THE PHARMACOLOGY OF OXYCODONE,

Studies *In Vitro*, *In Vivo* and in Humans

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Academic Dissertation

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To my wife Linda

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LIST OF ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxaleproprionate
ANOVA	analysis of variance
AUC	area under the curve
AUC%	percentage of area under the curve of the maximum area
BBB	blood-brain-barrier
cAMP	cyclic adenosine monophosphate
CFA	compete Freuds adjuvant
CHO	Chinese hamster ovary
CNS	central nervous system
CR	controlled-release
CYP 450	cytochrome P450
C6μ	C6 glioma cells expressing the rat μ-opioid receptor
DAMGO	[D-ala^2, N-Me-phe^4, gly^5-ol] enkephalin
DRG	dorsal root ganglion
EDTA	ethylenediaminetetraacetic acid
GTP	guanine tri-phosphate
i.c.v.	intracerebroventricular
i.g.	intragastric
i.t.	intrathecal
i.v.	intravenous
KA	kainate
LC	locus coeruleus
MPE%	percentage of the maximum possible effect
mRNA	messenger ribonucleic acid
NMDA	N-methyl-D-aspartate
NRPG	nucleus reticularis paragigantocellularis
Nor-BNI	nor-binaltorphimine
ORL	opioid receptor-like receptor
p.o.	per os

PAG	periaqueductal grey
PCR	polymerase chain reaction
PKC	protein kinase C
RVM	rostroventromedial medulla
s.c.	subcutaneous
SEM	standard error of mean
SNL	spinal nerve ligation
SP	substance P
STZ	streptozotocin
t.d.	transdermal

ABSTRACT

The antinociceptive properties of oxycodone and its metabolites were studied in models of thermal and mechanical nociception and in the spinal nerve ligation (SNL) model of neuropathic pain in rats. Oxycodone induced potent antinociception after subcutaneous (s.c.) administration in all models of nociception used in rats compared with morphine, methadone and its enantiomers. In the SNL model of neuropathic pain in rats, oxycodone produced dose dependent antinociception after s.c. administration. The antinociceptive effects of s.c. oxycodone were antagonized by naloxone but not by nor-binaltorphimine (Nor-BNI) a selective κ-opioid receptor antagonist indicating that the antinociceptive properties of oxycodone are predominantly μ-opioid receptor-mediated.

The antinociceptive activity of oxymorphone, noroxycodone, and noroxymorphone, oxidative metabolites of oxycodone, were studied to determine their role in the oxycodone-induced antinociception in the rat. Of the metabolites of oxycodone s.c. administration of oxymorphone produced potent thermal and mechanical antinociception. Noroxycodone had a poor antinociceptive effect and noroxymorphone was inactive. Oxycodone produced naloxone-reversible antinociception after intrathecal (i.t) administration with a poor potency compared with morphine and oxymorphone. This seems to be related to the low efficacy and potency of oxycodone to stimulate μ-opioid receptor activation in the spinal cord in μ-opioid receptor agonist-stimulated (GTP)γ[S] autoradiography, compared with morphine and oxymorphone. All metabolites studied were more potent than oxycodone after i.t. administration. I.t. noroxymorphone induced a significantly longer lasting antinociceptive effect compared with the other drugs studied.

The role of cytochrome P450 (CYP) 2D6-mediated metabolites on the analgesic activity of oxycodone in humans was studied by blocking the CYP2D6-mediated metabolism of oxycodone with paroxetine. Paroxetine co-administration had no effect on the analgesic effect of oxycodone compared with placebo in chronic pain patients, indicating that oxycodone-induced analgesia and adverse-effects are not dependent of the CYP2D6-mediated metabolism in humans. Although oxycodone has many pharmacologically active metabolites, they seem to have an insignificant role in oxycodone-induced antinociception in humans and rats.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following orginal publications, which are referred to in the text by their Roman numerals.

I. Lemberg K, Kontinen VK, Viljakka K, Kylanlahti I, Yli-Kauhaluoma J, Kalso E. Morphine, oxycodone, methadone and its enantiomers in different models of nociception in the rat. *Anesthesia and Analgesia. 2006;102:1768-1774.*

II. Lemberg K, Kontinen VK, Siiskonen A, Viljakka K, Yli-Kauhaluoma J, Korpi E, Kalso E. Antinociception by spinal and systemic oxycodone: why does the route make a difference? In vitro and in vivo studies in rats. *Anesthesiology. 2006;105:801-812.*

III. Lemberg K, Kontinen VK, Siiskonen A, Yli-Kauhaluoma J, Kalso E. Pharmacological characterization of noroxymorphone as a new opioid for spinal analgesia. *Anesthesia and Analgesia. 2008;106:463-470.*

IV. Lemberg K, Heiskanen T, Neuvonen M, Kontinen VK, Neuvonen P, Dahl ML, Kalso E. Does co-administration of paroxetine change oxycodone analgesia: an interaction study in chronic pain patients. *Submitted 2008.*

1. INTRODUCTION

In 1806 morphine was purified from opium. The new alkaloid was named after Morpheus, the Greek god of dreams. Opium is an extract of the opium poppy (Papaver somniferum) including thebaine, one of its over 20 distinct alkaloids. Oxycodone (14-dihydrohydroxycodeinone) is a semisynthetic opioid and a derivative of thebaine first synthesized in 1916 (Lenz et al. 1986). The molecular structure of oxycodone has similarities to other opium extracts e.g. codeine and morphine (Fig. 1).

Opioids have been used for the relief of pain for thousands of years. Today opioids including oxycodone are commonly used in the clinic to treat acute postoperative pain and chronic cancer pain. The use of opioids in the management of chronic non-cancer related pain remains a controversial issue. In the United States of America the medical use of opioids increased 400% in 5 years (from 1996 to 2000) (Davis et al. 2003). The increased consumption, particularly that of controlled-released (CR) oxycodone, has led to increased abuse (Cicero et al. 2005; Forrester 2007) and also oxycodone-related deaths (Baker and Jenkins 2008). In Finland, oxycodone is commonly used in the management of moderate to severe post-operative pain and cancer-related pain. The consumption of oxycodone has exceeded that of morphine in Finland (Hamunen et al. 2008).

Oxycodone has many pharmacologically active metabolites (Lalovic et al. 2006). Their pharmacological characterization may offer a possibility to develop new analgesics for clinical use. Because of the differences in the drug-metabolism between the rat and humans, pharmacokinetic evaluation should be performed not only in the rat but also in humans. The study of the spinal administration of oxycodone and its metabolites may increase our knowledge of the pharmacology and mechanisms of opioids on distinct areas of central nervous system (CNS). This is important, because opioids are increasingly used both i.t. and epidurally in the clinic.

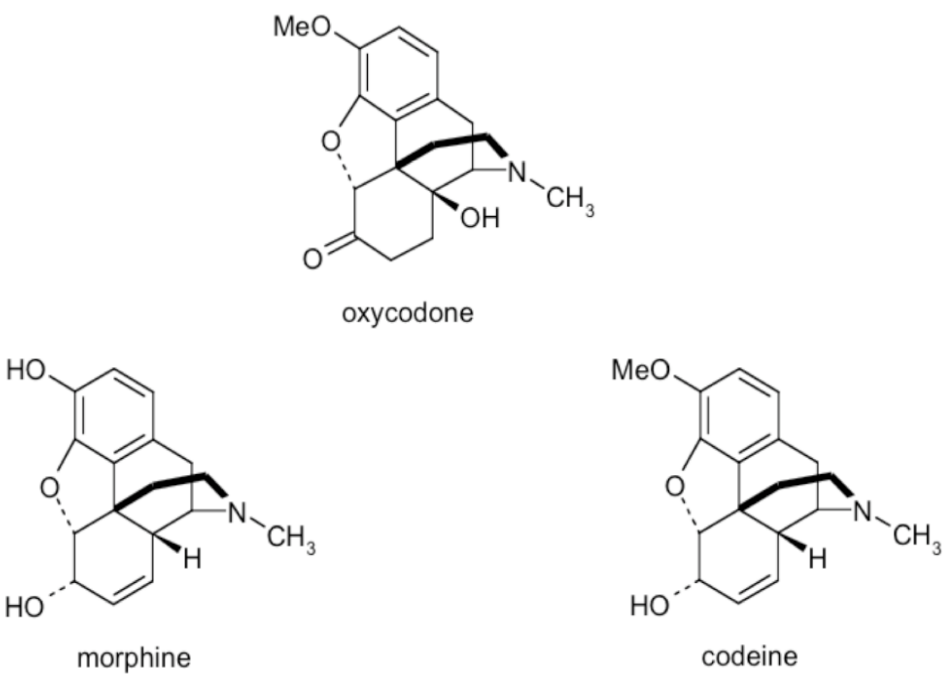

Fig. 1. Structural formulae of oxycodone, morphine and codeine.

2. REVIEW OF THE LITERATURE

2.1. Nociceptive pathways

Stimuli, that can potentially cause tissue damage (heat, cold, pressure, mechanical and chemical) are converted to electical potentials by primary afferent neurones that are specialized to respond to noxious stimuli (nociceptors) (Lawson 2002). The cell bodies of these neurones are located in the dorsal root ganglions (DRG). The painful stimuli activate nerve endings of both thinly myelinated A∂-fibres and unmyelinated C-fibres. A∂-fibres are high velocity conducting neurones compared with thin C-fibres. A∂-fibres are responsible for the sharp acute pain sensation. C-fibres conduct with a lower velocity because they lack the insulating myelin around the axon. C-fibres mediate the pain sensation with a delay, leading to more dull pain sensation compared with that of A∂-fibres. Large-diameter myelinated Aß-fibres are primary afferent neurones that are not nociceptive and do not function in the pain perception in normal conditions, but detect innocuous stimuli like touch, vibration and sensation from joints. Large–diameter Aß-fibre activity modulates pain perception, and can alleviate pain sensation by activating inhibitory interneurones in the spinal cord.

The first synapses of the primary nociceptive afferent neurones are located in the superficial layers of the dorsal horn of the spinal cord. Projection neurones (secondary afferents) recieve their neuronal input from the primary afferents in different layers of the dorsal horn. Projection neurones in lamina I (most superficial) of the dorsal horn, receive input from A∂-fibres, and also indirect input from C-fibres from the interneurones originating from deeper layers of the dorsal horn (Kandel et al. 2000).

Five separate ascending neural pathways (spinothalamic, spinoreticular, spinomesenchephalic, spinohypothalamic and cervicothalamic) transmit the nociceptive information from the spinal cord to supraspinal structures (Kandel et al. 2000). The spinothalamic tract is the most important pathway for nociceptive information, originating from from laminae I and V-VII directly to the thalamus. The thalamus functions as a gate for ascending information to the somatosensory cortex and the limbic areas like the anterior cingulate cortex of the brain. The spinoreticular tract

transmits information from the projection neurones of the laminae VII and VIII to the reticular formation of the medulla and also to thalamus. The important connection to periaqueductal grey (PAG) is mediated by the spinomesenchephalic tract, that orginates from laminae I and V of the dorsal horn of the spinal cord and has important connections to the PAG and to amygdala. The spinohypothalamic tract is an important modulator of the autonomic nervous system. The cervicothalamic tract arises from the cervical areas of the spinal cord and is connected to the thalamus.

2.2. Opioids and the opioidergic system

2.2.1. Opioid receptors

Opioid receptors belong to the superfamily of G-protein receptors that are coupled to pertussis toxin-sensitive GTP-binding proteins. μ-, δ-, κ- opioid receptors and the opioid receptor-like receptor (ORL) have been cloned (Kieffer et al. 1193; Evans et al. 1992; Chen et al. 1993a; Chen et al. 1993b; Yasuda et al. 1993; Meunier et al. 1995; Henderson and McKnight 1997). Pharmacological binding studies have suggested the existence of several opioid-receptor subtypes (Wolozin and Pasternak 1981), but specific genes for these have not been characterized. The opioid receptor subtypes may result from heterodimerization/oligomerization (Cvejic and Devi 1997; Jordan and Devi 1999; George et al. 2000) of the known receptors or receptor-RNA alternative splicing (Pan et al. 2005a; Pan et al. 2005b; Doyle et al. 2007a; Doyle et al. 2007b). The relevance of opioid receptor subtypes *in vivo* is unclear.

Table 1. Opioid receptors and their endogenous ligands. Examples of the synthetic agonists and antagonists are presented.

Receptor	μ	δ	κ	ORL
Endogenous agonists	β-endorphin endomorphins	met-enkephalin leu-enkephalin	dynorphin A dynorphin B	nociceptin/OFQ
Synthetic agonists	DAMGO	DPDPE SNC-80	U50488H	Ro 64-6198
Synthetic antagonists	β-funaltrexamine	naltrindole	nor-binaltorphimine	UFP-101

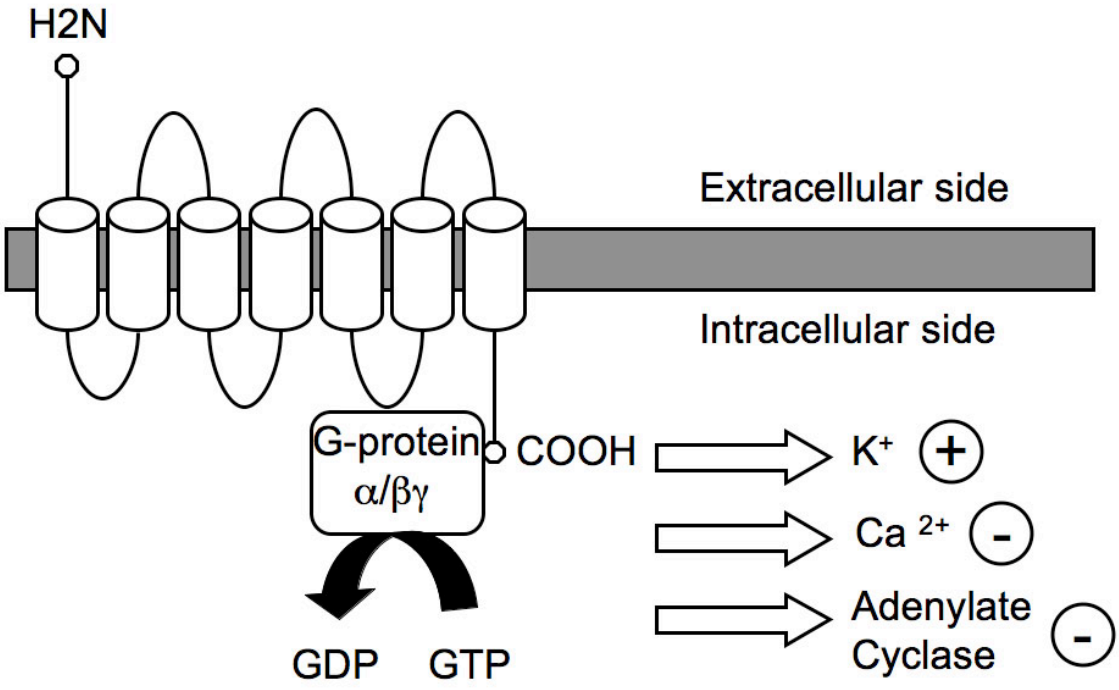

Fig. 2. Schematic illustration of the seven-transmembrane domain structure of the opioid receptor (modified from Chen et al. 1993b).

Opioid receptors are target molecules for endogenous opioids, as shown in Table 1. Opioid receptors have a similar seven-transmembrane domain structure (Fig. 2).

After a ligand binds to the extracellular domain of the opioid-receptor, a conformational change takes place in the receptor, which then activates $G_{i/o}$-proteins leading to the activation of effector-proteins. This leads to a decrease of intracellular cyclic adenosine monophosphate (cAMP) and hyperpolarization of the cell by increased K^+ efflux through K^+ channels. A decrease in Ca^{2+} influx decreases the release of excitatory neurotransmitters and pain transmission in the CNS. The role of opioid receptors in pain behaviour have been demonstrated in the studies with the opioid receptor knock-out mice devoid of functional opioid receptors (Table 2).

Table 2. The changes in the basal pain sensitivity in opioid receptor knockout mice. The data are modified from the original publications of 1) (Matthes et al. 1996; Sora et al. 1997; Fuchs et al. 1999; Sora et al. 1999; Qiu et al. 2000; Martin et al. 2003; Gaveriaux-Ruff et al. 2008). 2) (Zhu et al. 1999; Martin et al. 2003; Gaveriaux-Ruff et al. 2008). 3) (Simonin et al. 1998; Gaveriaux-Ruff et al. 2008). 4) (Nishi et al. 1997).*) Performed with combinatorial *MOR/KOR and MOR/DOR/KOR* mutants.

KO	Thermal (supraspinal)	Thermal (spinal)	Inflammatory	Visceral	Pressure	Morphine antinociception
MOR[1] (μ)	↔ or ↑	↔ or ↑	↔	↔	↑	↓
DOR[2] (δ)	↔ or ↑	↔	↑	↔	↔ or ↑	↔
KOR[3] (κ)	↔	↔	↔ or ↑	↑	↔	↔
ORL1[4] (ORL)	↔	↔		↔	↔	↔

Opioid receptors are located in both the central and peripheral nervous system. μ-Opioid receptors are found in the CNS in high densities, at both the spinal and supraspinal sites (Mansour et al. 1995). In the neurones, opioid receptors are located

mainly in the presynaptic cell surfaces compared with the postsynaptic ones (Besse et al. 1990). Spinal opioid receptors are produced in the dorsal root ganglions of the small afferent fibres of and then transported to central and peripheral nerve endings. This has been demonstrated with rhizotomy that decreases dramatically the amount of opioid receptors in the spinal cord, at the level of the section (Besse et al. 1990). In the spinal cord, high levels of opioid receptors are located in the terminals of pimary afferent C-fibres in the most superficial layers of the spinal cord (Besse et al. 1990; Stevens et al. 1991).

2.2.2. Pain modulation and the sites of opioid action

Antinociceptive effects of opioids in the CNS are mainly mediated by inhibition of the transmission of nociceptive input in the dorsal horn of the spinal cord and by activation of the descending inhibitory pathways (Rang et al. 2003).

Important pain modulative descending inhibitory pathways orginate from the PAG, located in the midbrain around the third ventricle and the cerebral aqueduct (Fig. 3). Descending tracts of PAG are connected to the dorsal horn of the spinal cord via serotonergic neurones of the nucleus raphe magnus in the rostroventral medulla. PAG contains a high density of opioid receptors, and direct administration of morphine to PAG produces antinociception in rats (Yeung et al. 1977). Noradrenergic inhibitory tracts orginate from the locus coeruleus (LC) in the pons, with connections to the dorsal horn of the spinal cord. Stimulation of LC has been shown to produce antinocecption in the rat (Segal and Sandberg 1977; Sandberg and Segal 1978; Jones and Gebhart 1986).

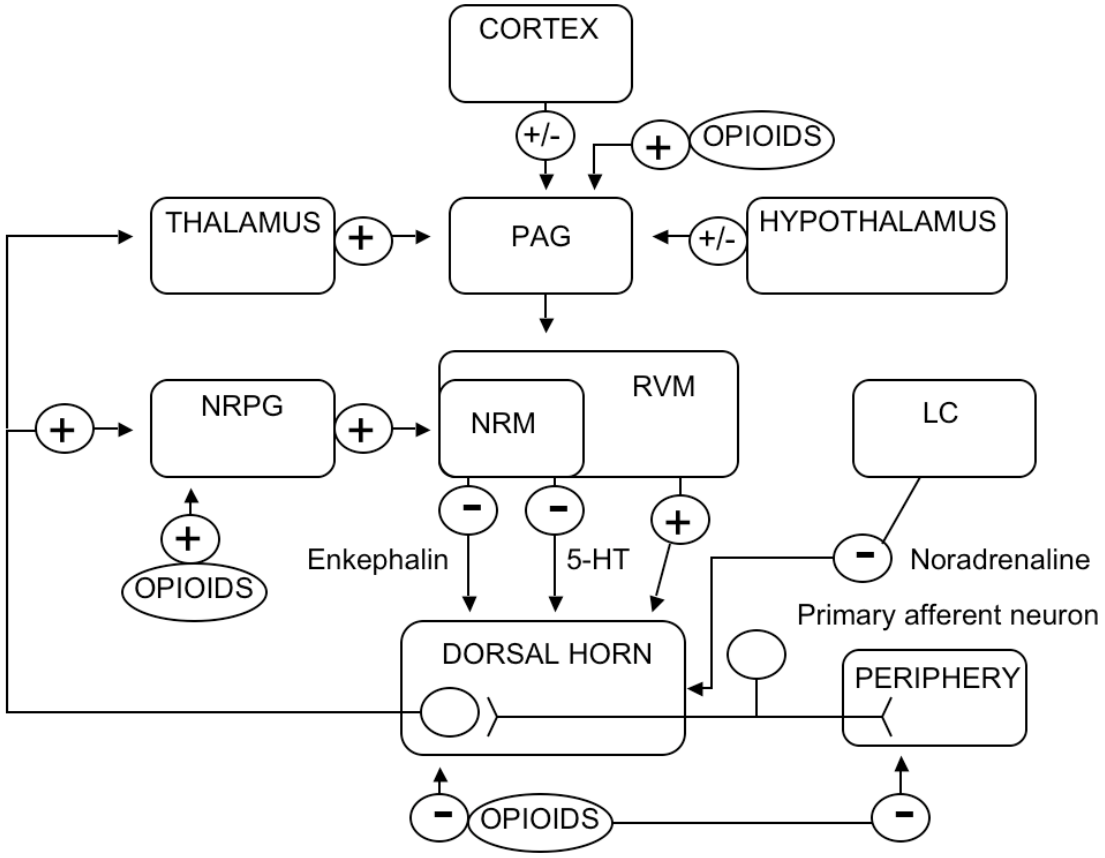

Fig. 3. Illustration of the pain transmission including the descending inhibition and facilitation with the main sites of opioid action. Periaqueductal grey matter (PAG), rostroventral medulla (RVM), locus ceruleus (LC), nucleus raphe magnus (NRM) and nucleus reticularis paragigantocellularis (NRPG). Modified from Rang et al. 2003.

The descending facilitatory tracts have also been characterized (Fig. 3). These neural tracts originate from the brain stem and modulate the spinal nociceptive input in a pro-nociceptive manner. Electrical or chemical stimulation of the rostroventromedial medulla (RVM) has been demonstrated to facilitate pain behaviour in rats (Rees et al. 1995; Almeida et al. 1999).

In the periphery, opioid receptors are functional only in inflammatory conditions. Also peripheral opioid receptors are produced in small afferent fibres of the DRG and transported to peripheral nerve endings via axonal transportation. This serves as a potential target for peripheral opioid administration (Cook and Nickerson 2005).

2.2.3. Adverse effects and tolerance

Typical adverse effects of opioids include constipation, nausea, vomiting, sedation, respiratory depression, dependence, tolerance and muscle rigidity, which are mediated via opioid receptors. In mice, the absence of functional μ-opioid receptors leads to lack of morphine-induced antinociception (Table 2) and also adverse effects like dependence, respiratory depression, inhibition of gastrointestinal transit, immunosuppression and hyperlocomotion (Matthes et al. 1996; Tian et al. 1997; Gaveriaux-Ruff et al. 1998; Matthes et al. 1998; Roy et al. 1998).

Opioid tolerance can be defined as a loss or decrease of analgesic effect following repeated administration of opioids. Chronic exposure of opioid agonists at the cellular level can lead to several changes in neurones. μ-Opioid receptor desensitization and internalization are also postulated to be important factors for the development of opioid tolerance (Whistler et al. 1999). Agonist-induced μ-opioid receptor internalization may protect against the development of tolerance. Morphine has been found to be a poor activator of μ-opioid receptor internalization or desensitization *in vitro* compared with DAMGO (He et al. 2002; Bailey et al. 2003). Poor receptor internalization after morphine administration leaves μ-opioid receptors on the cell surface in an active state. Co-administration of DAMGO (sub-analgesic dose) with morphine was found to increase μ-opioid receptor internalization and to confer protection against the development of morphine-induced tolerance in rats (He et al. 2002). However Bailey et al. failed to observe low concentrations of DAMGO increasing morphine-induced desensitization or internalization of μ-opioid receptors *in vitro* (Bailey et al. 2003). Duttaroy and Yoburn found that equianalgesic doses of opioids produce a different amount of tolerance *in vivo* (Duttaroy and Yoburn 1995). According to Duttaroy and Yoburn (1995) higher efficacy opioids like fentanyl and etorphine produced less tolerance to antinociceptive effects compared with morphine. In addition, the chronic administration of oxycodone and morphine produced more tolerance compared with the high efficacy agonist etorphine *in vivo* in mice (Pawar et al. 2007). In the same study oxycodone and morphine failed to downregulate μ-opioid receptors whereas etorphine caused ≈40% reduction in μ-opioid receptor density in the spinal cord (Pawar et al. 2007). The down-regulation of opioid receptors followed by a

chronic opioid administration seems to correlate poorly with opioid tolerance. Whether this has any clinical relevance needs to be further studied.

Binding of the μ-opioid receptor agonist to the μ-opioid receptor normally activates intracellular G_i- or G_o-proteins, leading to inhibition of the intracellular adenylyl cyclase pathway by activation of Gβγ-subunit (Laugwitz et al. 1993; Ikeda et al. 2000). This leads to hyperpolarization of the cell by increased K^+ influx to the cell through K^+ channels and by inhibition of voltage-dependent calcium channels. Chronically administered opioids are shown to produce cell excitation by loss of hyperpolarization, related to shift of the G-protein coupling to G_s-proteins and activation adenylyl cyclase (increased intracellular cAMP) (Wang et al. 2005b; Wang and Burns 2006). Attenuation or blockage of opioid tolerance has been described *in vivo* in rats with co-administration of NMDA-receptor antagonists with morphine, indicating an important role of NMDA-receptor activation in the development of opioid tolerance (Trujillo and Akil 1991; Manning et al. 1996).

Arrestins are intracellular proteins, which are shown to bind G-protein receptors after ligand-induced opioid receptor activation. Binding of β-arrestins to opioid receptors leads to increased uncoupling and internalization of opioid receptors (Marie et al. 2006). Studies with β-arrestin 2 knockout mice have shown prolonged and enhanced antinociceptive effect of morphine compared with wild-type mice (Bohn et al. 1999). β-Arrestin 2 knockout mice do not develop tolerance to morphine-induced antinociception in the hot-plate test (Bohn et al. 2000). This indicates an important role of β-arrestin in the supraspinal mechanisms of morphine tolerance in mice. The development of morphine tolerance is lesser compared with wild-type mice when studied in the tail-immersion test (spinal reflex) (Bohn et al. 2002).

2.3. Pharmacokinetics of oxycodone

The oral bioavailability of per os (p.o.) oxycodone is low in rats, which is an important finding considering the use of rats in a pharmacokinetic study (Chan et al. 2008). Significant sex-related differences in the bioavailability and pharmacokinetics of oxycodone in rats were reported by Chan et al. (Chan et al. 2008). Noroxycodone is the main metabolite of oxycodone in rats (Huang et al. 2005; Chan et al. 2008) and dogs

(Weinstein and Gaylord 1979). The CYP 2D1 that resembles CYP 2D6 in humans catalyses the oxidation of oxycodone to oxymorphone in rats (Cleary et al. 1994). Oxymorphone plasma concentrations were found very low after i.v. p.o. administrations in the rat (Chan et al. 2008). Previous studies have showed that after i.v. administration in rats and sheep the CNS concentration of unbound oxycodone is 3-6 and 2.5-times higher than in blood and after intragastric (i.g.) administration in rats 2-times higher than in blood (Boström et al. 2006; Lalovic et al. 2006; Villesen et al. 2006; Boström et al. 2008). This indicates that oxycodone is actively transported to the CNS. Recently, a transportter protein common with pyrilamine was suggested to operate as an influx transporter for oxycodone (Okura et al. 2008). In the blood-brain-barrier P-glycoprotein is an efflux transporter (Cordon-Cardo et al. 1989), which limits the concentration of drugs to the CNS. According to Hassan et al., oxycodone is a P-glycoprotein substrate in rats and mice (Hassan et al. 2007) like morphine and methadone (Letrent et al. 1999; Thompson et al. 2000). Opposite results also have been previously published (Boström et al. 2005).

In humans, the main oxidative metabolic route of oxycodone is N-demethylation at the 17-position by CYP 3A4/5 (CYP) 3A4/5 to noroxycodone (Pöyhia et al. 1991; Pöyhia et al. 1992b; Kaiko et al. 1996; Heiskanen et al. 1998; Lalovic et al. 2004; Lalovic et al. 2006). Noroxycodone is further metabolised to the reduced forms α- and β-noroxycodol (Fig. 4). A reductive metabolism of oxycodone occurs with a lower rate to α- and β-oxycodol (Fig. 4). Oxymorphone is a 3-O-demethylation metabolite of oxycodone and it is formed after oxidative reaction mainly catalysed by CYP 2D6 in humans (Otton et al. 1993). Oxymorphone is extensively conjugated and excreted in urine mainly as in the conjugated form (Cone et al. 1983; Pöyhia et al. 1992b; Kirvelä et al. 1996; Lalovic et al. 2006). The reductive metabolites of oxymorphone are α- and β-oxymorphol (Fig. 4). Noroxymorphone is formed mainly after O-demethylation of noroxycodone by CYP 2D6. Also, a minor route is by N-demethylation of oxymorphone to noroxymorphone via CYP 3A4 and 2D6 approximately with a 20- to 30-fold lower rate compared with noroxycodone O-demethylation (Lalovic et al. 2004).

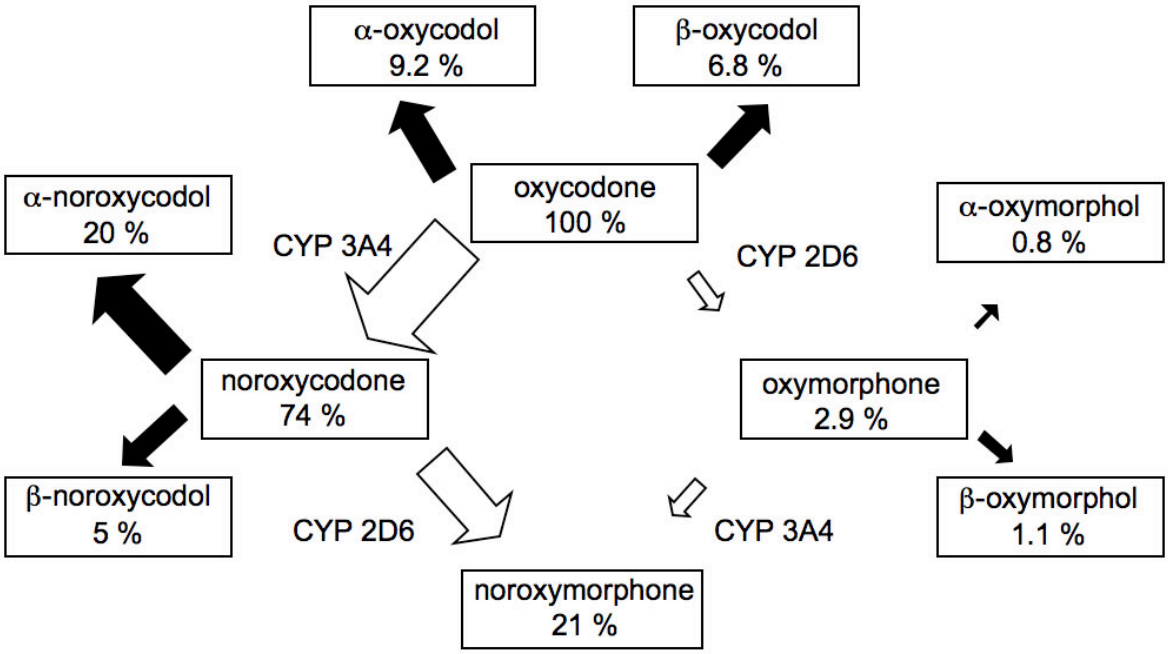

Fig. 4. An illustration of the metabolic pathways of oxycodone with the associated CYP-enzymes after oxidative (white arrows) and reductive (black arrows) reactions. The values represent percentages of plasma consentrations (C_{max} values, ng/ml) after a single 15 mg oral dose of oxycodone in humans. Modified from Lalovic et al. (2006).

Oxycodone has relatively high oral bioavailability in humans (60-87%) (Leow et al. 1992; Pöyhia et al. 1992b) compared with that of morphine (19-30%) (Osborne et al. 1990). Oxycodone is extensively metabolized in the liver and only 8 to 14 % is excreted in unchanged or conjugated forms to urine (Pöyhia et al. 1992b). According to Lalovic et al., oxycodone undergoes minimal oxidation in the intestinal mucosa during the first-pass oxidative metabolism compared with the liver (Lalovic et al. 2004). In healthy female volunteers, the clearance of oxycodone (on a weight-adjusted basis) was found to be 25% slower compared with men (Kaiko et al. 1996). In patients with renal and hepatic failure, the clearance of oxycodone was found to be significantly smaller compared with healthy patients (Kirvelä et al. 1996; Tallgren et al. 1997). Liukas et al. reported that elderly patients had two fold higher plasma oxycodone concentrations compared with younger patients after oral oxycodone administration (Liukas et al. 2008). Villesen et al. reported no differences between the age groups in the pharmacokinetics of oxycodone after i.v. administration (Villesen et al. 2007). The

plasma protein binding and physicochemical properties of oxycodone are reported to be similar to those of morphine (Pöyhia and Seppälä 1994; Peckham and Traynor 2006).

2.4. Pharmacodynamics of oxycodone and its metabolites

Oxycodone is significantly selective for the μ-opioid receptor compared with ∂- and κ-opioid receptors (Chen et al. 1991; Yoburn et al. 1995; Lalovic et al. 2006; Peckham and Traynor 2006). Depending on the study design used, the affinity of oxycodone for the μ-opioid receptor is 5-40 times lower compared with morphine (Chen et al. 1991; Lalovic et al. 2006; Peckham and Traynor 2006). Oxycodone also activates intracellular G-protein via μ-opioid receptors (Thompson et al. 2004; Lalovic et al. 2006; Peckham and Traynor 2006). In GTPγ[^{35}S] binding assay the potency of oxycodone compared with that of morphine has been found to be 4- to 8-times lower in membranes of C6μ and Chinese hamster ovary (CHO) cells transfected with human μ-opioid receptor (Lalovic et al. 2006; Peckham and Traynor 2006) and in the rat thalamus (Thompson et al. 2004). The antinociceptive effect of oxycodone can be antagonized with selective μ-opioid receptor antagonists β-funaltrexamine and clocinnamox *in vivo* in mice (Beardsley et al. 2004; Narita et al. 2008; Pawar et al. 2007). Reduced metabolites of oxycodone α- and β-oxycodol have a lower binding affinity for μ-opioid receptor and significantly lower potency in μ-opioid receptor agonist-induced GTPγ[^{35}S] binding assay compared with oxycodone (Lalovic et al. 2006).

Some *in vivo* studies have suggested that oxycodone interacts mainly with κ-opioid receptor (Ross and Smith 1997). The tolerance to the antinociceptive effect of oxycodone was reported to be κ-opioid receptor mediated after intracerebroventricular (i.c.v.) administration and by μ-opioid receptors after intravenous (i.v.) infusion (Nielsen et al. 2000). According to Nozaki et al., after systemic administration of oxycodone, the antinociceptive effect is mainly μ-opioid receptor-mediated in non-diabetic mice, but mediated via spinal but not supraspinal κ-opioid receptors in diabetic mice (Nozaki et al. 2005; Nozaki et al. 2006). It was further postulated that after s.c. administration of oxycodone in non- diabetic mice the antinociceptive activity of oxycodone is μ_1-opioid receptor-mediated (Nozaki and Kamei 2007). According to

Nozaki and Kamei, in diabetic mice, μ_1-opioid receptor activation can lead to indirect activation of κ-opioid receptors followed by increased release of dynorphin A (Nozaki and Kamei 2007). Ross et al. reported potentiation in the antinociceptive effect after i.c.v. administration of sub- antinociceptive doses of oxycodone and morphine in rats (Ross et al. 2000). Such a potentiation was not observed after s.c. administration in mice (Bolan et al. 2002). Nielsen et al. suggested that oxycodone has a high affinity for κ_{2b}-opioid receptors (Nielsen et al. 2007).

Previous *in vitro* studies have shown that nororoxycodone, the main metabolite of oxycodone, has four times poorer affinity for the μ-opioid receptor compared with oxycodone (Lalovic et al. 2006) and it shows 4-6 times lower GTPγ[^{35}S] binding measuring μ-opioid receptor-mediated G-protein activation in rat thalamus and CHO cell membranes transfected with human μ-opioid receptor compared with oxycodone (Thompson et al. 2004; Lalovic et al. 2006). Noroxycodone has a poor antinociceptive effect compared with oxycodone after i.c.v. administration in rats (Leow and Smith 1994) and oral and s.c. administration in mice (Weinstein and Gaylord 1979). The analgesic potency of noroxycodone has not been studied in humans. The metabolites of noroxycodone α- and β-noroxycodol were found to have an extremely low potency in μ-opioid receptor agonist-induced GTPγ[^{35}S] binding (Lalovic et al. 2006).

The CYP 2D6 mediated metabolite oxymorphone has a ≈45-fold higher affinity for the μ-opioid receptor compared with oxycodone (Lalovic et al. 2006; Peckham and Traynor 2006). The relative potency of oxymorphone to induce intracellular G-protein activation after μ-opioid receptor binding in GTPγ[^{35}S] binding assays in membranes of CHO cells transfected with human μ-opioid receptor is reported to be 8- to 30-fold higher compared with that of oxycodone (Thompson et al. 2004; Lalovic et al. 2006), 11-fold higher in C6μ cells and 30-fold higher in the rat thalamus (Thompson et al. 2004). Oxymorphone-induced antinociceptive effects have been characterized in animals (Briggs et al. 1995; Bolan et al. 2002) and in humans (Beaver et al. 1977). α- and β-oxymorphol are reduction products of oxymorphone. In a previous study by Lalovic et al., α- and β-oxymorphol were found to be 1.6- and 2.7 more potent compared with oxycodone in GTPγ[^{35}S] binding assay (Lalovic et al. 2006). After oral administration of oxycodone in humans, the plasma consentrations of α- and β-oxymorphol are even below the concentration of oxymorphone (Lalovic et al. 2006).

After oral administration of oxycodone, noroxymorphone a secondary oxidative metabolite of oxycodone, has been found in relatively high plasma concentrations in humans (Lalovic et al. 2006). Noroxymorphone shows 2-3-fold higher affinity for the μ-opioid receptor compared with oxycodone (Chen et al. 1991; Lalovic et al. 2006). The relative potency of noroxymorphone to induce intracellular G-protein activation after binding to the μ-opioid receptor in GTPγ[^{35}S] binding assay is 2-fold higher compared with oxycodone in membranes of CHO cells transfected with human μ-opioid receptor (Lalovic et al. 2006) and 7.3-fold higher in rat thalamus (Thompson et al. 2004). These results suggest that noroxymorphone could be a pharmacologically active metabolite of oxycodone.

2.5. The efficacy of oxycodone in different pain models

2.5.1. Nociceptive pain

Nociceptive pain is an essential warning mechanism that protects against tissue damage. Nociceptive pain arises from peripheral tissue injury (heat, cold, pressure or chemicals) leading to activation of nociceptors (A∂- and C-fibres). Inflammation is often associated with nociceptive pain.

In the models of acute nociception in rodents, oxycodone has been found to be more potent or equipotent compared with morphine after systemic administration (Pöyhia and Kalso 1992; Beardsley et al. 2004; Peckham and Traynor 2006). Pöyhia and Kalso reported that oxycodone is 2-4 times more potent compared with morphine after s.c. and i.p. administration in rats (Pöyhia and Kalso 1992). After i.t. (Plummer et al. 1990; Pöyhia and Kalso 1992; Nielsen et al. 2007) or i.c.v. (Ross and Smith 1997) administration in rats, oxycodone is significantly less potent compared with morphine.

These results from animal studies are in agreement with the clinical findings. In acute postoperative pain, the analgesic potency of oxycodone has been characterized in several studies (Kalso et al. 1991; Sunshine et al. 1996; Silvasti et al. 1998; Curtis et al. 1999; Reuben et al. 1999; Silvasti et al. 1999). Oxycodone has been found to be more potent or equipotent in the treatment of acute postoperative pain compared with

morphine (Kalso et al. 1991; Silvasti et al. 1998; Curtis et al. 1999). The analgesic potency of oxycodone seems to be related to its route of administration. After systemic administration, oxycodone has been found to be more potent compared with morphine (Kalso et al. 1991) whereas spinally administered oxycodone has a lower potency compared with morphine after epidural administration in humans (Backlund et al. 1997; Yanagidate and Dohi 2004).

2.5.2. Inflammatory pain

Tissue injury and inflammation lead to the release of peripheral pro-nociceptive mediators like serotonin, bradykinin, histamine, prostaglandins, leukotrienes and substance P from the damaged cells. Peripheral hyperalgesia occurs after sensitization of the peripheral nociceptors of the C- and Aδ-fibres by the inflammatory mediators (Kandel et al. 2000). The excessive and tonic stimulation of the spinal cord can lead to plastic changes in the CNS with the activation of N-methyl-D-aspartate (NMDA) receptor system leading to centrally mediated hyperalgesia (Kandel et al. 2000).

Morphine has an increased potency in inflammation compared to non-inflammatory conditions (Kayser and Guilbaud 1990; Hylden et al. 1991; Kayser et al. 1991; Perrot et al. 1998; Perrot et al. 2001). Peripheral inflammation alters the production of μ-, δ- and κ-opioid receptor messenger ribonucleic acid (mRNA) in DRG. Most importantly, μ-opioid receptors are up-regulated during inflammation (Zhang et al. 1998). *In vivo* studies have demonstrated a rapid induction of μ-opioid receptor mRNA production in DRG in the rat after complete Freuds adjuvant (CFA) administration, 1 hour after the injection (Puehler et al. 2004). In the same study, another peak of μ-opioid receptor mRNA up-regulation was observed 4 days after the injection (Puehler et al. 2004). In the carrageenan model for inflammation, up-regulation of μ-opioid receptor mRNA lasting for 1-3 days has also been observed (Ji et al. 1995). δ- And κ-opioid receptors have been shown to downregulate in inflammation (Zhang et al. 1998). Opioid receptors are also transported towards the peripheral nerve endings from DRG and it is enhanced during inflammation (Hassan et al. 1993; Mousa et al. 2001). Perrot et al. suggested that the increased potency of i.v. morphine in rats with inflammation is mediated by both central and peripheral sites of action (Perrot et

al. 2001). However when the induction of inflammation is repeated, the central mechanisms become more important (Perrot et al. 2001). According to Cook and Nickerson, the antihyperalgesic effects of opioids are mediated primarly via peripheral opioid receptors (Cook and Nickerson 2005). Recent studies have postulated an important role of peripheral opioid receptors for locally administered opioids in inflammation (Cook and Nickerson 2005; Nunez et al. 2007). Oxycodone has been poorly studied in the models of inflammation. The potency of oxycodone was increased in CFA-induced arthritis in male but not in female rats (Cook and Nickerson 2005). Oxycodone was found to be more potent in formalin-induced inflammation in rats compared with morphine (Meert and Vermeirsch 2005).

2.5.3. Neuropathic pain

Neuropathic pain can result from traumatic, inflammatory, infectious, ischaemic, metabolic or neoplastic damage in neurones. This can take place in either the peripheral or the central nervous system. Typical for neuropathic pain is the sensation of spontaneous burning pain and sometimes it is associated with allodynia (painful sensation triggered by a stimulus that is normally non-noxious). Recent studies have shown that allodynia is at least partly mediated through large diameter Aβ-fibres and thermal hyperalgesia through unmyelinated C-fibres (Campbell et al. 1988; Koltzenburg et al. 1992; Koltzenburg et al. 1994). In the spinal cord, postsynaptic responses are mainly mediated by glutamate (NMDA) and α-amino-3-hydroxy-5-methyl-4isoxaleproprionate (AMPA) receptors and kainate (KA) receptors (Yoshimura and Jessell 1990; Li and Zhuo 1998; Li et al. 1999). Several neuropeptides have also shown to have an important role in nociceptive signaling like substance P (SP) (Li and Zhuo 2001). After nerve injury, increased tonic input from nociceptive C-fibres leads to activation of the spinal glutaminergic system, further leading to central sensitization (Mao et al. 1995). Of opioids, methadone and both of its enantiomers have shown NMDA-antagonist properties *in vitro* (Gorman et al. 1997).

Several animal models of neuropathic pain have been developed to model nerve injury related neuropathic pain (Bennett and Xie 1988; Seltzer et al. 1990; Kim and Chung 1992; Xu et al. 1992). The animals show different pain behaviour in different

models (Kim et al. 1997). Differences in the symptoms seem to be related to different types of neuronal injury and variation in the inflammatory component between the models. Also, the response to opioids varies between the different models of neuropathic pain (Yamamoto and Sakashita 1999). Several mechanisms may interfere with the sensitivity of different neuropathic pain models to opioids. The number of μ-opioid receptors have been observed to increase or decrease in different models of neuropathic pain (Besse et al. 1992; Goff et al. 1998). In the spinal cord, increased phosphorylation of spinal μ-opioid receptors were observed after peripheral nerve injury (Narita et al. 2004a), due to activation of protein kinase C (PKC) (Narita et al. 2004b; Narita et al. 2007). In ethanol-induced neuropathy in the rat, [D-ala^2, N-Me-phe^4, gly^5-ol] enkephalin (DAMGO) induced μ-opioid receptor GTPγ[^{35}S] binding was decreased in the spinal cord, while κ- and δ-opioid receptor function was not altered (Narita et al. 2007). Recently, changes in the μ-opioid receptor related G-protein coupling from $G_{i/o}$ to G_s was observed in the spinal cord ipsilateral to the lesion in the SNL model of neuropathic pain in rats (Largent-Milnes et al. 2008). This seems to induce hyperpolarization of the neurones leading to excitation and reduced response to opioids.

Decreased opioid effects have been found in the models of neuropathic pain (Yamamoto and Yaksh 1992; Mao et al. 1995; Ossipov et al. 1995b; a; Pöyhia et al. 1999; Zhao et al. 2004), with relation to the route of administration (Lee et al. 1995). In mice with neuropathic pain like behaviour related to diabetes, oxycodone was found to be effective against thermal hyperalgesia (Nozaki et al. 2005; Nozaki et al. 2006; Nozaki and Kamei 2007). Nozaki et al. postulated, that oxycodone is more effective compared with morphine in the mice model of painful diabetic neuropathy (Nozaki et al. 2005; Nozaki et al. 2006). In streptozotocin (STZ) diabetic rats and rats with sciatic nerve ligation-induced neuropathic pain behaviour, s.c. oxycodone was found to be more potent compared with s.c. morphine (Narita et al. 2008; Nielsen et al. 2007). There was no difference in the oxycodone and morphine induced GTPγ[^{35}S] binding between the sham operated and sciatic nerve ligated mice (Narita et al. 2008).

Neuropathic pain is most commonly treated with tricyclic antidepressants and antiepileptics in the clinic. Opioids are not considered as a primarily option for treating non-malignant neuropathic pain or cancer-related (malignant) neuropathic pain (Finnerup et al. 2005). However, i.v. morphine, alfentanil and fentanyl, CR morphine and transdermal (t.d.) fentanyl have been found to be effective against postherpetic

neuralgia (Rowbotham et al. 1991; Pappagallo and Campbell 1994; Dellemijn and Vanneste 1997; Dellemijn et al. 1998; Raja et al. 2002). Oral oxycodone has been found to be effective in the management of neuropathic pain related to postherpetic neuralgia (Watson and Babul 1998) and in painful diabetic neuropathy (Gimbel et al. 2003; Watson et al. 2003).

2.5.4. Visceral pain

Pain from the viscera is different from pain of cutaneous origin. Studies on opioid receptor knock-out mice have indicated the κ-opioid receptors are important in visceral pain (Simonin et al. 1998). The κ-opioid receptor agonist have been found to be more effective in visceral pain compared with μ- and δ- opioid receptor agonists (Sengupta et al. 1996; Burton and Gebhart 1998; Simonin et al. 1998). However, in the clinic, selective κ-opioid receptor agonists are not available.

Recently oxycodone has been found to have a better analgesic effect compared with morphine in patients with chronic pancreatitis (Staahl et al. 2007) and in an experimental model for visceral pain in healthy volunteers (Staahl et al. 2006).

2.6. Other effects of oxycodone

The abuse potential and rewarding effects of oxycodone have been poorly characterized in the literature. Previous *in vivo* studies in rats have demonstrated that oxycodone has an abuse potential typical to potent μ-opioid receptor agonists, and it is comparable or higher compared with morphine and heroin (Beardsley et al. 2004; Leri and Burns 2005; Meert and Vermeirsch 2005). In mice, oxycodone and morphine both induced rewarding effects in the conditioned place preference test (CPP) in a dose dependent manner (Narita et al. 2007).

Oxycodone was found less potent to produce gastrointestinal depression after s.c. administration compared with morphine but induced more potently respiratory depression compared with morphine in rats (Meert and Vermeirsch 2005). Oxycodone-

induced CNS depression is mediated through opioid receptors and antagonized with the unselective opioid receptor antagonist naloxone (Pöyhia and Kalso 1992). In rats, systemically administered oxycodone produced more potent CNS depression compared with morphine (Pöyhia and Kalso 1992; Meert and Vermeirsch 2005).

An *in vitro* study by Sacredote et al. suggested that oxycodone does not suppress the immune system as much as morphine (Sacerdote et al. 1997). The clinical significance of this finding remains unclear.

In humans, morphine has been reported to produce more sedation and a greater decrease in the mean arterial blood pressure compared with oxycodone in equianalgesic doses (Kalso et al. 1991). Oxycodone seems to produce less hallucinations compared with morphine in humans (Kalso and Vainio 1990; Maddocks et al. 1996; Mucci-LoRusso et al. 1998). An insigficant rate of histamine release by oxycodone (Pöyhia et al. 1992a) compared with morphine (Rosow et al. 1982) can lead to less itching compared with morphine (Mucci-LoRusso et al. 1998).

3. AIMS OF THE STUDY

The aim of this study was to characterize the pharmacology of oxycodone and the role of its metabolites in oxycodone-induced antinociception in rats and humans. The specific objectives of this thesis were the following:

I. To find out if oxycodone or methadone and its enantiomers can produce more effective antinociception than morphine in various models of acute and chronic pain in rats.

II. To find out how the route of administration affects the antinociceptive potency of oxycodone and its metabolites compared with morphine.

III. To determine whether the effects of oxycodone are μ-opioid receptor-mediated.

IV. To assess the activation of the μ-opioid receptors in different regions of the CNS by oxycodone and oxymorphone compared with morphine.

V. To evaluate the role of cytochrome P450 2D6-mediated metabolism and the metabolites for the analgesic activity of oxycodone in humans.

4. MATERIALS AND METHODS

4.1. Ethical considerations

All animal studies were carried out according to the guidelines of the local authorities and the International Association for the Study of Pain (Zimmermann 1983). The protocols of the studies were approved by the institutional animal investigation committee and the provincial government of Southern Finland (Uudenmaan lääninhallitus, Helsinki, Finland). The smallest possible number of animals was used for ethical reasons. In all *in vivo* models used the animals were able to terminate the noxious stimulation. Also cut-off latencies were used when ever possible to avoid tissue damage. In the carrageenan model for inflammation animals were sacrificed immediately after the experiments.

Study IV was approved by the ethics committee of the Department of Surgery of the Helsinki University Central Hospital and the National Agency for Medicines, Finland. All patients provided a written informed content.

4.2. Animals

Male Sprague Dawley rats (Harlan, Horst, Netherlands and Taconic Europe, Ry, Denmark) weighing 175-250 g were used in these studies. Clear plastic cages were used for the housing of the animals. A 12 h/12 h artificial light-dark cycle and a free access to water and lab chow (served *ad libitum*) were used. Animals were housed individually after the i.t. cannulation to avoid damage of the cannulas. Before the *in vivo* behavioral tests, the animals were habituated in the testing environment for 30 min/day for 3 days, except in the spontaneous motor activity test, in which there was no habituation prior to the testing. The same animals were used 2-3 times with a 3-day washout. In the carrageenan model for inflammation rats were only used for a single experiment. The number of animals per group was 5-8 in behavioural tests and 5-6 in GTPγ[^{35}S] assay.

After the tests the animals were sacrificed by decapitation in CO_2 anaesthesia. All behavioural studies were performed following a blinded protocol.

4.3. Pain models

4.3.1. Models of acute nociception

The paw pressure- (Kayser and Guilbaud 1990), tail flick- (D'Amour and Smith 1941) and hot plate (Woolfe and Macdonald 1944) tests were used in studies I, II and III. The paw pressure test was performed with a paw pressure apparatus (Ugo Basile, Milan, Italy). The rats were individually wrapped in a towel during the test. The left hind paw of the rat was placed under the weight of the apparatus with a constantly increasing pressure. The test was terminated after a brisk foot withdrawal of the left hind leg as a sign of mechanical nociception. A Harvard Apparatus Ltd. hot plate (Edenbridge, Kent, U.K.) was used in the hot plate tests, with a temperature set at (52 ± 0.3 °C). Licking or shaking the hind paw or jumping were considered as signs of thermal nociception. Tail flick latencies were tested with a Ugo Basile (Comerio, Italy) apparatus. During the test, the rats were immobilized in transparent hard plastic tubes. The tests were repeated three times (with a 15 s-interval) at every time point. The withdrawal of the tail was considered as sign of thermal nociception. In the paw pressure test 45 g, 60 s in the hot plate test and 8 s in the tail flick test were used as a the cut-off to avoid tissue damage.

4.3.2. SNL model of neuropathic pain

The SNL model for neuropathic pain (ligation of the L5 and L6 spinal nerves) was performed under halothane anesthesia (2% in 50% O_2- 50% N_2O) as described by Kim and Chung (Kim and Chung 1992). L5 and the L6 spinal nerves were tied with a tight 6-0 silk ligature. Sutures and metal clips were used for closing the surgical wound. Animals with any neurological symptoms not typical to the model (paralysis of the

operated limb, difficulties in walking) were immediately sacrificed. Two weeks of recovery was allowed before the behavioural tests.

4.3.3. Models for mechanical and cold allodynia

In the SNL model for neuropathic pain, rats were placed under individual transparent plastic cages on a metal mesh and mechanical allodynia was studied with the von Frey filaments (Ren and Dubner 1993). The testing was performed as follows: the testing was started with the strongest filament (12.5 g). If no allodynia-like behaviour was observed with the strongest hair, 12.5 g was recorded as the threshold. Testing was continued to the next lighter filament until allodynia-like behaviour was observed. The lightest filament that produced a brisk foot withdrawal or lifting and licking of the paw of more than half of the stimulations was recorded (considered as allodynia-like behaviour).

To test cold allodynia, rats were placed as similarly as in the test for mechanical allodynia. The plantar surface of the ipsi- and contralateral paw were stimulated with a drop of acetone (Choi et al. 1994) three times at every time point. A brisk foot withdrawal or lifting and licking of the paw after the stimulation were considered as a allodynia- like behaviour, and the number of such behaviour was recorded.

4.3.4. Carrageenan inflammation

In Study III, the carrageenan model for inflammation (Winter et al. 1962) was used (Fig. 5). Predrug thresholds were studied 30 min before the carrageenan injection. Rats were placed as decribed in the behavioural testing in neuropathic pain models. The ventral surface of the inflamed paw was touched with a tip of the digital force gauge (Imada DPS-1 with a custom made metal tip, Imada CO, Nortbrook, IL, USA) until a paw withdrawal (a brisk foot withdrawal, shaking or licking of the paw) was observed and the force was recorded. The induction of inflammation was performed under enflurane anaesthesia. Rats were rapidly anesthetized (Enflurane, Abbot Scandinavia AB., Solna, Sweden). A s.c. injection of 0.2 mg λ-carrageenan (Sigma, St. Louis, MO,

USA) in 0.1 ml of saline into the left hindpaw was performed. The protocol of the study is illustrated in Fig. 5.

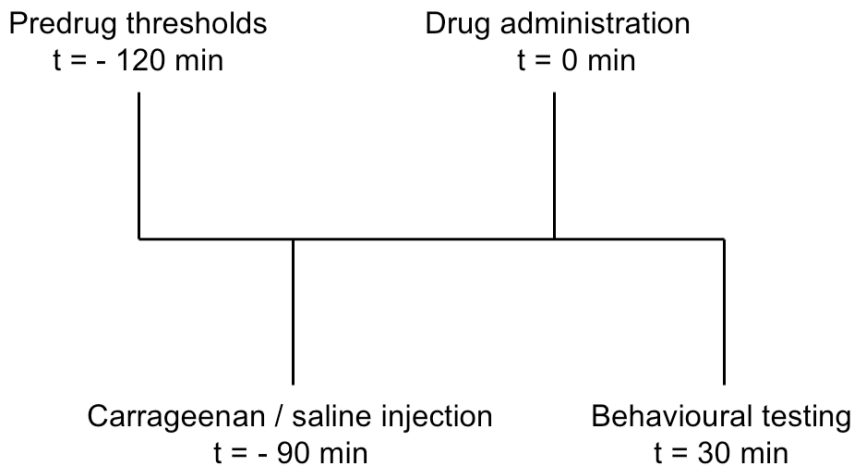

Fig. 5. Schematic diagram of the assessment of carrageenan inflammation in Study III.

4.4. Assessment of other opioid effects

In studies I and II, possible sedative effects of the drugs were tested with the spontaneous motor activity test. The rats were placed 30 min after s.c. drug injection one at a time in a measurement box (70 x 70 x 35 cm) that was covered and isolated from sounds and light. Photocells inside the box automatically detected horizontal and vertical movements of the rat. A 30-min period of testing was used and the first 15 min period was used for the analysis, because most of the activity takes place during that period.

A rotarod test apparatus (Palmer electric recording drum, UK; diameter 80 mm, speed 10 RPM) was used, to study changes in motor function and sedative effects of the drugs (Study III). The time the rat stayed on the rotating rod was calculated. Animals that stayed at least 120 s on the rotating rod before drug administration were included in the test. 120 s was set as the cut-off time in the test session.

4.5. Intrathecal cannulation

For i.t. administration of study drugs (studies II and III) i.t. cannulas were implanted. I.t. cannulation was performed under anaesthesia with s.c. injection of midazolam 5.0 mg/kg (Midazolam Alpharma®, Alpharma, Oslo, Norway) and Hypnorm® 1.0 ml/kg (fentanyl 0.2 mg/ml and fluanisone 10 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). A polyethene cannula (Portex Ltd, Hythe, Kent, UK) with a diameter of 0.61 mm was inserted through the cisterna magna to the lumbar subarachnoid space. The tip of the cannula was placed at 8 cm from the insertion as previously decribed by Yaksh and Rudy (Yaksh and Rudy 1976). The cannula was fixed with a suture to the paravertebral muscles and the skin was closed. Rats with any neurologic disturbances were immediately sacrificed. Five days after cannulation the placement of the cannula was verified with an i.t. administration of 10 μl of 10 mg/ml lidocaine (Lidocain®, Orion, Espoo, Finland). Only rats with reversible symmetrical paralysis of both hind limbs after injection were used in the experiments.

4.6. GTPγ[^{35}S] autoradiography

GTPγ[^{35}S] autoradiography was performed according to Laitinen and Jokinen (1998) and Laitinen (1999). All chemicals used in GTPγ[^{35}S] autoradiography were purchased from Sigma-Aldrich (USA), if not otherwise stated. The brains and the lumbar spinal cords were carefully dissected and stored at -80 °C. Sections (14 μm thick) were cut with a cryostat (Leica CM3050 S, Leica Microsystems, Nussloch, Germany) and mounted on poly-L-lysine-coated slides and stored at – 80 °C until used. The first preincubation was performed with 0.95 ml of 50 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4) per slide for 20 min at 20 °C. The second preincubation included (0.95 ml per slide for 60 min at 20 °C) with the same buffer and 48 mM GDP and 24 mM DPCPX (8-cyclopentyl-1,3-dipropylxanthine) (Tocris, UK). The third incubation (90 min at 20 °C) was performed using the buffer supplemented with 20 mM DTT, 48 mM GDP, 24 mM DPCPX 42 pM and GTPγ[^{35}S] (PerkinElmer Inc., Boston, MA, USA) supplemented with drugs studied. Nonspecific binding was determined in the presence of 10 μM unlabelled GTPγS. The

maximal binding was studied with DAMGO 10 μM. Non-μ-opioid receptor-mediated binding of the ligands was determined with administration of 10 μM naltrexone. Two separate 5 min washes were performed in ice-cold washing buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$). This was followed by a 30 second rinse in distilled water at 0 °C. Slides were dried in cool air. Slides were then exposed for five days to Kodak Bio Max MR film (Eastman Kodak, Rochester, NY, USA) with ^{14}C-plastic standards (GE Healthcare, Buckinghamshire, UK). The films were then manually developed and fixed. The analysis and quantification of the films were performed with Dage MTI-apparatus (DAGE-MTI Inc., Michigan City, USA) combined with MCID Elite™ M5+ version 4.0 (Imaging Research Inc., St. Catharines, Ontario, Canada). The binding densities in the studied CNS regions were converted to nCi/g values with the help of the simultaneously exposed standards. Nonspecific binding was subtracted from all values. The results were expressed as percentages of the 10 mM DAMGO-stimulated maximal values.

4.7. Drugs

Oxycodone hydrochloride, methodone hydrochloride and morphine hydrochloride were purchased from the University Pharmacy, Helsinki, Finland and naloxone and naltrexone hydrochloride from Sigma-RBI (Natick, MA, USA). Nor-binaltorphimine-hydrochloride (nor-BNI) and DAMGO were purchased from Tocris (Bristol, UK). (+)- And (-)-methadone hydrochloride, noroxycodone hydrochloride, oxymorphone hydrochloride, and noroxymorphone hydrochloride were synthesized in the Medicinal Chemistry Group of the Division of Pharmaceutical Chemistry of the Faculty of Pharmacy, University of Helsinki as described in the original articles (Sudies I-IV). The volume of i.t. drug solutions were 10 μl, followed by a 10 μl flush of saline. Saline served as a control. In Study IV, oxycodone (Oxycontin®, Mundipharma, Finland), morphine hydrochloride 4 mg/ml solution (Helsinki University Central Hospital Pharmacy, Helsinki, Finland) and paroxetine 20 mg (Seroxat® 20 mg, GlaxoSmithKline, Mayenne, France) were used.

4.8. Clinical Study (Study IV)

4.8.1. Study design

The study design of Study IV is illustrated in Fig. 6. Patients were first titrated to an acceptable level of pain relief with CR oxycodone tablets taken twice daily (12 -hour interval) and instructed to take oral morphine solution for breakthrough pain. Patients accepted for the study were randomized to take either placebo or paroxetine 20 mg orally once daily in the morning. Pain intensity (VAS 100 mm, at 8 AM, at 2 PM and at 8 PM), the adverse effects and the use of rescue medication were recorded in a pain diary daily. A 100 mm visual analogue scale (VAS) and an 8-point verbal rating scale for pain intensity (VRSpi) and a 5-point verbal rating scale for pain relief (VRSpr) were used for the assesmant of pain intensity and relief on the 7th day of both treatment-phases in the Pain Clinic. Also drug effects were assessed using a 100 mm Modified Drug Effect Scale (Kaiko et al. 1996). Blood samples were drawn for the measurement of oxycodone, three of its metabolites, paroxetine and for genotype analysis. Blood samples were collected in EDTA containing tubes followed with separation of plasma and storage at -20°C until analysis.

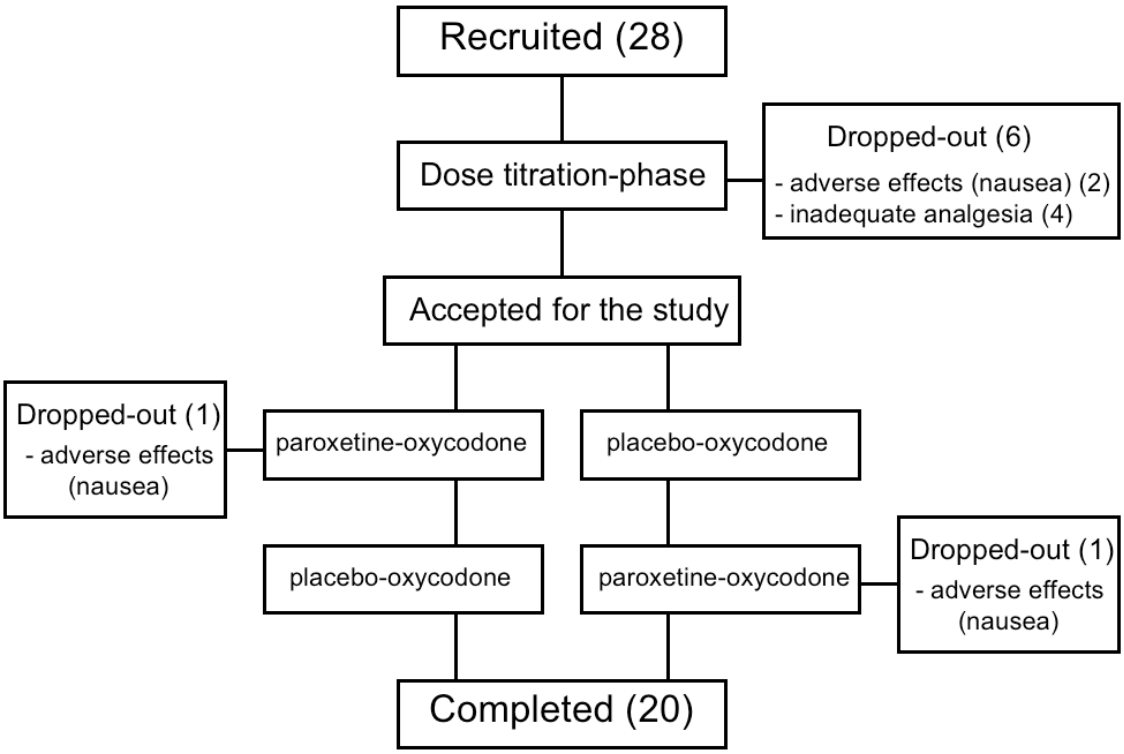

Fig. 6. The protocol of Study IV illustrated as a flow chart.

4.8.2. Determination of plasma oxycodone, three of its metabolites and paroxetine

A PE SCIEX API 3000 liquid chromatography-tandem mass spectrometry system (Sciex Division of MDS Inc., Toronto, Ontario, Canada) was used for the analysis. The limit of quantification was 0.1 ng/ml for oxycodone and oxymorphone, 0.25 ng/ml for noroxycodone, noroxymorphone, and 1.0 ng/ml for paroxetine (Neuvonen and Neuvonen 2008).

4.8.3. Patient genotyping

The *CYP2D6* alleles were analyzed using TaqMan® Pre-Developed Assay Reagents for allelic discrimination and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The *CYP2D6*5* allele (total deletion of the gene) was detected according to Herrsberger et al. (Hersberger et al. 2000) and the *CYP2D6* gene duplication as described by Steijns & van der Weide (Steijns and van der Weide 1998). Subjects were classified as poor (PM), extensive (EM) and ultrarapid metabolizers (UM) based on their allele distribution. *CYP3A4* detrimental alleles were identified according to van Schaik et al. (van Schaik et al. 2000; van Schaik et al. 2001) and *CYP3A4*4* allele (352A>G, Ile118Val) according to Wang et al. (Wang et al. 2005a). The *CYP3A5*2* (27289C>A, T398N; rs 28365083) and *CYP3A5*6* (14690G>A, splicing defect; rs 10264272) alleles were analysed as previously described by van Schaik et al. (van Schaik et al. 2000; van Schaik et al. 2001; van Schaik et al. 2002). The *CYP3A5*3* (6986A>G, splicing defect; rs 776746) allele was investigated according to (Mirghani et al. 2006). *ABCB1* polymorphisms were analysed with real-time polymerase chain reaction (PCR) by TaqMan kits (Applied Biosystems, for 1236C>T, rs1128503, Assay ID: C___7586662_10, for 3435C>T, rs1045642, Assay ID: C___7586657_1_, and for 2577G>A/T, rs2032582) with the following primers: GTA AGC AGT AGG GAG TAA CAA AAT AAC ACT, Reverse Primer GAC AAG CAC TGA AAG ATA AGA AAG AAC T, 2677G probe VIC-CCT TCC CAG CAC CT, 2677A probe FAM-CTT CCC AGT ACC TTC, 2677T probe FAM-CTT CCC AGA ACC TT).

4.9. Statistical analysis

Analysis of variance (ANOVA) for repeated measurements was used for the statistical analysis of significance between the differences of the study groups over time. In studies I and II paired t-test and non-parametric analyses of variance (the Kruskall-Wallis – and Mann-Whitney tests), of StatView 5.5 (SAS Institute Inc., Cary, NC), with Bonferroni correction for multiple comparisons were used for the statistical analysis. In

Study III non-parametric analyses of variance with Bonferroni and Tukey tests of Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA) were used for the statistical analysis. In Study IV paired t-test and Fisher's exact test (Prism 4.0, GraphPad Software Inc., San Diego, CA, USA) and ANOVA for repeated measures (StatView 5.0.1, SAS Institute, Inc., Cary, NC, USA) were used for the statistical analysis. $P<0.05$ was considered to represent a statistically significant difference. The results of the nociceptive tests where a cut-off value was used (paw pressure, hot plate and tail flick tests) are reported as mean of the percentage of the maximum possible effect (MPE%), calculated as: [(post value – pre value)/(cut off – pre value)] × 100%.

5. RESULTS

5.1. Oxycodone in models of acute nociception (Study I)

In the tail flick, hot plate and paw pressure tests, oxycodone (1.25, 2.5 and 5 mg/kg), morphine (2.5, 5 and 10 mg/kg), methadone (1.25, 2.5 and 5 mg/kg) and *l*-methadone (1.25, 2.5 and 5 mg/kg) induced dose-related antinociception after s.c. administration, with a maximum effect 30 min after the administration (Study I, Figs. 1, 2 and 3). After s.c. administration, the antinociceptive effects of oxycodone were more potent in all nociceptive tests compared with morphine (Study I, Figs. 1, 2 and 3). *d*-Methadone in the doses of 1.25, 2.5 and 5 mg/kg, was inactive in all tests. Taken together, in the models of nociception, morphine was less potent than oxycodone, methadone and l-methadone (Table 3).

Table 3. Lowest doses (mg/kg or μg*) of the study drugs that produced an effect of 50% MPE in the models of acute nociception after s.c. or i.t. (*) administration.

	Stimulus		
	Thermal		Mechanical
Drug	**Tail-flick**	**Hot-plate**	**Paw-pressure**
oxycodone	0.63, 20.0*	2.5	1.25
morphine	5.0, 1.0*	10.0	5.0
racemic methadone	2.5	5.0	1.25
l-methadone	1.25	2.5	1.25
d-methadone	> 5.0	> 5.0	> 5.0
oxymorphone	0.15, 0.63*	0.3	0.3
noroxycodone	25.0, 25.0*	> 25.0	> 25.0
noroxymorphone	> 25.0, 1.0*	> 25.0	> 25.0

5.2. Oxycodone in the SNL model of neuropathic pain (Study I)

In the SNL model of neuropathic pain, oxycodone (1.25, 2.5 mg/kg), morphine (5 and 10 mg/kg), methadone (1.25, 2.5 mg/kg) and particularly l-methadone (1.25 mg/kg) had a antiallodynic effect in the von Frey filament test for mechanical allodynia after s.c. administration (Study I, Fig. 4). The potency of morphine was lower compared with oxycodone, racemic methadone and l-methadone (Fig. 7).

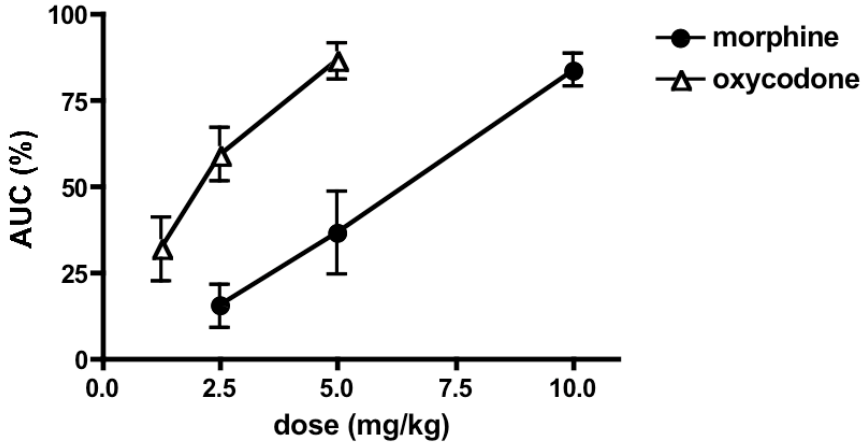

Fig. 7. The dose response curves for oxycodone and morphine after s.c. administration in the SNL model for neuropathic pain (mechanical allodynia tested with von Frey hairs). Results are given as the percentage of area under the curve of the maximum area (AUC) ± standard error of mean (± SEM).

Cold allodynia was tested by the acetone test. *l*-Methadone was more potent compared with morphine, oxycodone and methadone in the cold allodynia test (Study I, Fig. 5). *d*-methadone (1.25, 2.5 and 5 mg/kg) was inactive in these tests. A clear decrease in the spontaneous locomotor activity was observed with the antiallodynic doses of oxycodone (2.5 mg/kg), morphine (5.0 mg/kg) and *l*-methadone (1.25 mg/kg) in the spontaneous locomotor activity test. This would indicate sedation (Study I, Fig. 6). The potencies of

the studied opioids were reduced in the SNL model for neuropathic pain compared with nociceptive pain models after s.c. administration.

5.3. The influence of the route of administration on effects of oxycodone (Study II)

Oxycodone (0.63, 1.25 and 2.5 mg/kg) (Study II, Figs. 1a, 2a and 3a) and oxymorphone (0.15, 0.3, 0.63 and 1.25 mg/kg) (Study II, Figs. 1b, 2b and 3b) produced dose-related antinociception with a maximum effect 30 min after the s.c. injection in the models of acute nociception. A weak antinociceptive effect was observed after s.c. administration of noroxycodone 25 mg/kg in the tail-flick test (Study II, Figs. 1c, 2c and 3c). This indicates that noroxycodone has no role in oxycodone-induced antinociception after systemic administration in rats.

Dose-related antinociceptive effect was observed after i.t. administration of oxycodone (20, 100 and 200 μg) (Study II, Fig. 4a), oxymorphone (0.63, 1.25 and 2.5 μg) (Study II, Fig. 4b) and noroxycodone (25, 50 and 100 μg) (Study II, Fig. 4c). Oxycodone produced antinociception with a poor potency and a shorter duration compared with oxymorphone and noroxycodone (Table 3).

5.4. Pharmacodynamics of oxycodone in rats (Study II)

The antinociceptive effects of s.c. oxycodone (2.5 mg/kg) and oxymorphone (0.3 mg/kg) were antagonized with a s.c. administration of naloxone (1 mg/kg) (Study II, Fig. 5), indicating an opioid recptor-mediated effect. Also, the short-lasting antinociceptive effect of i.t. oxycodone (200 μg) was reversed by s.c. administration of naloxone (1 mg/kg) (Study II, Fig. 6). S.c. administration of the selective κ-opioid receptor antagonist Nor-BNI (10 mg/kg) failed to antagonize the antinociceptive effects of s.c. oxycodone (2.5 mg/kg) and oxymorphone (0.3 mg/kg) (Study II, Fig. 5).

5.5. Oxycodone induced G-protein activation (Study II)

In GTPγ[^{35}S] autoradiography, oxycodone, morphine and oxymorphone produced an opioid receptor-mediated G-protein activation in all studied rat brain regions and in the dorsal horn of the spinal cord, and it was abolished by 10 μM naltrexone (Fig. 8). Oxycodone had a lower efficacy and potency to activate G-proteins particularly in the dorsal horn of the spinal cord and PAG (Study II, Figs. 9e and f) compared with other study drugs. All drugs studied were partial agonists compared with DAMGO. Oxymorphone was the most potent of the study drugs in all the CNS regions studied with ≈10- to 100-fold greater potency compared with oxycodone to activate intracellular G-proteins.

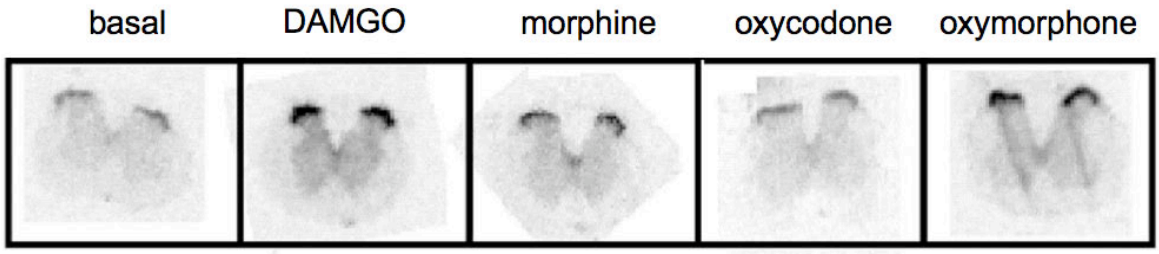

Fig 8. An example of autoradiographs, illustrating maximal agonist-stimulated GTPγ[^{35}S] binding of the drugs studied in the spinal cord. The μ-opioid receptors are predominantly located in the dorsal horn.

5.6. The role of noroxymorphone in the effects of systemic oxycodone in rats (Study III)

Subcutaneous noroxymorphone (5, 10 and 25 mg/kg) was inactive in the models of nociception (Study III, Figs. 2a, b and c) and inflammation (noroxymorphone 25 mg /kg) (Study III, Fig. 3.). In the carrageenan model for inflammation s.c. administration of oxycodone 2.5 mg/kg abolished all hyperalgesic behaviour, and the thresholds were comparable to predrug thresholds (Study III, Fig. 3.). No change in motor function or

any signs of sedation were seen after s.c. administration of noroxymorphone at the doses of 5, 10 and 25 mg/kg (Study III, Fig. 4.). Statistically significant decrease in the survival on the rotating rod was only observed after the highest dose (5 mg/kg) s.c. administrated oxycodone (Study III, Fig. 4.).

5.7. Intrathecal administration of noroxymorphone in the rat (Study III)

Noroxymorphone (1 and 5 μg/10 μl) induced a potent and longer-lasting antinociceptive effect compared with morphine (1 and 5 μg/10 μl) and oxycodone (200 μg/10 μl) after i.t. administration in rats (Table 3 and Fig. 9.) (Study III, Figs. 5a., 5b. and 8.). With 5 μg/10 μl of noroxymorphone, an MPE of 81% was still observed 12 h after the administration and an MPE of 17% was observed 24 h after the drug administration. The s.c. administration of naloxone (1 mg/kg) 15 min prior to i.t. administration of study drugs, significantly reduced the antinociceptive effect of noroxymorphone (5 μg) and morphine (5 μg) (Study III, Fig. 6.), indicating an opioid receptor-mediated antinociceptive effect.

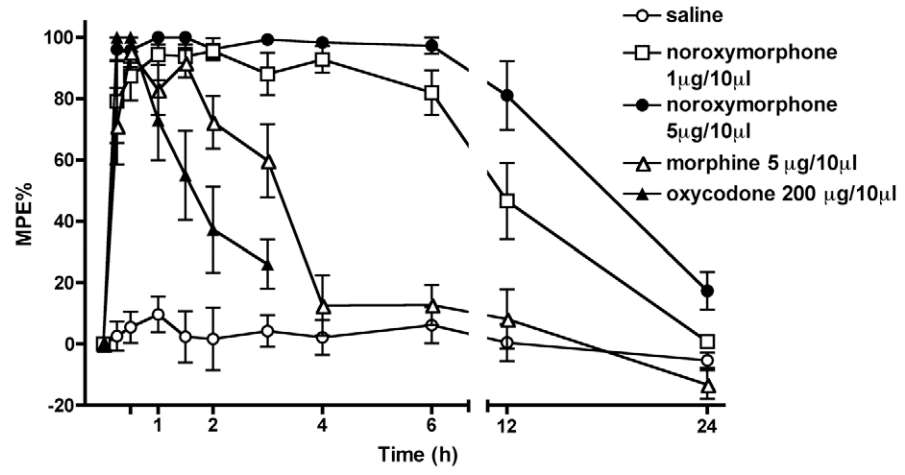

Fig. 9. Antinociceptive effects of oxycodone, morphine and noroxymorphone in the tail-flick test after i.t. administration in rats compared with a saline control. Results are given as MPE% ±SEM plotted over time (h).

5.8. The analgesic potency of oxycodone is independent of the CYP-2D6-mediated metabolism in humans (Study IV)

Twenty patients participated in the study (18 extensive metabolizers for CYP 2D6 and two ultra rapid metabolizers for CYP 2D6). The maximal concentrations of oxycodone and its oxidative metabolites after oral oxycodone administration in the placebo-control-group were: oxycodone> noroxycodone> noroxymorphone> oxymorphone (Study IV, Fig. 4). After co-administration of paroxetine, CYP 2D6-mediated metabolism of oxycodone was blocked and a statistically significant decrease was observed in the plasma concentrations of CYP 2D6-mediated metabolites oxymorphone and noroxymorphone. A statistically significant increase in the plasma concentration of noroxycodone was observed in the paroxetine-phase compared with that of placebo.

Co-administration of paroxetine with oxycodone had no statistically significant effect in the VAS-scales for analgesic effect (Study IV, Fig. 2). Also, the use of additional morphine was not changed in the paroxetine group indicating similar analgesic effect compared with placebo co-administration. Patients recorded more pain in the evenings compared with the mornings with no statistically significant difference between the study groups. No statistically significant differences were observed in any of the recorded adverse-effects (Study IV, Fig 5). Adverse-effects were also recorded in a pain diary for both seven days periods (placebo/paroxetine). The most common adverse effects were drowsinnes and nausea/vomiting (Table 4.). 25% of the patients informed about dizziness and headache during paroxetine co-administration, which were not present after placebo administration ($p=0.0471$). This seems to indicate that these differences may be adverse-effects of paroxetine itself.

Table 4. The most common adverse-effects during paroxetine- and placebo-phases given as a percentage (%), indicating the existence of adverse-effect in the study group. N.s. indicates an insignificant difference between the study groups.

	Adverse-effects (%)		
	Placebo	**Paroxetine**	**P value**
drowsiness	50	65	n.s.
nausea/vomiting	55	55	n.s.
constipation	20	25	n.s.
dizziness	0	25	0.0471
headache	0	25	0.0471
loss of appetite	15	20	n.s.
sweating	25	20	n.s.
tremor	5	10	n.s.
confusion	0	5	n.s.
respiratory distress	5	5	n.s.

6. DISCUSSION

6.1. Methodological considerations

The use of animals was justified because the pharmacology and effects of the study drugs needed to be studied *in vivo* in the complex living animal. This cannot be performed only *in vitro*, because procession of nociception and pain needs a complex neuronal network in living organisms including higher parts of the CNS. By using the same animals with a washout period the total number of animals was reduced.

Animal models of pain should simulate the clinical situation as exactly as possible and the predictive validity of the model used should be established. The tail flick test and hot-plate test have been found predictive in the study for the antinociceptive properties of opioids (Le Bars et al. 2001). Predictive validity of neuropathic pain models in rats has been found to be surprisingly good (Kontinen and Meert 2003). The main problem in animal models of neuropathic pain is that assessment of spontaneous pain is poor, and that it is a central component of human neuropathic pain conditions, such as painful diabetic neuropathy and postherpetic neuralgia (Otto et al. 2002; Nurmikko and Browsher 1990).

The pharmacokinetic studies should be also performed in humans because of the difference in drug metabolism between the rat and humans. In the Study IV, the pharmacokinetics of oxycodone was studied in chronic pain patients. Compared with the studies performed in healthy volunteers, our study design included limitations like heterogenity of the study group with multiple medications and differences in the origin of the chronic pain. Due to a limited number of available patients the group size was limited to 20 patients and the estimates for the sample size was not performed. However, by using a randomised, double-blind, placebo controlled 2-phase crossover study-design, comparisons between the treatment groups were possible.

6.2. Receptor pharmacology of oxycodone

The basic pharmacology of oxycodone has become clearer over the past few years. However, contradictory results of the pharmacodynamic profile of oxycodone have been published. According to Ross and Smith oxycodone was postulated to act as a κ-opioid receptor agonist *in vivo* (Ross and Smith 1997). More recently it was suggested that oxycodone is a κ_{2b}-opioid receptor agonist (Nielsen et al. 2007). However, the results of this thesis show that pretreatment with a selective κ-opioid receptor antagonist Nor-BNI does not antagonize the antinociceptive effect of oxycodone in rats (Study II, Fig. 5). According to the results of Narita and colleagues, the selective δ- or κ-opioid receptor antagonists naltrindol and Nor-BNI failed to reduce the antinociceptive effect of oxycodone in mice (Narita et al. 2008). In mice, the antinociceptive effect of oxycodone is abolished with the selective μ-opioid receptor antagonists β-funaltrexamine and clocinnamox (Beardsley et al. 2004; Narita et al. 2008; Pawar et al. 2007) and the non-selective antagonist naloxone (Study II, Fig. 5). Oxycodone has been proven to be a μ-opioid receptor agonist *in vitro*, although its affinity for the μ-opioid receptor is significantly lower compared with that of morphine (Chen et al. 1991; Yoburn et al. 1995; Lalovic et al. 2006; Peckham and Traynor 2006; Narita et al. 2008). Compared with morphine oxycodone activates intracellular G-protein via μ-opioid receptors with a lower potency (Thompson et al. 2004; Lalovic et al. 2006; Peckham and Traynor 2006). Oxycodone induces adverse effects in rodents, typical to μ-opioid receptor agonists (Study I, Fig. 6 and Study III, Fig. 4). Most importantly, in the human being, oxycodone behaves as a μ-opioid receptor agonist producing analgesia (Sunshine et al. 1996; Silvasti et al. 1998; Curtis et al. 1999; Reuben et al. 1999; Silvasti et al. 1999), dependence and euphoria (Cicero et al. 2005; Forrester 2007).

6.3. Oxycodone-induced analgesia is route dependent

Because μ-opioid receptor agonists are differentially capable of activating intracellular G-proteins (Traynor and Nahorski 1995; Selley et al. 1997; Thompson et al. 2004), the ability of oxycodone to activate intracellular G-proteins at the level of the spinal cord compared to other CNS regions was investigated in GTPγ[^{35}S] autoradiography (Sim et

al. 1995; Laitinen and Jokinen 1998; Laitinen 1999). The results of Study II show that the weak antinociceptive effect of oxycodone after spinal administration in the rat may result from its weaker ability to induce G-protein activation in the dorsal horn of the spinal cord compared with morphine and oxymorphone that seems to be related to its lower affinity for the μ-opioid receptor.

The results in studies I-III agree with the previous studies, indicating that oxycodone is effective after systemic administration in rats, but its potency is significantly reduced after i.t. administration (Plummer et al. 1990; Pöyhia and Kalso 1992). These findings seem to correlate also with studies in humans. The equianalgesic i.v. dose ratio of oxycodone in postoperative pain to morphine has previously been reported as 2:3 (Kalso et al. 1991), and epidurally administered oxycodone has a lower potency compared with morphine in humans (Backlund et al. 1997; Yanagidate and Dohi 2004).

After systemic administration oxycodone is efficiently transported across the blood-brain-barrier (BBB) compared with morphine. This leads to higher concentrations of oxycodone in the CNS compared with that of plasma in rats (Lalovic et al. 2006; Boström et al. 2008). The direct drug administration to CNS reduces the pharmacokinetic differences of the study drugs. The lower μ-opioid receptor binding affinity and G-protein activation potency of oxycodone compared with morphine, seem to correlate with lower analgesic potency after i.t. (Plummer et al. 1990; Pöyhia and Kalso 1992; Nielsen et al. 2007) and i.c.v. (Ross and Smith 1997) administration.

6.4. Oxycodone induced antinociception and analgesia after systemic administration

Oxycodone induced a potent antinociceptive effect in all used models of thermal and mechanical nociception in studies I-III and the potency of oxycodone was higher compared with morphine after s.c. administration. This is in good agreement with the previous study in humans (Kalso et al. 1991). Oxycodone induced a potent antiallodynic effect against both mechanical and thermal stimuli in the SNL model of neuropthic pain after s.c. administration in rats (Study I). Again, the potency of oxycodone was higher compared with morphine. These findings are supported by the results by Narita et al.

(2008). The antinociceptive effect of oxycodone was found to be higher compared with that of morphine in the sciatic nerve ligation model of neuropathic pain in mice (Narita et al. 2008). Compared with oxycodone and morphine, l-methadone had higher antiallodynic potency and d-methadone was found inactive (Study I).

In Study IV 11 of the 20 patients studied suffered from neuropathic pain. These and the rest of the chronic pain patients were titrated to an acceptable level of pain relief with oral oxycodone. This indicates that oxycodone is effective in the management of neuropathic pain. This is supported by the previous studies showing that oxycodone is effective in the management of chronic neuropathic pain related to postherpetic neuralgia (Watson and Babul 1998) and diabetic neuropathy in humans (Gimbel et al. 2003; Watson et al. 2003; Hanna et al. 2008).

6.5. The role of metabolites in oxycodone-induced antinociception and analgesia

In Study IV, CYP 2D6-mediated metabolism of oxycodone was effectively blocked with paroxetine, resulting in a decrease in the plasma concentration of oxymorphone. This caused no alteration in oxycodone-induced analgesia in the chronic pain patients indicating the negligible role of oxymorphone or other CYP 2D6-mediated metabolites in oxycodone analgesia. The blocking of CYP 2D6-mediated metabolism of oxycodone does not affect the oxycodone-induced psychomotor function or the subjective side-effects either (Heiskanen et al. 1998). These studies agree with Lalovic et al. who suggested that pupil constriction after oral oxycodone administration is mediated by oxycodone only (Lalovic et al. 2006). The plasma concentration of oxymorphone was found to be extremely low after oral administration of oxycodone in Study IV. Similar findings of other studies in humans (Pöyhia et al. 1991; Pöyhia et al. 1992b; Kaiko et al. 1996; Heiskanen et al. 1998; Lalovic et al. 2006) and rats (Huang et al. 2005) have been reported. The theory that O-demethylation to oxymorphone is necessary for the analgesic activity of oxycodone relates to its structural similarity between codeine. Codeine-mediated analgesia requires O-demethylation to morphine depending on CYP2D6 in humans (Caraco et al. 1996; Poulsen et al. 1996) and through CYP 2D1 in rats (Cleary et al. 1994).

In Study III we demonstrated that noroxymorphone cannot explain the difference in the potency of oxycodone after systemic and i.t. administration. After s.c. administration, noroxymorphone failed to produce antinociception in rats. Our findings with noroxymorphone are supported by the observations of Lalovic et al., who found that the CNS concentrations of noroxymorphone were very low after i.g. administration of oxycodone in rats, related to reduced permeability of noroxymorphone across the blood-brain-barrier (Lalovic et al. 2006). The CNS concentration of noroxymorphone is less than 1% of its plasma concentration (Lalovic et al. 2006). After i.t. administration in rats, noroxymorphone caused a potent and extremely long-lasting, opioid receptor-mediated, antinociceptive effect in tail-flick test compared with oxycodone and morphine (Study III, Figs. 5a and 8) This may be because of the low vascular absorption of noroxymorphone from the spinal fluid typical to high polarity molecules (Dickenson et al. 1990).

In humans and rats the main metabolite of oxycodone is noroxycodone which is formed by CYP 3A4/5 (Pöyhia et al. 1991; Pöyhia et al. 1992b; Kaiko et al. 1996; Heiskanen et al. 1998; Lalovic et al. 2004; Huang et al. 2005). The results of Study II clearly demonstrate that even a high dose of noroxycodone (25 mg/kg s.c.) produced only a small antinociceptive effect in rats, indicating no role in oxycodone-induced antinociception. This is in agreement with *in vivo* studies by Leow and Smith and also by Weinstein and Gaylord with noroxycodone, demonstrating a poor antinociceptive effect of noroxycodone compared with oxycodone after i.c.v., p.o. and s.c. administration in rodents (Weinstein and Gaylord 1979; Leow and Smith 1994). Noroxycodone has almost four times lower affinity to the μ-opioid receptor compared with oxycodone (Lalovic et al. 2006) and a 4-6 times lower GTPγ[^{35}S] binding in human μ-opioid receptor *in vitro* (Thompson et al. 2004; Lalovic et al. 2006). In Study IV, paroxetine increased the dose-adjusted mean area under the curve $AUC_{0\text{-}12h}$ of noroxycodone by 100% without affecting to analgesic properties of oxycodone. Induction of CYP 3A4-mediated metabolism of oxycodone by rifampicin has been suggested to reduce oxycodone-induced analgesia (Lee et al. 2006). Our results and those by others indicate that noroxycodone has a negligible role in antinociception and analgesia following systemic administration of oxycodone in rodents and humans.

6.6. Other pharmacological aspects

I.v. infusions of oxycodone in sheep and rats have shown the unbound concentration of oxycodone to be 2.5 and 3-6 times higher in the brain than in the blood, suggesting an active transport of oxycodone across the BBB to the CNS (Boström et al. 2006; Villesen et al. 2006; Boström et al. 2008). After i.g. administration in rats, the CNS concentration of oxycodone is twice as high as the plasma concentration (Lalovic et al. 2006). In sheep, the permeability of oxycodone across the BBB was 7 fold higher compared with that of morphine (Villesen et al. 2006). Recently Okura et al. postulated, that oxycodone is transported into the CNS by a common transporter with pyrilamine *in vitro* and *in vivo* (Okura et al. 2008). A recent study in rats also suggested that oxycodone is not a substrate for P-glycoprotein, an export transportter in the BBB (Boström et al. 2005) unlike morphine and methadone (Letrent et al. 1999; Thompson et al. 2000). The results of Boström et al. can be explained by a single low dose of oxycodone (infusion of 0.3 mg/kg for 60 min) used in that study (Boström et al. 2006). Morphine at low doses (1 mg/kg) has shown not to act as a substrate for P-glycoprotein but it is a substrate for P-glycoprotein at higher doses in mice (Thompson et al. 2000). The results by Boström et al. (2006) were strongly questioned by Hassan et al., who reported that oxycodone is a P-glycoprotein substrate in various *in vitro* and *in vivo* models (Hassan et al. 2007). Oxycodone was also shown to induce overexpression of P-glycoprotein after chronic oxycodone administration in rats (Hassan et al. 2007).

As morphine, oxycodone is a μ-opioid receptor agonist. It seems that oxycodone is able to compensate its significantly lower binding affinity for μ-opioid receptor compared with that of morphine by an active transportation to the CNS. Although the analgesic properties of oxycodone are mainly due to its own activity, CYP 3A4 inhibitors and inductors may alter the oxycodone-induced analgesia as a result of altered oxycodone concentration. The analgesic properties of oxycodone and drugs interfering with CYP 3A4 need to be further studied in the future.

7. CONCLUSIONS

I. Oxycodone, racemic methadone and l-methadone produced more potent antinociceptive effect after s.c. administration in the models of acute nociception and in the SNL model of neuropathic pain compared with morphine in rats. d-Methadone was found to be inactive.

II. The antinociceptive potency of oxycodone is low after i.t. administration compared with s.c. administration. Oxymorphone was found more potent compared with oxycodone after both s.c. and i.t. administrations. S.c. and i.t. noroxycodone showed a poor antinociceptive potency. S.c. noroxymorphone was found to be inactive, but when administered i.t., it induced a potent antinociceptive effect with a longer duration compared with oxycodone, oxymorphone, noroxycodone and morphine.

III. In rats the antinociceptive effect of oxycodone is μ-opioid receptor mediated.

IV. In all studied CNS regions oxycodone was less potent, compared with oxymorphone and morphine, to stimulate μ-opioid receptor-mediated GTPγ[^{35}S] binding particularly in the spinal cord and the PAG. The low i.t. potency of oxycodone in rats seems to be related to its low potency to stimulate μ-opioid receptor activation specially in the spinal cord compared to other study drugs.

V. Oxycodone-induced analgesia is not dependent on the CYP 2D6-mediated metabolism in humans.

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