonist ligands that have similar effects on receptor-mediated signaling may have dramatically different effects on intracellular trafficking of a G-protein-coupled receptor (Keith, Murray et al. 1996). For example, the opioids agonist morphine interacts differently with the μ-receptor than do other opioid agonists: morphine does not stimulate rapid internalization, even at high concentrations that strongly inhibit adenylyl cyclase. Lack of μ-receptor desensitization might explain why morphine has a higher potential to cause tolerance than DAMGO, methadone (Whistler, Chuang et al. 1999), fentanyl analogs (Bot, Blake et al. 1998) or endomorphine-1 (Horner and Zadina 2004). These results may elucidate how μ-agonists regulate the number and sensitization of their receptors. Administration of very high doses of certain opioids (phenantrene) might induce allodynic-hyperalgesic states more readily than others. In this context, switching from a phenantrene (e.g., morphine) to a piperidine derivate (e.g., fentanyl or sufentanil) is recommended (Yaksh and Harty 1988; Compton, Charuvastra et al. 2001; Angst and Clark 2006).

Moreover, opioid-induced internalization of μ-receptors in spinal interneurons (Trafton, Abbadie et al. 2000) was recently proposed to explain the anti-analgesia effect of remifentanil (Koppert, Angst et al. 2003). Extensive internalization and thereby inactivation of μ-opioid receptors induced by remifentanil (but not by morphine or

endogenous opioids) would reduce the number of functional receptors and consecutively reduce endogenous opioid-induced analgesia, similar to an acute withdrawal. This idea is supported by the absence of anti-analgesia following alfentanil, an opioid with a longer half-life, in the same model (Compton, Charuvastra et al. 2001; Koppert, Dern et al. 2001; Koppert, Angst et al. 2003).

This acute desensitization may, in fact, be a protective mechanism whereby cells adapt to avoid the development of physiological drug tolerance by rapidly attenuating receptor-mediated signaling. Those drugs that do not cause receptor internalization, such as morphine, may therefore have higher propensities to develop tolerance (Borgland 2001).

1.2.*3.2.* cAMP pathway

Activation of opioid receptors reduces cAMP levels. However, neuroadaptations resulting from long-term μ-agonist treatment can up-regulate adenylate cyclase activity, resulting in increased cAMP levels. Via presynaptic activation, increased cAMP level enhance release of excitatory neurotransmitters at the spinal level (Fairbanks and Wilcox 1997; Fairbanks and Wilcox 2000; Li and Clark 2002) and lead to increased transmission in mesencephalic PAG and other idbrain areas (Ingram, Vaughan et al. 1998).

1.2.*3.3.* NMDA system

Nowadays, the NMDA system attracts a great deal of attention. This system is a functionally important pro-nociceptive system, which can be activated by opioids or by tissue injury or surgery. It therefore plays a central role in the initiation and maintenance of central sensitization.

Current data suggest that while opioid-induced desensitization (pharmacological tolerance) and sensitization (OIH) are distinct processes, they may share common cellular mechanisms, including activation of the central glutamatergic system and Ca^{++}-regulated intracellular protein kinase C (PKC) (Mao, Price et al. 1995; Mao 2002). Furthermore, neural mechanism of opioid tolerance and OIH

may interact with the mechanisms of pathological pain (Mao, Pain, Clinical updates, 2008).

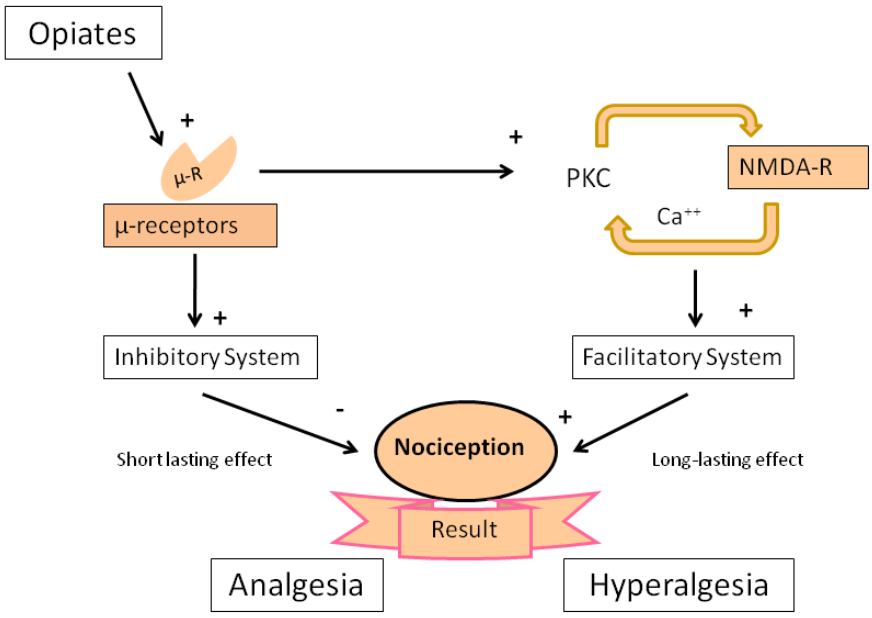


Figure 18: schematic view of the opposing effects of opiates on nociception Opiates activate not only pain inhibitory systems (eliciting analgesia) but also pain facilitatory systems (eliciting hyperalgesia) through glutaminergic NMDA receptors and activation of a protein kinase
Adapted from Rivat, 2002

(1) Postsynaptic μ-opioid receptor occupation by exogenous ligands may initiate PKC translocation and activation.

(2) Activation of PKC causes phosphorylation of NMDA receptors accompanied by removal of Mg^{++} blockade and increased Ca^{++} influx. With this blockage removed, even small amounts of EAAs could activate the NMDA receptors.

(3) The Ca^{++} influx causes a further increase of PKC activity, which contributes to the phosphorylation and inactivation of opioid receptors. In addition, Ca^{++}-mediated activation of neuronal NOS induces the generation of NO as well as regulation of relevant gene expression.

(4) NO may activate various protein kinases via cGMP and participate in the modulation of μ-opioid activated receptor. NO may diffuse out the neuron enhancing pre-synaptic release of endogenous EAAS resulting in a positive feed-back.

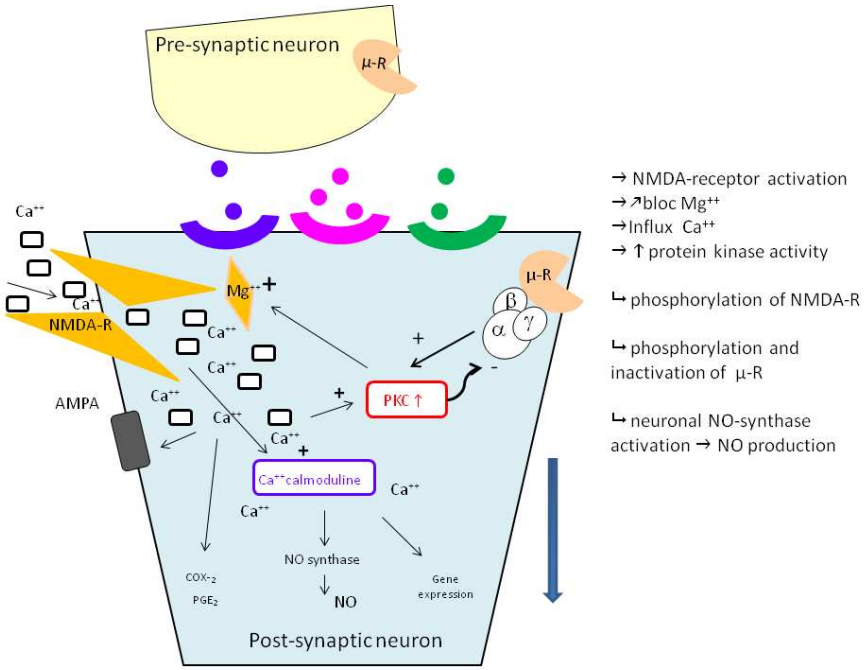


Figure 19: mechanistic model of spinal NMDA-receptor activation
Adapted from Mao, 1995 and Koppert, 2007

The role of NMDA-receptors can be summarized as follows:

1) The spinal NMDA-receptors system is a functionally important pronociceptive system which can become activated indirectly by opioids. Blocking NMDA receptors attenuate or prevent development of tolerance and OIH. Even at doses lacking analgesic effect, NMDA antagonists such as ketamine or MK-801 can prevent OIH development (Celerier, Rivat et al. 2000; Laulin, Maurette et al. 2002; Van Elstraete, Sitbon et al. 2005; Koppert and Schmelz 2007).

2) Chronic i.t. morphine induced a dose-dependent down-regulation of spinal glutamate transporters (GLUT) in the superficial dorsal horn.

This down-regulation was mediated through opioid receptors because it was blocked by naloxone (Mao, Sung et al. 2002).

3) One electrophysiological study using Xenopus laevis oocytes observed that remifentanil directly activated NMDA receptors. Remifentanil-induced currents were inhibited by MK-801 but not by naloxone or glycine or glutamate antagonists. In contrast, fentanyl did not stimulate NMDA receptors. Therefore, remifentanil appears to activate the NMDA receptor allosterically (Hahnenkamp, Nollet et al. 2004).

4) Prolonged morphine administration induces neurotoxicity via NMDA-mediated apoptosis within the dorsal horn of the spinal cord, predominantly in the superficial laminae. Increases in activated caspase-3 and mitogen-activated protein kinase (MAPK) were observed within the same region. The spinal adenylyl cyclase - protein kinase A (PKA) - MAPK pathway may contribute to the cellular mechanisms of morphine-induced apoptosis (Mao, Sung et al. 2002; Lim, Wang et al. 2005).

5) Neural mechanisms of persistent pain and opioid sensitization may cross exist through the NMDA system (Mao and Mayer 2001).

1.2.*3.4.* Protein kinases

As described above, activation of PKC causes phosphorylation of many receptors and ion channels, including μ-opioid and NMDA receptors. A role for PKC has been demonstrated in several animal models of OIH. Opiates evoke OIH in wild-type mice, but not in mice lacking PKC-γ (Zeitz, Malmberg et al. 2001; Celerier, Simonnet et al. 2004). Both PKC-γ and -ε are involved in OIH in rat pups (Sweitzer, Wong et al. 2004). At an extremely low dose, i.t. morphine elicits hyperalgesia without affecting cAMP levels. Pre-treatment with a selective inhibitor of PKC, but not PKA, combined with Ca^{++} channel blockade by i.t. nifedipine, antagonized hyperalgesia and prevented OIH. These results indicate a role for the G-protein/PKC pathway and Ca^{++} channels, but not G-protein signaling through cAMP, in OIH induced by low-dose morphine in rats (Esmaeili-Mahani, Shimokawa et al. 2008).

1.2.*3.5.* NO system

In addition to activation of NMDA receptors and increased Ca^{++} influx, activation of neuronal NOS induces generation of NO. Induction of the supraspinal isoform of NOS synthesis (nNOS1) reduces the anti-nociceptive potency of μ-agonists, and non-selective NOS inhibitors counteract development of tolerance (Dambisya and Lee 1996). Studies suggest a role of the NO system in opioid-induced pro-nociception, in acute postoperative pain as well as in acute inflammatory pain. NOS knockout mice show reduced development of OIH, and NOS inhibitors prevent development of OIH (Wong, Hsu et al. 2000; Li, Angst et al. 2001; Celerier, Gonzalez et al. 2006; Pol 2007).

1.2.*3.6.* Cyclooxygenase (COX) system

COX system has been implicated in opioid tolerance by interacting with NMDA and NO systems (Wong, Hsu et al. 2000). COX inhibitors act by inhibiting the cyclooxygenases enzymes (COX-1, COX-2) that synthesize and PGs, as well as lipoxygenases. COX inhibitors reduce central hyperalgesia in animals (Kang, Vincler et al. 2002) and human volunteers. While pain ratings are not affected, parecoxib and paracetamol significantly reduced the areas of secondary hyperalgesia to pinprick and touch (Koppert, Wehrfritz et al. 2004). Spinal ibuprofen prevents opioid withdrawal in the rat (Dunbar, Karamov et al. 2000). Moreover, hyperalgesia produced by i.t. NMDA is blocked by COX inhibitors (Malmberg and Yaksh 1992) and acute fentanyl-induced hypersensitivity is reversed by i.t. ketoralac (Kang, Vincler et al. 2002).

1.2.*3.7.* Spinal dynorphins and others peptides with opioid-antagonistic properties (“anti-analgesia”)

Dynorphin A, classified as an endogenous opioid (κ-agonist), possesses relevant pro-nociceptive properties, which result in part in activation of NMDA receptors (Faden 1992; Vanderah, Gardell et al. 2000; Vanderah, Ossipov et al. 2001). Moreover, with continuous infusion of μ-receptor agonists, spinal dynorphin levels increase, causing release of spinal excitatory neuropeptides such as CGRP

from primary afferents (Gardell, Wang et al. 2002), CCK (Watkins, Kinscheck et al. 1985; Ossipov, Lai et al. 2003; Xie, Herman et al. 2005; Yue, Tumati et al. 2008), neuropeptide FF (NPFF) (Simonin, Schmitt et al. 2006) and nociceptin (orphanin FQ) (Rizzi, Bigoni et al. 2000; Rizzi, Marzola et al. 2001). Blocking of these specific receptors was shown to potentate opioid analgesic effects.

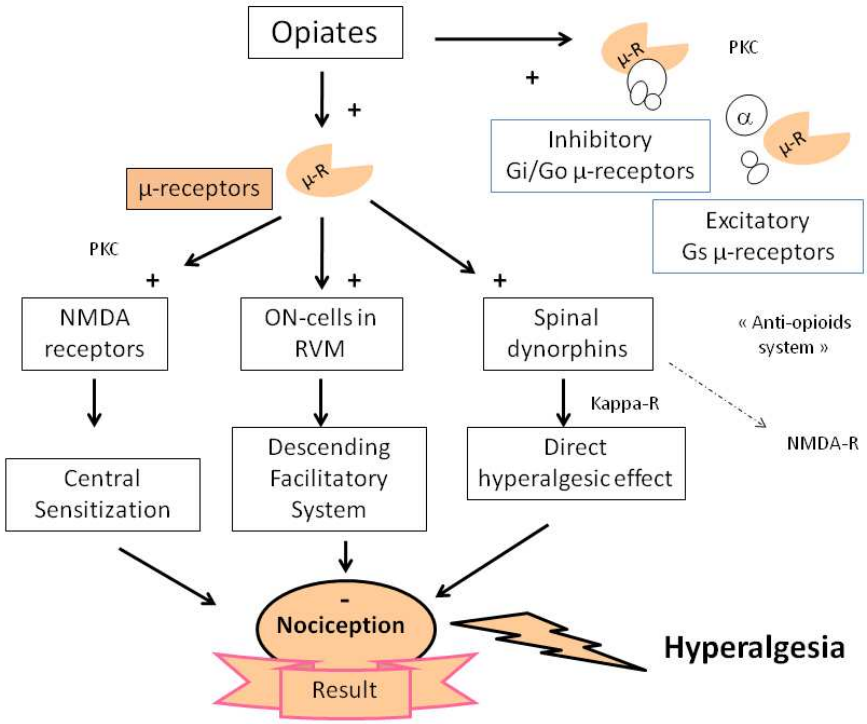


Figure 20: schematic summary of mechanisms of OIH
Adapted from Wilder-Smith, 2006

1.2.*3.7.* Descending facilitation

Descending inhibition originates in the PAG of the midbrain and the RVM. In the RVM, three classes of neurons can be distinguished based on their responses to painful stimuli: off-cells are inhibited, on-cells increase their firing rate and neutral cells do not respond. Off- and on-cells project to dorsal horn neurons to inhibit and facilitate, respectively, synaptic transmission of nociceptive inputs. The central analgesic effect of μ-agonists is attributed to inhibition of on-cells and

activation of off-cells. The RVM is therefore an important source of descending modulatory systems that both inhibit and facilitate pain at the level of the spinal cord.

Modulation of spinal input by brainstem descending pathways is implicated in the development of OIH and may be demonstrated through several mechanisms. To summarize the most interesting findings:

1) Lesioning the descending pathway to the spinal cord (dorsal lateral funiculus) or RVM lidocaine prevent the increase of excitatory neuropeptides and block opioid-induced pain (Vanderah, Suenaga et al. 2001; Gardell, Wang et al. 2002).

2) Activation of the descending pain facilitation pathway arises from the RVM, is mediated via opioid-sensitive on-cells, and is elicited in part by increased activity of CCK in the RVM. Interrupting this pathway abolishes abnormally-enhanced pain. CCK-2 antagonists can reverse OIH (Xie, Herman et al. 2005).

3) Morphine-induced elevation of spinal dynorphin content depends on descending influences and enhances stimulated CGRP release (Gardell, Wang et al. 2002). Furthermore, extended opioid exposure can increase CGRP and SP expression in the DRG. Spinal dynorphin antiserum reestablished the antinociceptive potency and efficacy of spinal morphine (Vanderah, Gardell et al. 2000). The same manipulations that block abnormal pain also block anti-nociceptive tolerance (Ossipov, Lai et al. 2005).

4) Noxious stimuli can activate 5-HT neurons in the RVM and accelerate the turnover of 5-HT in the spinal cord. Studies suggest that descending pain inhibitory or facilitatory pathways from RVM act in the spinal cord in acute and chronic pain states through activation of 5-HT_7 and 5-HT_3 receptors, respectively but also seems play a role in OIH, acting via 5-HT_3 and possibly 5-HT_2 receptors. Ondansetron, a widely used 5-HT_3 antagonist, blocks OIH (Dogrul, Ossipov et al. 2009).

5) In the plantar incision pain model, microinjection of lidocaine into the RVM completely reversed fentanyl-induced sensory

hypersensitivity and fentanyl-induced enhancement of incision-induced sensory hypersensitivity. RVM lidocaine also slightly reduced incision-induced sensory hypersensitivity in the absence of fentanyl pre-treatment. Spinal dynorphin content increased by 30% and 66% in fentanyl- and fentanyl/incision- treated rats (Rivat, Vera-Portocarrero et al. 2009). Spinal administration of dynorphin antiserum attenuated sensory hypersensitivity in fentanyl-treated rats.

6) Furthermore, ablation of NK-1-expressing spinal neurons by pre-treatment with SP-Saporin reduced sensory hypersensitivity in fentanyl-treated rats and, to a lesser extent, in fentanyl-treated rats with a surgical incision. These data support a role of NK-1 receptor-containing ascending pathways and of descending facilitatory pathways in fentanyl-induced hyperalgesia and in the fentanyl-induced hyperalgesia following a surgical incision (Vera-Portocarrero, Zhang et al. 2007; Bannister, Bee et al. 2009; Rivat, Vera-Portocarrero et al. 2009). Spinal administration of an NK-1 antagonist reversed OIH (King, Gardell et al. 2005). The NK-1 receptor may also interact with NMDA receptors to modify descending control.

1.2.*3.8.* Other systems

Although they are probably underappreciated, glial cells in the spinal cord also play a role in OIH. Following systemic morphine treatment, they become activated and enhance expression of multiple spinal chemokines and proinflammatory cytokines (e.g., IL-1) to oppose opioid analgesia, promote development of analgesic tolerance and pain enhancement (Johnston, Milligan et al. 2004). Such effects can occur via activation of an innate immune receptor expressed by glia and called toll like receptor 4 (TLR4) (Watkins, Hutchinson et al. 2009). TLR4 is recognized as a key glial activation receptor for the initiation and maintenance of chronic pain. The putative microglial inhibitor minocycline and the glial modulator propentofylline have been demonstrated to be involved in pain modulation (Raghavendra, Tanga et al. 2004; Hutchinson, Northcutt et al. 2008).

Nitrous oxide (N_2O), which is widely used as a component of anesthesia and possesses NMDA antagonist properties, has been demonstrated to prevent the enhancement of pain sensitivity induced

by nociceptive inputs and fentanyl and to oppose acute morphine tolerance (Richebe, Rivat et al. 2005).

Peripheral receptors, especially TRPV-1, also play a role in OIH. Morphine increased TRPV-1 immunoreactivity in the DRG and induced functional changes in the TRPV-1 receptor at the periphery which increased response to capsaicin. TRPV-1 knockout mice did not develop either tactile or thermal hypersensitivity to chronic morphine administration (Vardanyan, Wang et al. 2009) . A TRPV-1 antagonist was found to reverse OIH. This created interest in TRPV-1 antagonists as analgesics for some pain states (Niiyama, Kawamata et al. 2007; Niiyama, Kawamata et al. 2009) and in management of OIH (Knotkova, Pappagallo et al. 2008; Lambert 2009).

In addition, there may be a difference between particular opioids. D-methadone does not cause OIH, reduces morphine-induced OIH, enhances anti-nociception and abolishes sex-related differences, although both L-methadone and racemic methadone have hyperalgesic effects. This seems to be the result of d-methadone antagonism of the NMDA receptor (Holtman and Wala 2007). The μ- and δ-opioid receptors, like NMDA, are primarily located post-synaptically in dorsal horn neurons, specifically excitatory interneurons. These opioid receptor subtypes co-localize with NMDA receptors. Several studies indicate that δ-receptor agonists and antagonists can beneficially modulate pharmacological effects of μ-agonists. For example, δ-agonists can enhance the analgesic potency and efficacy of μ-agonists and diminish or prevent development of μ-agonist tolerance and physical dependence. Based on these observations, novel opioid ligands possessing mixed μ-agonist/δ-agonist or μ-agonist/δ-antagonist profiles could be a promising approach to analgesic drug development (Zhao and Joo 2006; Dietis, Guerrini et al. 2009).

1.2.*3.9.* Summary

In summary, the neurobiology of OIH is complex and several distinct underlying mechanisms may exist, but determining which mechanisms predominate in any given patient has important implications for the pain practitioner. Two predominant mechanisms

include: 1) activation of a spinal pro-nociceptive system via an NMDA receptor-mediated pathway (NMDA, PKC, NOS and COX) and 2) activation of an anti-analgesic system via descending facilitation of synaptic transmission in the dorsal horn (CCK, dynorphin). Based on this understanding, drugs acting at different points of the complex NMDA receptor cascade or descending facilitation system may oppose OIH regardless of their own analgesic potency. Future investigations probably will discover other mechanisms. **Therefore, we can ask the question of the physiological or pathological significance of this elaborated system through the evolution to counteract opioid mediated analgesia?**

1.2.*4.* Population pharmacokinetics: genetic and gender difference in opioid-mediated analgesia and hyperalgesia

It is remarkable that patients with similar pain conditions often require very different quantities of opioids. Factors that influence this variability include type of pain (e.g., nociceptive, inflammatory, or neuropathic), psychosocial condition and genetic disposition (e.g., gender, gonadal/hormonal status and ethnicity).

Individual genetic variations influence both the efficacy and side effects profiles of drugs used to treat pain conditions. μ-opioid receptor genetic variant in women reduces i.t. fentanyl analgesia (Landau, Kern et al. 2008) and poor metabolizers for CYP2D6 show a lower response rate to postoperative tramadol analgesia (Stamer, Lehnen et al. 2003). Likewise, the vulnerability to opioid dependence may be a partially inherited trait (Crowley, Oslin et al. 2003). In animal studies, susceptibility to OIH is highly strain-dependent (Liang, Liao et al. 2006). In humans, individuals homozygous for the met(158) polymorphism of the catechol-o-methyltransferase (COMT) gene have been reported to have increased pain sensitivity at baseline and after parenteral opioids (Jensen, Lonsdorf et al. 2009). Melanocortin-1 receptor gene variants displayed reduced sensitivity to noxious stimuli and increased analgesic responsiveness to morphine-6-glucuronide (M6G) in mice and humans (Mogil, Ritchie et al. 2005). Genetic differences among specific ethnic populations could also cause differences in metabolism, which could explain poor responsiveness or inability to tolerate particular opioids in certain ethnic groups (Smith 2009).

Sex-related differences are present in the analgesic and anti-nociceptive properties of opioids and in opioid-induced side effects, such as changes in respiration, locomotor activity, learning/memory, and addiction (Dahan, Kest et al. 2008). Typically, females are more sensitive than males to opioid agonists and experience respiratory depression and other adverse effects more easily. Males are therefore expected to require 30-40% higher doses of opioid analgesics to achieve similar pain relief (Pleym, Spigset et al. 2003;

Holtman and Wala 2005). In rats, OIH following systemic low-dose morphine was more pronounced in females than in males. Acquisition of tolerance was similar in male and female rats and abolished the sex difference in opioid-sensitivity (Holtman and Wala 2005).

With recent advances in genotyping methods, the list of genes suggested in pain processing and modulation (Lacroix-Fralish, Ledoux et al. 2007) and associated with persistent pain conditions (Diatchenko, Nackley et al. 2007) is rapidly increasing. It is realistic to assume that genetic approaches will discover in the near future novel hypotheses regarding the roles of genes in OIH.

1.2.*5.* Summary of chapter 1.2

OIH is a paradoxical response to an opioid agonist. In contrast to tolerance, which is a pharmacological concept related to the desensitization of the anti-nociceptive opioid pathway, OIH corresponds to an increase or sensitization in pain perception. Clinical distinction between OIH and tolerance is often difficult. Numerous mechanisms that contribute to OIH have been delineated. Clinical evidence supports the occurrence of OIH especially after high-dose opioids or an ultra-short acting μ-receptor agonist (remifentanil). OIH has been extensively documented in animals, even after one administration or extremely low doses of opioids. Clearly, opioid administration can aggravate injury-induced hypersensitivity. **However, few experimental studies have explored opiates in general anesthesia context**.

Section 2: Objectives of the thesis

Although the true incidence of OIH in clinical settings is unknown, there is sufficient clinical and experimental evidence of this phenomenon to engender caution when administering opioids. As summarized above, many animal models have been used in attempts to reproduce perioperative nociceptive conditions. The effects of various opioids and doses have been tested using different postoperative nociceptive thresholds. The majority of past studies observed animals during the postoperative recovery period. The evaluation of the nociceptive threshold was a behavioral assessment. Moreover, the nociceptive stimulus was applied in a conscious animal, but handling the animal may induce stress analgesia and leads to a bias. While all of these studies offer insight into the mechanistic aspects of OIH, none have evaluated the occurrence of OIH under general anesthesia.

Anesthesia may be considered a benign temporary state that has little consequence after patients regain consciousness. However, anesthetics delivered by various routes exert their effects via different molecular pathways. The route of administration and anesthetic compounds are selected based on the pharmacokinetic properties of the drugs, indication for preventing postoperative nausea and vomiting (Mukherjee, Seavell et al. 2003), type of surgery, age of the patient, or personal choice. Nevertheless, the question remains whether the choice of anesthetics influences the anti-nociceptive drug's properties.

For all these reasons and to closely mimic anesthetized surgical conditions, we have developed an animal model in which the effects of anti-nociceptive drugs usually used in the perioperative period were evaluated under anesthesia. The role of anesthesia and the interaction of anesthesia with analgesic drugs have been explored.

The thesis addresses the following questions:

1. whether OIH can develop under general anesthesia,

2. whether anesthetic drugs usually used to performed general anesthesia can influence opioid-induced analgesia (OIA) or OIH, and

3. what's the impact of surgical trauma and perioperative pain conditions on OIH development.

For this purpose, we aim at:

1. setting up an experimental model to mimic perioperative conditions,
2. validating outcome variables as objective, reproducible and comparable indices to assess nociception,
3. characterizing the experimental model and,
4. investigating mechanisms of OIH in the experimental model.

The work consists:

1. **To model an animal model reproducing the perioperative conditions** and exploring different dose of μ-receptor agonist sufentanil (Section 3 Chapter 3.1).

2.. **To evaluate whether minimum alveolar concentration (MAC) of volatile anesthetic that blocks adrenergic responses (BAR) can be used as an objective tool to assess anti-nociception under general anesthesia** (Section 3 Chapter 3.2).

3. **To investigate the excitatory cardio-circulatory effects of opioids under volatile anesthesia.** We assessed the effects of pharmacological agents with proven action against OIH development, including common clinical analgesics and anti-hyperalgesic drugs (Section 3 Chapter 3.3).

4. **To assess the influence of co-existing pain on the development of acute OIH following low (subanalgesic) or high doses of μ-opioid agonists.** Different nociceptive conditions were tested, including acute surgical pain and pre-existing chronic pain. Results were confirmed under the same nociceptive conditions in another experimental model of OIH: after administration of high-dose opioids (Section 3 Chapter 3.4).

Section 3: Experimental studies.OIH under general anesthesia.

Section 3.

3.1. Modeling the intraoperative situation

- **3.1.***1*. Definition of anesthesia: mechanisms of action
- **3.1.***2.* Modeling the intraoperative situation
- **3.1.***3.* **Experimental design: sevoflurane anesthesia**
  - 3.1.*3.1*. Animal preparation
  - 3.1.*3.2*. Determination of the MAC_{SEVO}
  - 3.1.*3.3.* Hemodynamic response: MAC-BAR_{SEVO}
  - 3.1.*3.4.* Painful stimuli
  - 3.1.3.*5.* Analgesic drug
  - 3.1.*3.6.* Statistical analysis
  - 3.1.*3.7.* Results
  - 3.1.*3.8*. Discussion
- **3.1.***4.* **Intravenous propofol anesthesia experiment**
  - 3.1.*4.1*. Materials and methods
  - 3.1.*4.2*. Results
  - 3.1.*4.3*. Discussion
- **3.1.***5.* **Summary of chapter 3.1**

3.2. Validation of the experimental model

- **3.2.***1.* **Is the MAC-BAR_{SEVO} an objective tool to assess anti-nociception in animals?**
  - **3.2.***1.1.* Different nociceptive stimuli and different end-points
  - **3.2.***1.2*. Materials and methods
  - **3.2.***1.3*. Results
  - **3.2.***1.4*. Discussion
- **3.2.***2.* **MAC-BAR-sparing effect of systemic clonidine**
  - **3.2.***2.1.* Systemic clonidine administration under sevoflurane and propofol anesthesia
  - **3.2.***2.2.* Systemic clonidine in different outcomes
  - **3.2.***2.3.* Spinal α_2-adrenoceptors: intrathecal α-antagonists

3.1. Modeling the intraoperative situation

Before approach the description of our experimental animal model, a review of basic concepts of anesthesia including anesthetic mechanisms of action, different anesthetic end-points as well as MAC concept will be exposed. This will facilitate the understanding of the choice of our experimental model.

3.1.*1.* Definition of anesthesia: mechanisms of action

Although more than 160 years have passed since the first successful public demonstration of anesthesia, the mechanisms of action of anesthetic drugs are still not completely understood. From discovery of the weak anesthetic potential of N_2O to ether, chloroform, xenon and halogenated gases such as halothane, enflurane, isoflurane (1981) and more recently desflurane (1992) and sevoflurane (1994), a large number of anesthetic drugs are now available. The i.v. hypnotics include barbiturates, ketamine, etomidate (1973) and more recently propofol (1977). In Europe and the US, propofol is widely used for its dose-dependent effects that allow titration from sedation to general anesthesia.

A definition of general anesthesia could be “a reversible, drug-induced loss of consciousnesses”. However, considering the patient’s best interests, anesthesia ideally provides three reversible conditions: unconsciousness, immobility and amnesia. Moreover, a wide definition of anesthesia could include control of pain (analgesia), muscle relaxation, suppression of reflexes, prevention of nausea and vomiting and even reduction of long-term effects such as postoperative cognitive dysfunction (Urban and Bleckwenn 2002). This wide definition highlights the fact that anesthesia needs to include more than one component.

In 1965, Eger et al. introduced the concept of **Minimum Alveolar Concentration (MAC)** as a way to compare equipotent concentrations of anesthetics. The 1.0 MAC is defined as the partial pressure of an inhaled anesthetic in the lungs at which 50% of non-

relaxed patients remain immobile at time of a skin incision (Eger, Saidman et al. 1965). This immobility component of MAC is widely accepted to result from the action of a volatile anesthetic at the spinal level (Antognini and Schwartz 1993; Rampil and King 1996). New information about sites of anesthetic action suggests that immobility may result from nonspecific anesthetic action within the spinal cord (Eger, Raines et al. 2008; Eger, Tang et al. 2008).

The MAC concept is now considered to be essential for general anesthesia and is applied every day in the operating room. The alveolar concentration is an interesting variable because, once it equilibrates, it represents the partial pressure of the inhaled anesthetic in the CNS, independent of diffusion or distribution to other tissues. An advantage of MAC is its reliability within an individual animal and within a species. MAC is modified by changes in temperature, atmospheric pressure and age (Miller, Anesthesia 4 ed).

In human patients, low concentrations (0.1-0.3 MAC) produce 'sedation', sensory distortion, sleepiness and memory loss, primarily by acting on the cortex. At **MAC awake** (0.3-0.5 MAC), patients are bordering on unconsciousness, and 50% lose the ability to respond to verbal commands.

Roizen and colleagues (Roizen, Horrigan et al. 1981) examined the ability of halothane, enflurane, opiates and spinal anesthesia to block cardiovascular and neuroendocrine responses to skin incision. They described the effect in terms of the MAC that blocked adrenergic responses (**MAC-BAR**). A positive response was arbitrarily defined as an increase of 10% or more in heart rate, blood pressure or norepinephrine levels. Different end-points depended on the anesthetic drug and concentration. A schematic dose-response curve summaries these concepts (Figure 21).

Many studies have observed that anesthetics act at different sites and that the concentration required to achieve certain end-points (e.g., unconsciousness and immobility) is related to their mechanisms. For volatile (inhaled) as well as i.v. anesthetics, sedation and amnesia arise from effects on cortical and subcortical regions of the brain. On the other hand, immobilization results predominantly from depression of spinal neurons for inhaled anesthetics but from both spinal and

supraspinal activity for i.v. anesthetics (Eger, Koblin et al. 1997; Sonner, Li et al. 1998; Nelson, Guo et al. 2002).

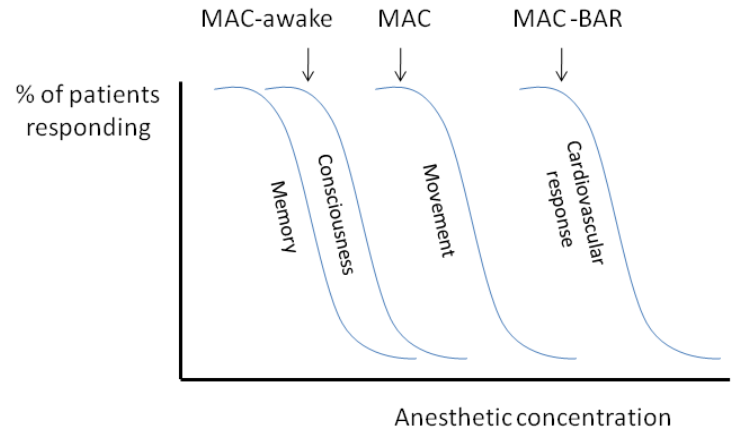


Figure 21: different anesthetic end-points related to the anesthetic concentration
Dose-response curves for various end-points. See glossary for definitions of ***MAC, MAC-awake and 'MAC-BAR****. Note that the curves for memory and consciousness are close to each other. Mac-awake might be greater during the noxious stimulation of surgery*
Adapted from Antognini, 2002

At the cellular level, anesthetics may alter the balance between inhibition and excitation of CNS neural transmission. Pre-synaptically, anesthetics may affect neurotransmitter release, whereas post-synaptically, they may change the frequency or amplitude of electrical impulses traversing the synapse. Chloride channels (associated with $GABA_A$ and glycine receptors) and potassium channels (K_{2P} and possibly K_V and K_{ATP}) remain the primary inhibitory ion channels. Excitatory ion channels include those activated by acetylcholine (nicotinic and muscarinic receptors), EAAs (AMPA, kainate and NMDA receptors) and serotonin (5-HT2 and 5-HT3 receptors) (Cheng and Kendig 2002; Gomez, Guatimosim et al. 2003; Perouansky, Hemmings et al. 2004). The post-synaptic $GABA_A$ receptors are a predominant target since this receptor channel is ubiquitous throughout the CNS. It might be particularly sensitive to i.v. anesthetics such as etomidate and propofol. The volatile anesthetics act on $GABA_A$ but likely recruit a different set of molecular targets, including ion channels and second messenger systems (Franks 2006;

Kopp Lugli, Yost et al. 2009; Sear 2009). Table 4 summarizes the latest knowledge of ion channel targets of anesthetics (Kopp Lugli, Yost et al. 2009).

Paradoxically, there is evidence that at relatively low concentrations (MAC-awake) halothane, isoflurane (Zhang, Eger et al. 2000) and desflurane (Sonner, Li et al. 1998) are associated with hyperalgesia. Barbiturates are also associated with hyperalgesia (Archer, Ewen et al. 1994). How inhaled anesthetics produce hyperalgesia is unknown. It has been hypothesized that low partial pressure of inhaled anesthetics can disrupt the modulating pathway and thereby decrease the threshold for perception of a noxious stimulus. Drasner postulated that halothane induces “a pharmacological disruption of the descending pain inhibitory pathway” which is involved in the analgesic effect of systemic opiates (Goto, Marota et al. 1996; Drasner 2001). Clinically effective concentrations of isoflurane have been proposed to modulate nociception via three mechanisms: 1) pro-nociceptive mechanisms requiring the descending spinal pathway, 2) anti-nociceptive mechanisms requiring descending noradrenergic neurons and 3) anti-nociceptive mechanisms generated intrinsically with the spinal cord neurons (Kingery, Agashe et al. 2002). Brain affects anesthetic requirements. During differential isoflurane delivery, larger concentrations are required to suppress movement when cranial delivery is applied (Antognini and Schwartz 1993; Borges and Antognini 1994). It is worth considering the clinical implications on recovery from anesthesia. When elimination causes the CNS concentrations to approach sub-anesthetic levels, will patients experience hyperalgesia?

In **summary**, anesthetic concentrations required for sedation and hypnosis are lower than those required for immobility. These anesthetic end-points are likely to result from action at different sites. The level of anesthesia is based on many different mechanisms and on multiple molecular biological targets.

Anesthetics	***GABA$_A$***	***K_{2P} channel***	***Glycine***	***NMDA***
Intravenous anesthetics				
Barbiturates	*(+)*	*No effect*	*(+)*	*(-)*
Propofol	*(+)*	*No effect*	*(+)*	*(-)*
Etomidate	*(+)*	*No effect*	*(+)*	*No effect*
Benzodiazepines	*(+)*	*No effect*	*(-)*	*No effect*
Volatile anesthetics				
Ether	*(+)*	*(+)*	*(+)*	*(-)*
Halogened hydrocarbons	*(+)*	*(+)*	*(+)*	*(-)*
Ketamine	*No effect*	*No effect*	*No effect*	*(-)*
Nitrous oxide	*No effect*	*(+)*	*(+)*	*(-)*
Xenon	*No effect*	*(+)*	*(+)*	*(-)*

Table 4: summary of mechanisms of various anesthetics
(+) potentiating effect, (-) inhibitory effect
Adapted from Kopp, 2009

3.1.*2.* Modeling the intraoperative situation

To model the intraoperative situation, important elements include the use of surgical anesthetics, ventilation and immobility of the animal, a stimulus that creates reproducible pain similar to surgical levels and an objective measure of the depth of anesthesia.

When Hecker introduced (Hecker, Lake et al. 1983) and Gomez de Segura (Gomez de Segura, Criado et al. 1998) reintroduced the use of MAC values for volatile anesthetics, they aimed to establish a reference to compare the anti-nociceptive potencies of analgesic drugs and their combinations in rodents. They described interactions between potent inhaled agents and the anesthetic potencies of analgesic drugs (such as sufentanil or synergistic effects of NSAIDS and morphine). The **MAC sparing-effect** defined as a decrease in MAC after an analgesic drug can determine the anesthetic potency of

the drug. Measurement of MAC depends on achieving a stable end-tidal anesthetic concentration, applying a standard noxious stimulus and observing whether movement occurs. Positive movement has been arbitrary defined as “gross and purposeful,” although other movement types could have been included. A pawing motion or turning of the head towards the stimulus are usually considered positive, while coughing, staining, chewing and stiffening are considered negative (Antognini and Carstens 2002). Many investigators also consider a simple withdrawal of the stimulated extremity as a negative response. What is important is that in any individual study, consistency is maintained in the definitions of positive and negative movement. Nevertheless, the functional endpoint(s) selected and the conditions under which they are measured remain often ambiguously defined. Functional endpoints are directly related to clinical endpoints such as immobility (**MAC**), suppression of the stress response to noxious stimuli (**MAC-BAR**) and amnesia and hypnosis (**MAC awake**). These entities are not single functional end-points, but rather comprise a whole set of functional endpoints. There are numerous limitations of using animals to model human behavior and human anesthesia. Assessing a reduced system may oversimplify the complex circuitry that underlies an anesthetic endpoint in the whole organism, and it is futile to argue whether one or the other is more “physiological”. Under these restricted considerations, the choice of experimental parameters aimed to mimic closely our anesthetist’s working conditions.

Using **MAC-BAR** of volatile anesthetics deserves attention for the following reasons: First, it provides a reliable quantification of the observed effect. Second, it avoids major biases such as false positive results because of stress analgesia induced by handling (Konarska, Stewart et al. 1989; De Kock and Meert 1997). Third, it mimics the intraoperative situation, as analgesics are frequently administered during anesthesia on the basis of indirect signs of nociceptive perception (e.g., increase in heart rate and arterial blood pressure). Finally, the model is more acceptable from an ethical point of view because the nociceptive stimulus is applied to an unconscious animal.

3.1.*3*. Experimental design: sevoflurane anesthesia

Adult male Wistar rats weighing 300-400 g were used for all experiments. Rats were maintained on a 12:12 h light-dark cycle and received food and water ad libitum. All tests were performed between 10 a.m. and 4 p.m. The guidelines for pain investigations in animals provided by The International Association for the Study of Pain were respected (Zimmermann 1983). All of the experiments were approved by the Institutional Animal Care and Use Committee of the Catholic University of Louvain.

We studied rats under mechanical ventilation and sevoflurane anesthesia.

3.1.*3.1.* Animal preparation

Rats were placed in an induction chamber with 8% sevoflurane (Abbott Laboratories, Chicago, USA) at a continuous oxygen flow of 3 L/min (sevoflurane vaporizer; Dräger, Lübeck, Germany) for 2-3 min, and then sevoflurane was reduced to 3%. After approximately 5 min, the rats were withdrawn from the induction chamber and positioned on their backs. Sevoflurane was then administered via a plastic cone.

A femoral artery and vein of one hind paw were catheterized with a fine catheter (PE-50) by a surgical cut down. A tracheotomy was performed, and a 16-gauge polyethylene catheter was inserted into the trachea. Correct positioning of the catheter was verified before it was connected to a small T piece with minimal dead space. Fresh gas flow to the T piece was adjusted to 1 L/min, and sevoflurane concentration was adjusted as required. At the end of the surgical preparation, mechanical ventilation was started with oxygen (Fi_{O2} 100%; Vt 10 ml/kg); respiratory rate 35-45 breaths/min) and adjusted for an end-tidal carbon dioxide (CO_2) of approximately 25-30 mmHg (Datex, AS3, Helsinki, Finland) and a maximum peak pressure of 25 mmHg.

Arterial blood pressure was continually monitored via the femoral catheter connected to a pressure transducer (Edwards Lifesciences, Germany), and the electrocardiograph was also continuously monitored. Arterial blood gases were measured at the end of the

experiment to ensure that the values were within normal limits of pH (7.35-7.45), oxygen pressure (Po_2 at least 90 mmHg) and CO_2 pressure (Pco_2 at 30-40 mmHg). Central core temperature was monitored by rectal measure and maintained between 37 ℃ and 38 ℃ by means of a heating light.

Only rats with a normal systolic arterial blood pressure (110-160 mm Hg) after initial instrumentation were included. During the study protocol, rats with persistently low systolic arterial blood pressure (inferior to 100 mm Hg and not responding to 2 ml of polygelin colloid solution) were excluded from the data analysis.

3.1.*3.2.* Determination of the MAC_{SEVO}

Inspiratory and end-expiratory sevoflurane concentrations were continuously measured by an infrared spectrometer (Datex AS3, Helsinki, Finland) and were calibrated before each manipulation. Samples were collected at the extremity of the endotracheal cannula (0.5 cm of the carina). After every step change in anesthetic concentration, at least 15 min was allowed to re-establish equilibrium (inspiratory equal to end-expiratory sevoflurane concentration) before a new noxious stimulus was applied.

3.1.*3.3.* Hemodynamic response: $MAC\text{-}BAR_{SEVO}$

From a practical standpoint, if anesthesia is defined as unconsciousness, amnesia and immobility are important end-points. The reduction of stress hormones is not absolutely required as an end-point, but it is preferable. MAC-BAR can be considered an “adequate” level of anesthesia (high level of spinal anesthesia), which abolishes the intraoperative neuroendocrine stress response (Roizen, Horrigan et al. 1981).

MAC values for the current study were established according to the method described by Eger and Roizen (Eger, Saidman et al. 1965; Roizen, Horrigan et al. 1981). The MAC-BAR is defined as the MAC that blocks the cardiovascular response to tail clamping. A 10% increase in systolic arterial blood pressure was considered to be a positive response, which served as a quantifiable and non-subjective measure. Changes below this threshold were considered to be

negative responses. In all cases, measurements were started at equilibrium 1.5 vol% sevoflurane. According to the outcome response (cardiovascular response), the sevoflurane concentration was then increased or decreased in increments of 0.2% until the response switched from positive to negative or vice versa. All measurements were performed during apnea to avoid hemodynamic variations associated with the different phases of mechanical ventilation.

3.1.*3.4.* Painful stimulus

Tail clamping was chosen as the noxious stimulus to mimic the surgical procedure. This technique is traditionally considered to be an extremely painful stimulus, giving results comparable to those obtained with the electrical currents (Laster, Liu et al. 1993; Antognini and Carstens 1998), and it is generally accepted to determine MAC in rodents. Tail clamping allows repeated quantification of a level of surgical analgesia that is not permitted with skin incision. The noxious (on-off) stimulus was applied with a long hemostat (8" Rochester Dean hemostatic forceps; Martin, Tuttlingen, Germany) clamped to the first ratchet lock, which was placed on the tail for 30 s. A 10% increase in systolic arterial blood pressure after clamping was considered to be a positive response, while changes below this threshold were considered as absence of responses.

3.1.*3.5.* Analgesic drug

The μ-opiate agonist sufentanil (Janssen-Cilag, Beerse, Belgium) was selected since it is widely used in anesthesia. The dosing and method of sufentanil administration were chosen in accordance to Hecker (Hecker, Lake et al. 1983). Sample sizes were 5/group, except for control and 0.07 μg/kg/min sufentanil groups, which included 10 rats. Five animals were included in each group excepted in the control, and sufentanil 0.07 μg/kg/h groups where ten animals were included. Basal (predrug) MAC-BAR$_{SEVO}$ was determined in every animal. Animals then received an equal volume or either saline or study drug: dose bolus in 0.5 ml followed by an infusion of saline or drug (1 ml/h). Measurements began 30 min after drug administration.

Sufentanil groups: group Suf0.005: bolus of 0.015 μg/kg followed by 0.005 μg/kg/min, group Suf0,025: bolus of 0.075 μg/kg followed by

0.025 µg/kg/min, group Suf0.07: bolus of 0.21 µg/kg followed by 0.07 µg/kg/min, group Suf0.1: bolus of 0.3 µg/kg followed by 0.1 µg/kg/min, group Suf0.5: bolus of 1.5 µg/kg followed by 0.5 µg/kg/min, group Suf1: bolus of 3 µg/kg followed by 1 µg/kg/min.

3.1.*3.6.* Statistical analysis

During each experiment, the first change in response was noted, and the mean of the two adjacent doses of sevoflurane (i.e., immediately before and after the change) was taken as the MAC-BAR_{SEVO}. Results presented are means ± SD. The normality of the data was assessed according to the Kolmogorov-Smirnov test. MAC values were compared using repeated-measures of analysis of variance (ANOVA), followed by post hoc analysis Tukey's test. A p value less than 0.05 was considered statistically significant.

3.1.*3.7.* Results

Anesth Analg; 92: S227, 2001 (ASA, 2001)
Eur J Anaesth, vol 20, suppl 30, 2003 (ESA, 2003)

The average MAC-BAR_{SEVO} value in control rats was 1.88 ± 0.2% (range, 1.05-2.85). Sufentanil, at the best dose in the present data (0.07 µg/kg/min) reduced MAC-BAR_{SEVO} by approximately 24%. As expected, large doses sufentanil (0.5 and 1 µg/kg/min) significantly reduced MAC-BAR_{SEVO} (80%). In contrast, a very low dose of sufentanil (0.005 µg/kg/min) significantly increased the MAC-BAR_{SEVO} to a similar extent (82%) (Figure 22).

To explore the evolution in time of this "paradoxical" phenomenon, a 5h-continuous infusion at rate 0.005 µg/kg/min was performed. The excitatory effect decreased 3 h after the infusion starting (Figure 23). The plasma concentrations of sufentanil were measured in four additional rats at 15, 30, 60, 120 min using the liquid chromatography technique (Waters 2795, Micromass Quattro micro, Mass Spectrometry Facility, salt Lake City, UT) (Figure 24).

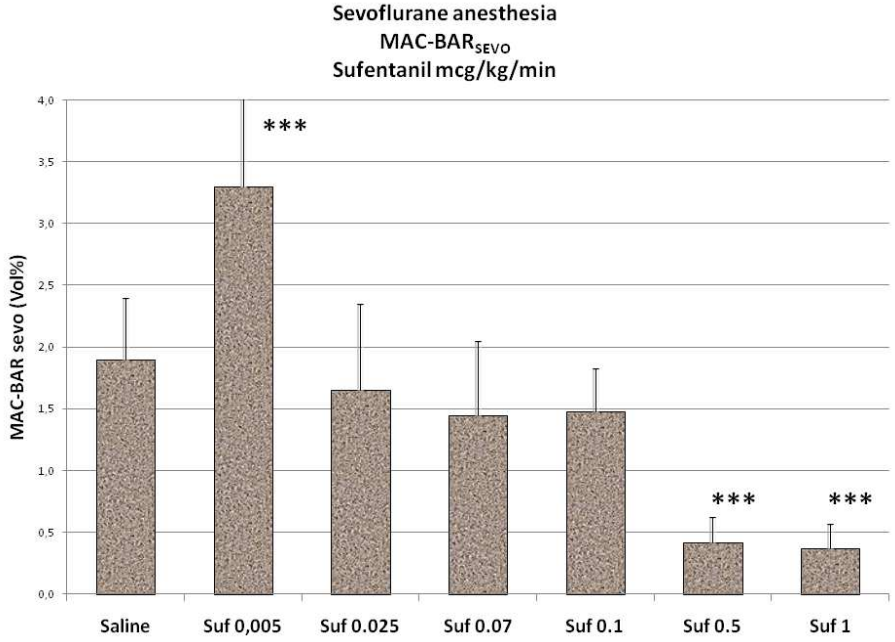


Figure 22: sevoflurane anesthesia and sufentanil
MAC-BAR$_{SEVO}$ in animals treated with different doses of i.v. sufentanil
Results are means ± SD. n = 5 to 10 per groups.
****: significant difference compared to saline controls (p<0.001)*

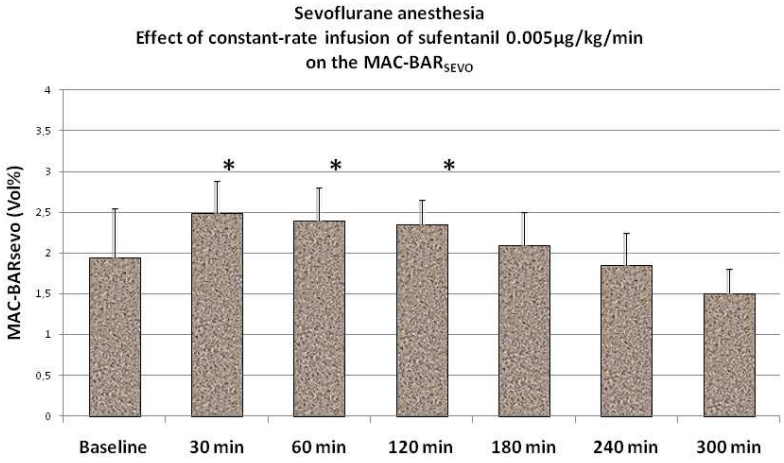


Figure 23 : constant-rate of sufentanil
MAC-BAR$_{SEVO}$ decreased 3h after a continuous infusion of sufentanil at 0.005 µg/kg/min. Results are mean ±SD. n=7.
**: significant difference compared to baseline (p<0.05)*

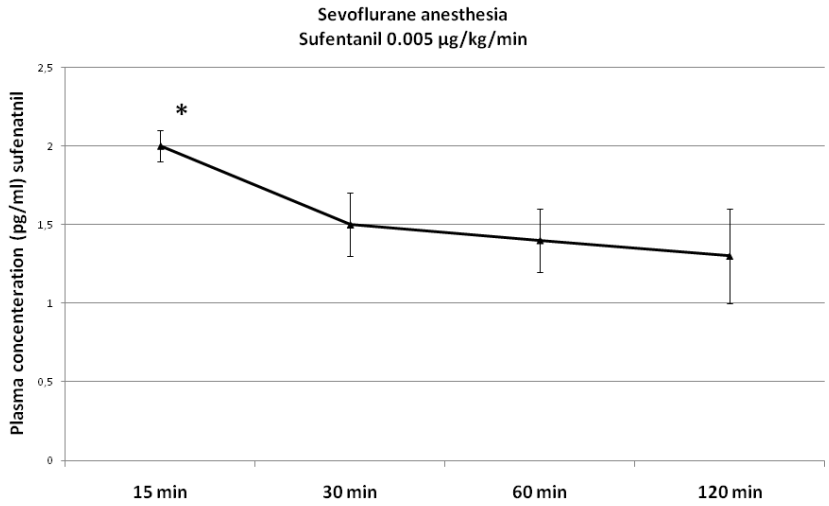


Figure 24: plasma concentration of sufentanil
I.v. bolus of 0.015 µg/kg followed by continuous infusion of 0.005 µg/kg/min. The plasma concentration of sufentanil was maintained at a steady state during 120 min. Values are mean±SD. n=4.
**: significant difference between times (p<0.05)*

3.1.*3.8.* Discussion

The results obtained on the MAC-BAR$_{SEVO}$ after administration of low dose of sufentanil raise questions. The dose of this µ-agonist was chosen based on the work of Hecker (Hecker, Lake et al. 1983), who evaluated the sufentanil-induced decrease of the MAC for halothane in rats. This group reported a sigmoidal dose-response curve with an abrupt steep response following the initial upward deflection of the curve. They found an additional 62% MAC reduction occurring between dose of 0.01-0.1 µg/kg/min. The ED50, defined as the dose required to produce a 50% decrease in MAC, was 0.07 µg/kg/min. This point was extrapolated from the linear portion of the curve. The animals who received similar doses showed a MAC reduction of 27%. In our study, rats that received a sufentanil dose of 0.07 µg/kg/min displayed a MAC reduction of approximately 23%. The MAC reduction afforded by the dose of 0.1 µg/kg/min was comparable, but higher doses (>0.5 µg/kg/min) produced nearly complete anesthesia when administered alone (±80% reduction of MAC-BAR$_{SEVO}$). Until this point, our data are in accordance with those of Hecker, though

the sigmoidal dose-response curve appears slightly displaced to the right.

More surprising is our observation that very low doses of sufentanil (0.005 μg/kg/min) significantly increase the MAC-BAR_{SEVO}. Such a paradoxical effect has been previously observed by Goto (Goto, Marota et al. 1996) and Drasner (Drasner 2001). The anti-nociceptive effects of nitrous oxide (N_2O) and morphine can be antagonized by halogen gas (halothane and isoflurane to a lesser extend). These results were confirmed by other authors (Cole, Kalichman et al. 1990; O'Connor and Abram 1995). The importance of this antagonism is linked to the gas used, with halothane being more potent than isoflurane. It is also end-point specific: a marked antagonism has been reported for the escape reaction to tail pressure (Kissin and Jebeles 1984) and the cardiac acceleration response to tail clamping in rats (Kissin, Kerr et al. 1984). In contrast, halothane and N_2O were additive in suppressing noxious stimulation-induced purposeful movements. These two observations may explain why this phenomenon was observed in our study and not in the one by Hecker. Sevoflurane may be more potent than halothane to reveal this paradoxical effect of sufentanil in a specific end-point of the MAC-BAR_{SEVO}. Interestingly, a genetic variability in MAC was observed. The underlying sensitivity to the noxious stimulus related to species can be reflected by the MAC, which may not be observed in other end-point conditions (Mogil, Smith et al. 2005). Furthermore, the presence of halothane increased the dose of i.v. morphine required to block the tail-flick reflex but enhanced the efficacy of i.t. morphine on this same behavior.

The question remains as to how low-dose sufentanil induced hyperalgesia under sevoflurane. Both Goto and Drasner suggest a disruption of the descending inhibitory pathway, a system involved in the analgesic effect of systemic opiates. Halogenated anesthetics could prevent such neural activation by decreasing the cerebral or spinal metabolic rate. Drasner also postulated that halothane induces “a pharmacologic transection of the spinal cord” (Drasner 2001). In our study, sevoflurane may have prevented recruitment of this noradrenergic descending inhibitory system and therefore unmasked

the excitatory properties of low-dose sufentanil, a pure μ opiate agonist.

3.1.*4.* Intravenous propofol anesthesia experiment

Anesth Analg; 92, 2001 (ASA, 2001)
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To explore this paradoxical excitatory effect of low-dose i.v sufentanil, the same doses of sufentanil were tested under i.v. propofol (2,6-disopropylphenol) anesthesia. This experiment could support or refute the hypothesis, as sevoflurane can unmask the excitatory or hyperalgesic properties of low-dose sufentanil. Propofol is a highly effective i.v anesthetic and is now widely used for general anesthesia and for sedation and in intensive care units.

3.1.*4.1.* Materials and methods

The equipment and monitoring, as well as administration of sufentanil, was similar to the previous experiment. A propofol 2% (Astra-Zeneca, Brussels, Belgium) infusion was started, at the end of surgical preparation, at a fixed dose of 150 mg/kg/h, in agreement with previous studies (Ewen, Archer et al. 1995; De Paepe, Belpaire et al. 2000). Sample sizes were 5/group.

The relative analgesic potency of the different doses of sufentanil was evaluated using the tail clamping technique with the stimulus applied every 5 min. A 10% increase in systolic arterial blood pressure was considered to be a positive response. Analgesic potency was determined based on the latency from injection of the analgesic until the animal became non-reactive to the noxious stimulus. At this moment, arterial blood was retrieved for measurement of propofol concentration. Immediately after collection, whole blood samples were hemolysed and stored at 4 ℃ until analysis by high performance liquid chromatography according to the method of Plumme (Plummer 1987).

The normality of the data was assessed according to the Kolmogorov-Smirnov test. Data were compared using repeated-measures of analysis of variance (ANOVA), followed by post hoc analysis Tukey's test. A p value less than 0.05 was considered

statistically significant. Variables included the time before loss of hemodynamic reaction and the arterial propofol concentrations at that moment.

3.1.*4.2.* Results

A duration of 41 min (range 29-49) of propofol infusion was necessary to suppress the hemodynamic reactions consecutive to noxious stimulation in control rats. A time duration necessary to achieve a level of anesthesia sufficient and to become unreactive to noxious stimulus. The concentration of propofol at this moment was 29 ± 3.3 µg/ml. The propofol-sparing effects of the different doses of sufentanil are presented in Figure 25.

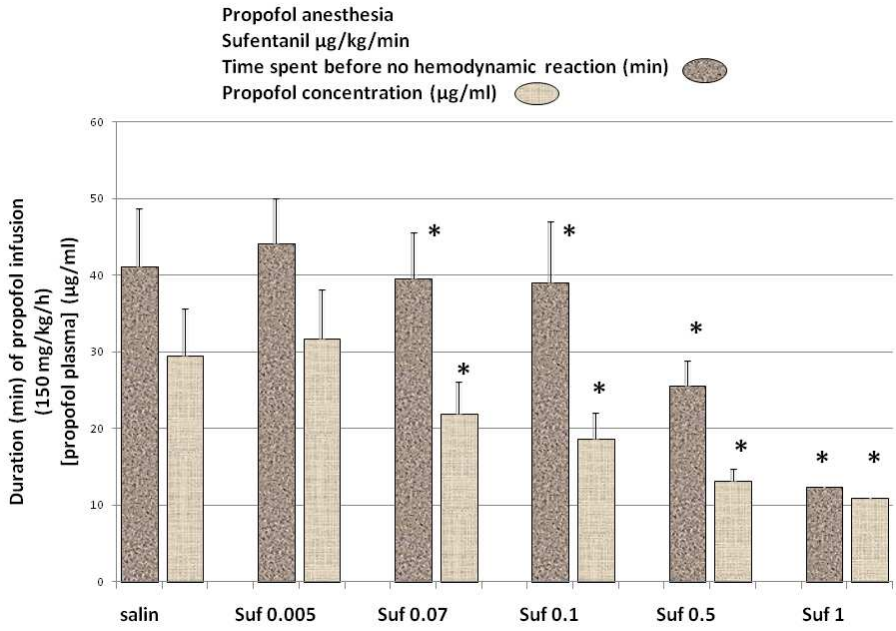

Figure 25: propofol anesthesia and sufentanil
A 41-min duration of propofol infusion was necessary to suppress the hemodynamic reactions consecutive to noxious stimulus in control rats. The [plasmatic propofol] at this moment was 29 ± 3.3 µg/ml. Different doses of sufentanil were tested. In dark grey color: minutes spent before loss of hemodynamic reaction. In light grey color: concentration of propofol measured at time of no hemodynamic reaction.
Results are mean±SD. n=5 to 10 per groups
**: significant difference compared to saline control (p<0.05)*

In contrast to animals anesthetized with sevoflurane, the dose of propofol required in the low-dose sufentanil group (0.005 μg/kg/min) was similar to controls.

3.1.*4.3.* Discussion

The results demonstrated an interesting interaction between anesthetic and analgesic drugs. The sparing effect of sufentanil appears linear under propofol anesthesia, but not under sevoflurane anesthesia.

The effects of sufentanil on the MAC-BAR_{SEVO}, especially the increase in MAC-BAR_{SEVO} after low doses of sufentanil (0.005 μg/kg/min), raise the question of why a hyperalgesic effect was not observed under propofol anesthesia. We have already hypothesized a possible disruption of the descending inhibitory pathway, which is involved in the pain modulation pathway. These results may have clinical implications. Experimental data show that low concentrations of inhaled anesthetics may increase pain perception in humans (Tomi, Mashimo et al. 1993). Moreover, recent publications have demonstrated that patients anesthetized with propofol have less postoperative pain and morphine use than those anesthetized with volatile anesthetics (Cheng, Yeh et al. 2008). As observed by Ben-David and Chelly (Ben-David and Chelly 2009), the pharmacological interaction of anesthetic drugs in perioperative period is not well understood and asks future investigations.

Propofol has been well documented as an allosteric potentiator and agonist of $GABA_A$ receptors, mechanisms which underlie its anesthetic effects. Propofol might also inhibit glutamate-mediated excitatory neurotransmission (Irifune, Takarada et al. 2003) and NMDA-receptor-mediated calcium increase (Grasshoff and Gillessen 2005). Furthermore, propofol at clinically-relevant concentrations blocked NMDA-mediated activation of MAPK- and event-related kinase (ERK)-mediated signaling, which normally would facilitate transcriptional events (Kozinn, Mao et al. 2006). This mechanism is GABA-independent. Nevertheless, in *vitro* studies volatile anesthetics inhibit also glutamate receptor function (Hollmann, Liu et al. 2001).

The unexpected phenomenon in these experiments is the pro-nociceptive effect of low-dose μ-agonists, highlighted by the concomitant administration of sevoflurane. When the dose of sufentanil increases, its anti-nociceptive effect becomes apparent. Experimental studies have observed that acute administration of μ-opioid agonists in rat can induce hyperalgesia, and the mechanism underlying this phenomenon is NMDA-dependent (Celerier, Rivat et al. 2000). This phenomenon is not observed with propofol, a general anesthetic acting by enhancement GABAergic neurotransmission and NMDA pathways. This might furnish supplemental and indirect arguments for the role of sevoflurane in OIH and for NMDA pathway implication.

3.1.5. Summary of chapter 3.1.

In attempt to explore whether OIH can occur under general anesthesia, we have developed an anesthetized animal model mimicking the perioperative nociceptive conditions.

Different doses of the μ-receptor agonist sufentanil were evaluated 1) under the halogenated vapor anesthetic sevoflurane 2) under intravenous propofol anesthesia. The relative anti-nociceptive potency of the different doses of sufentanil was evaluated using the tail clamping technique. A 10% increase in systolic arterial blood pressure (hemodynamic response) at the time of tail clamping was considered to be a positive response.

The anti-nociceptive sufentanil effects are modified by clinically effective concentrations of sevoflurane and are differently affected depending on the type of general anesthetic used, either volatile (sevoflurane) or i.v. (propofol). The mechanisms of action of anesthetics are based on multiple different molecular targets. Far from the simple definition of general anesthesia as “a reversible, drug-induced loss of consciousnesses,” anesthesia appears to be part of a complex interaction among all drugs used. This pharmacological interaction in the perioperative period is not yet adequately explored.

Furthermore, sevoflurane anesthesia unmasks the excitatory properties of low-dose sufentanil administration. OIH may occur after administration of a single dose of opioids. This effect should be considered in light of recent human reports highlighting the importance of intraoperative analgesia on postoperative pain perception. Our findings might have important implications for the paradigm including opioids as part of anesthesia. We therefore performed further investigation of this paradoxical phenomenon.

3.2. Validation of the experimental model

3.2.1. Is the MAC of sevoflurane an objective tool to assess anti-nociception in animals?

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Before investigating the mechanisms underlying the aforementioned observations, such as the "paradoxical" pro-nociceptive effect of low-dose sufentanil under sevoflurane anesthesia, these results should be confirmed with other end-points. Despite the numerous advantages of the anesthetized animal model, several questions remain unanswered: First, the reproducibility of the applied nociceptive stimulus and the precision of the outcome variable in anesthetized animals may be questioned. Moreover, whether this model is still valid for drugs having both analgesic and sedative or cardio-circulatory depressant properties (e.g. clonidine) is unknown. Finally, the significance of this nociceptive challenge is unclear, as the result does not simply represent the effect of the analgesic tested but rather the interaction between this drug and a halogenated vapor. Thus, we questioned whether sufentanil-induced pro-nociceptive effect, which we observed under sevoflurane anesthesia, could also be observed in other experimental end-point conditions.

3.2.1.1. Different nociceptive stimuli and different end-points

To evaluate the reliability of the minimum anesthetic alveolar concentration (MAC)-sparing effect as an objective measure of the antinociceptive properties of a drug, we conducted several experiments designed to evaluate different nociceptive stimuli (pressure or thermal) and different outcome variables (gross purposeful movement, paw withdrawal, and arterial blood pressure reactivity) and analyzed the significance of the results obtained.

Therefore, in a first set of experiments, we studied rats under **mechanical ventilation** and sevoflurane anesthesia. Outcome variables such as gross purposeful movements consecutive to tail clamping, paw withdrawal consecutive to increasing paw pressure (MAC), and cardio-circulatory reactivity (MAC-BAR) after these stimuli

were recorded. In a second set of experiment, sevoflurane-anesthetized rats under **spontaneous breathing conditions** were used. Thermal stimuli were compared with pressure. The MAC-sparing effect of several doses of sufentanil was evaluated. Finally, results were compared with those obtained in *awake animals*.

Stimuli	***Response***	***Ventilated animals***	***Spontaneous breathing animals***	***Awake habituated rats***
Clamp	*Behavior*	*MAC clamp*	*MAC clamp*	
	Hemodynamic	*MAC-BAR clamp*	*MAC-BAR clamp*	
Pressure	*Behavior*	*MAC pressure*		*x*
	Hemodynamic	*MAC-BAR pressure*		
Thermal	*Behavior*		*MAC thermal*	*x*
	Hemodynamic		*MAC-BAR thermal*	

Table 5: summary of different nociceptive stimuli and different outcome variables

3.2.*1.2.* Materials and methods

After approval by the Animal Care and Use Committee, eight adult male Wistar rats weighing 300–400 g were studied under each set of test conditions. The guidelines for pain investigations in animals provided by The International Association for the Study of Pain were respected.

3.2.1.2.1. Experimental groups

All tests were performed between 10 am and 2 pm. Only rats with a normal systolic arterial blood pressure (110–160 mm Hg) after initial instrumentation were included. During the study protocol, rats with persistently low systolic arterial blood pressure (<100 mmHg not responding to 2 ml of polygelin) were excluded from the data analysis.

Experiment 1: mechanical ventilation

In this experiment, different MAC_{SEVO} values were established according to the method described by Eger (Eger, Saidman et al. 1965) and Roizen (Roizen, Horrigan et al. 1981) in ventilated rats. The methodology was comparable to describe in chapter 3.1.3.

Briefly, rats were anesthetized with sevoflurane in an induction chamber. After approximately 5 min, the animals were removed from the induction chamber and positioned supine. Sevoflurane was then administered via a plastic cone. The femoral artery and vein of one hindpaw were catheterized with a fine tubing (PE-50) via surgical cutdown. Tracheotomy was performed and a 16 gauge polyethylene catheter inserted. At the end of this surgical preparation, mechanical ventilation was started with oxygen and sevoflurane and ventilation was adjusted. Arterial blood pressure via the femoral catheter was connected to a pressure transducer. Rectal temperature was monitored and maintained between 37 and 38℃ by mea ns of heating light. Inspiratory and end-expiratory sevoflurane concentrations were continuously measured by an IR spectrometer. After every step change in anesthetic concentration, an equilibration time (inspiratory sevoflurane concentration equal to end-expiratory) was allowed (at least 15 min) before a new noxious stimulus was applied.

A **noxious (on-off) stimulus** was applied with a long hemostat clamped to the first ratchet lock on the tail for 30 s. The tail was always stimulated proximal to a previous test site. For the **MACclamp$_{SEVO}$** (MAC of sevoflurane that blocks purposeful movements after tail clamp), the response was considered positive when a gross purposeful movement of the head, body, extremities, or combination of these was observed, whereas a negative response was a lack of movement or grimacing, swallowing, chewing, or tail flick. For the determination of **MAC-BARclamp$_{SEVO}$** (MAC of sevoflurane that blocks cardiovascular response to tail clamp), a 10% increase in systolic arterial blood pressure was considered as a positive response. No change or changes less than this threshold were considered as negative responses. In all cases the measurements were started at equilibrium 1.5 vol% sevoflurane. According to the outcome response, the sevoflurane concentration was then adjusted in decrements or increments of 0.2% until the negative response became positive or the positive response became negative. All measurements were performed in apnea to avoid hemodynamic variations consecutive to the different phases of mechanical ventilation.

A **(progressive) noxious stimulus** was applied using an analgesiameter (Randall Selitto Test Ugo Basile, Comerio, Italy). A linear increasing mechanical pressure was applied on the hind paw. For the **MACpressure$_{SEVO}$** (MAC of sevoflurane that blocks paw withdrawal), the response was considered positive when the animal withdrew its paw from the device; the pressure applied at this time was recorded. To avoid tissue hematoma and damage caused by the pressure, a maximum force of 400 g was allowed, based on the maximal pressure tolerated by a conscious animal. For the determination of **MAC-BARpressure$_{SEVO}$** (MAC of sevoflurane that blocks cardiovascular response to paw pressure), a 10% increase in systolic arterial blood pressure was considered as a positive response. No change or changes less than this threshold were considered as a negative response. The same procedure was applied as for MAC-clamp$_{SEVO}$ and MAC-BARclamp$_{SEVO}$.

The effects of different sufentanil doses were tested on the different MACs in ventilated rats.

Experiment 2: spontaneous breathing

In these experiments, we determined the MAC and MAC-BAR using **thermal and pressure stimuli** in spontaneously breathing rats. For this purpose, rats were equipped and monitored as in the first experiment except that no tracheotomy was performed. After instrumentation, the spontaneously breathing rats were placed in a special chamber encasing only the upper half of the body. Air tightness was achieved using a flexible seal maintained by a cord gently squeezed around the abdomen. Such equipment allowed easy testing of the hind paw reactions. The chamber contained an opening for anesthetic gases, a port for exhaust gases, and a small hole through which a fine catheter for gas sampling was introduced and threaded to the immediate vicinity of the animal's nostrils. Sevoflurane was administered in oxygen and directed into the chamber with an average flow of approximately 1 L/min. Anesthetic concentrations in the chamber were analyzed by infrared spectrometer. As for the previous manipulation, the measurements were started at equilibrium 1.5 vol% sevoflurane. Depending on the response, the sevoflurane concentration was then adjusted in

decrements or increments of 0.2% until the negative response became positive or the positive response became negative.

The thermal noxious stimulus (52°C) was applied using the device developed by Ozaki and Yaksh (Dirig, Salami et al. 1997) and was delivered to the hind paw with a cutoff of 20 s to avoid tissue injury. For the **MACthermal$_{SEVO}$** (MAC of sevoflurane that blocks paw withdrawal after thermal stimulus), the response was considered positive when the rat withdrew its hind paw before cutoff time. For the **MAC-BARthermal$_{SEVO}$** (MAC of sevoflurane that blocks cardiovascular response), the response was considered positive when the systolic arterial blood pressure increased by 10% or more before cutoff time. The same methodology and criteria were applied as in the first experiment. The thermal stimulus was replaced by a paw pressure stimulus delivered by the analgesia-meter.

The effects of sufentanil were also tested in spontaneously breathing rodents. Once the MAC value was determined, the concentrations of sevoflurane were progressively decreased to determine the concentration at which the unstimulated rat showed signs of spontaneous recovery (such as swallowing or movements of the head) (Table 6).

Experiment 3: awake habituated rats

The antinociceptive effects of the different doses of sufentanil facing a thermal or a pressure stimulus were also evaluated in awake habituated rats (Table 7).

3.2.1.2.2. Analgesic drug

The dose and the mode of sufentanil administration were conducted in accordance to the work by Hecker (Hecker, Lake et al. 1983). Rats (8 per group) received either a bolus of 0.015 µg/kg followed by 0.005 µg/kg/min (group Suf0.005), a bolus of 0.21 µg/kg followed by 0.07 µg/kg/min (group Suf0.07), a bolus of 0.3 µg/kg followed by 0.1 µg/kg/min (group Suf0.1) or a bolus of 3 µg/kg followed by 1 µg/kg/min (group Suf1).

3.2.1.2.3. Statistics

Results are presented as mean±SD. During each experiment, the first change in reaction was noted, and the mean of the two adjacent doses of sevoflurane (i.e., immediately before and after the change) was taken as the MACsevo or the MAC-BAR$_{SEVO}$. The normality of the data was assessed according to the Kolmogorov-Smirnov test. Different MAC values were compared using analysis of variance. In rats treated with sufentanil, repeated-measures analysis of variance followed by *post hoc* analysis using the Tukey test was applied. A p value < 0.05 was considered significant.

3.2.*1.3.* Results

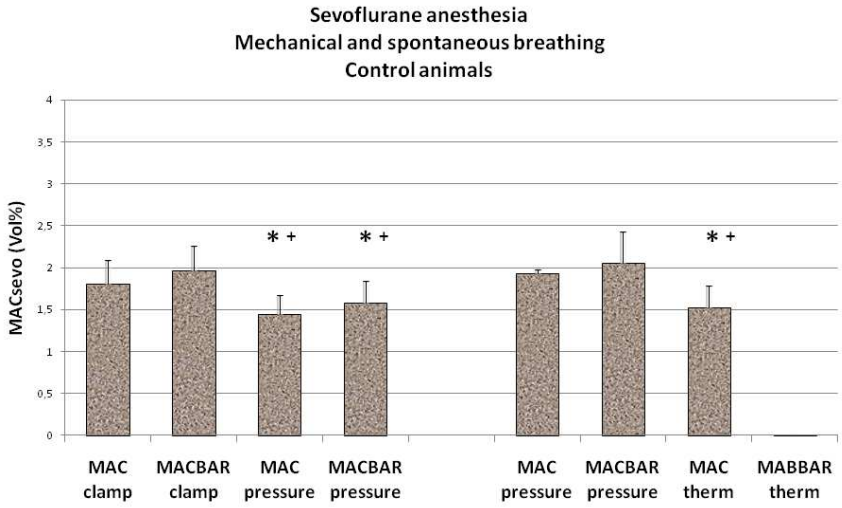

Figure 26: MACs in controls rats
Summary of the different MACsevo values recorded in control rats under mechanical ventilation (left) and spontaneous breathing (right)
Results are mean±SD. n=8 per group.
**: significant difference compared to MACclamp or MAC-BARclamp from ventilated animals (p<0.05)*
+: significant difference compared to MACpressure or MAC-BARpressure from spontaneous breathing animals (p<0.05)

One rat after instrumentation and two rats during the study protocol developed profound hypotension that could not be reversed by

polygelin administration. Three additional rats were therefore studied to maintain the initial population of eight rodents per group.

Experiment 1: mechanical ventilation

In ventilated rats, the MAC differed significantly according to the stimulus applied. The tail clamp was obviously a more important nociceptive stimulus than hind-paw pressure. However, no statistically significant difference was noted between MAC and MAC-BAR for these two stimuli (Figure 26).

In treated animals, the different MACs recorded before drug administration were comparable to the controls. The administration of small-dose sufentanil (0.005 µg/kg/min) had no effect on $MACclamp_{SEVO}$ and $MACpressure_{SEVO}$, whereas it significantly increased both MAC-BARs ($p< 0.05$). At larger doses, sufentanil significantly reduced both MAC in all test conditions ($p< 0.05$) (Figure 27).

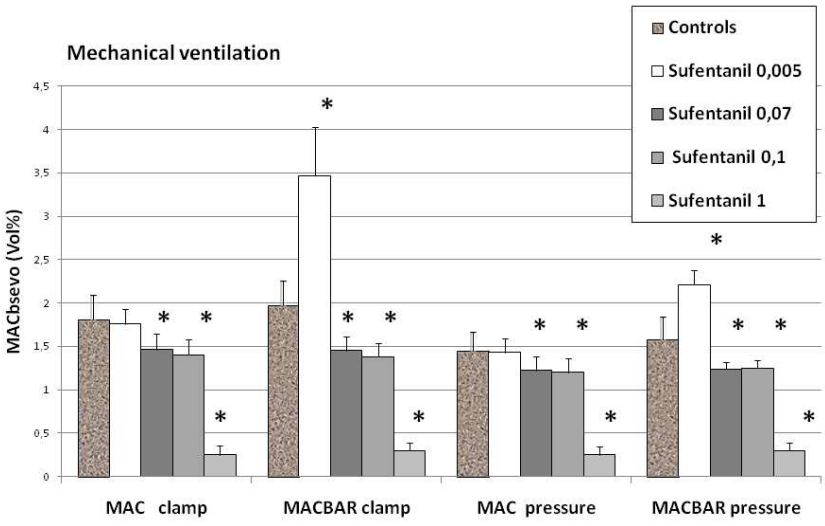

Figure 27: MACs: mechanical ventilation and sufentanil
Summary of different MACs recorded in ventilated rats with sufentanil administration.The administration of small-dose sufentanil had no effect on MACclamp and MACpressure whereas significantly increased both MAC-BARs. Larger doses significantly decrease both MACs in all conditions
Results are mean±SD. n=8 per group
**: significant difference compared to controls (p<0.05)*

Experiment 2: spontaneous breathing

The MAC value to suppress paw withdrawal consecutive to thermal stimulus is presented in Figure 26. MAC-BAR was impossible to determine using this stimulus because no blood pressure variations could be detected before or even after the cut-off time.

When the paw-pressure stimulus is applied to spontaneously breathing animals, the concentrations of volatile anesthetics required to suppress the withdrawal reaction are significantly larger than after thermal stimulus (Figure 28), and both MACs and the MACBARs for this stimulus are significantly larger than in ventilated animals (Figure 26).

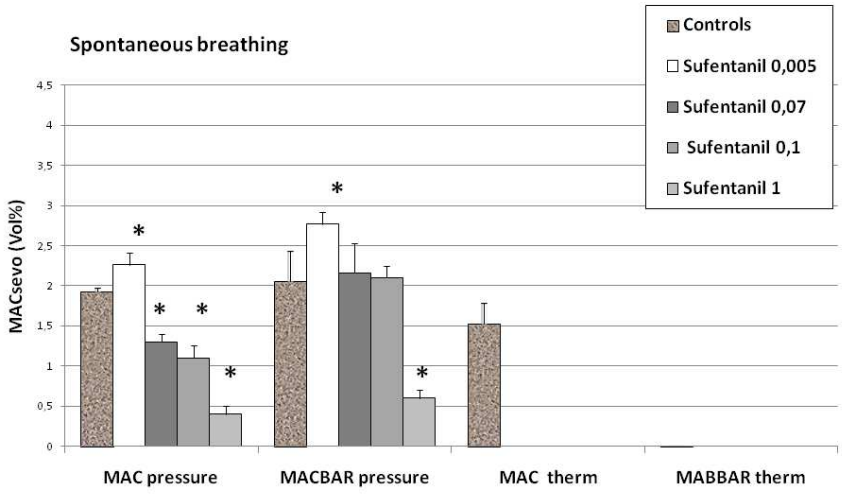

Figure 28: MACs: spontaneous breathing and sufentanil
Summary of different MACs recorded in spontaneously breathing rats with sufentanil administration. When the thermal stimulus was applied, no withdrawal reaction occurred after sufentanil administration. When the paw pressure was applied, small-dose of sufentanil produced significant increase of both MAC and MAC-BAR values. Larger doses reduced the MAC but were without significant effects on the MAC-BAR. The largest dose of sufentanil significantly reduced both MAC.
Results are mean±SD. n=8 per groups
**: significant difference compare to controls (p<0.05)*

Interestingly, the administration of sufentanil had important antinociceptive effects in the model because when a thermal stimulus was applied, no withdrawal reaction occurred, even at small expiratory concentrations of sevoflurane. When the paw pressure was applied, a small dose of sufentanil (0.005 µg/kg/min) produced significant increases of both MAC and MAC-BAR values ($p < 0.05$). Larger doses (0.07 and 0.1 µg/kg/min) reduced the MAC but were without significant effects on the MAC-BAR. The largest dose of sufentanil (1 µg/kg/min) significantly reduced both MAC ($p < 0.05$).

In the 1 µg/kg/min sufentanil group, no sign of spontaneous recovery was observed after 5 min of sevoflurane discontinuation. In the other groups, the concentration of anesthetics at which spontaneous recovery occurred was independent of the dose of sufentanil administered (Table 6).

	[sevoflurane] at which spontaneous recovery occurred
Controls	*1.04±0.11*
Suf 0.005 µg/kg/h	*1.1±0.14*
Suf 0.07 µg/kg/h	*1.02±0.10*
Suf 0.1µg/kg/h	*1.04±0.12*
Suf 1 µg/kg/h	*No recovery after 5 min Sevo discontinuation*

Table 6: [Sevoflurane] at which spontaneous recovery occurred
The sevoflurane concentration was independent of the dose of sufentanil administrated. This indicates that the small doses were inferior to those required to obtain a cataleptic effect and that the results obtained are pertinent to an effect on nociception. This is not the case when 1 µg/kg/h sufentanil (very high dose) is used.

Experiment 3: awake habituated animals

Latencies or weight reached before withdrawal by awake, habituated rats submitted to a thermal or a pressure noxious stimulus are presented in Table 7.

	Controls	***Suf0.005***	***Suf0.07***
Thermal testing: Latencies (sec)	*11±2.1*	*12.6±1.9*	*8.6±3.0*
Paw pressure: Pressure (g) reached before withdrawal	*95±22*	*88±26*	*82±19*

Table 7: withdrawal reaction in awake animals

Repeated measures

Tail clamping is a generally accepted technique to determine MAC in rodents (Eger, Saidman et al. 1965; Laster, Liu et al. 1993; Antognini and Carstens 1998). Nevertheless, some concerns may arise from repeated measurements. Repeated noxious supramaximal stimuli may indeed induce some degree of central sensitization that might interfere with the results independently of any treatment.

To test for possible sensitization after repetitive tail clamping, additional rats were equipped according to the study protocol. In each rat, 30 measures (tail clamping) were performed over a period of 150 min, starting from the caudal to the rostral extremity. Five successive MAC-BARclamp$_{SEVO}$ were determined in the same rat. Results of repetitive stimulation of the tail are presented in Figure 29. Significant reduction of the MAC-BARclamp$_{SEVO}$ occurred after 150 min and after approximately 40 successive tail clamps. We observed that the MAC measurements remained constant after four consecutive determinations. Significant changes only occurred after the fifth determination and approximately 30 measurements.

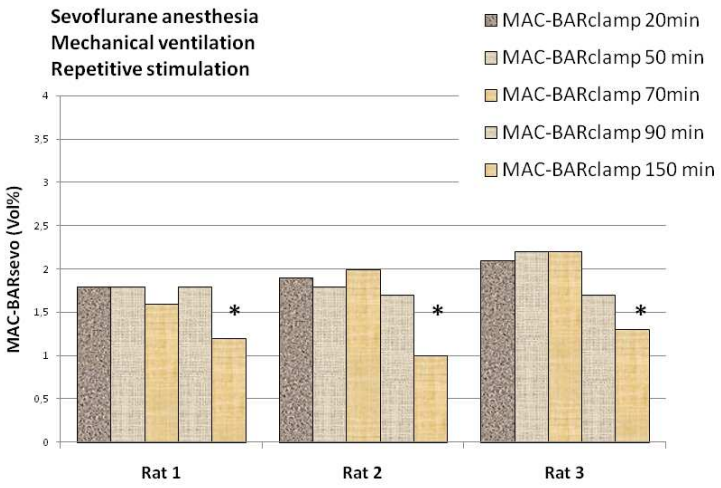

Figure 29: repeated measures
MAB-BAR$_{SEVO}$ in animals during mechanical ventilation and repetitive tail clamping. Significant changes occurred after approximately 30 measurements.
**: significant difference compare to the first measure ($p<0.05$)*

3.2.*1.4.* Discussion

Our data confirm that this particular rat model of nociception under general anesthesia provides objective, reproducible, and comparable outcome variables. Interestingly, from a technical point of view, it is applicable to spontaneously breathing animals. Moreover, it can discriminate between a sedative and an antinociceptive effect when the concentrations of vapors associated with spontaneous recovery are recorded. Nevertheless, it is clearly not just a simple measure of the antinociceptive potency of a drug. All the observations made were the results of the interactions between the halogenated vapor and the tested analgesics.

We used three different stimuli: tail clamping, paw pressure, and heat. Without doubt, the most intense of these is tail clamping. Consequently, the largest MAC values were recorded with this stimulus. The anesthetic concentration required varies within a moderate range depending on the severity of the stimulus (Eger, Saidman et al. 1965). Paw pressure, a progressive submaximal stimulus, is an interesting alternative to tail clamping. It is easily quantified and consequently, highly reproducible. It also introduces a new objective variable, the pressure for which the withdrawal reaction occurs. Thermal stimulus as applied in our study is probably the most gentle one. It did not trigger any cardio-circulatory modification. However, it remains important to consider because it involves different sensory afferences than the mechanical stimuli. Furthermore, thermal nociceptive stimulation is highly sensitive to the antinociceptive effect of opiates.

We recorded two different outcome variables, MAC and MAC-BAR. The concept of the MAC-BAR was first introduced by Roizen (Roizen, Horrigan et al. 1981). In this model, instead of a gross purposeful movement, a 10% increase in blood pressure, heart rate, or rate pressure product and a 10% increase in noradrenergic levels are considered positive responses. According to these authors, the MAC-BAR is a multiple of the MAC. This can be understood taking into account that volatile anesthetics exert a direct and predominant action at the spinal cord level and hence block several spinal nociceptive reflexes such as withdrawal movements or increases in

arterial blood pressure and heart rate (Antognini and Schwartz 1993; Rampil, Mason et al. 1993; Antognini and Berg 1995). We compared the MAC with the MAC-BAR (based here on systolic arterial blood pressure) for the following reasons: First, cardio-circulatory data are technically objective variables that are easy to record. Second, the use of the MAC-BAR mimics clinical situations where the decision to deepen the anesthetic level is based on cardio-circulatory reactivity consecutive to a noxious stimulus.

Our results did not show any significant differences between the two variables in control rats. Surprisingly, when considering the results obtained after sufentanil administration, the two variables did not vary in a strictly parallel fashion, indicating that the MAC-BARs could not always simply be substituted for the MAC values. Sufentanil is a potent and specific μ-opiate agonist that is often administered during anesthesia because of its analgesic and cataleptic properties. We therefore feel it is important, in our model, to discriminate between the antinociceptive and the sedative properties. Using a small dose range of sufentanil, the different MACs varied independent of the concentrations of sevoflurane associated with spontaneous recovery. This indicates that these small doses were inferior to those required to obtain a cataleptic effect and that the results obtained are pertinent to an effect on nociception. This was not the case when 1 μg/kg/min sufentanil was used.

When considering the MAC-BARs, unexpected results were recorded with the smallest dose of sufentanil; a significant increase of the MAC-BAR was noted in both mechanically ventilated and spontaneously breathing rats. It is not the first time that opposite effects between halogenated vapors and opioids have been reported in animal models (Kissin and Jebeles 1984; Goto, Marota et al. 1996). It is probable that the experimental conditions used have unmasked on opiate-induced excitatory effect on cardiovascular reactivity to noxious stimuli. This might be in accordance with the observation that opioids concomitantly induce inhibitory (i.e., antinociceptive) and excitatory (i.e., hyperalgesia) effects (Celerier, Rivat et al. 2000; Crain and Shen 2001). With regard to this peculiarity, Antognini (Antognini and Carstens 2002) also linked the dissociation between the MAC

and the MAC-BAR to the hyperalgesic properties of the tested anesthetic.

Another methodological point to discuss is the comparison between mechanically ventilated and spontaneously breathing animals. The same stimulus (paw pressure) was applied in both conditions. In unmedicated rats, both MACs and MAC-BARs were larger in spontaneously breathing rats. This finding might be explained by the difference in the sampling of the halogenated vapors for measurements; it is clear that sample collection at the level of the carina through a hermetically sealed catheter is more accurate than measurements of gas contained in an induction chamber. Nevertheless, a 30% difference is large for this poorly soluble anesthetic, and accuracy of gas measurements can hardly explain the observations with sufentanil. In spontaneously breathing animals treated with the smallest dose, significant increases in both MAC-BAR and MAC were noted. Moreover, the larger doses (0.07 and 0.1 µg/kg/min) of sufentanil had no effect on the MAC-BAR of nonventilated rats. The explanation probably lies in the underlying conditions of the rats. Rats in the mechanically ventilated group had undergone a more extensive surgical procedure (a tracheotomy) before MAC determination. Consequently, more intense pain was likely to have induced some recruitment of the endogenous opiate system before measurement, which might account for the different results observed. This indicates that the absence of tracheotomy is not a simple technical matter but rather places rats in a different condition that can influence nociceptive challenge.

The analysis of the results of nociceptive challenges in awake rats helps in the understanding of the significance of testing anesthetized animals. When thermal testing is used, the small doses of sufentanil do not have significant antinociceptive effects. This is in contrast with observations in animals under sevoflurane anesthesia and indicates that this volatile vapor produces mainly a potentiating effect of the antinociceptive properties of µ-opiates agonists.

In conclusion, the assessment of the MAC-sparing effect provides several reliable and quantifiable variables that allow comparisons between different doses and different analgesic substances.

However, the observations made are not simply the result of the antinociceptive effects of the tested drug but rather the combination of complex interactions between this drug and the volatile vapor. This clearly indicates that this methodology cannot be substituted to the classic nociceptive challenges in awake animals to assess the antinociceptive potency of a drug. However, it is not without interest because it provides information on the analgesic-hypnotic interaction in anti-nociception at a time when the balance between opiates and anesthetic is heatedly debated in human anesthesia.

3.2.*2.* MAC-BAR-sparing effect of systemic clonidine administration

Annual Meeting of SARB, 2001
Anesth Analg; 92: S227, 2001 (ASA, 2001)
Docquier et al, Anesth Analg 2003; 97:1033-9

Clonidine, a non-selective α_2-adrenergic agonist, is administrated both acutely and chronically for a variety of indications. Clonidine is widely used intraoperatively because it produces a MAC-sparing effect for anesthetic agents, provides sedation, decreases hemodynamic fluctuations caused by anesthesia and surgery, and prevents postoperative shivering (Kamibayashi and Maze 2000; Smith and Elliott 2001). In addition, because of its analgesic properties, clonidine may improve postoperative pain and decrease the amount of other analgesic agents (e.g., opioids) needed.

Moreover, α_2-adrenergic agonists enhance analgesia from spinal opioids. In animals, this interaction occurs both pre- and post-synaptic to the primary afferent synapse in the spinal cord and is clearly synergic when both drugs are administrated intrathecally. In contrast, the epidural administration interacts in an additive manner (Ossipov, Harris et al. 1990; Eisenach, De Kock et al. 1996). Clonidine has been used for treatment of neuropathic pain, severe cancer pain and the symptoms of opioid withdrawal (Koppert, Sittl et al. 2003; Gowing, Farrell et al. 2009). Surprisingly, clonidine seems also able to induce paradoxical pain hypersensitivity in rats (Quartilho, Mata et al. 2004). α_2-Adrenoceptors are located on primary afferent terminals (both at peripheral and spinal endings), on neurons in the superficial laminae of the spinal cord, and within several brainstem nuclei implicated in analgesia. This supports the possibility of analgesic action at peripheral, spinal, and brainstem sites (Pertovaara, Kauppila et al. 1991; Guo, Jiang et al. 1996). Nevertheless, data obtained from volunteers and patients with acute postoperative or chronic pain favor a preferential spinal site of action for its antinociceptive effect (De Kock, Crochet et al. 1993; De Kock, Eisenach et al. 1997) (De Kock, Lavand'homme et al. 2005).

There are similarities in the second messenger systems between opioids and α_2-agonists: 1) both exert a potent presynaptic effect on

the release of transmitter from small primary afferents and 2) both are coupled by a G-protein to increase potassium conductance which leads to a hyperpolarisation of the membrane (Dunbar and Yaksh 1997). However, minimal cross-tolerance of one to the other is seen after chronic administration (Lameh, Eiger et al. 1992). Clonidine mimics endogenous norepinephrine release from descending noradrenergic pathway (Eisenach, De Kock et al. 1996). Furthermore, the opioids stimulate the neurons of PAG and RVM and modulate via descending inhibitor pathway the nociceptive message.

For these reasons, i.e. the evident interrelationship between endogenous opioid and adrenergic systems, we decided to evaluate the administration of clonidine (systemic and spinal) in our animal model. First, in the following study, we will evaluate systemic clonidine administration and question: 1) the MAC-BAR-sparing effect of clonidine under general anesthesia (sevoflurane *versus* propofol), 2) under different outcome variables, 3) the underlying mechanism mediating the MAC-BAR-sparing effect observed. Second, later in the manuscript, we will assess intrathecal administration of clonidine and other related drugs in the expression of OIH.

3.2.*2.1.* Systemic clonidine administration under sevoflurane and propofol anesthesia

Adult male Wistar rats, weighing 300-400 g were used for all experiments. After institutional animal care committee approval, the effects of clonidine and α-adrenergic antagonists were recorded. All tests were performed between 10:00 am and 4:00 pm.

3.2.*2.1.1.* Materials and methods

Animal preparation The equipment and monitoring were similar to the previous experiments. See chapter 3.1.3.

Experimental model At the end of surgical preparation, sevoflurane (Abbott Laboratory, Chicago, IL) anesthesia was preceded with either sevoflurane or propofol anesthesia. The propofol 2% (Astra-Zeneca, Brussels, Belgium) infusion was started at a fixed dose of 150 mg/kg/h, in agreement with previous experiments.

Rats received one dose of clonidine (5, 7.5, 10, 15 or 20 µg/kg) (Boerhinger, Ingelheim, Germany) or saline in a 0.5 ml bolus, followed by an infusion of 0.9% NaCl (1 ml/h). Sample sizes were 5/group, except for control and 10 µg/kg clonidine which included 10 rats. Measurements began 30 min after drug administration.

In sevoflurane anesthesia, the MAC-BAR of sevoflurane using the clamp stimulus was recorded in ventilated rats as in first experiment (chapter 3.1.3). In propofol anesthesia, the relative analgesic potency of the different doses of clonidine was evaluated using the tail clamping technique with the stimulus applied every 5 min. A 10% increase in systolic arterial blood pressure was considered to be a positive response. Analgesic potency was determined based on the latency from injection of the analgesic until the animal became non-reactive to the noxious stimulus. At this moment, arterial blood was retrieved for measurement of propofol concentration

3.2.*2.1.2*. Results

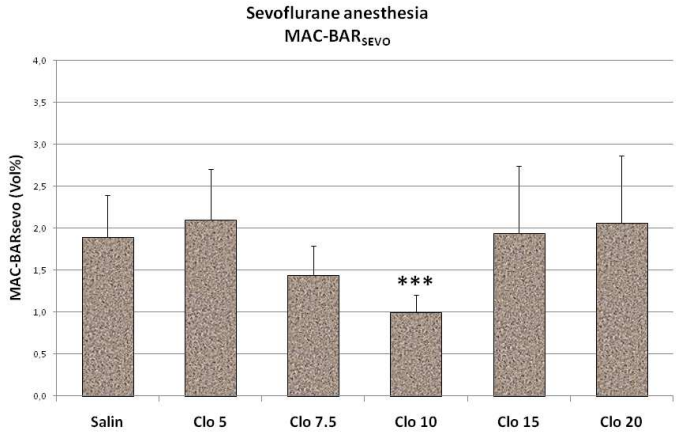

Figure 30: sevoflurane anesthesia and clonidine
MAC-BAR$_{SEVO}$ in animals treated with different doses of i.v. clonidine
Results are means ± SD, n = 5 to 10 per groups
****: significant difference compared to saline controls (p<0.001)*

The dose-response curve for the effects of clonidine on MAC-BAR$_{SEVO}$ was an U-shaped function: the lowest (5 µg/kg) and highest (15-20 µg/kg) doses were ineffective, while the intermediate dose (10 µg/kg) reduced the MAC-BAR$_{SEVO}$ by 45% compared to the controls.

In contrast to the results obtained under sevoflurane anesthesia, the dose-response curve of propofol is linear (Figure 30 and 31).

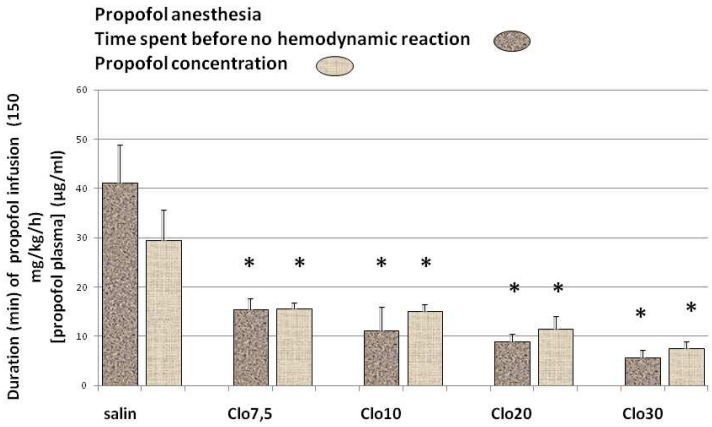

Figure 31: propofol anesthesia and clonidine
A duration of 41 min of propofol infusion was necessary to suppress the hemodynamic reactions consecutive to noxious stimulation in control rats. Different doses of clonidine were tested. In dark grey color: minutes spent before loss of hemodynamic reaction. In light grey color: concentration of propofol measured at time of no hemodynamic reaction in different sufentanil groups.
Results are mean±SD. n=5 to 10 per groups.
**: significance difference compared to saline control (p<0.05)*

3.2.*2.1.3*. Discussion

Under sevoflurane anesthesia, i.v. clonidine produces an U-shaped effect on reduction of the MAC-BAR_{SEVO} (i.e., low and high doses are ineffective). At the most effective dose (10 µg/kg), the α_2 -adrenergic agonist reduced the volatile anesthetic requirement by approximately 45%, which is consistent with the results of the halothane MAC-sparing (Bloor and Flacke 1982; Maze, Birch et al. 1987). Systemic clonidine can induce hemodynamic effects by interacting with central noradrenergic receptors and imidazolin receptors. Nevertheless, the reactivity of the sympathetic system appears, at least partially intact following i.v. clonidine, and enhanced reactivity of the vascular smooth muscle has been reported in anesthetized dogs undergoing post-ganglionic nerve stimulation after i.v. clonidine (15-30 µg/kg). According to the previous findings, it seems unlikely that the MAC-BAR_{SEVO} modifications observed after systemic administration in our

model are exclusively related to the hemodynamic properties of this drug. In agreement with human data, clonidine reduced propofol requirements. A dose of 10 μg/kg clonidine allows a ~50% reduction of propofol. In contrast to the results obtained under sevoflurane anesthesia, the dose-response curve of propofol is linear. Another argument considers the effects of clonidine, a partial α-adrenergic agonist that produces anti-nociception by mimicking the effect of the descending pain inhibitory pathway. Specifically, clonidine's anti-nociceptive effect is mainly through spinal mechanisms of action, but a central mechanism (inhibition at the locus coeruleus) is also involved. We observed a difference in the shape of the anti-nociceptive dose-response curve of clonidine depending on the general anesthetic used; clearly indicating that sevoflurane affects the analgesic efficacy of clonidine.

3.2.*2.2.* Systemic clonidine administration in different outcomes

3.2.*2.2.1.* Materials and methods

Animal preparation The equipment and monitoring were similar to the previous experiments. See chapter 3.2.1.2.

Experimental model The effect of clonidine on different MACs and MAC-BARs were recorded in ventilated (tracheotomy) and in spontaneously breathing animals. The antinociceptive effects of clonidine to thermal or a pressure stimuli were also evaluated in awake habituated animals. Clonidine was given at the dose of 5, 7.5 and 10 μg/kg. For determination of different MACs, the response was considered positive for: the MACclamp (MAC of sevoflurane that blocks purposeful movements after tail clamp) when a gross purposeful movement of the head, body, extremities or combination of these was observed; the MACpressure (MAC of sevoflurane that blocks paw withdrawal), when the animal withdrew its from the analgesia-meter; MAC-BARclamp (MAC of sevoflurane that blocks cardiovascular response to tail clamp) and MAC-BARpressure (MAC of sevoflurane that blocks cardiovascular response to paw pressure), as a 10% increase in the systolic arterial blood pressure; MACthermal (MAC of sevoflurane that blocks paw withdrawal after thermal

stimulus) when the rat withdrew its hind paw before cutoff time and finally for MAC-BARthermal when the systolic arterial blood pressure increased by 10% or more before cutoff time.

Statistical analysis Results are presented as mean±SD. During each experiment, the first change in reaction was noted, and the mean of the two adjacent doses of sevoflurane (i.e., immediately before and after the change) was taken as the MACsevo or the MAC-BAR_{SEVO}. The normality of the data was assessed according to the Kolmogorov-Smirnov test. Different MAC values were compared using repeated-measures analysis of variance followed by *post hoc* analysis using the Tukey test was applied. A p value < 0.05 was considered significant.

3.2.*2.2.2.* Results

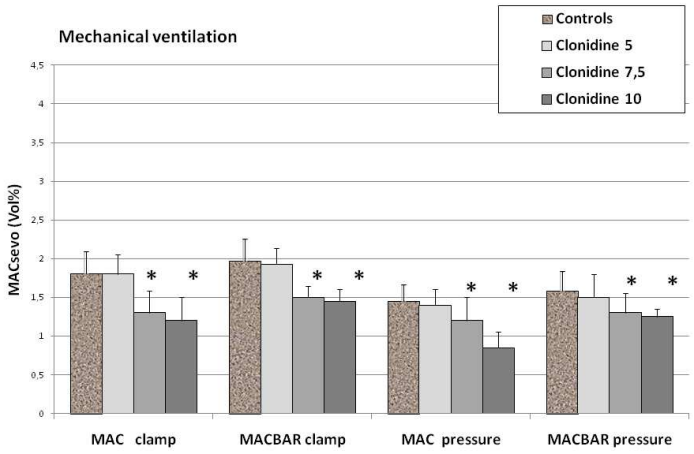

Figure 32: MACs: mechanical ventilation and clonidine
Summary of different MACs recorded in ventilated rats with clonidine administration. Results are mean ± SD, n=8 per group
**: significant difference compared to controls (p<0.05)*

Doses of 7.5 and 10 µg/kg of clonidine significantly reduced both MAC and MAC-BAR indifferently. When using the analgesia-meter, the largest pressure applied in treated rats before withdrawal was comparable to that recorded in controls.

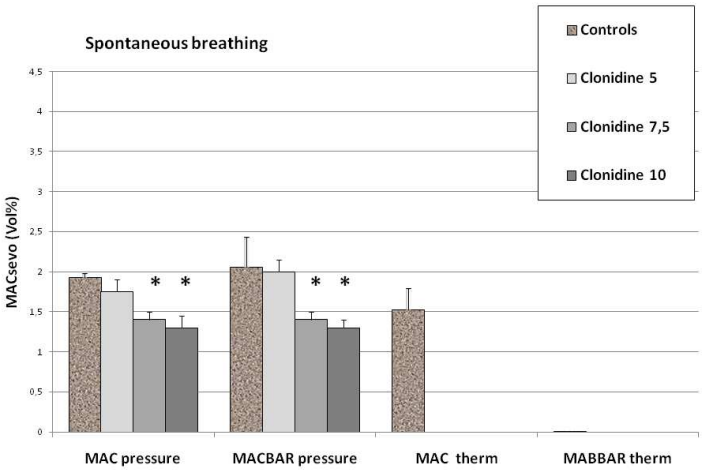

Figure 33: MACs: spontaneous breathing and clonidine
Summary of different MACs recorded in spontaneous breathing rats with clonidine administration. Results are mean ± SD, n=8 per group.
**: significant difference compared to controls (p<0.05)*

	Controls	***Clo5***	***Clo10***
Thermal testing: Latencies (sec)	*11±2.1*	*10.7±3.1*	*9.0±3.4*
Paw pressure: Pressure (g) reached before withdrawal	*95±22*	*98±27*	*118±21*

Table 8: withdrawal reaction in awake rats

3.2.*2.2.3.* Discussion

Our results did not show any significant differences between the two variables in control rats and even in rats treated with clonidine, a drug that interferes with the control of blood pressure. The α_2-adrenergic agonist clonidine, at its optimal dose (10 µg/kg), reduced both the MAC and the MAC-BAR by approximately 40%. These results provide arguments for a specific antinociceptive rather than a hemodynamic effect. The MAC reduction obtained is in accordance with the values obtained by others (Bloor and Flacke 1982; Maze, Birch et al. 1987). In our model, clonidine had no major effect on systolic arterial blood pressure. It is not surprising that the MAC-BAR was unaffected by the hemodynamic properties of clonidine (De Kock, Le Polain et al. 1993; Quintin, de Kock et al. 1998).

Another methodological point to discuss is the comparison between mechanically ventilated and spontaneously breathing animals. The same stimulus (paw pressure) was applied in both conditions. In unmedicated and clonidine treated rats, both MACs and MAC-BARs were larger in spontaneously breathing rats. The explanation probably lies in the underlying conditions of the rats. Rats in the mechanically ventilated group had undergone a tracheotomy before MAC determination. This indicates that the tracheotomy is not a simple technical matter but can influence nociceptive challenge.

When thermal testing is used, clonidine doesn't have significant antinociceptive effects. This is in contrast with observations in animals under sevoflurane anesthesia and indicates that this volatile vapor produces mainly a potentiating effect of the antinociceptive properties of both μ-opiates and α_2-adrenoceptor agonists.

3.2.*2.3.* Intrathecal α-antagonists

3.2.*2.3.1.* Materials and methods

Animal preparation Forty adult male Wistar rats were implanted with chronic lumbar intrathecal (i.t) catheters as described by Yaksh (Yaksh and Rudy 1976). Briefly, rats were anesthetized, and an i.t. catheter (PE-10 tubing) was inserted through a small hole in the cisterna magnum and advanced 8 cm caudally such that the tip lay in the i.t. space around the lumbar enlargement. Rats showing neurologic deficits were immediately killed by an overdose of pentobarbital. Correct placement of the spinal catheter was assessed by injection of 500 μg of lidocaine. Animals that did not display complete motor blockade were excluded. After 4 to 5 days' recovery, the reduction of the minimum alveolar concentration of sevoflurane that blocks cardiovascular response to a noxious stimulus (MAC-BAR_{SEVO}) in response to clonidine or an α-adrenergic receptor antagonist alone or combined was evaluated.

Drugs The α-adrenergic receptor antagonists phentolamine mesylate, yohimbine hydrochloride, and prazosin hydrochloride were obtained from ICN Biomedicals (Belgium).

Experimental model The methodology for determination of MAC-BAR_{SEVO} was comparable to the one used in previous work (Chapter 3.1.3.). Basal (predrug) MAC-BAR_{SEVO} was determined in every animal. Rats were then divided into two main groups (n=20) according to a computer-generated randomization list. They received an equal volume of either i.v. saline or i.v. clonidine 10 µg/kg. These two main groups were divided into four equal subgroups (n=5) for i.t. drug administration. Rats in these subgroups received either i.t. saline or one of the following i.t adrenergic antagonists: the nonselective α_1/α_2-antagonist phentolamine 50 µg, the more selective α_2-antagonist yohimbine 100 µg, and the α_1-antagonist prazosin 30 µg. These drugs were injected in a total volume of 15 µl, followed by 10 µl of saline to flush the i.t. catheter. Pretreatment was performed 20 min before either i.v. saline or an i.v. bolus of clonidine 10 µg/kg. MAC-BAR_{SEVO} determination in pretreated rats started 30 min after unmedicated MAC-BAR_{SEVO} determination and was determined according to the same methodology used for basal (predrug) MAC-BAR_{SEVO} determination. In a second set of experiments, additional rats (two per condition) were studied according to the same protocol to test the effects of the α-antagonist dose used, but given by the i.v. route, on the MAC-BAR_{SEVO}-sparing effect of clonidine.

Statistics The MAC-BAR_{SEVO} for each rat was calculated according to the following: during each experiment, the first change in reaction (i.e., from reaction to pain stimulus to no reaction) was noted, and the mean of the two adjacent doses of sevoflurane (i.e., immediately before and immediately after the change) was taken as the MACbarsevo. Comparison of these values was based on ANOVA followed by Dunnett tests (StatSoft; Statistica, Tulsa, OK). Results presented are means±SD. A p value of <0.05 was considered as statistically significant.

3.2.*2.3.2.* Results

The average MAC-BAR_{SEVO} value before i.t. treatment was 1.95± 0.6 (n=60). This value was comparable to that obtained in i.t. saline-treated animals (2.1± 0.8). The administration of i.v. 10 µg/kg clonidine reduced the MAC-BAR_{SEVO} to 1.07±0.4 (45% reduction). The α-adrenergic antagonists phentolamine and yohimbine alone had

no particular effect on the MAC-BAR_{SEVO}. In contrast, prazosin significantly reduced the MAC-BAR_{SEVO} (83%). In animals treated with i.v. clonidine, i.t. injections of phentolamine and yohimbine totally reversed the MAC-sparing effect of the drug. Moreover, significantly higher MAC-BAR_{SEVO} values (+32%) were recorded in yohimbine-treated animals when compared with the controls. The association of i.v. clonidine with i.t. prazosin resulted in a complete loss of any hemodynamic reaction consecutive to noxious stimuli. The i.v. administration of the different α-antagonists did not modify the MAC-BAR_{SEVO} of saline- or clonidine-treated animals (1.97±0.8 vs 0.9±0.5).

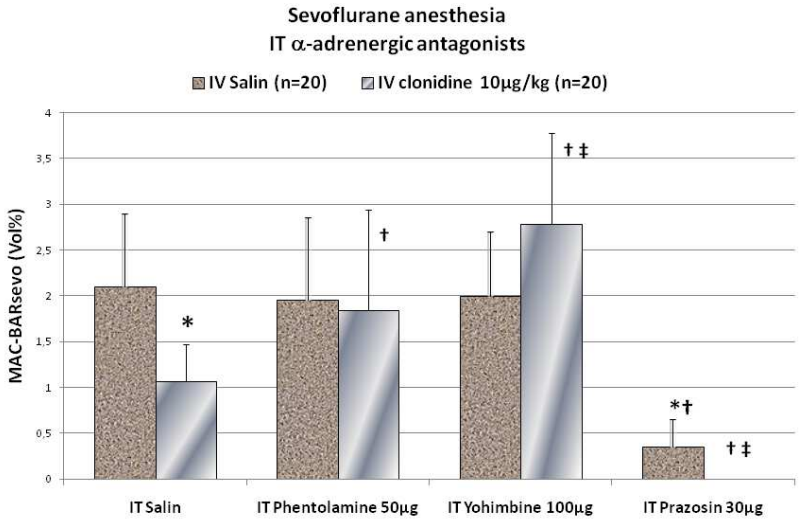

Figure 34: MAC-BARsevo and α-adrenergic antagonists
MAC-BARsevo and i.t. pretreatment of α-adrenergic receptor antagonists. Mean ± SD.
**: significant difference compared to saline control (p<0.01)*
†: significant difference compared to clonidine 10 µg
‡: significant difference compared to saline control (p<0.05)

No significant decrease in arterial blood pressure was observed after systemic administration of the α_2-adrenoceptor agonist clonidine or after the i.t injections of various α-adrenergic antagonists. I.v. administration of α-adrenergic antagonists at the same doses failed to induce any significant hemodynamic effect in anesthetized animals.

Hemodynamic data	*Baseline SABP*	*SABP after IT drugs*	*Baseline SABP*	*SABP after IT drugs*
	IV saline	**IV saline**	**IV clo 10 µg/kg**	**IV clo 10 µg/kg**
IT saline	*132±22*	*138± 36*	*124±23*	*126±45*
IT phentolamine	*124±38*	*131±42*	*132±37*	*122±28*
IT yohimbine	*122±32*	*125±31*	*121±27*	*130±18*
IT prazosin	*138±22*	*131±39*	*129±22*	*124±25*

Table 9: hemodynamic data
Systolic arterial blood pressure (SABP) after systemic administration of the α_2-adrenoceptor agonist clonidine and intrathecal injection of various α-adrenergic antagonists
Results are means ±SD. No significant was noted

3.2.*2.3.3.* Discussion

Our results show that systemic clonidine significantly reduced MAC-BAR_{SEVO}. Because this effect is totally reversed by the IT administration of specific α_2- (yohimbine) and unspecific α-(phentolamine) adrenergic receptor antagonists, and because the i.v. administration of these antagonists is ineffective, we can hypothesize that the MAC-BAR-sparing effect of systemic clonidine relies on a spinal action. The dose of 10 µg/kg of clonidine was chosen in accordance with a previous work that we conducted with the same model (Anesth Analg 2001; 92:S227). In this work, we demonstrated that the clonidine dose-response curve for MAC-BAR_{SEVO} was U-shaped. Clonidine 10 µg/kg was the most efficient dose that provided a 45% reduction of MAC-BAR_{SEVO}. Such a result is close to the one obtained by other authors who considered the clonidine effect on the MAC (purposeful movements) of halothane (Bloor and Flacke 1982; Maze, Birch et al. 1987).

We found that the i.t. administration of phentolamine and yohimbine completely reversed the clonidine MAC-sparing effect. Because systemic resorption of these antagonists did not account for the observed effect, systemic clonidine most likely interferes with the spinal nociceptive hemodynamic reflex. These findings are in marked contrast to those reported by Kita (Kita, Kagawa et al. 2000), who found a supraspinal, not a spinal, involvement of α_2-adrenergic receptors in the anesthetic-sparing and hemodynamic-stabilizing effects of systemic clonidine in rats. This is surprising, considering

that both we and Kita et al. used relatively similar methods and end points. It is to be noted, however, that the dose of clonidine used by Kita et al. was significantly larger (100 vs 10 µg/kg), and the time elapsed before MAC-BAR determination was also longer. Furthermore, the spinal penetration of the α_2- adrenergic antagonist chosen by these authors, rauwolscine, may be less important than for yohimbine. That may contribute to the discrepancy between the data from Kita and data found in the literature. First, Rampil and King (Rampil and King 1996) demonstrated that spinal neurons are involved in the purposeful movements consecutive to noxious stimulation (MAC) under volatile anesthesia. Similarly, Antognini and Berg (Antognini and Berg 1995) reported that the brain had little effect on MAC-BAR for isoflurane in goats investigated with selective blood perfusion. Finally, brain-dead organ donors experience hypertension and tachycardia at skin incision (Pennefather, Dark et al. 1993).

Another interesting observation from our data concerns the effects of the different α-adrenoceptor antagonists alone. Several years ago, Sagen and Proudfit (Sagen and Proudfit 1984) investigated the effects of the i.t. α-adrenergic antagonists yohimbine, phentolamine, WB4101, and prazosin on nociception (tail-flick and hotplate tests) in naïve, awake rats. These authors demonstrated a decrease in nociceptive threshold leading to a hyperalgesic effect correlated with the relative potency of the antagonist used for the α_2-adrenergic receptor. Their observations reinforced the results of Proudfit and Hammond (Proudfit and Hammond 1981) and argue for tonically active inhibitory noradrenergic descending systems. We did not observe hemodynamic hyperactivity consecutive to noxious stimuli in sevoflurane-anesthetized rats after i.t. administration of the various α-adrenergic antagonists. We propose the following explanations. First, the outcome variables were different: Sagen and Proudfit and Proudfit and Hammond recorded a motor response after a noxious stimulus, and here we considered a hemodynamic reaction in paralyzed animals. Unpublished data from our laboratory that considered the hemodynamic and motor responses to paw pressure in rats under sevoflurane anesthesia indicate that both reactions are concomitant. A second explanation could be an interaction between sevoflurane and the noradrenergic descending pain inhibitory system, the spinal α_2-adrenergic receptors, or both. Previously, we have demonstrated

that sevoflurane had a negative influence on the antinociceptive effects of clonidine, sufentanil, and their combination. Several authors had already pointed out an interaction between volatile vapors (halothane) and the antinociceptive effects of nitrous oxide or morphine (Goto, Marota et al. 1996; Drasner 2001). Drasner, in his discussion, hypothesized a “pharmacological disruption of the descending pain inhibitory pathway” induced by halothane. Accordingly, the lack of increase in the hemodynamic responsiveness after noxious stimulation might be attributed to disruption of the descending pain inhibitory pathway. Nevertheless, there is no argument in our data to ascertain this hypothesis. More difficult to explain from our findings are the effects of i.t. prazosin alone or after systemic clonidine. Prazosin is a potent α_1-adrenergic antagonist. It is, however, difficult to relate the significant reduction in hemodynamic reaction consecutive to noxious stimuli simply to a suppression of the spinal α_1-adrenergic activity. I.t. injection of the α_1-agonists phenylephrine and methoxamine also produces analgesia, which suggests that the analgesia induced by i.t. injection of norepinephrine is mediated by both α_1- and α_2-adrenergic receptor subtypes (Reddy, Maderdrut et al. 1980). Prazosin is not only an α_1-antagonist, but it is also a relatively potent inhibitor of cyclic nucleotide phosphodiesterase (Hess 1975) and an α_{2A} subtype-specific adrenergic agonist. These other properties could account for the observed effect.

In conclusion, our results demonstrate that a spinal mechanism is involved in the MAC-BAR-sparing effect of systemic clonidine. Furthermore, spinally administered α-antagonists display effects in rats under sevoflurane anesthesia that differ from those reported in the literature in awake animals.

3.2.*2.4.* Conclusion of the results

Clonidine, a α_2-adrenergic agonist is widely used at surgery time. It improves postoperative pain and decreases the amount of other analgesic agents. It mimics endogenous norepinephrine release from descending noradrenergic pathway.

The results concerning clonidine in our experimental setting provide arguments for 1) a specific antinociceptive rather than a hemodynamic effect of systemic clonidine administration, 2) a spinal mechanism involved in the MAC-BAR-sparing effect of systemic clonidine, 3) the underlying conditions of experimental animal preparation (tracheotomy) can influence nociceptive challenge, 4) the anesthesia modifies the antinociceptive properties of clonidine. We hypothesized that sevoflurane may influence the recruitment of the noradrenergic descending inhibitory system and modify therefore the antinociceptive properties of the drug.

3.2.*3.* Summary of chapter 3.2.

Sufentanil and clonidine anti-nociceptive effects are differently affected depending on the type of general anesthetic. At clinically effective concentrations of sevoflurane, the anti-nociceptive effects of sufentanil and clonidine are modified in a dose-dependent manner. Anesthesia appears to be part of a complex interaction among all drugs used.

Under sevoflurane anesthesia, the assessment of MAC-sparing effect provides several reliable and quantifiable variables that allow comparisons between different doses and different analgesic substances. The MAC-BAR measure allows to mimic clinical situation where the decision to deepen the anesthetic level is often based on cardio-vascular reactivity consecutive to a nociceptive stimulus. Moreover, it provides information on the analgesic-hypnotic interaction in anti-nociception.

Furthermore, under sevoflurane anesthesia, the MAC-BAR significantly increases following administration of the very low dose of sufentanil. In awake animals, the same dose does not have any effect. Sevoflurane probably inhibits the recruitment of the noradrenergic descending inhibitory system, rendering apparent an excitatory effect of sufentanil. This pronociceptive effect should be considered in light of recent human reports highlighting the importance of intraoperative analgesia on postoperative pain perception (Aubrun, Langeron et al. 2003). We therefore performed further investigations of this paradoxical phenomenon.

3.3. Investigations of underlying OIH mechanisms

3.3.1. MAC-BAR$_{SEVO}$ increase after low dose of sufentanil: an opioid-induced excitatory effect

Docquier et al, Br J Anesth 2004; 93:408-13

In previous studies, we defined the MAC-sparing effect as an objective tool to assess anti-nociception in animals, and we demonstrated that spinal mechanisms are involved in the MAC-BAR-sparing effect of systemic clonidine administration. We observed that a very low dose of the μ-opiate agonist sufentanil paradoxically increased the MAC-BAR$_{SEVO}$. We speculated that this phenomenon could be an excitatory opioid-induced effect revealed by sevoflurane administration. Recently, a great deal of attention has been paid to these opioid-induced excitatory effects. Although opioids are undoubtedly the most potent and useful analgesics for alleviating pain in humans, they appear to concomitantly induce both inhibitory and excitatory effects (Celerier, Rivat et al. 2000).

Using a pharmacological approach, the present study intended to determine the similarities, if any, between this opioid-induced excitatory hemodynamic phenomenon unmasked by sevoflurane and the OIH observed in awake animals (Rivat, Laulin et al. 2002). To accomplish this, several drugs with proven efficacy in opioid hyperalgesia (Sotgiu, Biella et al. 1998; Celerier, Rivat et al. 2000; Crain and Shen 2001) were tested for their efficacy to reverse this opioid-induced hemodynamic hyper-reactivity. These included the NMDA antagonists ketamine and MK801, the opioid antagonists naloxone and COX-1 and the COX-2 inhibitors ketorolac and meloxicam.

3.3.1.1. Materials and methods

Animal preparation

Adult male Wistar rats weighing 300-400 g were used for all experiments. Rats were maintained on a 12:12 h light-dark cycle and

received food and water *ad libitum*. After institutional animal care committee approval, the effects of the different drugs on the opioid-induced hemodynamic hyper-reactivity consecutive to noxious stimuli in sevoflurane anaesthetized rats were recorded. Animals designed to receive intrathecal (i.t.) medications were implanted with chronic lumbar intrathecal catheters as described by Yaksh and Rudy (Yaksh and Rudy 1976). Briefly, rats were anesthetized with sevoflurane and an intrathecal catheter (PE-10 tubing) was inserted through a small hole in the cisterna magnum and advanced 8 cm caudally such that the tip lay in the intrathecal space around the lumbar enlargement. The animals were allowed to recover over a period of 4–5 days. Rats showing neurological deficit were immediately killed by an overdose of pentobarbital. Then the correct placement of the spinal catheter was assessed by injection of lidocaine 500 µg. Animals that did not display complete motor blockade were excluded. During the study, the tests were performed between 10 a.m. and 4 p.m.

The methodology for determination of MAC-BAR_{SEVO} (minimum alveolar concentration of sevoflurane that blocks cardiovascular response to noxious stimulus) was comparable to the one used in previous work (Chapter 3.1.*3*).

Experimental groups

Six animals were studied for each condition. Basal (predrug) MAC-BAR_{SEVO} was determined in every animal. Rats then received drugs known to interfere with acute OIH as ketamine, MK801, naloxone, ketorolac, meloxicam or saline 15 min after the predrug MAC-BAR_{SEVO} measurement. A second MAC determination followed, and 15 min later the excitatory dose of sufentanil (Janssen-Cilag, Beerse, Belgium) was administered. The excitatory dose of sufentanil, as assessed by a previous investigation, consisted of a bolus of 0.015 µg/kg followed by a continuous infusion of 0.005 µg/kg/min. After predrug (control group) MAC-BAR assessment, the animals were randomly assigned to receive one of the following drugs: i.v. ketamine (0.25, 0.5 mg/kg), i.t. ketamine (250 µg), i.v. MK-801 (0.5, 1 mg/kg), i.t. MK-801 (30 µg) (dose chosen according to Ishizaki and colleagues) (Ishizaki, Yoon et al. 1995), i.v. naloxone (0.1, 0.5 mg/kg), i.v. ketorolac 10 mg/kg, i.t. ketorolac (50, 100 µg) or i.t.

meloxicam (100, 150 µg) (Boerhinger Ingelheim, Ingelheim, Germany).

MAC-BAR$_{SEVO}$ determination in treated animals was carried out 30 min after the previous evaluation (predug) and was performed according to the same method. Only rats with a normal systolic arterial blood pressure (110–160 mmHg) after initial instrumentation were included. During the study protocol, animals presenting with persistent low systolic arterial blood pressure (<100 mmHg not responding to 2 ml Haemacel fluid supplementation) were excluded from data analysis.

Statistical analysis

MAC-BAR$_{SEVO}$ for each rat was calculated as follows. During each experiment, the first change in reaction (i.e. from reaction to pain stimulus to no reaction) was registered and the mean of the two adjacent doses of sevoflurane (i.e. immediately before and immediately after the change) was taken as the MAC-BAR$_{SEVO}$. The normal distribution of the data was assessed by the Kolmogorov–Smirnov test. Different MAC values were compared using repeated measures of analysis of variance, followed by post hoc analysis using Tukey's test. The same tests were applied for intra- and inter-group comparison of the change in systolic arterial blood pressure. Results are presented as mean±SD, and $p<0.05$ was considered statistically significant.

3.3.*1.2.* Results

The average MAC-BAR$_{SEVO}$ value (predrug MAC-BAR$_{SEVO}$) before treatment was 1.9±0.3 vol%. Administration of i.v. sufentanil 0.005 µg/kg/min to saline controls significantly increased this value to 3.2±0.3 vol% ($p<0.05$).

Effects of drugs on MAC-BAR$_{SEVO}$

With the exception of naloxone, all drugs reduced the predrug MAC-BAR$_{SEVO}$. Both i.v. ketamine 0.5 mg/kg and i.t. ketamine reduced MAC-BAR$_{SEVO}$ by approximately 70%. Intravenous MK-801 1 mg/kg and i.t. MK-801 30 µg reduced MAC-BAR$_{SEVO}$ by approximately 40%

and 76%, respectively. Of the COX inhibitors, i.t. meloxicam 150 µg was the most efficacious with a 90% reduction (Figure 35 and 36).

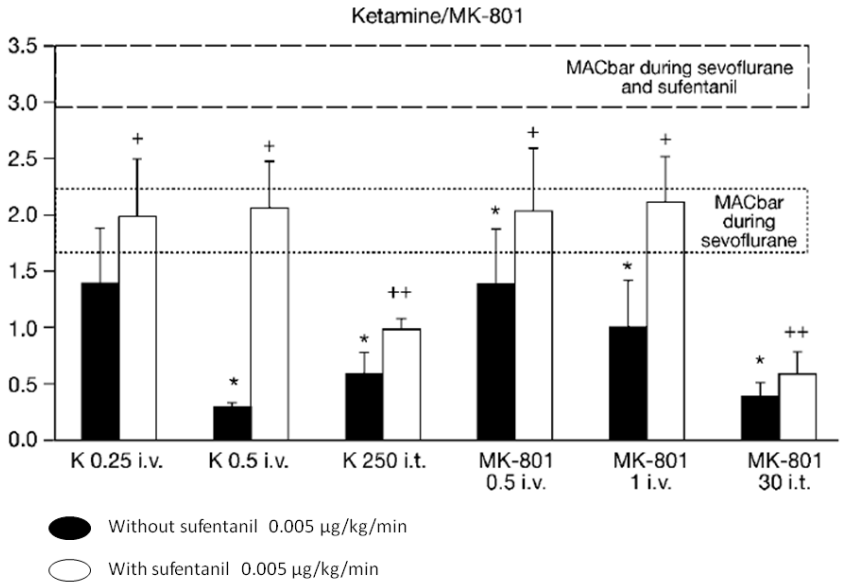

Figure 35: NMDA antagonists
MAC-BAR$_{SEVO}$ (vol%) in animals (six per condition) treated with i.v. (0.25, 0.5 mg/kg) or i.t. (250 µg) ketamine (K), i.v. (0.5, 1mg/kg) or i.t. MK-801 (30 µg) without and with sufentanil (0.005 µg/kg/min).
MAC-BARsevo and MAC-BAR$_{SEVO}$ with sufentanil in saline-treated animals are presented in the horizontal boxes (means±SD)
***: significant difference compared to saline control animals (p<0.05)*
+: significant difference compared to saline-sufentanil-treated animals (p<0.05)
++: significant difference compared to saline control and saline-sufentanil-treated animals (p<0.05)

Effects of drugs on sufentanil-induced cardio-circulatory hyper-excitability

When considering the effects of the agents that significantly reduced MAC-BAR$_{SEVO}$, two patterns are observed on the excitatory effect of sufentanil. With i.t. ketamine, MK-801 (Figure 35), ketorolac 100 µg and meloxicam 150 µg (Figure 36), MAC-BAR was lower than during sevoflurane without sufentanil in control animals (sevoflurane without sufentanil) as well as in sufentanil-treated control animals (sevoflurane + sufentanil). In contrast, with i.v. ketamine (0.5 mg/kg), MK-801 (Figure 35) and ketorolac (Figure 36), while MAC-BAR was

also lower than during sevoflurane without sufentanil in control animals (sevoflurane without sufentanil), in the sufentanil treated animals MAC-BAR was lower than during sevoflurane + sufentanil and comparable to, but not lower than, MAC-BAR during sevoflurane without sufentanil. Intravenous naloxone 0.5 mg/kg completely prevented the sufentanil-induced increase in MAC-BAR_{SEVO}. None of the drugs significantly affected systolic arterial pressure.

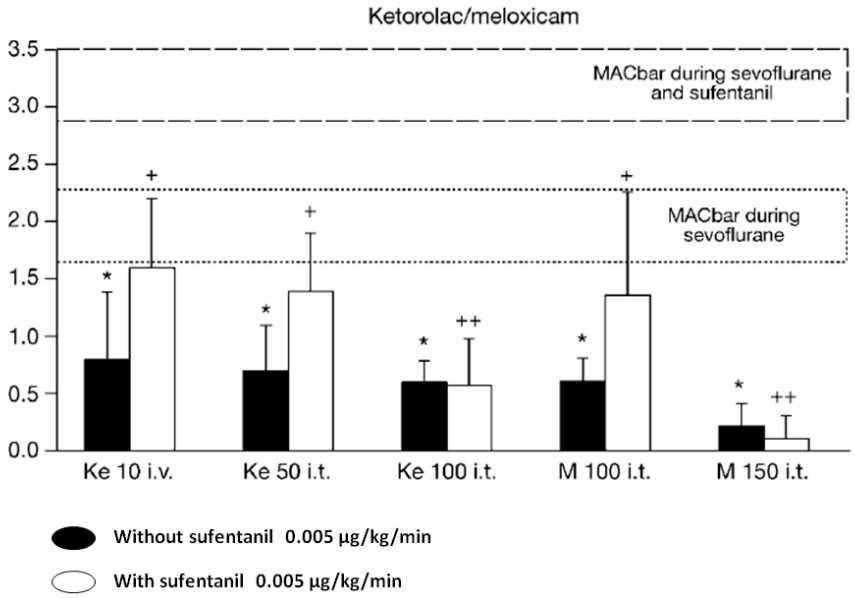

Figure 36 : NSAIDs
$MAC\text{-}BAR_{SEVO}$ (vol%) in animals (six per condition) treated with i.v. (10 mg/kg) or i.t. (50, 100 µg) ketorolac (Ke) and i.t. meloxicam (M) (100, 150 µg) without and with sufentanil (0.005 µg/kg/min).
$MAC\text{-}BAR_{SEVO}$ and $MAC\text{-}BAR_{SEVO}$ with sufentanil in saline-treated animals are presented in the horizontal boxes (mean±SD)
***: significant difference compared to saline control animals (p<0.05)*
+: significant difference compared to saline-sufentanil-treated animals (p<0.05)
++: significant difference compared to saline control and saline-sufentanil-treated animals (p<0.05)

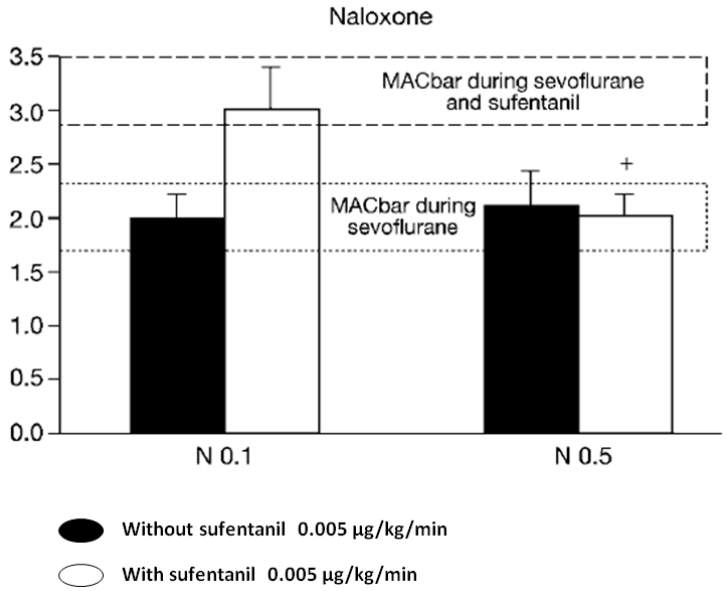

Figure 37 : naloxone
MAC-BAR$_{SEVO}$ (Vol%) in animals (six per condition) treated with i.v. naloxone (N) (0.1, 0.5 mg/kg) without and with sufentanil. MAC-BAR$_{SEVO}$ and MAC-BAR$_{SEVO}$ with sufentanil in saline-treated animals are presented in the horizontal boxes (means±SD).
+: significantly different (p<0.05) from saline-sufentanil-treated animals.

3.3.*1.3.* Discussion

Our results show that both non-specific and specific antagonists at NMDA receptors, ketamine and MK-801 prevent the sufentanil-induced increase in cardiocirculatory reactivity in response to a noxious stimulus in animals under sevoflurane anesthesia. This inhibitory effect is particularly evident when these drugs are administered spinally. The opioid receptor antagonist naloxone completely suppressed sufentanil-induced increase in MAC-BAR. Concerning the mechanisms involved, our pharmacological challenge supports an excitatory reaction occurring at the spinal level initiated by the opioid receptor and mediated by the NMDA receptor. This is in accordance with recent theory of the biphasic effects of opioids on nociceptive perception (Celerier, Laulin et al. 1999; Crain and Shen 2000). According to this theory, opioids concomitantly induce both inhibitory effects (i.e. antinociceptive effects) and excitatory effects (i.e. pronociceptive, hypersensitivity or hyperalgesic effects, pruritus

and nausea). The cardiocirculatory hyperactivity following noxious stimulation appears to be another opioid-induced excitatory manifestation revealed by the experimental setting used.

Non-competitive antagonists at the N-methyl-D-aspartate receptor, ketamine and MK-801 were used because the excitatory neurotransmitter glutamate plays a pivotal role in the development and maintenance of opioid-induced excitatory effects via these receptors (Feng and Kendig 1995). For example, ketamine has been demonstrated to prevent opioid hyperalgesia in animals and humans (Celerier, Rivat et al. 2000; Guignard, Bossard et al. 2000; Rivat, Laulin et al. 2002). In our study, the selective NMDA antagonist MK-801 was used in order to determine that the effect observed after ketamine administration is related to antagonism at the NMDA receptor. This was done because ketamine can act on several receptor systems, such as the opioidergic (μ, δ, κ) and cholinergic (muscarinic and nicotinic) (Hustveit, Maurset et al. 1995) systems, involves the monoaminergic system (Pekoe and Smith 1982) and shows local anesthetic effects by blockade of the sodium channels (Clements and Nimmo 1981). Ketamine reduces the excitability in superficial dorsal horn neurons by blocking sodium and voltage-gated potassium currents (Schnoebel, Wolff et al. 2005). Concerning the routes of administration (i.v. versus i.t.), it is interesting to point out that whereas i.v. ketamine and MK-801 both reverse sufentanil-induced increase in MAC-BAR$_{SEVO}$, their 'specific' sevoflurane MAC-BAR-sparing effect is lost. In contrast, i.t. ketamine and MK-801 inhibit the exacerbated hemodynamic reactivity but maintain their sevoflurane MAC-BAR-sparing effect. This finding argues in favor of a preferential spinal site for MAC-BAR. These data are in agreement with a previous study where we demonstrated a spinal site of action for the MAC-BAR-sparing effect of i.v. clonidine (Docquier, Lavand'homme et al. 2002). It also highlights the important role played by spinal NMDA receptors in the processing of spinal polysynaptic reflexes (Maruoka, Ohno et al. 1997), and particularly of cardiocirculatory responses following noxious stimulation as already assessed for pain perception (wind-up phenomenon) (Dickenson 1995; Nadeson, Tucker et al. 2002).

Low doses (0.5 mg/kg) of the specific opioid antagonist naloxone are devoid of effect on the MAC-BAR$_{SEVO}$ whereas it totally prevents the sufentanil-induced increase in this response. Hence the recorded excitatory manifestation is mediated by opioid receptors. This observation is consistent with the work of Crain and Shen (Crain and Shen 2001). Using a multidisciplinary approach based on nociceptive neurons in culture, behavioural assays in mice and clinical trials on post-surgical pain patients, these authors demonstrated a specific effect of low doses of naloxone or nalmefene on Gs-coupled excitatory opioid receptor functions together with markedly enhanced morphine antinociceptive potency and simultaneous attenuation of opioid tolerance and dependence by co-treatment with these opioid antagonists.

The results obtained with non-steroidal anti-inflammatory drugs (NSAIDs) are of particular interest. Ketorolac (the nonspecific COX-1 COX-2 inhibitor) and meloxicam (the more selective COX-2 inhibitor) significantly reduced MAC-BAR$_{SEVO}$ in control and sufentanil-treated animals. Such an observation is not surprising. While it is clear that NSAIDs exert their analgesic effect by interacting with local inflammatory processes, strong evidence also exists for a central analgesic effect (Cashman 1996) or, rather, an anti-hyperalgesic effect as previously demonstrated in animal models (Malmberg and Yaksh 1992; Yamamoto and Nozaki-Taguchi 1996; Kang, Vincler et al. 2002). Spinal administration of NSAIDs provides efficient analgesia in humans (Eisenach, Curry et al. 2002) and an intraoperative anesthetic-sparing effect of systemic NSAIDs has been described in surgical patients (Moss, Baysinger et al. 1992). A spinal interaction between cyclooxygenase and NMDA receptor activity seems to partly account for this central effect of NSAIDs (Sotgiu, Biella et al. 1998) which may explain the positive results we observed in our model on sufentanil-induced hemodynamic hyper-reactivity. It has been demonstrated that COX-2 expressed in the central nervous system colocalizes with glutamate in excitatory neurones and that NMDA receptor activation results in increased prostanoid synthesis (Kaufmann, Worley et al. 1996).

In conclusion, using a pharmacological approach, we were able to confirm the similarity between opiate-induced hyperalgesia in awake

animals and an increased cardiocirculatory reactivity following a noxious stimulus in rodents under sevoflurane anesthesia and low-dose opioid. Does the present study indicate that sevoflurane exacerbates the excitatory effects of the opioids? From a theoretical point of view, this appears unlikely because volatile anesthetics inhibit rather than stimulate NMDA-mediated excitatory neurotransmission (Sear 2009). It appears that sevoflurane exacerbates both excitatory and inhibitory effects of opiates on nociception because, in our previous study with this model (Docquier, Lavand'homme et al. 2003), we observed significant MAC-sparing effects using doses of sufentanil that were completely ineffective in awake animals.

3.3.*2.* Other molecular mechanisms of OIH

Annual Meeting ASA, 2004
Annual Meeting ESA, 2006

OIH is postulated to be related to activation of excitatory pathways by opioids. The possible mechanisms of pro-nociceptive opioid effects are numerous. A range of molecular mechanisms are likely involved at all levels of the nociceptive system. A great deal of experimental studies strongly supports a role of the spinal cord as the neuroplastic site OIH genesis. The mechanisms include up-regulation of the cAMP pathway, spinal PGs and NMDA receptor, or the participating intracellular second messenger system such protein kinase C (PKC) and the NO pathway. An anti-analgesic pathway has been also suggested through spinal dynorphin levels increase.

In our experimental anesthetized animal model, the previous findings support 1) a spinal site of action for the MAC-BAR-sparing effect, 2) that the excitatory opioid-induced effect is a hyperalgesic opioid-induced phenomenon occurring at the spinal level, 3) initiated by the opioid and mediated by the NMDA receptor.

Other mechanisms suspected to be implicated in OIH were explored using intrathecal route. Intrathecal NK-1 receptor inhibitor (SP), α_2-adrenoceptor agonist such clonidine (an α_{2A}-AR dependent agent) and ST-91 (an α_{2c}-AR preferring agonist), protein kinase inhibitor and serum anti-dynorphin were evaluated in low-dose sufentanil-induced hyperalgesic effects.

3.3.*2.2.* Materials and methods

Adult male Wistar rats (n = 4-10/group) were implanted with i.t. catheters under sevoflurane anesthesia. After 1 week of recovery, rats were anesthetized with sevoflurane in O_2, according the methodology previously described (Chapter 3.1.3). Tests were performed between 10 a.m. and 4 p.m. MAC-BAR_{SEVO} to tail clamping stimulus was recorded at baseline, after i.t. drug administration and following administration of low-dose sufentanil.

The following drugs were administrated i.t. in a total volume of 10 µl (doses based on previous studies), followed by 10 µl of saline to flush the catheter:

1) *L-732,138*, a tachykinin NK-1 receptor selective antagonist (Tocris Cookson Ltd, UK) was administered at 50 µg (n=9) (King, Gardell et al. 2005).

2) The α_2-A-preferring adrenergic agonist *clonidine* (Tocris Cookson Ltd, UK) (n=15) or the α_2-nonA-preferring adrenergic agonist *ST-91* (gift from Boerhinger Ingelheim, Ingelheim, Germany) (n=11) were administered at 15 µg.

3) *Lavendustin A* (Sigma-Aldrich), a potent PK inhibitor that reduces binding of ATP to kinases, was administered at 1 µg (Sato, Takano et al. 2003).

4) *Dynorphin A antiserum* was administrated at 200 µg. With the collaboration of laboratories of CHEX (experimental surgery) and IMEX (experimental immunology), we attempted to produce an anti-dynorphin serum. Dynorphin A (1-13) antiserum was raised by repeated injections of dynorphin A (1-13) (Bachem) coupled to bovine serum albumin (KLH/BSA) to two rabbits. After observing an efficacious immunization, the antibody preparation serum was purified by affinity chromatography. The concentration of antibodies were then quantified and diluted to 200 µg/5 µl. Control serum was collected from rabbits of the same strain that had not been exposed to the antigen.

The results concerning NK-1 antagonist and α_2-agonists analyzed the effects of i.t drugs and i.v. sufentanil administration on the initial MAC-BAR$_{SEVO}$ (increase or decrease) according to equation (equation 1):

$$Drug\ Effect = \frac{(MACBAR\,drug) - (MACBAR\,baseline)}{MACBAR\,baseline} X\,100$$

$$Suf\ Effect = \frac{(MACBAR\,suf\ 0.005) - (MACBAR\,baseline)}{MACBAR\,baseline} X\,100$$

The normal distribution of the data was assessed by the Kolmogorov–Smirnov test. Student-t test was then performed to check the i.t. drug and sufentanil effects were different from 0. The results are expressed in % as mean ± SE.

The results concerning experiments with protein kinase inhibitor and dynorphin antiserum were analyzed by repeated measures of analysis of variance, followed by post hoc analysis using Tukey's test. Results are expressed as means ± SD, $p < 0.05$ considered significant.

3.3.*2.3.* Results

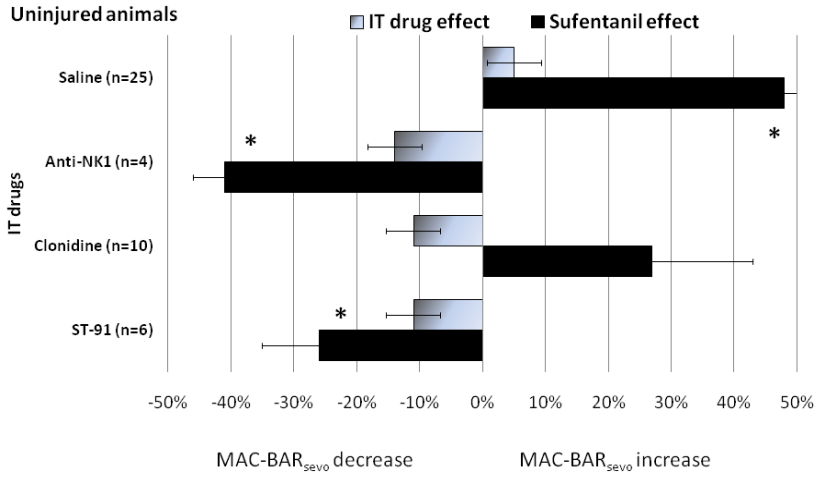

Figure 38: α_2-adrenoceptor agonists and NK-1 receptor antagonist
Effects on the MAC-BAR$_{SEVO}$ of i.t administration of NK-1 receptor selective antagonist, clonidine (an α_{2A}-AR dependent agent) and ST-91 (an α_{2c}-AR preferring agonist) without and with sufentanil administration
Effects are calculated according to equation 1 and expressed in %
**: significantly different from 0*

The different drugs intrathecally administrated prevent sufentanil-induced MAC-BAR$_{SEVO}$ increase in response to a noxious stimulus in animals under sevoflurane anesthesia. They have no significant antinociceptive effect *per se.* Only NK-1 receptor inhibitors and α_2-agonists show a moderate MAC-BAR-sparing effect. After clonidine pretreatment, low-dose sufentanil increase the MAC-BAR$_{SEVO}$ but the effect is not significant. In contrast, after ST-91 and NK-1 antagonist

pretreatment, low-dose sufentanil provides a significant sparing-effect. Dynorphin antiserum prevents sufentanil-induced hyperalgesic effect whereas control serum has no effect.

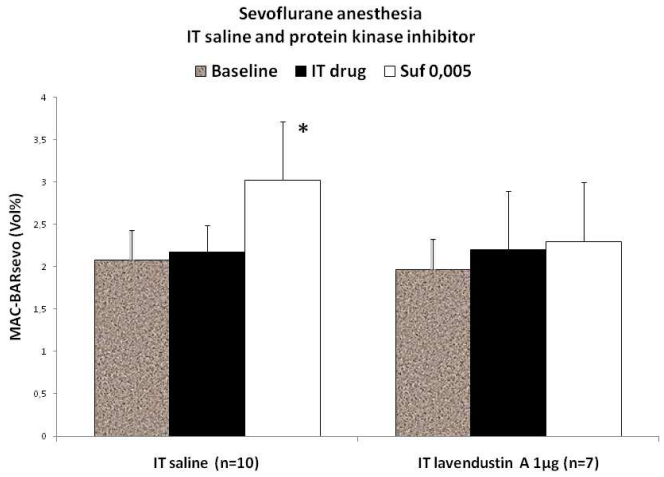

Figure 39 : protein kinase inhibitor
Effect of i.t. lavendustin, a potent protein kinase inhibitor.
Results were expressed as mean ± SD
**: significant difference compared to MAC-BAR$_{SEVO}$ baseline (p<0.05)*

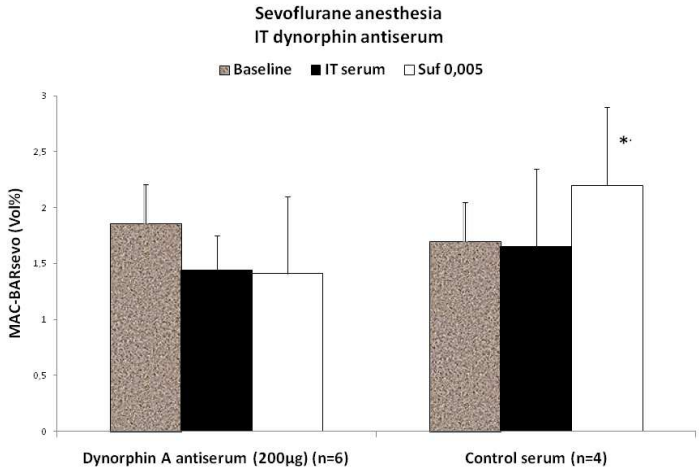

Figure 40: anti-dynorphin serum
Effect of i.t. anti-dynorphin serum and control serum administration
Dynorphin A antiserum was raised by repeated injections of dynorphin A coupled to bovine serum albumin to rabbits. Control serum was collected from rabbits of the same strain that had not been exposed to antigen
Results were expressed as mean ± SD
**: significant difference compared to MAC-BAR$_{SEVO}$ baseline (p<0.05)*

3.3.*2.4.* Discussion

Different systems known to be implicated in OIH (e.g., dynorphin, PK and SP) are tested using intrathecal delivery of specific inhibitors. All treatments prevent the sufentanil-induced hyperalgesic reactivity in response to the noxious stimulus in animals under sevoflurane anesthesia.

In addition to the NMDA (Price, Mayer et al. 2000; McCartney, Sinha et al. 2004) and COX systems (Samad and Abdi 2001; Kang, Vincler et al. 2002), the pain sensory pathway and opioid-induced paradoxical pain involve SP signaling (Trafton, Abbadie et al. 1999; Vera-Portocarrero, Zhang et al. 2007). As glutamate, substance P plays an important role in mediating central sensitization. SP is an excitatory neurotransmitter synthesized by primary afferent nociceptors and released into the spinal cord after noxious stimulation. SP causes a prolonged depolarization of dorsal horn neurons, enhances their response to input from C-fibers and participates in wind-up. The release of SP in the dorsal horn appears to potentiate NMDA receptor activation, which may affect OIH (Marvizon, Martinez et al. 1997; Wu, Guan et al. 2004) . A role of NK-1 neurotransmission in OIH has also been demonstrated (King, Gardell et al. 2005). Our results show that NK-1 inhibitor prevents OIH and produces a significant anti-nociceptive effect when added to low-dose sufentanil.

Norepinephrine modulates the transmission of nociceptive information in the dorsal horn and induces antinociception (Fairbanks, Stone et al. 2002; Quartilho, Mata et al. 2004; Crassous, Denis et al. 2007). This action is mediated principally through α_2-adrenergic receptors (α_2-ARs). Alpha$_2$-AR agonists can enhance analgesia from intraspinal opioids. This interaction is additive in humans and synergistic in animals occurring both at pre- and post-synaptic level of the primary afferent synapse in the spinal cord. Alpha$_2$-AR agonists have both common regulatory organization and similarities in the second messenger with opioids. The antinociceptive potency of α_2-AR varies across pain states and through α_2-AR subtype receptors activated. Alpha$_{2A}$ and α_{2C}-subclasses are observed. They differ in both affinities for NE and activation kinetics. The α_{2C}-AR has greater affinity for NE

than the α_{2A}-AR and the α_{2C}-AR show slower deactivation after NE stimulation (Bunemann, Bucheler et al. 2001). Experiments using antibodies specific for the α_{2A}- and α_{2C}-subtypes show that both are concentrated in the superficial dorsal horn but are associated with different axonal population (Stone, Broberger et al. 1998; Olave and Maxwell 2003). The α_{2A}-AR is found in axons that contain substance P and CGRP which are likely to be terminals of nociceptive pathway primary afferents, although the α_{2C}-AR are present on presynaptic excitatory interneuron terminals (Olave and Maxwell 2003). NE can influence NK-1 projection neurons through both an action on primary afferent terminals (substance P, CGRP) via α_{2A}- AR and a presynaptic action in axon terminals (glutaminergic) via α_{2C}-AR. The analgesic action of i.t. α-adrenoceptor agonists is due to reducing of glutamate and SP release from central afferent terminals and to hyperpolarizing dorsal horn neurons (Ueda, Oyama et al. 1995; Woolf 2007). These phenomena are related to the inhibition of Ca^{++} channels on the presynaptic membrane and to an increase in the conductance of inwardly rectifying K^{+} channels in dorsal horn neurons, respectively. On the other hand, the α_2-adrenoceptor is coupled to a Gi protein, which reduces the activity of adenylyl cyclase, simultaneously suppressing both the production of cAMP and the activity of PKA (Karim and Roerig 2000). Moreover, clonidine's effect is associated with a dose-dependent reduction of phosphorylation of NMDA (NR1 subunit) in the dorsal horn in the spinal dorsal horn in a rat model of neuropathic pain (Roh, Kim et al. 2008). Our results show that α_{2A}- and α_{2C}-subtype agonists prevent OIH in the experiment model. Moreover, the α_{2C}-subtype agonists with low-dose sufentanil administration show a significant antinociceptive effect. The α_2-subtype-ARs influence substance P and glutaminergic transmission but α_{2C}-subtype located on terminals axons are likely presynaptic and glutaminergic to nociceptive cells. These findings confirm the primordial role of glutaminergic/NMDA system in the mechanisms of OIH.

Protein tyrosine kinases (PKs) are widely distributed throughout the CNS. They play an important role in signaling pathway for many extracellular molecules and modulate neuronal excitability (Sato, Takano et al. 2003). PKs are recognized as a major mechanism

underlying the regulation of NMDA receptor function (Zou, Lin et al. 2002; Goebel, Alvestad et al. 2005). Furthermore, μ-opioid receptor stimulation triggers the activation of NMDA receptor by increasing intracellular PKC activity. PKC reduces the anti-nociceptive effect of morphine, and PKC inhibitors attenuate the morphine-induced EAA release (Wu, Wen et al. 2006). Therefore, PKs represent a key element that links opioid receptor activation and the recruitment of the glutamatergic/NMDA system implicated in the promotion of pain. The lavendustin, a potent protein kinase inhibitor prevents sufentanil-induced hyperalgesia.

Spinal dynorphins (DYN) contribute to hyperalgesia following tissue and nerve injury by mediating a cascade involving the spinal release of EAAs and PGs (Koetzner, Hua et al. 2004). They are implicated in opioid-induced pain and anti-nociceptive tolerance (Gardell, Wang et al. 2002). Although control serum of the same strain rabbits doesn't any effect on sufentanil-induced excitatory effect, the dynorphin antiserum prevents OIH development.

In conclusion, we have characterized the role of spinal dynorphin and protein kinase in OIH development. SP and NE modulate both nociceptive pain transmission in the dorsal horn and sufentanil-induced pronociceptive effects. Moreover, SP receptor inhibitor and α_{2C}-subtype agonist when given with low-dose sufentanil show a significant antinociceptive effect demonstrating a relevant role of SP and glutaminergic pathway in the mechanisms of OIH.

3.3.*3.* Summary of molecular mechanisms implicated in the anesthetized animal model of OIH

Using the MAC-BAR-sparing effect of sevoflurane as an objective tool to assess antinociception in rodents, we observed that a very low dose of μ-agonist sufentanil paradoxically increased the MAC-BAR of sevoflurane. This excitatory effect is revealed by sevoflurane. Sevoflurane might exacerbate both inhibitory and excitatory effects of opioids on nociception because significant MAC-sparing effects were observed using doses of sufentanil that were completely ineffective in awake animals. Moreover, this excitatory effect is a sufentanil-induced hyperalgesic effect. Different pathway and intracellular signaling known to be implicated in OIH mechanism were evaluated (e.g., NMDA system, COX, PK, dynorphin, SP). From this pharmacological challenge, similarities between opioid-induced hyperalgesia in awake animals and the sufentanil-induced excitatory effect under general anesthesia are obvious. Figures 41 and 42 summarize the pathways which seem to be involved in our experimental model.

-Even at very low dose, opioid receptor activation (1) may initiate a pronociceptive effect. Intravenous **naloxone**, a **μ-receptor antagonist** completely reverses that sufentanil-induced pronociceptive effect.

-Postsynaptic opioid receptor occupation by exogenous sufentanil ligand may activate an intracellular cascade. Opioids are known to translocate and activate GTP-mediating protein-mediated protein kinase. The activation of PK (2) causes phosphorylation of many receptors and ion channels including both μ-opioid receptors (which are still inactivated) and NMDA receptors (3) with neutralization of Mg^{++} block yielding to the increase of intracellular Ca^{++} levels. The Ca^{++} influx contributes to increase PKC activity and activate a neuronal NO-synthase inducing NO generation. NO may diffuse out the post-synaptic neuron and thereby enhances the pre-synaptic release of endogenous glutamate, resulting to a positive feed-back. Ca^{++} may also play a key part in the regulation of an inductible form of cyclo-oxygenase-2 activity (5).

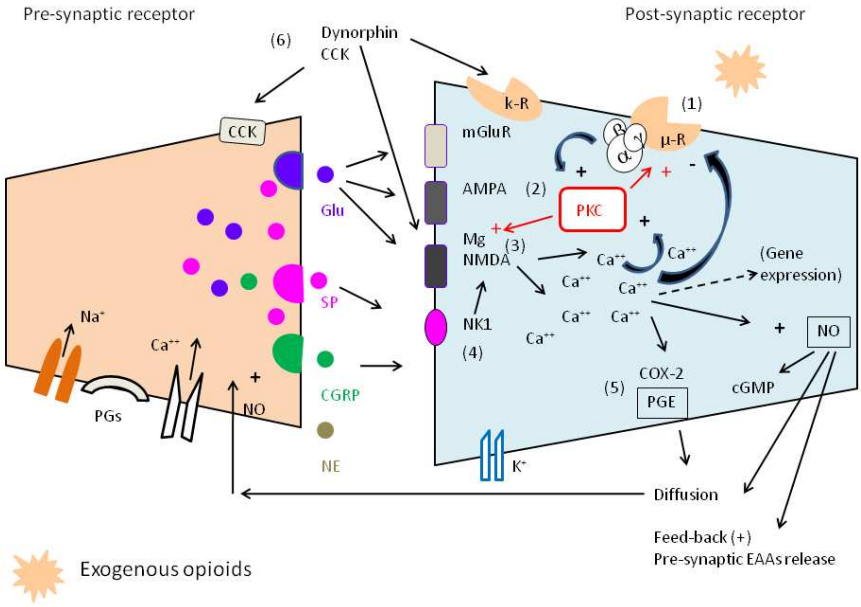

Figure 41: mechanistic model of OIH
Schematic presentation of different pathways implicated in OIH development.

-**Antagonists at the NMDA receptor** (3), ketamine and MK-801 prevent OIH occurrence. The excitatory neurotransmitter glutamate is known as a pivotal role in the development and maintenance of opioid-induced hyperalgesic effects.

-Beside glutamate, substance P is also an excitatory neurotransmitter in the dorsal horn through its binding to NK-1 receptor. Intrathecal **NK-1 receptor antagonist** (4) prevents OIH. SP causes a prolonged depolarization of dorsal horn neurons, enhances their response to input from C-fibers and participates in wind-up phenomenon. The release of SP in the dorsal horn appears to potentiate NMDA receptor activation, which may affect OIH (Marvizon, Martinez et al. 1997; Wu, Guan et al. 2004).

-**COX-1/COX-2 inhibitors** (5), ketorolac and meloxicam also prevent OIH development. COX-2 expressed in CNS co-localizes with glutamate in excitatory neurons and NMDA receptor activation results in increased prostanoid synthesis.

-The inhibitory effect, and by consequence the prevention of OIH phenomenon is particularly evident when these drugs are administered spinally supporting the fact that hyperalgesic effect occurs at the spinal level.

-**Protein kinase C** (2) has a central role in this processing. Blockage of PKC-induced intracellular cascade prevents OIH development.

-Moreover, **spinal dynorphins** (6) are also involved. Intrathecal anti-dynorphin serum inhibits OIH development. Dynorphins are endogenous κ-opioid agonist which can promote antinociception under certain circumstances. Increased expression of spinal dynorphin may be pronociceptive increasing the release of excitatory neurotransmission from PAFs (Faden 1992; Gardell, Wang et al. 2002).

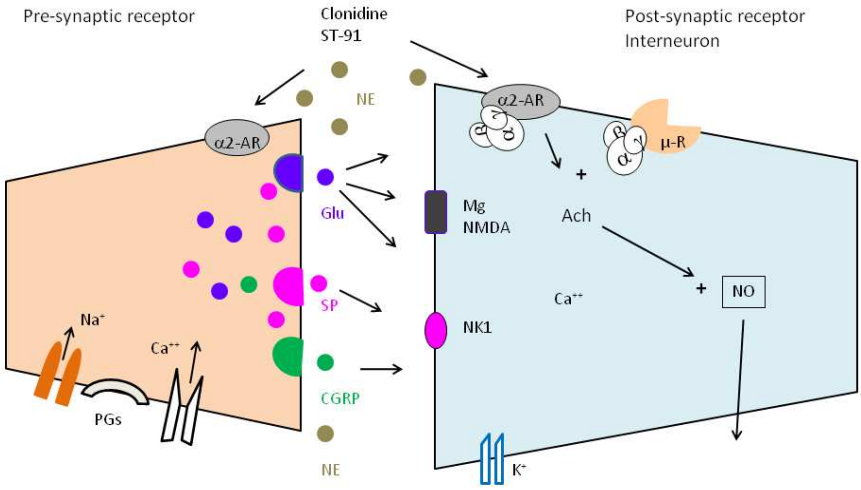

Figure 42: schematic presentation of mechanism NE analgesia

-**Alpha$_2$-AR agonists**, clonidine and ST-91 administration mimic the endogenous NE-induced antinociception and promote analgesia. Descending noradrenergic pathway releases NE to cause analgesia directly and to stimulate acetylcholine (Ach) release (Eisenach, De Kock et al. 1996). Alpha$_2$-AR receptors are coupled to G-proteins which induce hyperpolarization by decreasing the Ca^{++} influx and

increasing the K^+ efflux. It is well known that alpha$_2$-AR agonists potentiate analgesia from intraspinal opioids. Moreover, this interaction occurs both pre- and post-synaptic to the primary afferent synapse in the spinal cord. Different α_2-subtypes are implicated in this analgesic effect. Intrathecal α_2-AR agonists prevent sufentanil-induced hyperalgesia. In contrast to clonidine, a α_{2A}-AR preferring agonist, the ST-91, a more specific α_{2c}-AR subtype adds to low-dose sufentanil to produce a significant antinociceptive effect. As aforementioned, the α_{2A}-ARs are located on axons that contain SP and CGRP although the α_{2C}-ARs are likely present on presynaptic excitatory glutaminergic interneurons. This observation confirms the relevant role of glutaminergic pathway in our OIH model.

3.4. Can a pre-existing pain condition influence the hyperalgesic effects of sufentanil?

Docquier et al, submitted

In other words, can underlying central synaptic plasticity induced by acute and chronic pain condition modify the paradoxical anti-analgesic effect of sufentanil in anesthetized animal?

Peripheral tissue injury and nerve damage induce sensitization of sensory processing and produce profound morphological and pharmacological perturbations once the central pro-nociceptive excitatory cascade is initiated. Moreover, neurogenic and inflammatory hyperalgesia and anti-nociceptive effect of morphine, two seemingly-unrelated phenomena, are interrelated by common neural substrates which activate NMDA receptors and subsequent intracellular events leading to CNS neuronal changes (Mao, Price et al. 1995; Mayer, Mao et al. 1999; Simonnet and Rivat 2003).

Recent studies have demonstrated that perioperative opioid administration worsens hyperalgesia induced by local inflammation (carrageenan-elicited tissue damage) or by paw incision and increases postoperative pain (Christensen and Kayser 2000; Rivat, Laulin et al. 2002; Richebe, Rivat et al. 2005). Neuropathic pain has been classified according either to the etiological diagnosis of neuropathy (e.g., painful diabetic neuropathy, post-herpetic neuralgia, post-traumatic neuralgia, etc.), or to the anatomical site of the lesion (e.g., central pain, peripheral neuralgia). Neuropathic pain, an invalidating chronic pain condition, involves a mixture of pathophysiological mechanisms, a complex assortment of spontaneous and elicited pain states and a somewhat unpredictable response to analgesics (Hansson 2003). Efficacy of opioids in patients with neuropathic pain shows conflicting results. Inter-individual differences in the extension and nature of nerve lesions are likely to contribute variability in results (Jensen, Madsen et al. 2009). Numerous therapeutic strategies for neuropathic pain have been suggested, reflecting the advanced comprehension of physiopathological mechanisms in the past few years (Moulin, Clark

et al. 2007; Jensen, Madsen et al. 2009). Although opioid analgesics are widely used in chronic pain, few reports have explored acute OIH in pathological neuropathic states.

A better understanding of the acute paradoxical pronociceptive opioid-induced effect is mandatory because the clinical implications are still unknown: e.g. the effectiveness of opioid treatment in chronic pain patients, the possible facilitation of the development of persistent post-surgical pain in some patients (Angst and Clark 2006; Wilder-Smith and Arendt-Nielsen 2006; Koppert and Schmelz 2007).

Using the experimental setting of the hyperalgesic effect of low-dose sufentanil, the present study sought to investigate whether acute OIH is expressed differently in the presence of different pain states, such as acute incisional pain or chronic neuropathic pain.

The observations were evaluated with another animal model describing hyperalgesia elicited by administration of high doses of fentanyl in awake status and presenting similar co-existing acute and chronic pain conditions.

	Opioids	***Noxious stimulus***	***Response***	***Measure***
Docquier et al	*Low dose sufentanil infusion*	*Stimulus on/off Tail clamp*	*MAC-BAR$_{SEVO}$*	*Acute During sevoflurane anesthesia*
Adapted from Simonnet et al	*High dose fentanyl administration*	*Thermal noxious stimulation Hot-plate test*	*Paw Withdrawal Latency (PWL)*	*Long-lasting Hours and days after opioid administration*

Table 10: two animal models with OIH
Summary of different outcomes and experimental settings in two animal models describing an opioid-induced hyperalgesic effect

3.4.*1.* Sufentanil-induced hyperalgesic effect in the anesthetized animal model

3.4.*1.1*. Materials and methods

Animal preparation After institutional Animal Care Committee approval, experiments were performed on adult male Wistar rats

weighing 250-350 g. During all the time requested for experiments, animals were housed in group of 2 to 3 rats and maintained on a 12h: 12h light-dark cycle and had free access to food and tap water.

The animals were allocated into 4 groups: uninjured (controls), an acute postoperative pain group undergoing an abdominal incision (Abd Inc), chronic neuropathic pain group induced either by a mechanical lesion of the sciatic nerve (Partial Sciatic Nerve Ligation-PSNL) or by metabolic insult inducing by diabetes mellitus status (diabetic).

In the *abdominal incision* pain model, the animals, underwent under volatile anesthesia a 5 cm longitudinal abdominal incision through the skin and the muscle made on the midline of the abdomen (linea alba) starting at the xiphoid cartilage. The peritoneal cavity was opened to expose the underlying intestine during 3 to 4 min. The wound was then sutured in two layers, muscles and skin with 2-0 silk. Animals were then included into the study protocol 60 min or 24 h after surgical incision.

Chronic neuropathic condition (PSNL) was induced by a *partial ligation of one sciatic nerve* at least 3 months before the present study and performed as described by Seltzer (Seltzer, Dubner et al. 1990). From the time of surgical ligation until the study, paw withdrawal threshold (PWT, in g) to hind paw application of Von Frey filaments was regularly assessed and only animals who displayed a significant mechanical allodynia defined as PWT < 10 g were used. (Average PWT before nerve ligation was 30 g) The neuropathic animals were included in the study protocol when neuropathic pain state was well established, around 12 to 14 weeks after nerve ligation. *Chronic diabetic condition* resulted from a single intraperitoneal injection of streptozotocin (50 mg/kg weight) which effect is to kill pancreatic β cells and to induce insulin deficiency (Courteix, Bardin et al. 1994). Clinical diabetic status was assessed by blood glucose measurement (average hyperglycemia was 586±43 mg/dL). Development of peripheral neuropathy was confirmed by the presence of mechanical hyperalgesia to the application of von Frey filament (average PWT of 11 g). The animals were included in the

study protocol between 4 and 6 weeks after i.p. streptozotocin injection.

Experimental model As previously described, the MAC-BAR_{SEVO} was evaluated facing to the ON/OFF mechanical tail clamping noxious stimulus. After baseline MAC-BAR_{SEVO} determination in each animal, the low doses of sufentanil were administrated.

The methodology for determination of MAC-BAR_{SEVO} (minimum alveolar concentration of sevoflurane that blocks cardiovascular response to noxious stimulus) was comparable to the one used in previous work (Chapter 3.1.*3*).

First basal MAC-BAR_{SEVO} was determined in every animal. Subsequently, animal received a bolus of sufentanil (0,015 µg/kg) followed by continuous infusion of 0,005 µg/kg/min and MAC-BAR_{SEVO} was determined after equilibrium of 15 min. Sufentanil administration was then increased to 0.07 µg/kg/min and preceded by a bolus of 0.21 µg/kg. After equilibrium, a novel measure of MAC-BAR_{SEVO} was realized. At the end of experiment, the animals were euthanized by intravenous injection of an overdose of pentobarbital.

Drugs Opioids drugs were provided from commercial solutions and diluted in saline as needed. Fentanyl (50µg/ml) and sufentanil (5µg/ml) were purchased from Janssen-Cilag, Beerse, Belgium and Pancuronium bromure from Organon. Streptozocin was provided by Sigma-Aldrich. Sevoflurane was obtained from Abbott Laboratories, Chicago, USA and delivered by Sevoflurane vaporizer (Dräger, Lubeck, Germany).

Statistical analysis The effects of sufentanil on the initial MAC-BAR_{SEVO} (increase or decrease) have been calculated according to the following equation (equation 2):

$$Suf\ 0.005 = \frac{(MAC - BAR\,suf\ 0.005) - (MAC - BAR\,baseline)}{MAC - BAR\,baseline} X\,100$$

$$\text{Suf } 0.07 = \frac{(\text{MAC} - \text{BAR suf } 0.07) - (\text{MAC} - \text{BAR baseline})}{\text{MAC} - \text{BAR baseline}} \text{X}100$$

The normality of the distribution of sufentanil effects in different subgroups was assessed by Kolmogorov-Smirnov and QQ plots test. Student *t*-test was then performed to check if sufentanil effects were significantly different from 0. The sufentanil effects were expressed in % as mean ± SD. The baselines of different subgroups of pre-existing pain were compared using Mann-Whitney test.

In each group, the MAC-BAR-sparing effect of 0.005 µg/kg/min sufentanil administration was calculated. A MAC-BAR_{SEVO} increase of at least 20% from baseline value after opioid administration was considered as relevant and clear hyperalgesia. The percentage of animals displaying a MAC-BAR_{SEVO} increase after sufentanil administration more than 20% was noted (Table 11).

3.4.*1.2.* Results

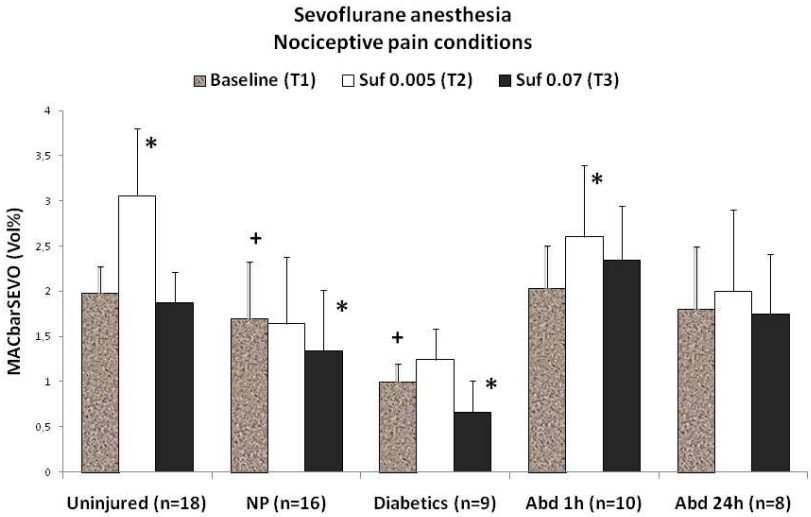

Figure 43: low-dose sufentanil administration in different pain conditions
MAC-BAR_{SEVO} at baseline and after sufentanil administration in anesthetized animals Comparison among uninjured animals, neuropathic animals and animals with an abdominal incision The effects of sufentanil on the initial MAC-BAR_{SEVO} (increase or decrease) have been calculated according to equation 2.
**: significant difference compared to the baseline ($p<0.05$)*
+: baseline significantly different compared to the baseline of uninjured animals ($p<0.05$)

In uninjured control animals (n=18), infusion of a low dose of sufentanil (0,005 µg/kg/min) increased significantly the MAC-BAR$_{SEVO}$ instead of having a MAC-BAR sparing effect (3.1 ± 0.7 instead of 1.9 ± 0.3; p=0.0001) (Figure 43). 77 % of the animals displayed at least 20% increase of basal MAC-BAR$_{SEVO}$ after sufentanil infusion (Table 11). Calculated MAC-BAR$_{SEVO}$ sparing effect was -55% and +26%, respectively under continuous infusion of sufentanil 0.005 and 0.07 µg/kg/min.

Pain conditions	***MAC-BAR-sparing effect of sufentanil 0.005 µg/kg/min***	***% of animals with >20%MAC-BAR increase***
Uninjured	*-55%*	*77%*
NP	*4%*	*25%*
Diabetics	*-24%*	*55%*
Abd 1h	*-28%*	*60%*
Abd 24h	*-11%*	*25%*

Table 11: low-dose sufentanil administration in anesthetized animals MAC-BAR-sparing effect and percentage of animals displayed at least 20% increase of basal MAC-BAR$_{SEVO}$ after sufentanil infusion

The baseline of MAC-BAR$_{SEVO}$ was significantly lower in diabetic and neuropathic animal groups than in control group. Under acute pain condition, a sufentanil-induced pronociceptive effect was observed after early abdominal incision (n=5) (at 1 h post-surgery, p=0.025) but not later (n=3) (24 h post-surgery, p=0.16). The MAC-BAR$_{SEVO}$ sparing effect ranged from - 28% to -11% at respectively 1 h and 24 h after abdominal surgery. Under continuous infusion of sufentanil at increased rate of 0.07 µg/kg/min, MAC-BAR$_{SEVO}$ sparing effect was + 4% and + 9% at respectively 1 h and 24 h after surgery. One hour after incision, 60% of the animals displayed at less 20% increase in MAC-BAR$_{SEVO}$ but at 24 h after abdominal incision, only 25% of the animals show a pronounced excitatory cardiocirculatory effect under subanalgesic doses of sufentanil. Pre-existing chronic pain secondary to partial nerve ligation (n=16) and diabetic neuropathy (n=9) prevented the development of pronociceptive effect induced by sufentanil. In animals with partial nerve ligation, MAC-BAR$_{SEVO}$ sparing effect was +4% and +36% and in diabetic rats, MAC-BAR$_{SEVO}$

sparing effect was -24% and +35%, respectively under continuous infusion of sufentanil 0.005 and 0.07 µg/kg/min.

3.4.*2.* High dose of fentanyl administration in awake animals

3.4.*2.1.* Materials and methods

Animal preparation The animals were allocated into 4 groups: uninjured (controls), an acute postoperative pain group undergoing an abdominal incision (Abd Inc), chronic neuropathic pain group induced either by a mechanical lesion of the sciatic nerve (Partial Sciatic Nerve Ligation-PSNL) or by metabolic insult inducing by diabetes mellitus status (diabetic). A supplemental group of animals underwent an abdominal incision but without administration of fentanyl preoperatively. The procedure of these different experimental pain conditions was performed as described above.

Experimental model According to the method previously reported by Celerier (Celerier, Rivat et al. 2000), animals received four subcutaneous injections of fentanyl 60 µg /kg per injection (or a total dose of 240 µg /kg) at 15 min interval.

The basal nociceptive threshold to a thermal stimulus was evaluated by paw withdrawal latency to radiant heat application in awake animals. The amperage delivered to the light source, thereby the intensity of the stimulus, was monitored to remain constant and a 20 sec cut-off time was used to limit possible tissular damage. Both paws were tested in alternance with a 10-min time interval between testing. To obtain an average paw withdrawal latency three to four trials were realized. Results from both paws were pooled together in controls, abdominal incision, diabetic neuropathy and also peripheral mononeuropathy induced by partial sciatic nerve ligation. In the PSNL model, at 12 weeks and later, stable thermal hyperalgesia is observed in both ipsilateral and contralateral paw and reduced withdrawal latencies do not differ between both hind paws (Takaishi, Eisele et al. 1996).

Development of antinociception and/or hyperalgesia was assessed by latency to hind paw withdrawal (PWL). PWL was evaluated one day

before fentanyl injection (baseline), at 4 hours (day 0), and then at day 1, day 2, day 3 and day 4 after fentanyl administration.

Statistical analysis Analyses of paw withdrawal latency to thermal stimulus were expressed as mean ± SD (in second). The statistical model used was a mixed model allowing estimations despite missing data. The individual was considered as a random effect and conclusion might given for the whole population.

The model is: $Y_{ijk} = J_i + G_j + (GJ)_{ij} + \tau_k + \varepsilon_{ijk}$

where: Y_{ijk} is the individual response in sec (PWL)
J_i the day
G_j the group
$(GJ)_{ij}$ the interaction between the group an the day
τ_k the individual effect

EFFECT	*F-value*	*p-value*
Day	*45.72*	*<0.001*
group	*9.77*	*<0.001*
*Day*group*	*4.83*	*<0.001*

Table 12: description of statistical model
The three effects are significant

In each group an *Algesic Index* (AI) was determined as previously described [= (Σ nociceptive threshold values at D_{+1} to D_{+4} - baseline value) x number of days] (Celerier, Rivat et al. 2000). Moreover, we defined clear thermal hyperalgesia as latencies less than 20% of baseline, as this level appeared to describe relevant effects and we calculated the percentage of animals displaying clear thermal hyperalgesia.

3.4.*2.2.* Results

High dose of fentanyl produced biphasic time-dependent effects on thermal threshold: an early transitory analgesic effect (Day 0, 4 hours) followed by a long-lasting hyperalgesia (from Day 1 to Day 4) in uninjured control rats (n=24) (Figure 44 and 45). Calculated *Algesic Index* Day 1 to Day 4) was -11.81 (Table 13). Further, 50 % of the animals in control group (average percentage from Day 0 to Day 4)

displayed a paw withdrawal latency (PWL) <20% of the baseline (Table 12).

The baseline thermal latencies of PSNL and diabetic rats were significantly different from control animals. In the presence of mechanical nerve lesion (n=14), an early analgesic effect of fentanyl appeared but not the development of delayed hyperalgesia. With diabetes mellitus status (n=10), animals do not presented with the early opioid analgesic effect but with a transitory hyperalgesic effect expressed by significant reduced latency of thermal threshold at day 2 and 3. Calculated *Algesic Index* was +2.8 and -4.67 in PSNL and diabetic group respectively.

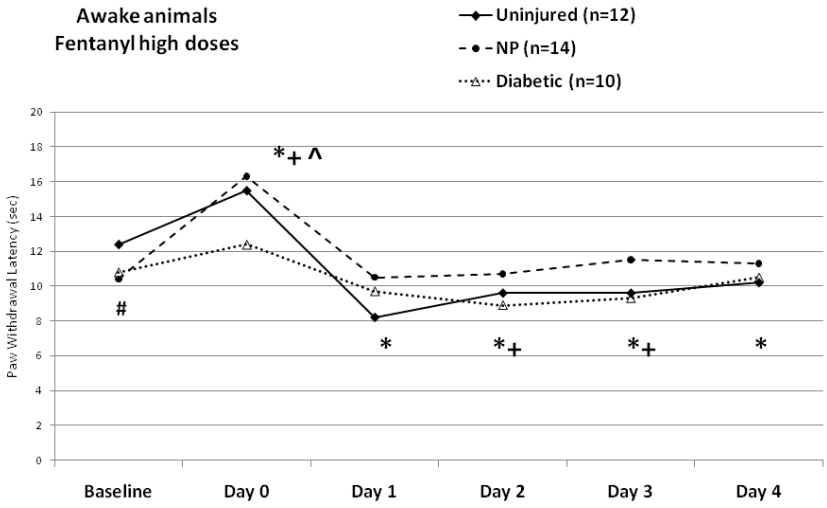

Figure 44: high doses of fentanyl in awake animals
Paw withdrawal latency (sec) to a thermal stimulus in uninjured versus neuropathic animals.
**: significant difference compared to baseline in uninjured group (p<0.05)*
#: baselines of neuropathic and diabetic animals are different compared to the baseline of uninjured animals (p<0.05)
^: significant difference compared to baseline in neuropathic group (p<0.05)
+: significant difference compared to baseline in diabetic group (p<0.05)

Under acute pain conditions, such as an abdominal incision (n=6), early analgesic effect of fentanyl administration was not observed but the animals presented with delayed long-lasting hyperalgesia from day 1 until day 4. The calculated *Algesic Index* was -11.98. 26% of

animals displayed clear OIH. The supplemental group of animals who had undergone an abdominal incision without perioperative fentanyl administration (n=6) displayed early analgesia but delayed hyperalgesia did not appear. None individuals developed hyperalgesia.

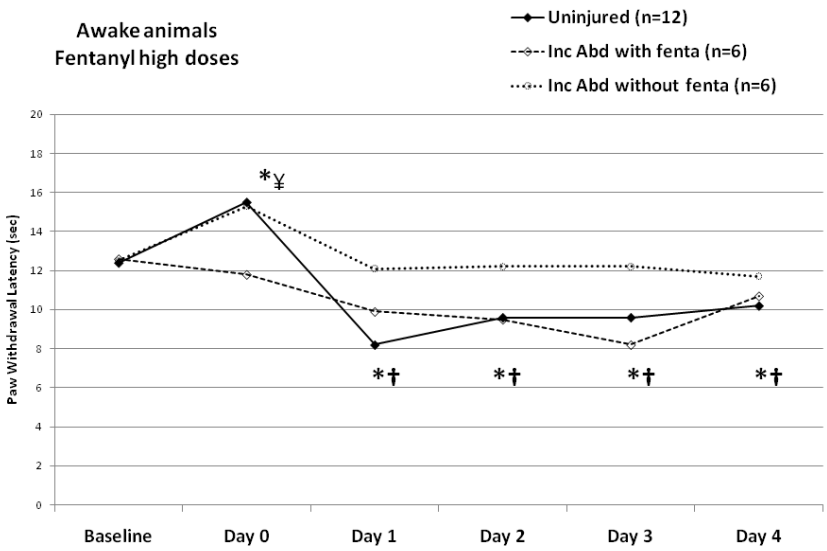

Figure 45 : high doses of fentanyl in awake animals
Paw withdrawal latency (sec) to a thermal stimulus in uninjured animals versus in animals with an abdominal incision
**: significant difference compared to baseline in uninjured group (p<0.05)*
¥: significant difference compared to baseline in abdominal incision without fentanyl (p<0.05)
†: significant difference compared to baseline in abdominal incision with perioperative fentanyl administration (p<0.05)

Pain conditions	***Algesic index***	***% of animals with <20% PWL decrease***
Uninjured	*-11.8*	*50%*
NP	*2.64*	*2.8%*
Diabetics	*-4.67*	*20%*
Abd Inc with F	*-11.98*	*26%*
Abd Inc without F	*-2.06*	*0%*

Table 13: high dose fentanyl and pain conditions
Fentanyl experiment in different pain conditions: Algesic index and percentage of animals displayed at least 20% decrease in paw withdrawal latency to thermal stimulus

3.4.*3*. Discussion

The originality of the study lies in simultaneous observations of pro-nociceptive effects following the systemic administration, at either very low doses (subanalgesic) or at high doses of μ-opioid agonists that are usually used in clinical practice. Because administration of any μ-opioid agonist triggers pain facilitatory processes, the impact of this effect on co-existing hyperalgesic states involving a preexisting CNS sensitization deserves to be studied. In clinical practice, the usual management of intraoperative and postoperative pain by opioid analgesics leads to circulating concentrations that can temporarily be either sub-analgesic or supra-analgesic and might therefore worsen a concomitant or pre-existing pain condition.

The models we used mimic perioperative pain conditions. First, in the experiment, animals undergo an abdominal incision, and the analgesic and hyperalgesic effects of opioids are assessed by application of a noxious stimulus in a remote region (i.e. tail clamping or paw withdrawal latency to a thermal stimulus). In contrast, in other studies, the noxious stimulus was applied to the injured tissues. In these previous studies, high-dose fentanyl demonstrated a bi-phasic pattern with an early analgesic effect followed by hyperalgesia, which was exaggerated after plantar incision (Richebe, Rivat et al. 2005) or carrageenan injection (Rivat, Laulin et al. 2002). Similarly, subanalgesic doses of morphine enhanced the hyperalgesia induced by paw pressure in arthritic rats (Kayser, Besson et al. 1987). Second, the use of the MAC-BAR to assess anti-nociceptive potency of analgesic drugs mimics clinical situations where the decision to deepen the anesthetic level is sometimes based on cardio-circulatory reactivity in response to a noxious stimulus. Moreover, cardio-circulatory data are easy to record in experimental conditions and are objective (Gomez de Segura, Criado et al. 1998).

Although models of opioid-induced pro-nociceptive effects are very different methodologically, similarities are apparent in these models, suggesting that common mechanisms may underlie different pain conditions. Furthermore, such results support the use of aggravated hemodynamic responses to noxious stimuli in the presence of subanalgesic doses of opioid as a validated model for acute OIH

(Docquier, Lavand'homme et al. 2003). Regardless, the aim of the study is not to compare two animal models with different methodologies but to observe the trend of opioid-induced effects under different nociceptive conditions, such as acute or chronic pain condition, in two validated animal models displaying opioids-induced pro-nociceptive effects.

Under acute pain in our study, opioid administration always induced a pro-nociceptive effect but didn't show any analgesic effect. After perioperative administration of high-dose fentanyl during abdominal incision, our observations were similar to results previously reported in the literature for acute pain conditions, except that a transient analgesic effect was not apparent in our model. The lack of a transient analgesic effect could be related to the degree of tissue injury (abdominal incision versus plantar incision of the paw) as well as to the doses of fentanyl administered. Fentanyl effects, both analgesic and hyperalgesic, are dose-related, and we limited the total fentanyl dose to 240 μg/kg to reduce the risk for major respiratory depression. In contrast, Richebe and colleagues (Richebe, Rivat et al. 2005) administered a total fentanyl dose up to 400 μg/kg after plantar incision. Furthermore, abdominal incision without perioperative opioid administration induced early analgesia but was not followed by delayed hyperalgesia. This reinforced the idea that perioperative opioid administration can exaggerate the incision pain effect, inhibiting stress analgesia and inducing a long-term pro-nociceptive effect. A short-lasting (within the first hour after tissue injury), local spinal release of the EAAs glutamate and aspartate has been reported after plantar incision in rats (Zahn, Sluka et al. 2002). Abdominal incision produces more extensive tissue damage, which activates not only local but multi-segmental pro-nociceptive systems in the spinal cord, similar to post-surgical events in humans (Wilder-Smith and Arendt-Nielsen 2006). The temporary release of EAAs and subsequent activation of NMDA receptors may reinforce the excitatory effects of sufentanil subanalgesic doses, thus accounting for the early increase in the MAC-BAR_{SEVO} observed at 1 h after abdominal incision. Spinal activation of NMDA receptors has been previously reported to mediate the excitatory effects of sub-analgesic doses of sufentanil because i.t. administration of NMDA antagonists blocked the phenomenon (Docquier, Lavand'homme et al. 2004).

At 24 h after abdominal incision, subanalgesic doses of opioids did not induce a significant pronociceptive effect (only 25% of the animals displayed a significant hyperalgesia versus 60% at 1 h after incision). Using a similar model of abdominal incision and assessing opioid analgesic effect in a remote region (thermal tail flick), Ho et al (Ho, Wang et al. 1999) demonstrated that surgical pain significantly slowed the development of acute morphine tolerance. The authors suggested that the underlying mechanism could be an endocrine response via a stress-induced activation of the hypothalamus-pituitary-adrenal (HPA) axis. Surgery-related and postoperative pain may account for the effects we observed 24 h after surgery, such as no development of sufentanil hyperalgesia. Moreover, the NMDA system, implicated in pro-nociceptive states after acute pain (Pogatzki, Niemeier et al. 2002; Brennan, Zahn et al. 2005), is already engaged (24 h or more) and cannot be further stimulated; therefore the pro-nociceptive effect induced by low-dose opioids cannot be observed. Different impacts of pain duration are already observed. A time-dependent pattern appears: at 1 h after surgery, the hemodynamic pattern of MAC-BAR$_{SEVO}$ response does not differ significantly from controls, perhaps because it is too soon after surgery. At 24 h later, various pro-nociceptive pathways are fully implicated, which could explain the different responses at these two time points.

In the presence of chronic neuropathic pain, neuronal plastic adaptations have strongly modified both the anti- and pro-nociceptive central processes. Some of these changes account for the poor efficacy of morphine and other opioids to alleviate neuropathic pain, as reported in both patients (Chu, Clark et al. 2006) and in experimental conditions (Dickenson and Suzuki 2005). However, there is some controversy surrounding this topic because opioids are commonly used in chronic neuropathic patients with various degree of success, as in an experimental model of peripheral mononeuropathy (Eisenberg, McNicol et al. 2005). In our results, the early analgesic effect of high-dose of fentanyl in rats with partial nerve ligation is consistent with previous findings (*Algesic Index* 1.8). Moreover, a long-lasting OIH effect does not occur. In same way, although baseline MAC-BAR$_{SEVO}$ response does not differ from controls, pro-nociceptive doses of sufentanil not only failed to induce an excitatory

effect, but they were associated with a sparing effect. The persistence of neuropathic pain is associated with the recruitment of central pro-nociceptive processes, which may be insensitive to further activation by opioid administration. Common intracellular mechanisms involving NMDA receptor activation have already been highlighted in neuropathic pain and development of morphine tolerance (Mao, Price et al. 1995). Activation of spinal NMDA receptors contributes to thermal hyperalgesia following peripheral nerve injury (Seltzer, Cohn et al. 1991) and to mechanical allodynia in diabetic rats (Malcangio and Tomlinson 1998). As previously discussed, both sub-analgesic doses of sufentanil (Docquier, Lavand'homme et al. 2004) and high-dose fentanyl induced pro-nociceptive effects (Celerier, Rivat et al. 2000) that are partly mediated by NMDA receptors because they are blocked by NMDA antagonists. More recently, an increase of intracellular PKCγ activity associated with NMDA receptor activation has been reported within the spinal cord of mononeuropathic animals (Ohsawa, Narita et al. 2000). This is thought to contribute to thermal hyperalgesia after partial nerve ligation. PKCγ has been identified as a key element that links opioid receptor activation and the recruitment of pro-nociceptive systems after high-dose fentanyl (Celerier, Simonnet et al. 2004) or extremely low dose morphine (Galeotti, Stefano et al. 2006) . Finally, partial nerve ligation is associated with an up-regulation of spinal dynorphins, which activates the spinal κ-opioid system, but also induces opioid receptor tolerance (Xu, Petraschka et al. 2004). Although the role of dynorphins in the mechanisms underlying acute OIH is unclear, spinal dynorphin content seems to increase acutely following the administration of high-dose fentanyl (Rivat, Neuroscience Meeting 2006). Thus, many systems implicated in pro-nociceptive states may also be involved in the paradoxical OIH effect.

With short-term (4-6 weeks) experimental diabetes, animals do not exhibit marked fiber degeneration or regeneration in their peripheral or cutaneous nerves, and authors therefore conclude that this is a relevant model of chronic pain (Freshwater, Svensson et al. 2002) . In our observations, diabetic animals did not show early analgesic or hyperalgesic effects of fentanyl. One explanation could be altered sensitivity to the noxious stimulus used. Although all animals displayed mechanical allodynia, baseline latencies were lower than in

control animals. The presence of thermal hyperalgesia as feature of diabetes-induced neuropathy is controversial (Courteix, Bardin et al. 1994); some animals even present with thermal hypoalgesia (Fox, Eastwood et al. 1999). A second explanation might be, as reported previously, the loss of systemic morphine efficacy against acute nociceptive stimuli in diabetic rats (Courteix, Bardin et al. 1994) due to alterations of the endogenous opioid system (Courteix, Bourget et al. 1998; Chen and Pan 2003). These could include impaired μ-opioid receptor and G-protein coupling. The different results of fentanyl-induced analgesia observed after neuropathic and diabetic injury are also dependent on the etiology of the damage. Under sevoflurane anesthesia, MAC-BAR$_{SEVO}$ in diabetic animals is significantly lower than in the control group. However, the response is hemodynamic, and diabetic context may influence vascular reactivity and produce poor health status (Fox, Eastwood et al. 1999). Thus, these results must be interpreted cautiously. Regardless, when the opioid-induced effect is compared to the baseline MAC-BAR$_{SEVO}$ in the same diabetic animals, low-dose sufentanil administration does not induce pro-nociceptive effects. The most relevant observation is that a co-existing neuropathic pain condition, either peripheral mononeuropathy or diabetic polyneuropathy, prevented the development of acute opioid-induced hyperalgesia, regardless of the particular model used.

In **conclusion**, we have demonstrated a pro-nociceptive effect of opioid administration using two different paradigms: 1) cardio-circulatory excitatory effects induced by very low (sub-analgesic) doses of sufentanil, and 2) hyperalgesic response to thermal noxious stimuli following high (analgesic) doses of fentanyl. Furthermore, we have investigated the impact of co-existing pain conditions on OIH. In the presence of incision pain, sub-analgesic and so-called “analgesic” opioid doses display pro-nociceptive effects whereas the early analgesic effect of high doses is blunted. In contrast, once the pro-nociceptive processes have been triggered by co-existing chronic neuropathic pain or by incision pain lasting 24 h, acute pro-nociceptive effects of opioids do not occur, and therefore opioid administration does not seem to worsen pre-existing pain. The contribution of this study was to demonstrate similar results in two different models of pro-nociceptive opioid-induced effects. However, in diabetic conditions, results should be interpreted with caution.

Importantly, our results demonstrated that common mechanisms underlie tissue injury-induced hyperalgesia and paradoxical OIH. The relationship between central sensitization induced by chronic pain and OIH requires further investigation. At present, the pathophysiological mechanisms hypothesized to underlie OIH might seem speculative or too limited, but additional studies using a pharmacological approach are warranted to better elucidate these mechanisms.

Section 4: Conclusion

4.1. General discussion of the studies

Perioperative pain management remains a challenge in clinical practice. Acute postoperative pain is source of suffering and disability. Moreover, poorly relieved postoperative pain may have long-term consequences such as the development of chronic pain syndrome.

Opioids are widely used in daily perioperative pain management. Acute postoperative pain relies on central nervous system sensitization which results from surgical procedure and associated tissue damages. A great debate is now opened to know if opioids which may induce antinociceptive effect (OIH) defined as a sensitization of nociceptive pathway, consequently might enhance sensitization related to tissue injury. At the present time, there is sufficient clinical and experimental evidence to engender caution when administering opioids in perioperative conditions. Nevertheless, the true incidence of OIH in clinical settings is still unknown as well as the real clinical implications of this phenomenon (e.g. increased susceptibility to persistent postsurgical pain).

This thesis focused on the expression of OIH in perioperative conditions. Many animal models have been used in attempts to reproduce perioperative nociceptive conditions. While all of these studies offer insights into the mechanistic aspects of OIH, none have assessed the phenomenon under general anesthesia. For these reasons, we have developed an anesthetized animal model mimicking our clinical practice. The MAC-BAR (hemodynamic response) was chosen as a reference to test the anti-nociceptive potency of analgesic drugs and to allow the evaluation of the interactions between opioids and anesthetic drugs. Several questions were investigated.

The first study was designed to determine whether OIH can occur under anesthesia. Different doses of the μ-receptor agonist sufentanil were evaluated under the halogenated vapor anesthetic sevoflurane. Very low doses of sufentanil infusion significantly increased the MAC-BAR of sevoflurane. This hyperalgesic effect was not observed under

propofol anesthesia. Volatile halogenated anesthetics unmask the phenomenon probably by blocking descending noradrenergic inhibitory pathways.

The second study investigated whether the assessment of MAC-sparing effect provides reliable, quantifiable and objective measure of the anti-nociceptive effect of a drug. Different outcome variables and stimuli were evaluated. The sufentanil hyperalgesic effect was reproducible in different experimental conditions. In animals anesthetized with sevoflurane and ventilated through a tracheotomy, the hyperalgesic effect of low-dose sufentanil was observed using the MAC-BAR for tail clamping and paw pressure. In spontaneously breathing animals (without tracheotomy), the pronociceptive effect remained apparent using MAC-BAR and MAC (movement response) face to paw pressure. Therefore, the sufentanil excitatory effect is reproducible under various experimental conditions. It is worth noting that, tracheotomy represents more than a simple technical matter, it is a different nociceptive challenge.

The third study questioned whether the mechanisms underlying sufentanil paradoxical effect observed in anesthetized animals were similar to those implicated in OIH reported with high doses of opioids in awake animals. Several compounds with proven efficacy in the literature against OIH were effective to prevent the hyperalgesic effect of sufentanil in our model, such as: naloxone (non-specific μ-receptor antagonist), ketorolac (non-specific COX-1/COX-2 inhibitor) and meloxicam (more selective COX-2 inhibitor), ketamine and MK-801 (non-competitive, selective NMDA antagonists). It is worth noting that intrathecal administration of the drugs caused a significant MAC-BAR-sparing effect, arguing in favor of a preferential spinal site for MAC-BAR. Furthermore, i.t. administration of anti-dynorphin serum, PK inhibitor, or SP antagonist also inhibit the development of the hyperalgesic sufentanil effect. By consequence, the excitatory effect of sufentanil in our experimental setting relies on the same mechanism known to be involved in OIH. Furthermore, these observations support the fact that hemodynamic hyperreactivity may be an expression of OIH under anesthesia.

The next question focused on the impact of concomitant acute or chronic pain condition on the expression of sufentanil pro-nociceptive effect observed in anesthetized animals. In the presence of acute pain, low-dose sufentanil supported its pro-nociceptive effects. Conversely, once pro-nociceptive processes have already been triggered by co-existing chronic neuropathic pain or by incision pain lasting 24 h, the acute hyperalgesic effects of sufentanil do not occur. Most likely, the hyperalgesic sufentanil effect shares underlying mechanisms common to those of injury-induced hyperalgesia.

4.2. Conclusions and perspectives

This thesis should be considered as new insight into the investigation of the paradoxical phenomenon of OIH in a perioperative context. While animal models can help to elucidate underlying mechanisms and test new treatment approaches, it remains to relate these findings back to the clinical situation.

From the present investigations, it seems clear that anesthetic management has serious implications in pain management and therefore may have an impact on the future of our patients. All the pharmacological interactions occurring among anesthetic drugs in perioperative period are not yet fully understood and certainly deserve further investigations (Bonnet and Marret 2005). Anesthesia is more than simply a loss of consciousness. Furthermore, because of the large interindividual variability, we should individualize the perioperative management of our patients.

Despite progress in understanding the neurobiology of opioids, a clinical challenge remains: opioids are potent analgesic drugs, but they have paradoxical pro-nociceptive effects, even after single administration. Opioids have opposing effect on nociception: they activate not only pain inhibitory pathways eliciting antinociceptive effects but also pain facilitatory processes favoring hyperalgesia. We have to keep this reality in mind when administering opioids. Moreover, in perioperative conditions, where acute sensitization induced by tissue injury is developing, opioid treatment may initiate latent pain sensitization that could facilitate the development of chronic pain. Aggressive treatment of pain with opioids predisposes

patients to greater levels of pain at later times by inducing a new biological state associated with high neuronal activity (nociceptive memory). This may make patients prone to derangement, which in a clinical context may translate into increased vulnerability to pain (latent sensitization). Consequently, high dose opioid treatment should be proscribed and we should favor association of opioids with other analgesic drugs (playing a synergy) as well as the association of opioids with "protective" medications i.e. antihyperalgesic drugs which are able to modulate central sensitization. The term of 'balanced anesthesia' should become synonym with 'balanced analgesia', i.e. association of analgesic and antihyperalgesic medications.

The paradoxical phenomenon induced by opioids administration may be considered as a positive sign of system adaptation. By nature, a physiologic balance exists between inhibition and activation, i.e. excitation. As described in Chapter 1.1, the endogenous opioid peptide system is very complex. Many molecular and cellular activities have developed as phylogenetically-ancient survival strategies. Endogenous morphine coupled to constitutive NO activity has shown a downregulation (inhibitory effect). This inhibition is followed by hyperactivity or enhanced activation, resulting from the rebound from inhibition. This activation itself represents a physiologically-relevant phenomenon allowing organisms to have enhanced pain sensitivities following a depression of this sense. This raises the question of whether the hyperalgesic effect inducing by exogenous opioids in experimental or clinical settings might also represent a physiological event that would allow the nociceptive pathway to return to a greater vigilant and protective level. This process might therefore be considered as a positive sign of adaptation, maintaining cellular function in states of biological readiness. Other senses can develop a parallel phenomenon to protect themselves. For example, in auditory perception, there is a neural habituation to deleterious tinnitus (Bessman, Heider et al. 2009). Therefore, if we consider the "paradoxical" phenomenon of OIH such as a protective physiological process, adequate opioids use may become easier. Moreover, the question rises how far it is judicious to prevent it?

To date, we do not know the true incidence of OIH in clinical practice, and evidence of OIH at very low opioid doses is limited. Therefore, to bring about improvements in management of perioperative pain and nociception, a shift from symptom-based to mechanism-based approaches may be required to specifically investigate pain and its mechanisms. Prospective studies are needed that will use objective quantitative sensory testing with pain threshold and pain tolerance evaluation before and after opioid administration. Moreover, the clinical studies should investigate the effects of interactions between analgesic and anesthetic drugs on early pain and chronic pain outcome. Furthermore, animal models can help us to understand the underlying mechanisms and to explore clinically-relevant pharmacological approaches, particularly in genetic search settings. The near future probably will give us evidence-based genetic approaches for tailoring individual treatments and therapies for common pain conditions.

Finally, the perception and the modulation of pain is a highly complex, involving a network of immune, genetic, psychological, emotional and motivational subsystems. It is still unknown whether perioperative pain management in surgical patients could start earlier in the preoperative stage. The interconnected psychological, emotional and motivational subsystems are potent modulators of nociceptive pathways (Wobst 2007; Eippert, Bingel et al. 2009). Therefore, why not take advantages of these natural antinociceptive mechanisms (opioidergic or DNICs)? Personal attention to the patient as a whole and an individual, emotional support, positive suggestions and even hypnosis may probably help us to reduce opiates use and to improve the care of our patients in the stressful surgical context.

Personal contributions

Articles

I.” Spinal α2-adrenoceptors are involved in the MAC-BAR-sparing effect of systemic clonidine in rats “
Docquier M-A, Lavand’homme P, Collet V, De Kock M.
Anesth Analg 2002; 95: 935-9

II. “Can determining the minimum alveolar anesthetic concentration of volatile anesthetic be used as an objective tool to assess antinocicepotion in animals?”
Docquier M-A, Lavand’homme P, Ledermann C, Collet V, De Kock M.
Anesth Analg 2003; 97:1033-9

III. “Questioning the cardiocirculatory excitatory effects of opioids under volatile anaesthesia”
Docquier M-A, Lavand’homme P, Boulanger V, Collet V, De Kock M.
Br J anaesth 2004; 93:408-13

IV. “Influence of co-existing pain on acute opioid-induced excitatory effect following subanalgesic and high dose of μ-opioid agonists”
Docquier M-A, Collet V, De Kock M, Lavand’homme P.
Submitted

Poster – presentation

-“The type of general anesthetic used, intravenous or volatile, modifies the interaction between clonidine and sufentanil in rats”.

Presentation at 2001 Annual Meeting of SARB (Society for Anesthesia and Resuscitation of Belgium)

-“In contrast with sevoflurane, propofol does not counteract the analgesic effect of low doses sufentanil in rats”.

Presentation at 2001 Annual Meeting of ASA (American Society of Anesthesiologists), New Orleans

-“Spinal but not intravenous cyclo-oxygenase inhibitors reverse hyperalgesia consecutive to administration of opioids in the rats”.

Presentation at the 2001 Annual Meeting of ASA, New Orleans

-“In contrast with normal rat, neuropathic rat does not display opioid hyperalgesia”

Presentation at 2002 Annual Meeting of ASA, Orlando

-“Surgical incision prevents the development of acute opioid hyperalgesia in rats”

Presentation at 2003 Annual Meeting of ESA (European Society of Anaesthesiology), Glasgow

Presentation at 2003 Annual Meeting of SARB, Belgium

-“Plasticity of spinal NMDA system mediating opioid hyperalgesia in normal and neuropathic conditions”

Presentation at 2004 Annual Meeting of ESA, Lisboa

-“Involvement of spinal tyrosine kinase in an acute opioid-induced hyperalgesia model in rats”

Presentation at 2004 Annual Meeting of ASA, Las Vegas

-“Spinal dynorphin antiserum prevents acute opioid hyperalgesia in rats”

Submitted to2005 Annual Meeting of ESA, Vienna

-“Implication of spinal COX in acute opioid hyperalgesia mechanisms in normal and neuropathic rats”

Presentation at 2006 Annual Meeting of ESA, Madrid

-“Role of spinal Substance P in expression of acute opioid hyperalgesia under normal and neuropathic conditions”

Presentation at 2006 Annual Meeting of ESA, Madrid

-“Analgesic and hyperalgesic effect of single intrathecal dose of morphine under normal and neuropathic conditions”

Presentation at 2007 Annual Meeting of ESA, Munich

-“Effect of single high dose of spinal clonidine under normal and neuropathic conditions”

Presentation at 2007 Annual Meeting of ESA, Munich

-“Development of mechanical hypersensitivity in young versus old individuals in an animal model of persistent postoperative pain”

Presentation at 2008 Annual Meeting of ESA, Copenhagen

-“Hyperalgesic effect of Fentanyl high dose on a SMIR animal model”

Presentation at 2008 Annual Meeting of ESA, Copenhagen

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