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Naloxone : actions of an antagonist

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Naloxone: actions of an antagonist

PROEFSCHRIFT

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dr. L.J.S.M. Teppema

*It's too late to change events
It's time to face the consequence
For delivering the proof
In the policy of truth*

Depeche Mode – Policy of Truth

To all of those who don't fit in

The investigations in this thesis were performed in the Anesthesia and Pain Research Unit under the supervision of Prof. Dr. A. Dahan, except for those in Chapter 2, which were performed in the Animal Research Laboratory of the Department of Anesthesiology under the supervision of Dr. L.J.S.M. Teppema. Chapter 5 was a collaboration between the Anesthesia and Pain Research unit (human experiments) and the laboratory of Dr. Benjamin Kest of Staten Island University, NY, USA (animal experiments).

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SECTION I

Introduction

CHAPTER 1

Introduction

1.1 Background

The opioid antagonist naloxone has a special place in pharmacology – it has no intrinsic action of its own, but it is able to save lives in the case of life threatening side-effects caused by other drugs. Naloxone is an antagonist for all opioid receptors, but most specifically for the μ -opioid receptor, which is the receptor through which opioids such as morphine and fentanyl exert their effects. Those effects include first and foremost analgesia, but also nausea and vomiting, sedation and life-threatening respiratory depression. It is in the case of the latter effect that naloxone can be life-saving, as it is able to reverse respiratory depression.

Paradoxically, naloxone, as an antagonist, was a side product of the search for an opioid agonist, one without addictive properties. For many centuries, the addictive properties of opium (and later morphine) were the cause of severe medical and social problems. However, there was (and still is) no alternative to morphine when it comes to analgesia. The solution to this problem was expected to come in the form of a non-addictive opioid agonist and since the start of the twentieth century, scientists have been working to find such a compound. This search has been fruitless with regard to a non-addictive opioid agonist, but has produced several opioid antagonistic drugs.

Minor alterations to a drug's chemical structure can change an agonist into an antagonist. The first opioid antagonist, N-allylnorcodeine was discovered in 1915, by changing a methyl group in the codeine molecule to an allyl group.¹ After this discovery, however, the research in non-addictive opioids lay dormant for a while and it would take until 1944 for a second member of the opioid antagonist class, N-allylnormorphine (or nalorphine), to be characterized. Nalorphine showed antagonism for morphine induced respiratory depression,² but was later found to be a μ -opioid receptor agonist as well, with severe dysphoric side-effects (due to its agonism of the κ -opioid receptor).³ Further experimentation with nalorphine's chemical structure finally yielded N-allylnoroxymorphone, or naloxone, in 1960.⁴

1.2 Respiration

Naloxone is best known for its use in opioid induced respiratory depression and it is therefore that the first part of this thesis is dedicated to its use in respiratory studies. Opioid induced respiratory depression is clinically recognized by an increase in arterial P_{CO_2} .⁵ This is caused by a reduction of both tidal volume and respiratory frequency, which is in turn caused by activation of the μ -opioid receptors in the respiratory control centers of the central nervous system.⁶ This μ -opioid receptor activation leads to a decreased sensitivity of the chemoreceptors, characterized in a right and downward shift of the $\dot{V}_I$ - P_{ET,CO_2} -response curve. In other words, opioids cause the chemoreceptors to be less sensitive to carbon dioxide (CO_2), which is one of their main stimuli. Naloxone

can antagonize this effect through competitive antagonism at the μ -opioid receptor and is thus able to save lives by reversing respiratory depression. It is always important to keep resuscitated patients under surveillance, as naloxone's duration of action is often shorter than that of the opioid. This means that renarcotization can occur easily, especially with longer acting opioids such as morphine, heroin and methadone. The duration of naloxone's reversal is highly dependent on the opioid used, and therefore it is important that we characterize naloxone's behaviour in different opioids.

1.3 Pain and hyperalgesia

Essentially, pain is a physiological signalling system: it alerts the brain that something is wrong in the body and thus urges the body to protect itself from further harm. There is a purely physical component to pain, called nociception. This is the conduction of a signal from a nociceptor (a receptor responsive to painful stimuli) or a damaged nerve in the peripheral nervous system on to the central nervous system.⁷ But nociception alone is not pain. Pain also has an emotional component, which consists of our response to a painful stimulus. This response is highly variable and depends on both individual and cultural factors.⁸ Analgetic drugs, such as opioids, influence one or both of these components and thus cause us to feel less pain. Opioids are renowned for their analgetic qualities – they still form the gold standard in pain therapy. Less recognized is that they may also increase pain sensitivity.

This so-called 'Opioid induced Hyperalgesia' (OIH) has proven to be a growing problem in pain management and has therefore been the focus of much research over the past decade.⁹ OIH can in general be defined as an increased pain response due to the use of opioids.¹⁰ For a long time, OIH has been mistaken for opioid tolerance, as both conditions require higher opioid dosing. But at present the general hypothesis is that OIH may be the result of an central sensitization process.¹¹ This is probably caused by activation of N-methyl-D-aspartate (NMDA) receptors. Heightened activity of protein kinase C removes the magnesium 'lock' off the NMDA-receptor, thereby activating the receptor. This ultimately leads to a higher pain perception. It has been suggested that μ -opioid receptor activation could activate protein kinase C.¹² If naloxone could prevent this activation, it could perhaps be used in the prevention and treatment of opioid induced hyperalgesia.

1.4 Addiction

Opioid addiction remains a social and medical problem. Initial attempts to manufacture an opioid without addictive properties were in vain. The obvious solution was then to try and block the μ -opioid receptor, using opioid antagonists such as naloxone.¹³ This causes acute withdrawal syndrome in opioid dependent patients, which can either be a goal of the therapy (in detoxification settings) or a threatening side-effect

(after an opioid overdose).¹⁴ Due to its short elimination half-life, naloxone is not the first choice in maintenance therapy for opioid dependent patients.¹⁵ It is however most famous for its use in the treatment of opioid overdose. Patients overdosing on heroin have a severe respiratory depression, which often results in a comatose state. In those patients, naloxone can make the difference between life or death.

1.5 Aims

With its antagonism of the μ -opioid receptor, there are several applications of naloxone worth investigating. This thesis is specifically aimed to answer the following questions:

- In chapters 2, 3 and 4, the possibility to reverse opioid-induced respiratory depression with naloxone, is explored and the question whether this differs between different opioids is studied.
- In chapter 5, the question is addressed whether naloxone can be used to abolish opioid induced hyperalgesia.
- Chapter 6 is an elaboration upon the roles naloxone can play in the treatment of opioid addiction.

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SECTION II

Respiration

Differential effect of morphine and
morphine-6-glucuronide on the control of
breathing in the anesthetized cat

Luc J. Teppema, Eveline L.A. van Dorp, Babak Mousavi Gourabi, Jack W. van Kleef
& Albert Dahan
Anesthesiology 2008; 109: 689–697

2.1 Introduction

In animals and humans, morphine's metabolite, morphine-6-glucuronide (M6G), activates the μ -opioid receptor causing typical opioid behaviour.¹⁻³ This includes analgesia (or antinociception), miosis, respiratory depression and nausea/vomiting. M6G is present in the blood of patients after just a single dose of morphine but its contribution to morphine analgesia and toxicity (e.g., sedation and respiratory depression) only becomes significant after long-term morphine treatment and/or in patients with renal impairment (as the primary route of M6G clearance is via the kidneys).⁴⁻⁷ Several studies by Pasternak and co-workers indicate the existence of a unique M6G receptor responsible for its analgesic activity. First of all, in morphine-insensitive mice, M6G analgesia is uncompromised⁸ and M6G shows no analgesic cross-tolerance in mice made tolerant to morphine.⁸ Furthermore, studies into labelled M6G binding to bovine tissue indicate the existence of a high and a low affinity component. The low-affinity component corresponds to labelling of traditional μ -opioid receptors, while the high-affinity component shows selectivity to M6G.^{9,10} More evidence for a separate M6G receptor comes from another study, in which 3-methoxynaltrexone (3mNTX) is an opioid receptor antagonist selective for the M6G binding site. In CD-1 mice and rats, 3mNTX displaces the M6G dose-response (analgesia) curve without affecting the curve for morphine.^{10,11} Finally, rats treated with antisense probes against exon 1 of the μ -opioid receptor gene (*Oprm1*) display reduced morphine analgesia but normal M6G analgesia. Similar observations were made for probes targeting specific G-protein α subunits^{8,12,13} and for *Oprm1* gene knockout mice. The evidence from these last studies is less compelling, as *Oprm1* gene knockout mice do not display any G-protein activation.¹⁴ Furthermore, the effect of M6G analgesia in this mouse strain was not reproduced by others.¹⁵ Interestingly, the M6G opioid receptor seems equally sensitive to heroin.^{8-13,16}

Animal and human studies indicate that M6G produces less respiratory depression than morphine at equi-antinociceptive/analgesic doses.¹⁷⁻¹⁹ This is an important feature of a potent opioid analgesic, as respiratory depression is a potentially lethal side effect of acute opioid administration.²⁰ Possibly, the different effects of M6G and morphine on respiration reflects activation of distinct μ -opioid receptors with different effects on the ventilatory control system. The current study was designed to quantify the respiratory effects of M6G versus morphine and to assess whether the effect of M6G is related to the earlier classified unique M6G-receptor. We initially measured the effects of morphine and M6G on the dynamic ventilatory response to carbon dioxide (CO₂) in the anesthetized cat and next investigated the effect of the M6G-receptor selective antagonist 3mNTX on respiratory depression induced by morphine and M6G. The ventilatory responses were analysed using a two-compartment model of the respiratory controller, reflecting the peripheral and central chemoreflex pathways.²¹⁻²⁴ These studies provide information about the sites of action of M6G, morphine and 3mNTX with respect to their dynamic and steady-state effects on the ventilatory CO₂ response curves.

2.2 Materials and Methods

The experiments were performed after approval of the protocol by the local Ethical Committee for Animal Experiments (UDEEC, Leiden University Medical Center, The Netherlands). Eighteen purebred (European shorthair) cats (eight males/ten females; mean ($\pm$ SD) body weight $3.3 \text{ kg} \pm 1.0 \text{ kg}$) were sedated with 10 mg/kg intramuscular ketamine hydrochloride. Next, the animals were anesthetized with a gas containing 0.7 to 1.4% sevoflurane and 30% oxygen (O_2) in nitrogen (N_2). The right femoral vein and artery were cannulated, after which 20 mg/kg α -chloralose and 100 mg/kg urethane were slowly administered intravenously. Subsequently the volatile anesthetic was withdrawn. Approximately one hour later, an infusion of an α -chloralose-urethane solution was started at a rate of 1.0 to $1.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ α -chloralose and 5.0 to $7.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ urethane. This regimen leads to conditions in which the level of anesthesia is sufficient to suppress pain withdrawal reflexes but light enough to preserve the corneal reflex. The stability of the ventilatory parameters was studied previously, and they were found to be similar compared to those in awake animals, and to be stable over a period of at least six hours.²⁴⁻²⁶ We use a feline experimental model as it allows the application of the dynamic end-tidal forcing technique which is an important requirement for studying ventilatory control in a reliable fashion. A second argument for using this technique is that cat data are often easily comparable to human data.

To measure inspiratory and expiratory flow, the trachea of the animals was cannulated and connected to a Fleisch Nr. 0 flow transducer (Fleisch, Lausanne, Switzerland), which was attached to differential pressure transducer (Statham PM197, Los Angeles, CA, USA). The flow transducer was connected to a T-piece of which one arm received a continuous fresh gas flow of $5 \text{ l}\cdot\text{min}^{-1}$. Three computer-controlled mass flow controllers (Bronkhorst High-Tech, Veenendaal, The Netherlands) composed desired inspiratory gas mixtures of O_2 , CO_2 and N_2 . The inspiratory and expiratory fractions of O_2 and CO_2 were measured with a Datex Multicap monitor (Datex-Engstrom, Helsinki, Finland). The temperature of the animals was controlled within 1°C and ranged among cats between 38 and 39°C . All signals were recorded digitally (sample frequency 100 Hz) and stored on a breath-to-breath basis on a computer for further analysis.

Study Design

The dynamic ventilatory response to CO_2 was studied with the Dynamic End-tidal Forcing (DEF) technique.^{21-24,27} Step-wise changes in end-tidal $P_{\text{ET},\text{CO}_2}$ at a constant end-tidal P_{ET,O_2} (110 mmHg) were applied. Each DEF run started with a steady-state period of 2 minutes during which $P_{\text{ET},\text{CO}_2}$ was maintained at 4 mmHg above resting values. Thereafter, the $P_{\text{ET},\text{CO}_2}$ was elevated by 7.5 mmHg for 7 minutes and then lowered to the initial value and kept constant for another 7 minutes. In order to avoid irregular breathing at $P_{\text{ET},\text{CO}_2}$ values close to the apneic threshold, we adjusted a clamped baseline $P_{\text{ET},\text{CO}_2}$ at a level approximately $3\text{-}4 \text{ mmHg}$ higher than the apneic

threshold during any given experimental condition (i.e., in control and after each drug infusion).

Initially, the effect of four cumulative M6G doses (0.15 mg/kg, 0.3 mg/kg, 0.6 mg/kg and 0.9 mg/kg) followed by two cumulative 3mNTX doses (0.1 and 0.2 mg/kg) on ventilation, with P_{ET,CO_2} clamped 4 mmHg above resting, was tested in two cats. This was done to determine the M6G and 3mNTX doses to be used. Next, we performed three separate studies.

Study 1 In this study, the effect of the intravenous infusion of morphine (0.15 mg/kg) followed by 3mNTX (0.2 mg/kg iv) and subsequently M6G (0.8 mg/kg iv) on the dynamic ventilatory response to CO_2 was assessed in six cats.

Study 2 Here, we obtain ventilatory CO_2 responses in six cats after the iv infusion of M6G (0.8 mg/kg), followed by 3mNTX (0.2 mg/kg iv) and lastly morphine (0.15 mg/kg iv).

Study 3 Finally, the effect of just 3mNTX (0.2 mg/kg iv) was assessed in four cats.

In all studies, three to four control DEF runs were obtained prior to any drug infusion (control runs); after each drug infusion and a pause of about 20–30 minutes, two to four DEF runs were performed. M6G was obtained from CeNeS Ltd. (Cambridge, United Kingdom), morphine from Pharmachemie BV (Haarlem, The Netherlands), and 3mNTX from Sigma BV (Zwijndrecht, The Netherlands).

Data and Statistical Analysis

The steady-state relation between inspired minute ventilation ($\dot{V}_i$) and P_{ET,CO_2} is linear down to apnea and described by:^{21–24,27}

$$\dot{V}_i = (G_c + G_p) \cdot (P_{ET,CO_2} - B)$$

with G_c : sensitivity of the central chemoreceptors

G_p : sensitivity of the peripheral chemoreceptors

B : apneic threshold (extrapolated P_{ET,CO_2} at $\dot{V}_i = 0 \text{ l} \cdot \text{min}^{-1}$).

When applying rapid changes in end-tidal P_{CO_2} at constant end-tidal P_{O_2} it is possible to quantify the contributions of the peripheral and central chemoreflex loops to total ventilation. This is based on the difference in response times and dynamics of the two chemoreflexes in response to a change in end-tidal P_{CO_2} .^{21–24}

The central chemoreflex loop displays a relative large time delay (average response time in the cat is 8 s) with slow dynamics (average time constant in the cat is 100 s); the response time of the peripheral chemoreflex loop is on average 4 s with a time constant of about 10 s.^{21,22,24} To estimate G_c , G_p and B , we fitted the ventilatory responses

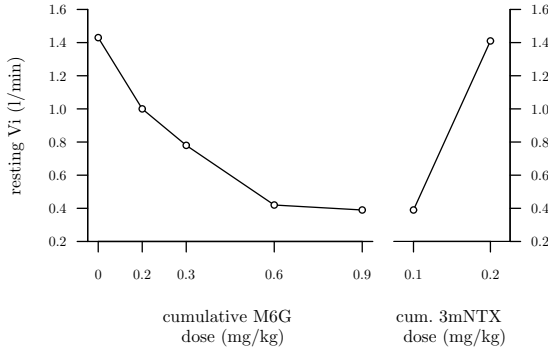


Figure 2.1: Effect of four cumulative M6G doses, followed by 2 cumulative 3mNTX doses on resting ventilation in one cat. The data were obtained at a clamped P_{ET,CO_2} of 45 mmHg. Only at M6G doses of 0.6 mg/kg and higher was a reduced response to CO_2 observed (data not shown).

to a two-compartmental model using a least-squares fitting routine as described previously.^{21–24} In the fitting procedure parameters G_p and B were not restricted to values equal to or greater than zero. Occasionally a negative optimal value for G_p was obtained which then was set to zero in the statistical analysis.

Initially the data were tested for normality using the Kolmogorov-Smirnov test. As all data were normally distributed, to determine the level of significance of the treatment effects, we next performed an analysis of variance on the group data. A separate analysis was performed on the data from studies 1, 2 and 3. Post-hoc comparisons were made with the Bonferroni-test. In order to correct for multiple comparisons, P-values < 0.05 were considered significant. The analysis was performed using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). Values reported are means $\pm$ SD.

2.3 Results

The M6G and 3mNTX doses used in the study were based on the effects of incrementing doses of the two drugs on resting ventilation as observed in two cats (see figure 2.1 for the results in one animal). M6G produced a dose-dependent depression of resting ventilation. The M6G dose causing a depression similar to that observed with 0.15 mg/kg morphine^{20,21,23} was 0.8 mg/kg. We therefore used an M6G dose of 0.8 mg/kg in the subsequent studies. 3mNTX produced no effect at 0.1 mg/kg but displayed full reversal of the depressed resting ventilation at a dose of 0.2 mg/kg.

To get an appreciation of the quality of the DEF experiments and data fits, we plotted four examples obtained in one cat from study 2 in figure 2.2. The top diagrams show the applied steps into and out of end-tidal P_{CO_2} . In the bottom graphs, each dot represents one breath. The slow central (V_c) and fast peripheral (V_p) components are shown, together with the least-squares model fits (the thick lines through the data points). As can be seen by visual inspection, the model adequately describes the data. In these examples, M6G increased the apneic threshold (B) and reduced the ventilatory CO_2 sensitivity of the peripheral chemoreflex loop (G_p) without affecting the ventilatory CO_2 sensitivity of the central chemoreflex loop (G_c). The subsequent infusion of

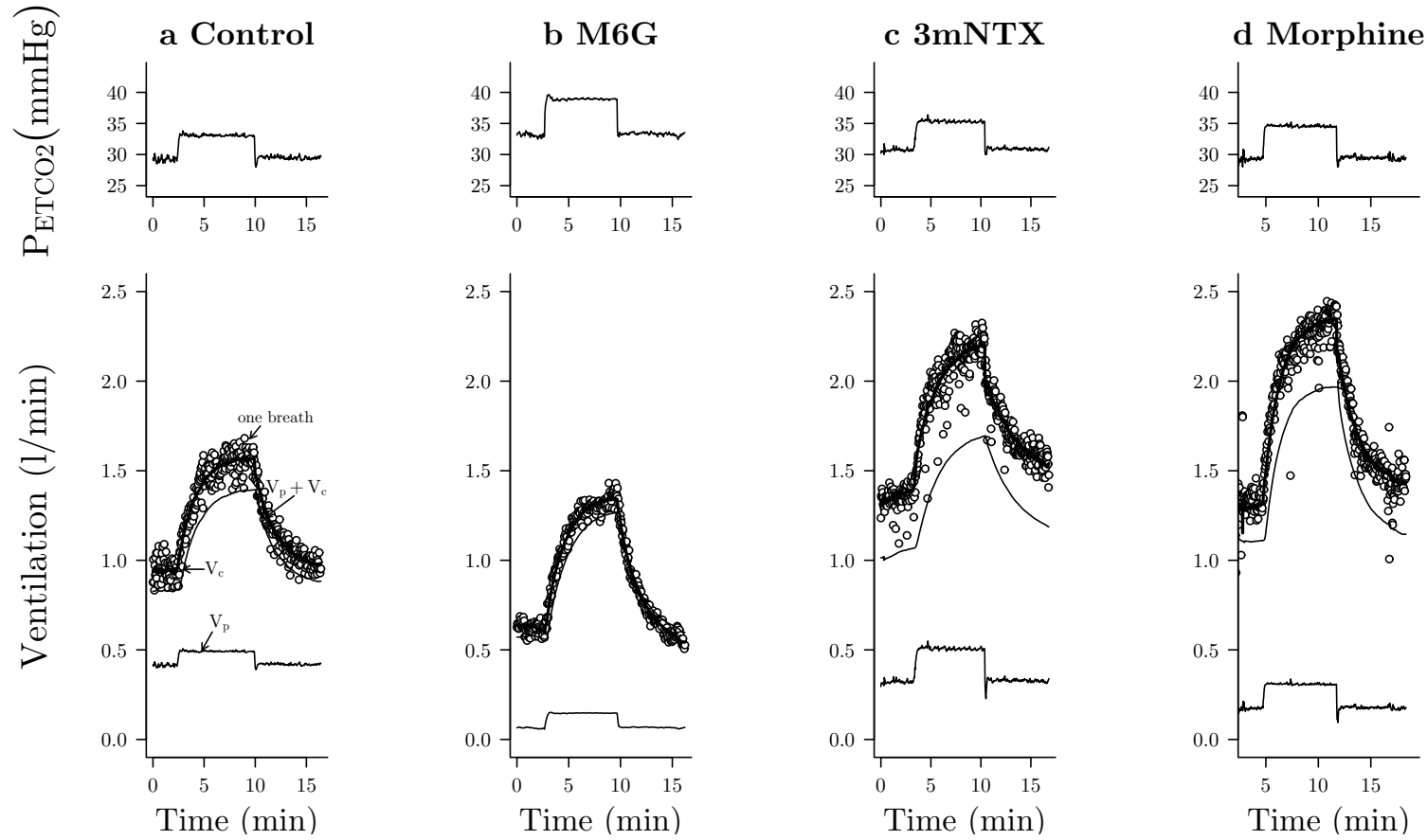


Figure 2.2: Example of the dynamic ventilatory responses to end-tidal partial pressure of CO_2 ($P_{\text{ET},\text{CO}_2}$) and data fits in one cat. One control response (A), one response after 0.8 mg/kg morphine-6-glucuronide (M6G; B), one response after a subsequent dose of 3-methoxynaltrexone (3mNTX; C), and one response after a subsequent dose of 0.15 mg/kg morphine (D) are shown. The top panels show the input function to the system (i.e., $P_{\text{ET},\text{CO}_2}$). In the lower panels, each open circle represents one breath. The line with the fast dynamics is the estimated output of the peripheral chemoreflex loop (V_p); the thin line with the slow dynamics is the estimated output of the central chemoreflex loop (V_c). The sum of V_p and V_c is the thick line through the data points. Parameter values for the shown data fits are as follows: (A) apneic threshold = 23.6 mmHg, central CO_2 sensitivity = $0.13 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, peripheral CO_2 sensitivity = $0.020 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; (B) apneic threshold = 28.7 mmHg, central CO_2 sensitivity = $0.13 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, peripheral CO_2 sensitivity = $0.011 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; (C) apneic threshold = 22.8 mmHg, central CO_2 sensitivity = $0.13 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, peripheral CO_2 sensitivity = $0.04 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; (D) apneic threshold = 22.5 mmHg, central CO_2 sensitivity = $0.16 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, peripheral CO_2 sensitivity = $0.025 \text{ l}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$.

3mNTX caused the return of apneic threshold to control values and increased peripheral CO₂ sensitivity to values greater than control. Finally, the infusion of morphine after 3mNTX did not further influence any of the model parameters.

Study 1 In the 6 cats of study 1 we performed 22 control experiments, 20 after morphine, 19 after 3mNTX and 16 after M6G. Treatment effects were observed on the apneic threshold, central and total CO₂ sensitivities with no effects on peripheral CO₂ sensitivity and the ratio peripheral/central CO₂ sensitivity (figure 2.3). Morphine caused a significant increase of the apneic threshold from 27.5 ± 3.6 mmHg to 31.5 ± 2.2 mmHg, and reduced the central and total CO₂ sensitivities from 0.13 ± 0.06 to 0.07 ± 0.04 and from 0.16 ± 0.07 to 0.08 ± 0.04 l·min⁻¹·mmHg⁻¹, respectively ($P < 0.01$). After the infusion of the opioid antagonist 3mNTX the apneic threshold reduced to values below baseline (24.8 ± 2.5 mmHg), central CO₂ sensitivity increased to a value in between morphine and M6G (0.11 ± 0.04 l·min⁻¹·mmHg⁻¹) and total CO₂ sensitivity returned to control values (0.11 ± 0.05 l·min⁻¹·mmHg⁻¹). Infusion of M6G after 3mNTX had no further effect on any of the model parameters.

Study 2 In the 6 cats of study 2 we performed 27 control experiments, 17 after M6G, 18 after 3mNTX and 17 after morphine. Treatment effects were observed for all model parameters except central CO₂ sensitivity (figure 2.4). M6G caused a significant increase of the apneic threshold from 26.3 ± 5.7 mmHg to 34.2 ± 25.0 mmHg, and reduced the peripheral and total CO₂ sensitivities from 0.031 ± 0.013 to 0.013 ± 0.017 l·min⁻¹·mmHg⁻¹ and from 0.16 ± 0.02 to 0.13 ± 0.05 l·min⁻¹·mmHg⁻¹, respectively ($P < 0.01$). The ratio peripheral/central CO₂ sensitivity was reduced from 0.26 ± 0.13 to 0.09 ± 0.13 . Infusion of the opioid antagonist after M6G caused full return to baseline levels of the apneic threshold (26.5 ± 5.1 mmHg), the peripheral and total CO₂ sensitivities (0.024 ± 0.017 and 0.17 ± 0.05 l·min⁻¹·mmHg⁻¹, respectively), and the ratio peripheral/central CO₂ sensitivity (0.18 ± 0.11). Infusion of morphine after 3mNTX had no further effect on any of the model parameters.

Study 3 In the 4 cats of study 3, we performed 30 experiments (15 control and 15 after 3mNTX). Infusion of 0.2 mg/kg 3mNTX had no systematic effect on any of the estimated model parameters (figure 2.5).

2.4 Discussion

Morphine (0.15 mg/kg) affects the control of breathing by increasing the apneic threshold and by reducing central ventilatory CO₂ sensitivity. These effects are fully antagonized by 3mNTX and subsequent infusions of M6G are without further effect. M6G (0.8 mg/kg) on the other hand, causes an increase of the apneic threshold together with a reduction of the peripheral CO₂ sensitivity without affecting central CO₂ sensitivity. This indicates a preferential effect of M6G within the peripheral chemoreflex

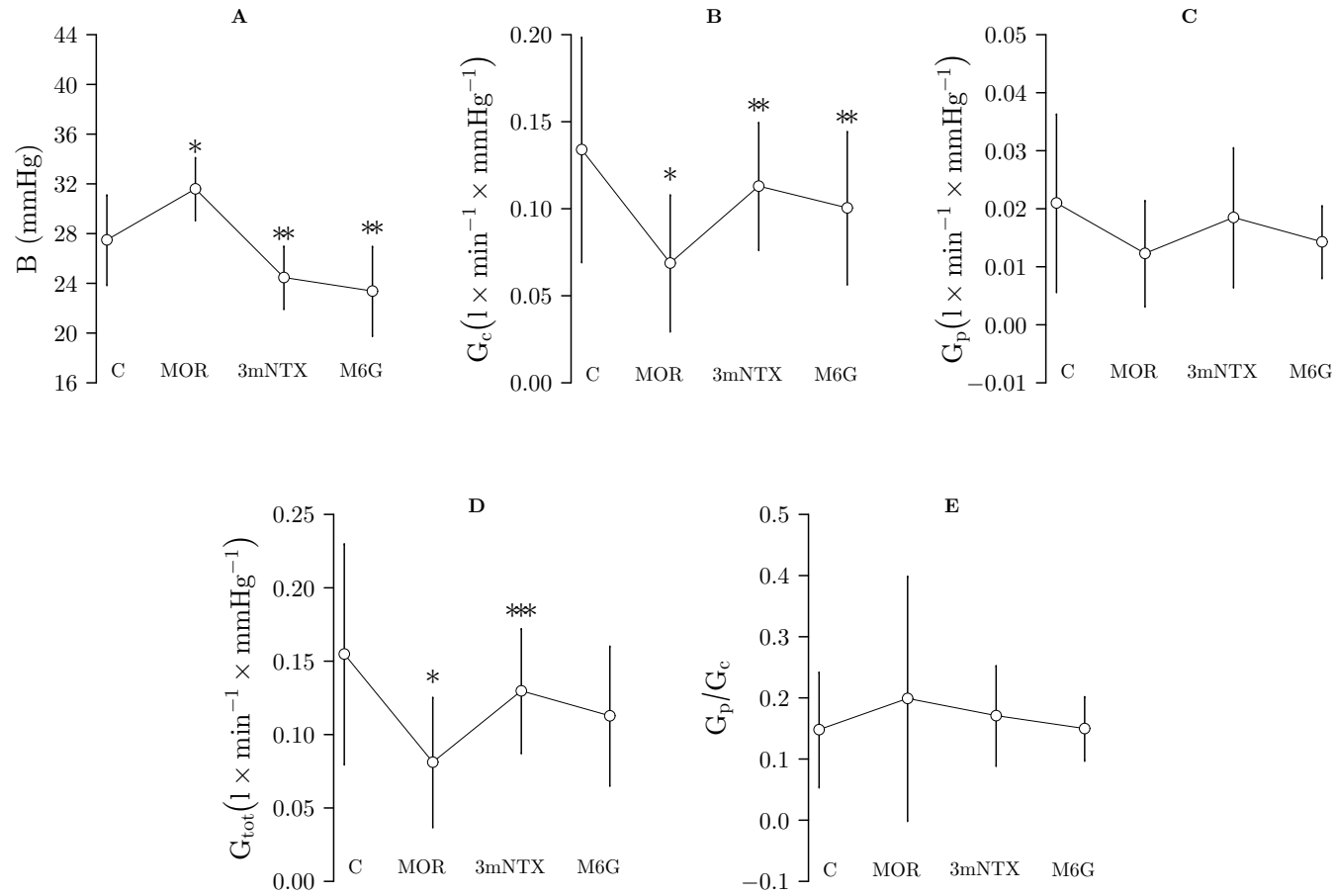


Figure 2.3: Study 1: Effect of morphine (MOR) followed by 3-methoxynaltrexone (3mNTX) and morphine-6-glucuronide (M6G) on apneic threshold (B ; figure A), central CO_2 sensitivity (G_c ; figure B), peripheral CO_2 sensitivity (G_p ; figure C), total CO_2 sensitivity (sum of peripheral and central CO_2 sensitivity; figure D), and ratio of peripheral to central CO_2 sensitivity (figure E). Data were obtained in six cats. Values are the mean of the cat means $\pm$ SD. Treatment effects were obtained for apneic threshold ($P < 0.001$), central CO_2 sensitivity ($P < 0.001$), and total CO_2 sensitivity ($P < 0.001$). * $P < 0.01$ versus control. ** $P < 0.01$ versus morphine and control. *** $P < 0.01$ versus morphine. C = control.

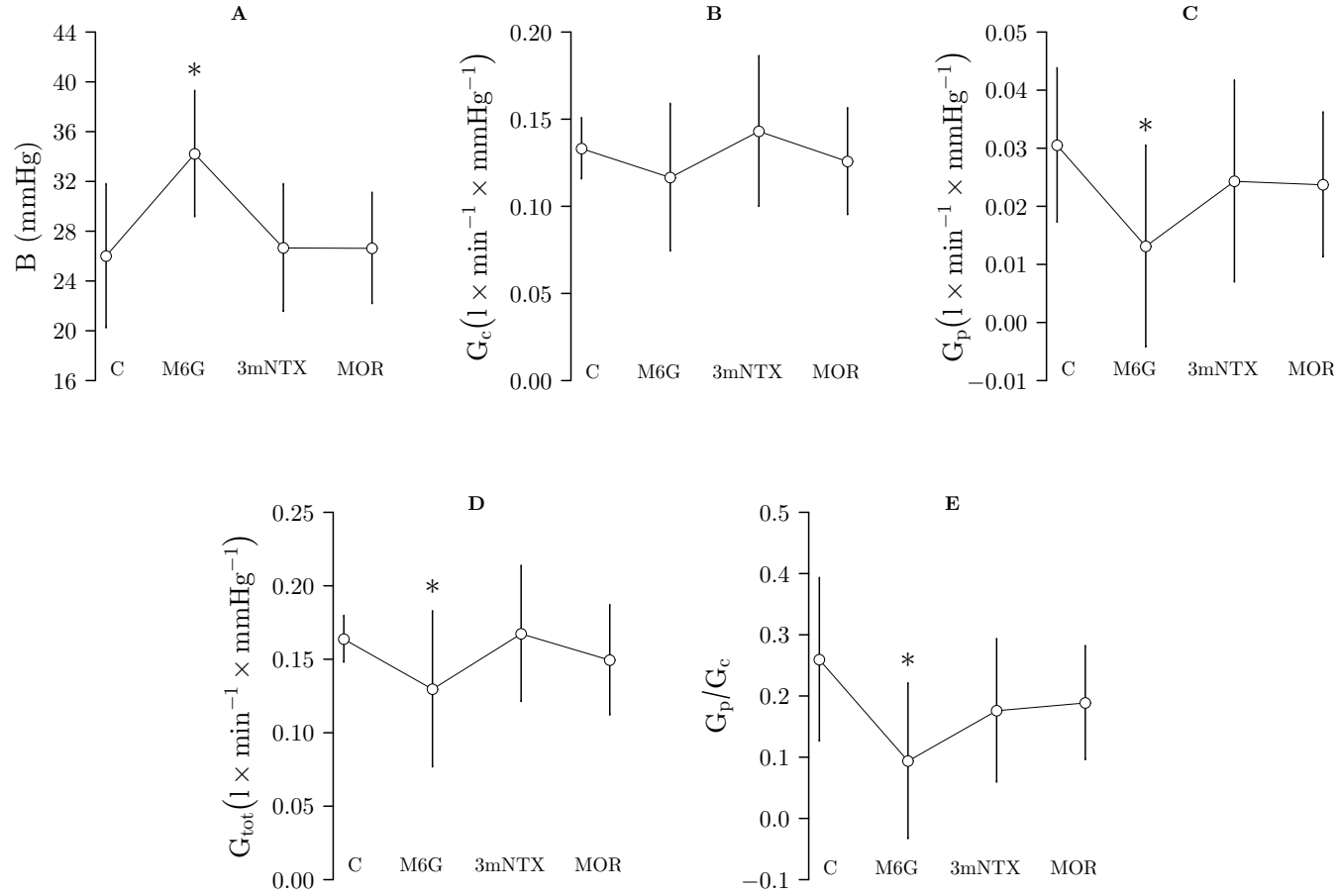


Figure 2.4: Study 2: Effect of morphine-6-glucuronide (M6G) followed by 3-methoxynaltrexone (3mNTX) and morphine (MOR) on apneic threshold (B ; figure A), central CO_2 sensitivity (G_c ; figure B), peripheral CO_2 sensitivity (G_p ; figure C), total CO_2 sensitivity (G_{tot} ; sum of peripheral and central CO_2 sensitivity; figure D), and ratio of peripheral to central CO_2 sensitivity (G_p/G_c ; figure E). Data were obtained in six cats. Values are the mean of the cat means $\pm$ SD. Treatment effects were observed for apneic threshold ($P < 0.001$), central CO_2 sensitivity ($P = 0.01$), peripheral CO_2 sensitivity ($P = 0.001$), total CO_2 sensitivity ($P < 0.001$), and ratio of peripheral to central CO_2 sensitivity ($P = 0.001$). * $P < 0.01$ versus control and 3mNTX. C = control.

loop. These effects of M6G are fully antagonized by 3mNTX and subsequent infusions of morphine are without further effects. Finally, 3mNTX (0.2 mg/kg) has no effect on the apneic threshold and the peripheral and central CO₂ sensitivities when given without prior opioid infusion.

We used a M6G dose that was 5.3 times greater than the morphine dose. The M6G dosing was based on our pilot experiments in two cats showing that at 0.8 mg/kg M6G causes a reduction in resting ventilation of similar magnitude as 0.15 mg/kg morphine. This observation was later confirmed: the morphine and M6G ventilatory CO₂ response curves intersect at 38 mmHg (just above the metabolic hyperbola), a value close to the mean clamped end-tidal P_{CO₂} value in our study (figure 2.6). In contrast to our observation of greater morphine potency, animal studies usually show that M6G is the more potent drug with respect to antinociception (cf. Kilpatrick and Smith³ and references cited therein) and respiratory depression. For example, in mice, rats, dogs and neonatal guinea pigs morphine:M6G potency ratios for respiratory depression vary from 1:4 after intraperitoneal or intravenous injections to 1:10 after intracerebroventricular injection.²⁸⁻³¹ Apparently the cat forms an exception to this rule which may be related to the absence of an effect on the CO₂ sensitivity of the central chemoreflex loop, the major component of total chemical drive.

In the present study morphine had no effect on the peripheral CO₂ sensitivity (see figure 2.3). This contrasts with earlier studies on morphine using a similar cat model,^{21,22,27} as well as with our observation that morphine failed to affect the ratio peripheral/central CO₂ sensitivity (G_p/G_c , figure 2.3). This latter observation together with the reduction of central CO₂ sensitivity suggests an effect of morphine on neuronal structures common to both the peripheral and central chemoreflex pathway (such as the respiratory centers in the ventrolateral medulla). Some effect of morphine on the peripheral chemoreflex is expected. There are indications for the presence of opioid-receptors in cat carotid bodies: 98% of type I carotid body cells exhibit enkephalin immunoreactivity,³² and naloxone enhances the response to hypoxia as measured from single or paucifiber preparations of carotid body afferents.³³ With the above taken into account, we believe that our current study may have been underpowered to observe a morphine effect on the peripheral CO₂ sensitivity ($P = 0.07$ versus control). However, we cannot exclude that study-differences in the effect of morphine on the peripheral chemoreflex loop are also partly related to differences in the genetic background of the cats we used in our studies: mongrel cats in our previous studies versus inbred animals in the current study.^{21,22,27}

Compared to morphine, M6G showed important differences in its effect on ventilatory control. At the relatively high dose tested, M6G increased the apneic threshold by 8 mmHg, while the peripheral CO₂ sensitivity decreased by more than 60% without any effect on central CO₂ sensitivity (morphine reduced the central CO₂ sensitivity by about 50%; see also figure 2.6). There are several possible explanations for the differ-

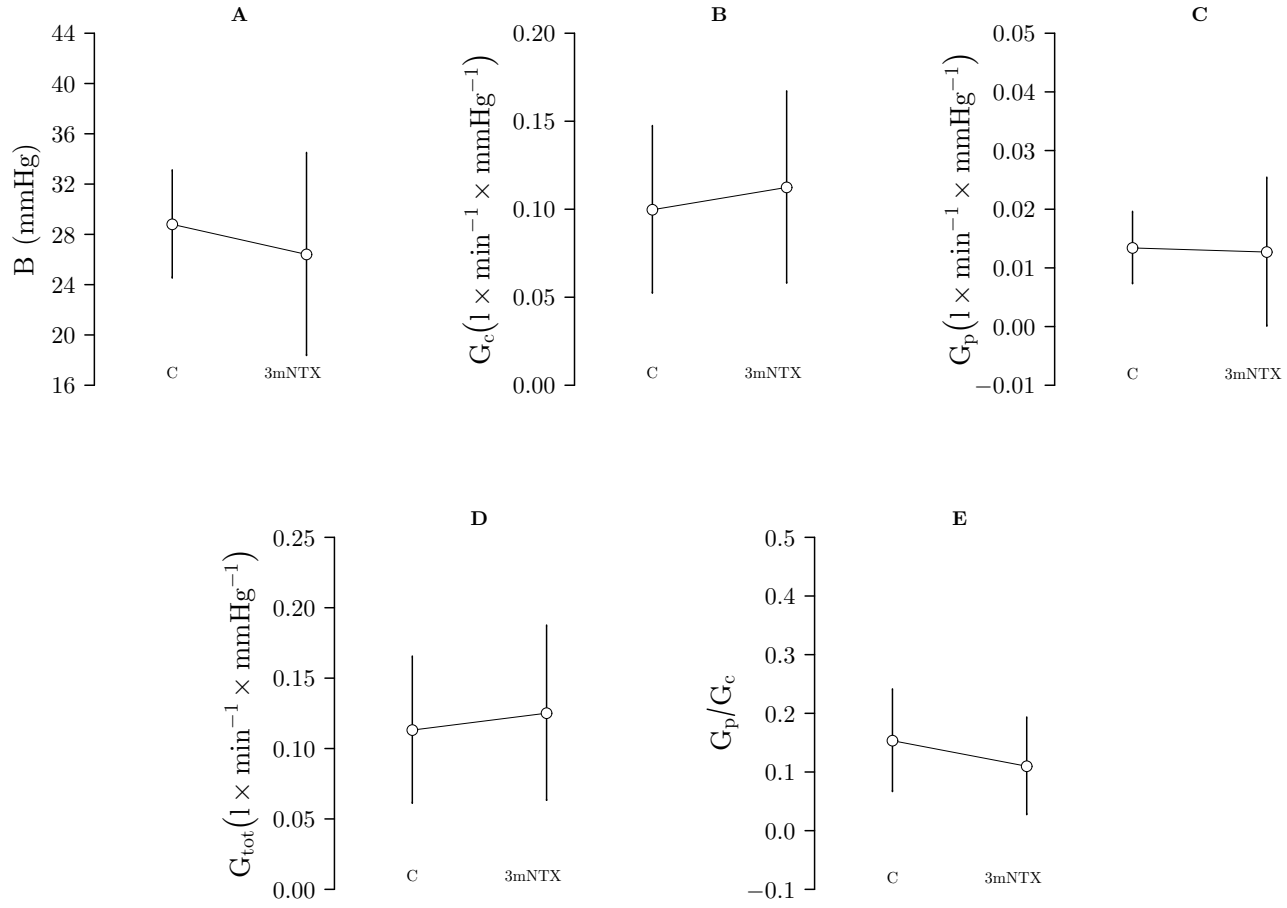


Figure 2.5: Study 3: Effect of 3-methoxynaltrexone on apneic threshold (B; A), central CO₂ sensitivity (G_c ; B), peripheral CO₂ sensitivity (G_p ; C), total CO₂ sensitivity (G_{tot} ; sum of peripheral and central CO₂ sensitivity; D), and ratio of peripheral to central CO₂ sensitivity (G_p/G_c ; E). Data were obtained in four cats. Data are the mean of the cat means $\pm$ SD. C = control, 3mNTX = 3-methoxynaltrexone.

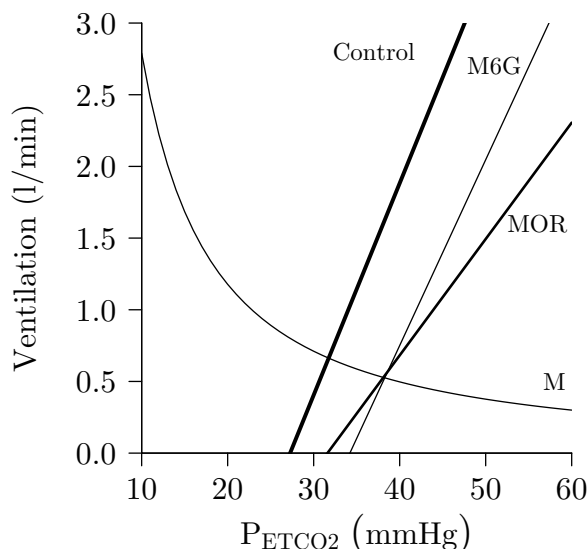


Figure 2.6: Effect of 0.15 mg/kg morphine and 0.8 mg/kg morphine-6-glucuronide (M6G) on the ventilatory response to CO_2 . The control response is also given. During anesthesia, resting ventilation occurs at the intersection of the ventilatory CO_2 response curve and the metabolic hyperbola (M). While resting ventilation did not differ between the two drugs, the end-tidal partial pressure of CO_2 ($P_{\text{ET},\text{CO}_2}$) to reach a ventilation level of $2 \text{ l}\cdot\text{min}^{-1}$ was 7 mmHg (approximately 1 vol%) greater for morphine than for M6G (50 versus 57 mmHg). This is an indication that M6G produces less respiratory depression than morphine at the drug doses used.

ence in respiratory behaviour between the two opioids. In contrast to morphine, M6G may not have crossed the blood-brain barrier in sufficient amounts but exerted its effect at the carotid bodies. M6G is much more polar than morphine,³⁴ and consequently passes the blood-brain barrier much slower than morphine.³⁵ However, at the dose used and the relatively low volume of distribution and clearance, there will be a large M6G concentration gradient across the blood-brain barrier.³⁶ This will result in sufficient passage of M6G into the brain to cause a central effect. Furthermore, although opioid receptors are assumed to exist in the carotid body (see paragraph above) there are no studies that directly demonstrate the actual existence of μ -opioid receptors in the carotid chemoreceptors. Of interest to our discussion is the observation that in our anesthetized cat model, we were unable to observe an effect of morphine (0.15 mg/kg) on the steady-state ventilatory response to hypoxia.²² In humans, an experimental opioid that does not cross the blood-brain barrier has no effect on the ventilatory response to acute hypoxia,³⁷ while intrathecal morphine has a profound and long-lasting effect on this same response.³⁸ In summary, we suggest that an appreciable amount of the M6G that we infused did cross the blood-brain barrier and consequently may have affected the ventilatory control system for a large part at central sites (i.e., within the central nervous system).

Another possibility for the observed differences between morphine and M6G is that while morphine acts at the classical μ -opioid receptor, ubiquitously present on the neuronal substrates of the ventilatory control system, M6G acts at the proposed unique M6G receptor,^{8-13,16} which is then present in the peripheral chemoreflex pathways and/or brainstem neurons that control the apneic threshold but not within the central chemoreflex pathway. An important feature of this opioid receptor system is its selective antagonism by 3mNTX.^{10,11} However, we were unable to demonstrate 3mNTX

selectivity for M6G-induced respiratory depression. 3mNTX antagonized both morphine and M6G-induced respiratory changes and administration of either opioid after 3mNTX was without effect. One can contend that we missed a distinctive effect of 3mNTX between morphine and M6G because we did not perform dose-response studies. There are however strong arguments to dismiss this suggestion. In mice intracerebroventricular infusion of 2.5 ng 3mNTX significantly lowered the analgesic actions of M6G without affecting morphine analgesia (see fig. 2 of Brown *et al.*¹⁰). Five to six times higher doses of 3mNTX were required to reduce morphine analgesia to the same effect.¹⁰ We observed that at the lowest dose at which 3mNTX caused full reversal of M6G respiratory effect (0.2 mg/kg) full reversal of morphine respiratory effect already occurred. Since the dose-response of 3mNTX appeared to be very steep (no effect at 0.1 mg/kg; see figure 2.1) we decided not to test the effect of 3mNTX on M6G or morphine at doses < 0.2 mg/kg.

Hence, our data permit the conclusion that in contrast to the data obtained in mice and rats on analgesia,^{8-13,16} our data do not suggest the presence of a unique 3mNTX-sensitive M6G receptor in the ventilatory control system of the cat. In agreement with our findings, in rhesus monkeys, 3mNTX was able to antagonize the antinociceptive effects of heroin as well as morphine.³⁹ Note, however, that our design is unable to exclude the existence of a separate (3mNTX-insensitive) M6G binding site. It may well that such a binding site may need to be pursued in less complex systems than the ventilatory control system.

There are several alternative explanations for our observations. First of all, morphine and M6G interact with distinct subpopulations of the μ -opioid receptor, which are differentially expressed on the various neuronal substrates of the ventilatory control system. These subpopulations may be splice variants of the μ -opioid receptor gene. In mice, at least fifteen of such variants arising from alternative splicing have been identified.⁴⁰ Another explanation could be that morphine and M6G, acting at the same opioid receptor, may activate different G-proteins. This in turn causes differences in signalling events and consequently divergence in behavioural responses.⁴¹ The differences in respiratory effect could also be due to differences in distribution of morphine and M6G within the brain compartment.⁴² Finally, morphine and M6G may differentially activate excitatory pathways within the ventilatory control system. This may be similar to the hyperalgesic responses observed after M6G infusion but not morphine in mice lacking the μ -opioid receptor.^{15,28}

A final point of criticism may be that in the current study we found larger pre-drug (i.e., baseline) values for the peripheral and central CO₂ sensitivities compared to some of our previous studies (cf. e.g., DeGoede *et al.*²¹). In both awake and anesthetized animals and in humans the variability in ventilatory CO₂ and hypoxic sensitivities is considerable (20 to 30%),^{43,44} and this applies particularly to the relative contributions of the peripheral and central chemoreceptors to the total ventilatory CO₂ response.⁴⁵ By

itself the ratio peripheral/central CO₂ sensitivity is insensitive to the depth of anesthesia.⁴⁶ This does not exclude, however, that the depth of anesthesia in our present animals may have been somewhat less because, compared to our previous studies, we adapted premedication (reducing the ketamine dose) and the inhalational and intravenous anesthesia (using sevoflurane rather than halothane for maintenance and reducing the chloralose-urethane dose). This then may have resulted in larger baseline ventilatory CO₂ sensitivities than in some of our previous studies. Other causes for the observed differences may be biological variability related to genetic components (e.g. the use of inbred animals in our current study). It is important to note, however, that irrespective of the baseline parameter values, the chosen anesthetic regimen results in a stable preparation and steady experimental conditions over several hours (over more than six hours).²⁵

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Naloxone reversal of buprenorphine induced respiratory depression

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3.1 Introduction

Long-acting opioids are important tools in the treatment of postoperative acute pain and chronic cancer and non-cancer pain. When selecting one of the available compounds, not only must the analgesic properties be considered, but also the safety profile of the drug. In general, opioids are well tolerated. Among the typical opioid side effects, however, respiratory depression is of special importance because of the risk of fatal outcome for the patient.

Buprenorphine is a potent analgesic (a hundred-fold more potent than morphine) with μ -agonistic, opioid receptor-like 1 (ORL-1) receptor agonistic, and κ -antagonistic opioid properties. In patients, buprenorphine is used for treatment of acute and chronic pain via various administration modes, such as intravenous, transdermal, sublingual, epidural, or spinal administration. In humans, buprenorphine behaves as a typical μ -opioid receptor agonist, showing analgesia, euphoria, sedation, respiratory depression, and pupillary constriction.^{1,2} Buprenorphine has a high affinity for opioid receptors and it has slow receptor association and dissociation compared to other opioids.³ After an intravenous infusion of 0.2 – 0.4 mg/70 kg, the duration of action of buprenorphine is approximately 6 – 8 hours. Data obtained in opioid-naive volunteers indicate that buprenorphine causes dose-dependent respiratory depression that levels off at greater buprenorphine doses (i.e., plateau or ceiling of respiratory effect).⁴

Surprisingly few studies have addressed the ability to reverse the respiratory effects of opioids in general and buprenorphine in specific. Just two studies, dating from the 1980s, as well as some anecdotal data, suggest that the respiratory depression from buprenorphine is resistant to antagonism by naloxone.^{5–7} Relatively low bolus doses of intravenous naloxone have no effect, whereas high doses (2.5 – 10 mg) causes only partial reversal of the respiratory effects of buprenorphine. These results may be explained by the short duration of action of a bolus dose of naloxone (resulting from a rapid elimination), combined with the high affinity of buprenorphine for μ -opioid receptors. Consequently, a bolus dose of naloxone may be unable to displace buprenorphine from the opioid receptors. The buprenorphine-naloxone data contrast data on the ability to reverse fentanyl-induced respiratory depression, which is considered relatively easy. Short naloxone infusions up to 0.4 mg cause full reversal of fentanyl-induced respiratory depression in patients during halothane-N₂O anesthesia.⁸

We performed a series of experiments to study the influence of naloxone on buprenorphine-induced respiratory depression. Our aim was to obtain a naloxone-dosing regimen that would cause full reversal of buprenorphine-induced respiratory depression. Initially (study 1), we assessed the effect of 0.8 mg naloxone (or placebo) on 0.2 mg intravenous buprenorphine-induced respiratory depression in healthy volunteers. In a subsequent study (study 2), we explored which naloxone dose causes full reversal of 0.2 mg intravenous buprenorphine induced respiratory depression. To do so, we tested various naloxone doses in the range from 0.5 to 7 mg in separate subjects. In another

study (study 3), we assessed the effect of a continuous naloxone (or placebo) infusion on 0.2 and 0.4 mg intravenous buprenorphine-induced respiratory depression.

3.2 Materials and Methods

Subjects

A total of 67 male and female subjects (age range: 20 – 30 years; weight: 54 – 93 kg) participated in and completed the studies after approval of the protocols was obtained from the Human Medical Ethics Committee (Commissie Medische Ethiek, Leids Universitair Medisch Centrum, Leiden, The Netherlands). We obtained oral and written consent. All subjects were healthy and did not have a history of illicit drug use or psychiatric illness. All women were taking oral contraceptives. Subjects were asked to have a normal night of sleep and not to eat or drink for at least six hours before the study. They were comfortably seated in a hospital bed for the duration of the study. They were naive with respect to the nature of the studies but were informed regarding the risk of participating. All subjects were students at Leiden University and received a financial reimbursement for their participation (75 – 100 euros depending on the study).

Apparatus

Inspired and expired gas flows were measured with a pneumotachograph (Hans Rudolph, Myandotta, MI, USA) connected to a pressure transducer and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump (stroke volume 1,000 ml, at a frequency of 20 min⁻¹). The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture with a flow of 45 l·min⁻¹ from a gas mixing system, consisting of three mass flow controllers (Bronkhorst High Tech BV, Veenendaal, The Netherlands) with which the flow of oxygen (O₂), carbon dioxide (CO₂), and nitrogen (N₂) could be set individually at a desired level. A personal computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal O₂ and CO₂ concentrations (P_{ET,O₂} and P_{ET,CO₂}, respectively) to follow a specified pattern in time, independent of the ventilatory response (i.e., dynamic end-tidal forcing).⁹ In studies 1 – 3, end-tidal partial pressure of CO₂ (P_{ET,CO₂}) was clamped at 53 mmHg throughout the measurements (approximately 8 mmHg above resting values), while end-tidal partial pressure of O₂ (P_{ET,O₂}) was maintained at a normoxic value of 110 mmHg. The O₂ and CO₂ concentrations and the arterial hemoglobin-O₂ saturation were measured with a Datex Multicap gas monitor near the mouth (Datex-Engstrom, Helsinki, Finland) and a Masimo pulse oximeter (using a finger probe) (Masimo, Irvine, CA, USA), respectively. The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). P_{ET,O₂},

P_{ET,CO_2} , inspired minute ventilation ($\dot{V}_i$), and O_2 saturation were collected on a breath-to-breath basis and stored on disk for further analysis.

Study Design and Data Analysis

The studies were placebo-controlled and had a double blind design. The hospital pharmacy delivered the buprenorphine hydrochloride (Reckitt Benckiser Health-care Ltd., Hull, United Kingdom), naloxone hydrochloride (manufactured by the pharmacy), and placebo (0.9% NaCl). Randomization and preparation of the syringes was performed by a physician not involved in the study. Randomization lists were obtained from www.randomization.com.

All buprenorphine and naloxone doses are per 70 kg. All bolus infusions were given over 90 s. Each subject participated once in any of the studies. Values reported are mean $\pm$ SEM, unless otherwise stated.

Study 1. Sixteen subjects participated in this study. All received 0.2 mg intravenous buprenorphine (at $t = 0$) followed by 0.8 mg naloxone (in eight subjects) or placebo (in eight subjects) at $t = 120$ minutes. At the following time periods, steady state ventilation ($\dot{V}_i$) was measured (measurement period 7 minutes): -10 minutes (10 minutes before drug infusion), 15, 75, 140, 180, 240, 300, 360, 420, and 480 minutes. Analysis of variance and post hoc t-tests were performed to detect a significant effect of naloxone on ventilation at the $P < 0.05$ level.

Study 2. Twenty-four subjects participated in this study. All received 0.1 mg buprenorphine at $t = 2$ minutes, followed by a continuous infusion of 0.1 mg/h for one hour (total dose = 0.2 mg in 60 minutes). At $t = 32$, X mg naloxone was given, one half as bolus and one half infused over 30 minutes. The following total naloxone doses (X) were tested: 0, 0.5, 1, 2, 3, 4, 5, 6, and 7 mg. Each dose was tested in two subjects, except for placebo, which was tested in eight subjects. Breathing was measured continuously from 2 minutes before buprenorphine infusion until 90 minutes after the start of infusion. The breath-to-breath data were averaged over one minute periods. An ensemble average (mean of the one minute subject means) was performed on the data of the eight subjects receiving the buprenorphine-placebo combination, allowing the calculation of buprenorphine-placebo induced respiratory effect at various time points. To quantify the respiratory effect of naloxone relative to placebo, we used the following formula on the data of each subject who had received the buprenorphine-naloxone combination:

$$R(z) = \frac{\dot{V}_{\text{naloxone}}(z) - \dot{V}_{\text{placebo}}(z)}{\dot{V}_{\text{baseline}} - \dot{V}_{\text{placebo}}(z)}$$

- with z : time period ranging from $t = 61$ to $t = 63$ minutes
 (−1 to +1 minute at the end of the continuous naloxone infusion)
- $\dot{V}_{\text{placebo}}(z)$: mean minute ventilation in the placebo group during period z
- $\dot{V}_{\text{naloxone}}(z)$: mean minute ventilation during period z after naloxone
- $\dot{V}_{\text{baseline}}$: mean ventilation of the 2 minutes before the buprenorphine infusion

This analysis will yield a quantitative measure of reversal, with 0 indicating no reversal (naloxone no better than placebo) and 1 indicating full reversal (response returned to pre-buprenorphine level).

Study 3 Thirty-two subjects participated in this study.

Study 3.1 Sixteen received 0.1 mg intravenous buprenorphine at time $t = 2$ minutes, followed by a continuous infusion of 0.1 mg/h for one hour (total dose = 0.2 mg in 60 minutes). At time $t = 32$ minutes, 2 mg naloxone ($n = 8$) or placebo ($n = 8$) was infused, followed by a continuous infusion of 4 mg/h for two hours.

Study 3.2 Sixteen other subjects received 0.2 mg intravenous buprenorphine at time $t = 2$ minutes, followed by a continuous infusion of 0.2 mg/h (total dose = 0.4 mg in 60 minutes) for one hour. At time $t = 32$ minutes, 3 mg naloxone ($n = 8$) or placebo ($n = 8$) was infused, followed by a continuous infusion of 4 mg/h for two hours. The bolus naloxone dose was 50% greater than that of study 3.1. This was based on a pilot study in three subjects that showed the need for a greater initial dose of naloxone after 0.4 mg but not after 0.2 mg buprenorphine.

Ventilation was initially measured continuously from 2 minutes before buprenorphine infusion until 120 minutes after the start of infusion. Subsequently measurements were made at 30 minute intervals until $t = 240$ minutes, after which hourly measurements were performed until $t = 420$ minutes. The breath-to-breath data were averaged over one-minute periods. An ensemble average was performed in the naloxone and placebo data groups. The values were compared with baseline ventilation ($\pm$ its 95% confidence interval).¹⁰ When the mean ventilation value equalled or crossed (baseline ventilation $- 1 * 95\%$ confidence interval), we somewhat arbitrarily assumed that ventilation had returned to pre-drug baseline.

3.3 Results

All subjects completed the studies without major side-effects. The most frequent side effects were sedation (which occurred in all subjects) and nausea (which occurred in 46 of the 67 subjects).

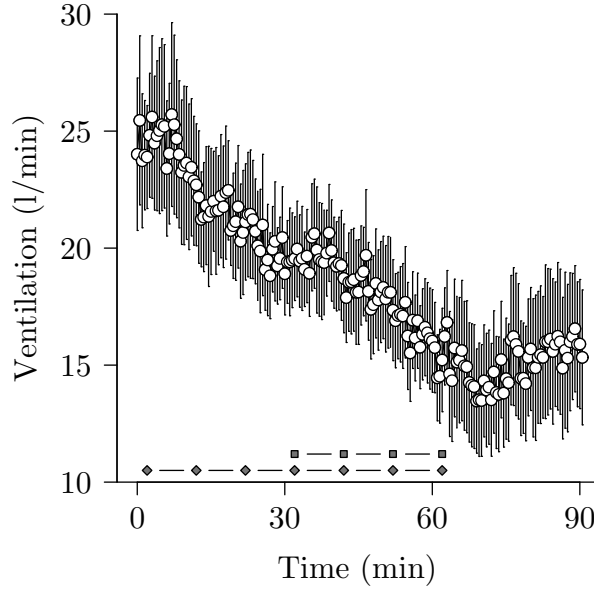


Figure 3.1: Effect of placebo on 0.2 mg buprenorphine-induced respiratory depression. Values are the mean values $\pm$ SEM ($n = 8$) of the 1-minute averages of individual subjects. Diamonds represent buprenorphine infusion; squares represent naloxone infusion.

Study 1

In the placebo group, buprenorphine decreased ventilation from $24.2 \pm 1.7 \text{ l}\cdot\text{min}^{-1}$ to $13.6 \pm 3.4 \text{ l}\cdot\text{min}^{-1}$ at $t = 75 \text{ min}$; in the naloxone group, ventilation decreased from $26.5 \pm 2.1 \text{ l}\cdot\text{min}^{-1}$ to $14.4 \pm 1.7 \text{ l}\cdot\text{min}^{-1}$ at $t = 75$ (not significant, analysis of variance). After infusion of $800 \mu\text{g}$ naloxone, ventilation at none of the measurement times differed between placebo and naloxone groups (analysis of variance). To detect a small effect of naloxone on ventilation unobserved in the pooled data analysis, we calculated the difference in ventilation from $t = 75$ to $t = 180$ minutes. In the placebo group, the change in ventilation was $0.2 \pm 0.5 \text{ l}\cdot\text{min}^{-1}$, versus $2.2 \pm 0.7 \text{ l}\cdot\text{min}^{-1}$ in the naloxone group. This difference did not reach the level of significance ($P = 0.08$, one-tailed Student t-test, assuming a larger response in the naloxone group).

Study 2

The mean effect of buprenorphine-placebo on minute ventilation is given in figure 3.1 and in figure 3.2 (grey area). Baseline ventilation was $24.0 \pm 3.3 \text{ l}\cdot\text{min}^{-1}$ at a fixed $P_{\text{ET},\text{CO}_2}$ of $52.9 \pm 0.9 \text{ mmHg}$. Peak depression of ventilation occurred at $t = 71$ minutes after the start of the buprenorphine infusion, reaching a value of $13.5 \pm 1.5 \text{ l}\cdot\text{min}^{-1}$. Relative to baseline, peak depression was $62 \pm 11\%$ of baseline, indicating a reduction of baseline ventilation by 38%. To get an impression of the naloxone data, we plotted representative data of two subjects given 2 and 6 mg naloxone in figure 3.2. The subject receiving 2 mg showed full reversal back to baseline (reversal = 1). In contrast, the subject given the higher naloxone dose showed little reversal (reversal = 0.1). In figure 3.3, we plotted the individual dose-reversal data for time frame 61 – 63 minutes. The data show that full reversal $\pm 20\%$ was obtained at doses between 2 and 4 mg naloxone

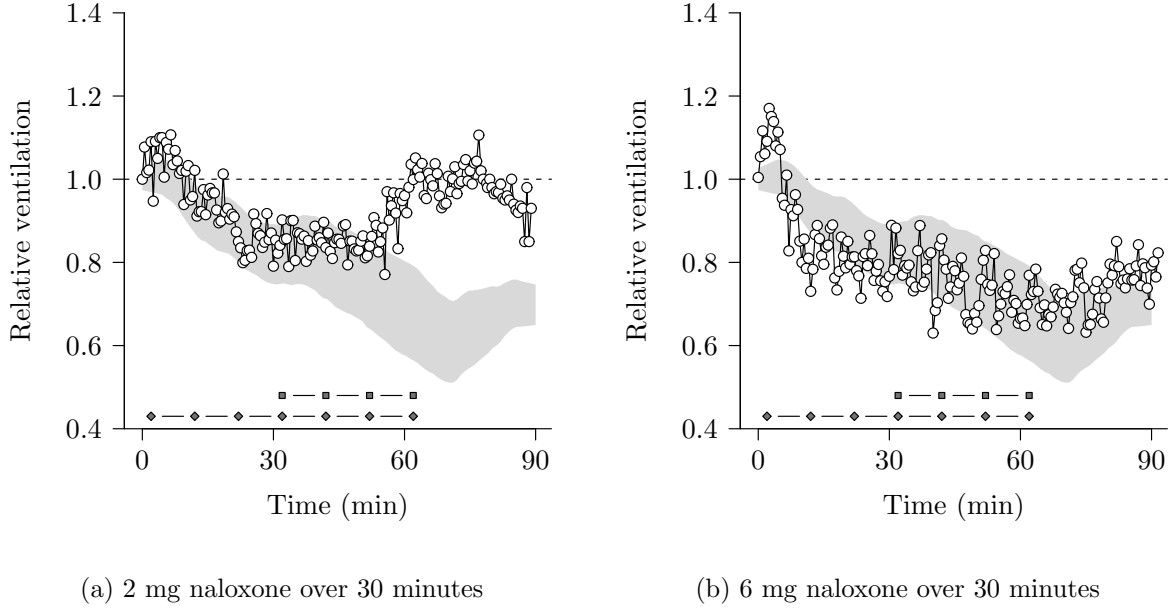


Figure 3.2: Individual plots of the actions of different naloxone doses on 0.2 mg buprenorphine-induced respiratory depression. Gray field in the background is the result of the placebo group. Diamonds represent buprenorphine infusion; squares represent naloxone infusion.

but that at higher doses, reversal gradually declined. We calculated the naloxone dose causing 50% reversal was 0.95 ± 0.09 mg and the dose causing the return to 50% depression was 5.20 ± 0.94 mg naloxone. Using NONMEM,¹¹ a sigmoid $E_{\max}$ function incorporating an inhibitory component was fitted to the data:

$$Y = \frac{X_1^\gamma}{(1 + X_1^\gamma)} - \left[\frac{1}{(1 + X_2^\gamma)} \right]$$

with Y : reversal
 X_1 : dose/ D_1
 X_2 : dose/ D_2

D_1 is the naloxone dose causing 50% reversal, and D_2 is the naloxone dose causing the return to 50% depression. Values obtained are $D_1 = 0.95 \pm 0.09$ mg, $D_2 = 5.20 \pm 0.94$ mg and $\gamma = 4.77 \pm 0.22$ (median $\pm$ SE). See also figure 3.3.

Study 3

Baseline ventilation averaged to 21.9 ± 2.5 l·min⁻¹ (data from studies 3.1 and 3.2 combined). The effects of both doses of buprenorphine (0.2 and 0.4 mg) were successfully reversed by a continuous infusion of naloxone at the dose chosen by us, which was, at least partly, based on the data from study 2.

Study 3.1. See figure 3.4a. A buprenorphine dose of 0.2 mg caused a rapid decrease in ventilation. Before naloxone or placebo infusion ($t = 32$ minutes), ventilation was 84

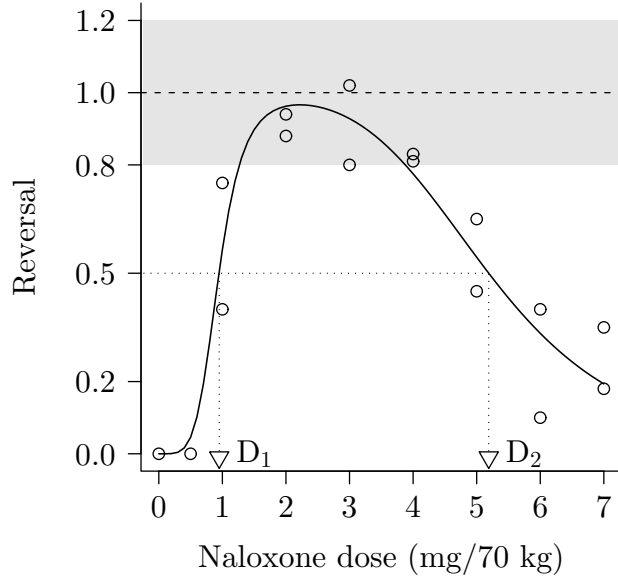


Figure 3.3: Influence of 0 (placebo) and 0.5 – 7.0 mg naloxone on 0.2 mg intravenous buprenorphine-induced respiratory depression. Circles are values of individual subjects (2 subjects received 0.5 mg naloxone, 8 subjects received placebo). Gray field indicates the area of full reversal $\pm 20\%$. Note the biphasic nature of the naloxone response.

± 3 and $79 \pm 5\%$ of baseline, respectively. In the placebo group, ventilation declined further to a nadir of $57 \pm 6\%$ of baseline at $t = 120$ minutes. In the naloxone group, the nadir was $78 \pm 4\%$ of baseline at $t = 48$ minutes (at the same time period, ventilation was $61 \pm 5\%$ of baseline in the placebo group). From that point on, ventilation increased to reach baseline values (i.e., baseline ventilation $- 1 \times 95\%$ confidence interval) at $t = 70$ minutes. Ventilation did not differ from baseline during the remainder of the naloxone infusion. After termination of the naloxone infusion (at $t = 152$ minutes), ventilation decreased, but it never reached the level observed in the placebo group.

Study 3.2. See figure 3.4b. A rapid decrease in ventilation occurred after the initiation of the 0.4 mg buprenorphine infusion. Before naloxone or placebo infusion ($t = 32$ minutes), ventilation was $62 \pm 5\%$ and $64 \pm 5\%$ of baseline, respectively. In the placebo group, ventilation declined further to a nadir of $40 \pm 3\%$ of baseline at $t = 150$ minutes. In the naloxone group, the ventilation nadir was $61 \pm 5\%$ of baseline at $t = 34$ minutes (ventilation of the placebo group was $66 \pm 7\%$ at $t = 34$ minutes). From that point on, ventilation increased to reach baseline values at $t = 93$ minutes. Ventilation did not deviate from baseline during the remainder of the naloxone infusion. After termination of the naloxone or placebo infusion (at $t = 152$ minutes), the changes in ventilation were similar to those observed in study 3.1.

3.4 Discussion

In our studies we observed that an intravenous dose of naloxone of 0.8 mg had no effect on respiratory depression induced by the opioid analgesic buprenorphine. We next explored the naloxone dose-response relation and observed that increasing doses of

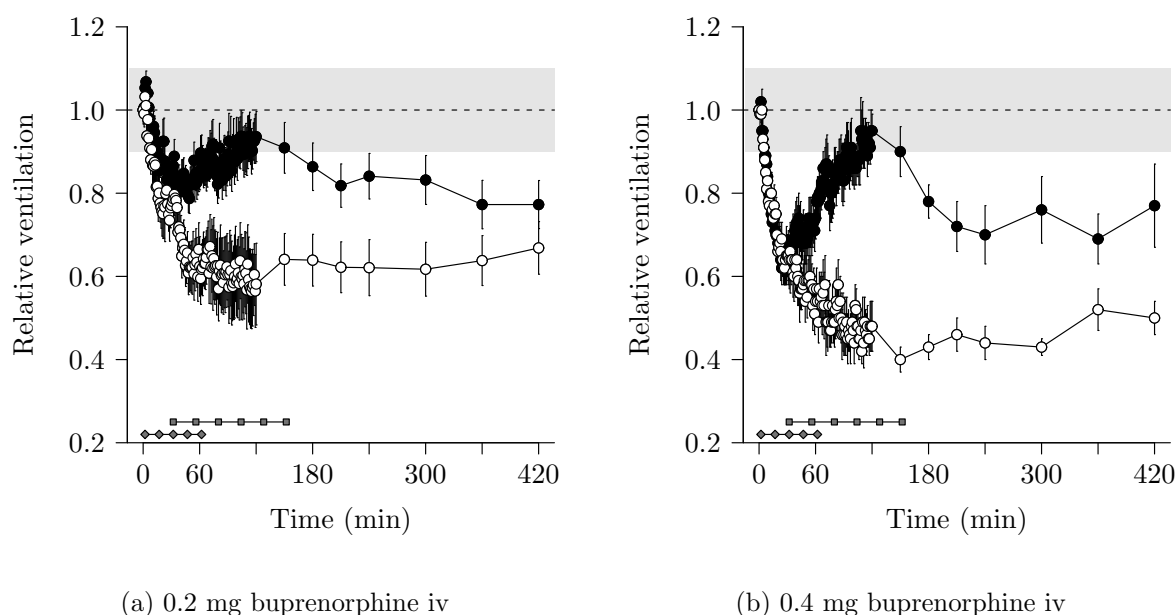


Figure 3.4: Influence of a continuous infusion of naloxone and placebo on buprenorphine-induced respiratory depression. Black circles represent naloxone ($n = 8$ per treatment); open circles represent placebo ($n = 8$ per treatment). Mean ventilation data are relative to baseline $\pm$ SEM. The gray area represents the 95% confidence interval of predrug baseline ventilation. The diamonds represent buprenorphine infusion ($t = 2$ until $t = 62$ minutes), the squares represent naloxone infusion ($t = 32$ until $t = 152$ minutes).

naloxone caused full reversal of buprenorphine respiratory depression (2 – 4 mg naloxone given in 30 minutes). Further increasing the naloxone dose (5 – 7 mg), however, caused a decline in reversal activity. The form of the dose-response relation is best described by a bell-shape or inverse U. Taking into account these data, we designed a naloxone infusion scheme intended to cause full reversal of the respiratory depression from 0.2 and 0.4 mg buprenorphine. A naloxone bolus dose of 2 – 3 mg, followed by a continuous infusion of 4 mg/h, caused full reversal within 40 – 60 minutes. Renarcotization did occur upon the termination of the naloxone infusion. These data indicate that reversal of buprenorphine-induced respiratory depression is possible but depends on the naloxone dose and its inverse U-shaped dose-response relation. That is, reversal is possible within a specific naloxone dose window. Furthermore, because respiratory depression from buprenorphine may outlast the effects of naloxone boluses or short infusions, a continuous infusion of naloxone may be required to maintain reversal of respiratory depression. Note that the design of studies 2 and 3 was such that it mimics the clinical situation in which a possible respiratory effect from a buprenorphine transdermal patch must be reversed by naloxone. A subcutaneous depot of buprenorphine will persist upon the removal of the patch. During the existence of this depot and the need for reversal, naloxone and buprenorphine will then be released or administered simultaneously to the blood (as they are in studies 2 and 3).

All opioids that interact with the μ -opioid receptor system depress respiration.¹² The

extent of respiratory effect is highly variable and is related to the specific opioid, the opioid dose, the administration mode, concurrent medication, underlying disease, pain and the state of arousal (these two factors vary over time), genetics, and exogenous stimulatory factors. Because the occurrence of overt and sometimes life-threatening respiratory depression is often unpredictable, the ability to induce rapid opioid reversal is of evident importance. In contemporary medicine, naloxone has become the drug of choice for treatment of opioid-induced respiratory depression. Naloxone is a nonspecific opioid receptor antagonist (i.e., it antagonizes the μ -, κ -, and δ -opioid receptors) with a relatively short duration of action resulting from rapid elimination; its half-life in plasma is approximately 30 – 45 minutes.¹³

There is ample evidence that buprenorphine, like other μ -opioid receptor agonists, produces significant respiratory depression at clinical doses (figs. 3.1 and 3.4), although we recently showed that buprenorphine-induced respiratory depression, unlike other μ -opioids, shows an apparent maximum in effect (ceiling).^{4,14} Interestingly, only sparse data from the literature has addressed the issue of reversal of buprenorphine induced respiratory depression.⁵⁻⁷ The picture that emerges from these few studies is that even at relatively large bolus naloxone doses, little (i.e., only partial) reversal of the respiratory effects of buprenorphine is observed. For example, a recent short report indicates that an incremental naloxone dose of 2.4 mg has an effect on 0.4 mg buprenorphine-induced respiratory depression no greater than placebo in patients during sevoflurane-N₂O anesthesia.⁷ An older study by Gal⁵ showed only partial reversal of 0.3 mg buprenorphine with 5 and 10 mg intravenous naloxone (given as single bolus). The inability to obtain full reversal in these two studies may be related to various factors, such as anesthesia (anesthesia must be considered a serious complication when studying opioid-induced respiratory depression due to the complex opioid-anesthetic interaction on breathing),^{15,16} the lack of sensitivity of the respiratory model applied to assess naloxone-buprenorphine interaction, the use of single naloxone doses, and finally, the use of an overly high dose of naloxone (fig. 3.3).

The resistance to naloxone reversal is related to the high affinity of buprenorphine for the μ -opioid receptor.^{1,3} This high affinity explains why relatively high doses of naloxone (2 – 4 mg) are needed before reversal is observed. The need for a continuous infusion in this process (upon termination of the naloxone infusion, there was a rapid return of respiratory depression; fig. 3.4) implies the need for continuous supply of naloxone to the opioid receptor sites in the brain involved in respiratory depression. Otherwise, the naloxone bolus is rapidly washed out from the brain compartment and eliminated from the body. We believe that the use of a single dose of naloxone infusion to reverse opioid-related overdose has several disadvantages that are unrelated to the opioid involved: renarcotization due to the short duration of action of naloxone, the inability to titrate to effect causing the return of pain, and sympathico-excitation. An infusion regimen aimed at a prolonged and steady state naloxone plasma concentration may overcome these shortcomings. For example, continuous (eleven hour) naloxone

infusion after high-dose fentanyl anesthesia caused reversal of respiratory depression without causing renarcotization, pain, or sympathico-excitation.¹⁷

An interesting observation in studies 3.1 and 3.2 is that higher ventilation levels were recorded after naloxone treatment than after placebo treatment at times when naloxone is washed out from the brain and possibly also from the body (fig. 3.4 at $t = 240$ minutes). This is probably due to washout from the brain compartment of buprenorphine which was replaced by naloxone at the μ -receptor or at nonspecific binding sites (i.e., some buprenorphine was lost without replacement).

Naloxone doses exceeding the maximal effective dose (> 4 mg) lead to a decrease in (0.2 mg) buprenorphine reversal efficacy (fig. 3.4a). Because the number of subjects was limited (just two subjects per naloxone dose over the dose range from 0.5 to 7 mg), we consider this observation preliminary. Evidently, further studies are needed. In a first attempt, we performed a set of experiments after 0.4 mg buprenorphine and applied various naloxone doses (one dose per subject; duration of naloxone infusion 30 minutes) and observed a similar bell-shaped dose-response relation, albeit full reversal was not reached (A. Dahan, unpublished observation, September 2004 – January 2005). Our unexpected observation is most probably specific to buprenorphine and its interaction with naloxone. Buprenorphine has a long history of showing bell-shaped dose-response curves with respect to its analgesia and side effect profile.¹ Most of these observations were made in animals. For example, rodents display a bell-shaped buprenorphine dose-response relation in various antinociceptive assays (electrical pain, heat pain, visceral pain, and spinal nerve ligation).^{18,19} In humans too, there are indications of the existence of a bell-shaped dose-response curve with respect to analgesia. For example, two patients treated with buprenorphine (0.03 – 0.04 mg/kg) for postoperative pain showed improved pain relief after 0.4 mg naloxone infusion, probably due to shifting of the bell-shaped dose-analgesia curve to the right.²⁰ A rightward-shift of the bell-shaped buprenorphine dose-response curve after the infusion of an opioid-antagonist (naltrexone) has been observed in rats using an electrical pain test.²¹ A bell-shaped curve for buprenorphine's analgetic effect was never observed in experimental human studies and clinically, complete analgesia is reached with buprenorphine.

The mechanism of the bell-shaped curve remains unknown. Some argued that the form of the curve is related to the type and intensity of (experimental) pain administered.²¹ Others suggested non-competitive auto-inhibition, in which there are two receptor subpopulations, one mediating the agonistic properties at low dose, the other mediating the antagonistic properties at high dose.^{3,18,19,21} Finally, Lutfy *et al.*²² suggest the contribution of the ORL-1 receptor. They showed that buprenorphine, but not morphine, given to mice activates ORL-1 receptors, compromising (antagonizing) analgesia from μ -opioid receptors. The latter theory seems unplausible, however, when one takes into account that the sparse literature that exists on the respiratory effects of stimulation of ORL-1 receptors shows respiratory depression rather than stimulation.²³ The existence

of two μ -opioid receptor subpopulations as described above could theoretically explain our findings with high-dose naloxone causing the antagonism of the receptors mediating the antagonistic effects of buprenorphine. We are not aware, however, of any observation of these two receptor subpopulations in *in vitro* or *in vivo* animal studies. The results of our studies demonstrate that the specific dose and mode of administration of naloxone to restore breathing and to maintain it at an adequate level are complex matters that require further study. Our data show that even after administration of large boluses of naloxone or boluses plus brief infusions, respiratory depression induced by buprenorphine recurred and persisted for the duration of the study (seven hours in study 3). Additional studies are required to define the dose and the mode of administration of naloxone to restore breathing and to maintain it at an adequate level in the clinical setting, which is complicated by acute and chronic pain, gender effects,²⁴ high doses of opioids, long-acting opioids, and various sustained-release preparations of opioids.

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Different time-effect profiles for naloxone
reversal of morphine and M6G-induced
respiratory depression

4.1 Introduction

In clinical practice, opioids remain the cornerstone of pain therapy. One of their great disadvantages however is the number of side-effects they cause, such as sedation, nausea, vomiting and respiratory depression. Especially the latter is highly important, as it can lead to coma or even death of the patient. Opioid antagonists, such as naloxone, enable reversal of opioid-side effects. Reversal is not only dependent on the pharmacokinetics and pharmacodynamics characteristics of naloxone itself but also on the pharmacokinetics (PK) and pharmacodynamics (PD) of the opioid that requires reversal.^{1,2} However there is limited knowledge on the complex interaction of naloxone and the various opioids used in clinical practice. Despite the widespread clinical use of naloxone in opioid overdose, few studies addressed naloxone reversal of opioid-induced respiratory depression.³⁻⁷

In our laboratory, we recently modeled the interaction of buprenorphine and naloxone regarding buprenorphine-induced respiratory depression.⁸ To our knowledge, there are no other PK/PD models on reversal of opioid-induced respiratory depression. In the current study, we performed a series of experiments with naloxone, morphine (MOR) and morphine-6-glucuronide (M6G), investigating the effects of these opioids and several naloxone doses on ventilation. The aim of this study was to characterize naloxone's reversal of respiratory depression induced by these two opioids. Taking the pharmacokinetic parameters from previous studies⁸⁻¹⁰ and using minute ventilation at a constant carbon dioxide (CO₂) level as an end-point for the pharmacodynamic analysis, we performed a pharmacodynamic modelling study.

4.2 Methods

Subjects

A total of 56 male and female volunteers (age range: 18 – 34 years, weight range: 50 – 95 kg) participated in 2 main studies (study A and study B, for study design, see table 4.1). They were recruited after approval of the protocol by the local Human Ethics Committee. Oral and written consent was obtained from all subjects. Before participation, all subjects were screened at the Pre Operative Screening Unit of the Anesthesiology Department of the Leiden University Medical Center (LUMC). All subjects were healthy and did not have a history of drug or alcohol abuse. All women were taking contraceptives. On the study day itself, subjects were asked to refrain from alcoholic beverages for at least twelve hours prior to the study, and from any food or drink for at least eight hours prior to the study. After arrival in the laboratory, subjects were seated comfortably in a hospital bed.

Subgroup	Study A (M6G)	Study B (MOR)
1	0 μg (placebo) 400 μg	0 μg (placebo) 400 μg
2	25 μg 100 μg	200 μg

Table 4.1: Schematic overview of the study design

Apparatus

For the whole duration of the study, peripheral oxygen saturation (S_pO_2) of the subjects was monitored using a fingerprobe with a Masimo Signal Extraction Pulse Oximeter (Masimo Co., Irvine, CA, USA). Subjects breathed through a face mask (Intersurgical, Wokingham, United Kingdom). In- and expired gas flows were measured with a pneumotachograph (Hans Rudolph, Myandotta, MI, USA) connected to a pressure transducer and integrated to yield a volume signal. The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture, with a flow of $45 \text{ l}\cdot\text{min}^{-1}$, from a gas-mixing system consisting of three mass-flow controllers (Bronkhorst High-Tech BV, Veenendaal, The Netherlands), that could be used to individually set the flow of nitrogen (N_2), oxygen (O_2) and CO_2 at a desired level.

A personal computer provided signal control to the mass-flow controllers so that the inhaled gas mixtures could be adjusted to be able to force the end-expiratory levels of O_2 and CO_2 , regardless of the ventilatory response (dynamic end-tidal forcing technique). End-tidal O_2 concentration (P_{ET,O_2}), end-tidal CO_2 concentration (P_{ET,CO_2}), tidal volume (V_T), respiratory frequency (f), inspired minute ventilation ($\dot{V}_i$) and peripheral oxygen saturation (S_pO_2) were collected and stored on disk for further analysis. The in- and expired O_2 and CO_2 concentrations were measured with a Datex Multicap gas monitor (Datex Engstrom, Helsinki, Finland). The software for steering the respiration and for collecting the above mentioned parameters (ACQ and ResReg) was custom-built locally in the LUMC.

At the start of the experiment, before drug infusion, resting values (P_{ET,O_2} , P_{ET,CO_2} , $\dot{V}_i$) for the volunteers' breathing were obtained. Next, subjects' respiration was stimulated by gradually increasing P_{ET,CO_2} until a inspired minute ventilation ($\dot{V}_i$) of between 20 and $30 \text{ l}\cdot\text{min}^{-1}$ was reached. Once steady state was accomplished, $\dot{V}_i$ values were stored on disk for further analysis. These values were taken as baseline values for the other measurements. The P_{ET,CO_2} at which ventilation was increased to about $20\text{--}30 \text{ l}\cdot\text{min}^{-1}$ (on average 7.0 kPa), was kept constant for the duration of the measurements.

Study design

Both studies were placebo controlled and had a single blind design. Both studies had two subgroups – so there were four subgroups (A1, A2, B1 and B2), with seven arms in total (see Table 4.1). Initially, only the experiments in subgroups A1 and B1 were performed. Due to the unexpected results found in those studies, we decided that more naloxone doses would need to be studied before we could draw any firm conclusions. Hence the need for experiments in subgroups A2 and B2. Randomization took place within the four subgroups, but subjects were kept naive with respect to the particular drugs they would receive in their subgroup. They were told they would either receive morphine or M6G, followed by either a placebo or a dose of ‘antidote’.

M6G was supplied by CeNeS Ltd (Cambridge, UK). The hospital pharmacy delivered morphine (manufactured by Teva Pharmachemie, Haarlem, The Netherlands), naloxone (manufactured by the pharmacy) and placebo (0.9% NaCl, manufactured by the pharmacy). All drug doses are per 70 kg, and all doses were administered as bolus injections over 90 s. Before the start of experiments, an anti-emetic drug (ondansetron, 4 mg iv) was administered to all subjects.

Study A1 Sixteen subjects participated in this study. All received a bolus dose of 21 mg morphine-6-glucuronide (M6G) at $t = 0$ minutes followed by 400 μg naloxone (in eight subjects) or placebo (in eight subjects) at $t = 55$ minutes. Continuous measurement of respiration started at $t = 45$ minutes and continued until $t = 145$ minutes. Subsequent measurements of steady-state ventilation (measurement period ± 7 minutes) took place at time points 180, 210 and 240 minutes.

Study B1 Sixteen subjects participated in this study. All received a bolus dose of 10.5 mg morphine at $t = 0$ minutes, followed by 400 μg naloxone (in eight subjects) or placebo (in eight subjects) at $t = 30$ minutes. Respiration was measured constantly from two minutes before morphine infusion until $t = 90$ minutes. Subsequent steady state ventilation was measured at time points 150, 180 and 210 minutes.

Study A2 Sixteen subjects participated in this study, which was the follow-up study to study A1. All subjects received an M6G bolus dose of 21 mg at $t = 0$ minutes, which was followed by a naloxone dose at $t = 55$ minutes of either 25 μg (eight subjects) or 100 μg (eight subjects). Respiratory measurements were continuous from $t = 45$ minutes until $t = 120$ minutes.

Study B2 Eight subjects participated in this study, which was the follow-up study to study B1. In this study, subjects received a morphine bolus dose of 10.5 mg, which was followed by a bolus dose of naloxone 200 μg at $t = 30$ minutes. Respiration was measured constantly from two minutes before morphine infusion until $t = 120$ minutes.

Data Analysis

Descriptive analysis

Plotting and descriptive statistical analyses were conducted using the statistical package R (version 2.8).¹¹ The breath-to-breath data, obtained from the pneumotachograph using the ACQ and ResReg software were averaged over one minute periods. An ensemble average was performed on all groups separately. We compared baseline ventilation levels and the levels of ventilation just before administration of the antidote, within the studies, using a one-way ANOVA.

Time of maximum reversal (i.e., time at which ventilation levels were highest), $t_{\max}$, as well as the corresponding levels of ventilation, $\dot{V}_{\max}$, were computed for each individual and averaged per group. These were considered ‘summary measures’ and compared using a one-way ANOVA within the studies.¹² Then $t_{\max}$ and $\dot{V}_{\max}$ were compared between the two studies using a Student t-test. In all comparisons, a $p < 0.05$ was considered significant.

Pharmacokinetic Models

Because in this study no blood samples were taken, we assume that morphine, M6G and naloxone concentrations can be described by earlier established pharmacokinetic models.^{8–10}

Pharmacodynamic Model

The differential equations describing morphine or M6G (M) and naloxone (N) molecules binding receptors (R) are¹³

$$\begin{aligned}\frac{d[MR]}{dt} &= k_{\text{on},M} \cdot [M] \cdot [R] - k_{\text{off},M} \cdot [MR] \\ \frac{d[NR]}{dt} &= k_{\text{on},N} \cdot [N] \cdot [R] - k_{\text{off},N} \cdot [NR].\end{aligned}\tag{4.1}$$

If $k_{\text{off},N}$ is large we may assume $k_{\text{on},N} \cdot [N] \cdot [R] - k_{\text{off},N} [NR] = 0$, or $[NR] = [N] \cdot [R] / C_{50,N}$, with $C_{50,N} = k_{\text{off},N} / k_{\text{on},N}$. Furthermore, with $[RT] = [R] + [RM] + [RN]$, and the normalization of $[MR]$ and $[NR]$ by setting $[RT] = 1$ (without loss of generality), we have

$$[NR] = \frac{[N]/C_{50,N}}{1 + [N]/C_{50,N}} \cdot (1 - [MR]).\tag{4.2}$$

Naloxone dose (μg)	0	25	100	400
Age (yr) (range)	24.0 (18–34)	21.5 (18–27)	20.9 (18–24)	22.3 (19–34)
Weight (kg)	70.6 ± 11.8	65.9 ± 6.6	70.4 ± 9.5	65.6 ± 9.03
Height (cm)	176 ± 8.8	171 ± 9.2	180 ± 8.0	173 ± 5.5
Sex (m:f)	3:5	3:5	4:4	5:3
Baseline ventilation	28.65 ± 4.16	25.36 ± 2.6	23.71 ± 1.5	23.79 ± 4.7

Table 4.2: Baseline characteristics for study A; group means with standard deviations

For an adequate description of the data, we introduced a steepness parameter γ for naloxone and wrote

$$[NR] = \frac{([N]/C_{50,N})^\gamma}{1 + ([N]/C_{50,N})^\gamma} \cdot (1 - [MR]). \quad (4.3)$$

The delay between drug concentration in the blood and at the receptor site(s) was characterized by parameters $t_{1/2,k_{e0}}$ for each drug.

Finally, ventilation $\dot{V}$ was assumed to be dependent on $[MR]$ according to

$$\dot{V} = \dot{V}_0 \cdot (1 - [MR]). \quad (4.4)$$

where $\dot{V}_0$ is the baseline (pre-drug) value. Because $[MR]$ was normalized, $0 < [MR] < 1$ and $0 < \dot{V} < \dot{V}_0$.

Statistical Analysis

The pharmacodynamic data were analyzed using NONMEM VI¹⁴. Model parameters were assumed to be log-normally distributed across the population, except the Hill parameter γ , which was assumed to lie between 0 and 20 via the inverse logit transformation, to avoid extremely large values of γ causing numerical problems. Residual error was assumed to be additive with variance σ^2 .

4.3 Results

All 56 subjects completed the study without major side-effects. Their baseline characteristics, divided by subgroup, are shown in table 4.2 and 4.3. Minor side-effects that were reported included ‘heavy feeling’ (exclusively in study A), nausea, but no vomiting (in both studies) and sedation (exclusively in study B). All symptoms were mild and did not need further treatment.

Naloxone dose (μg)	0	200	400
Age (yr) (range)	21.9 (19–24)	23.0 (19–26)	22.4 (20–27)
Weight (kg)	67.8 ± 17.4	71.4 ± 9.1	67.6 ± 16.6
Height (cm)	174 ± 10.8	175 ± 7.9	179 ± 8.9
Sex (m:f)	2:6	3:5	4:4
Baseline ventilation	22.19 ± 6.7	25.28 ± 1.8	20.60 ± 2.3

Table 4.3: Baseline characteristics for study B; group means with standard deviations

Descriptive analysis

Baseline ventilation averages can be found in tables 4.2 and 4.3. Because there were significant differences between the M6G subgroups (the placebo subgroup differed significantly from the 100 and 400 groups, one-way ANOVA $p=0.026$), we did all further descriptive analyses using the relative ventilation values (i.e., absolute ventilation values divided by the baseline ventilation). This led to the graphs seen in figures 4.1 and 4.2, where the mean relative ventilation values are shown.

We observed that after administration of the opioids, ventilation levels fell quickly to about 65% of baseline ventilation (M6G per subgroup: 67.7, 65.8, 66.3 and 65.4 %; MOR per subgroup: 69.1, 67.1 and 70.4 %) just before administration of the antidote. Comparison of these values for both studies separately showed no significant differences (study A, one-way ANOVA: $p=0.635$ and study B, Student t-test: $p=0.358$). Comparison of both studies (using a pooled average for study A and study B: $65.4 \pm 1.7\%$ and $70.4 \pm 2.3\%$ respectively) showed no significant differences either (Student t-test, $p=0.075$), suggesting that at the doses used in this study, morphine and M6G cause the same amount of CO_2 -driven respiratory depression. A comparison of $t_{\max}$ -values shows a mean $t_{\max}$ of 44.39 ± 2.73 minutes for the M6G groups (pooled average) and a mean $t_{\max}$ of 13.33 ± 1.2 minutes, a significant difference of 31 minutes ($p < 0.001$). So maximum reversal is reached far later in the M6G group than in the morphine groups.

PK/PD analysis

Initially, all 56 subjects were included in the PK/PD analysis. However, three outliers were identified. In study A (M6G), ID 25 was an outlier with respect to $C_{50,N}$. The data from this subject were not discarded, but $C_{50,N}$ was fixed to 10. ID 31 from study A was also an outlier and therefore γ was fixed to 20. In study B, ID 5 was an outlier and those data were discarded.

Morphine, M6G and naloxone pharmacokinetic parameters for a similar group of subjects have been published earlier.^{8–10} For the present re-analysis, weight was introduced as a covariate. Dose was proportional to weight. For the pharmacodynamic param-

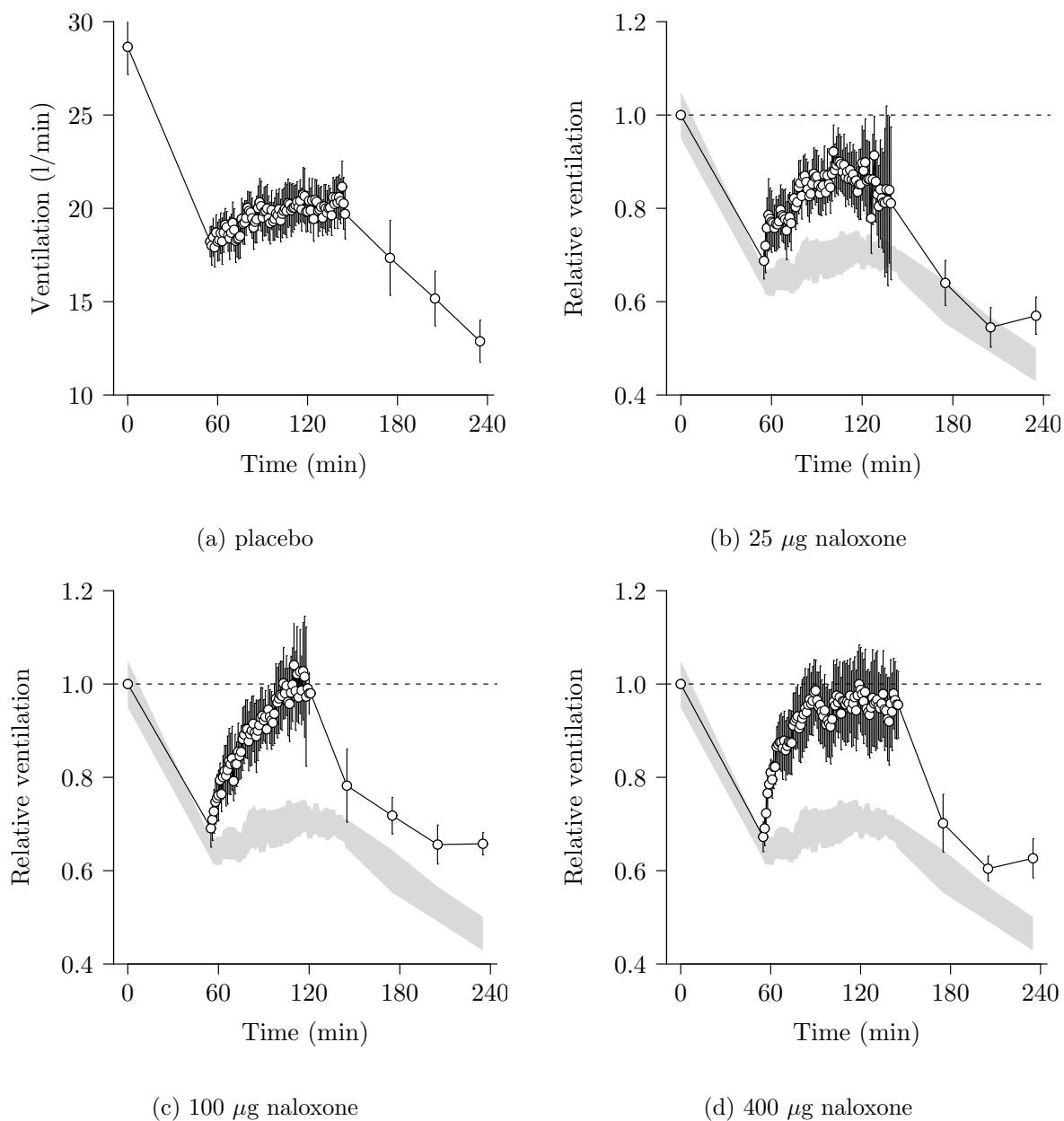


Figure 4.1: Actions of different naloxone doses in study A (M6G). Figure (a) represents the absolute group average $\pm$ SEM for the placebo group. Figures (b), (c) and (d) show separate group means $\pm$ SEM, relative to baseline ventilation. Grey field in the background is the relative mean of the placebo group.

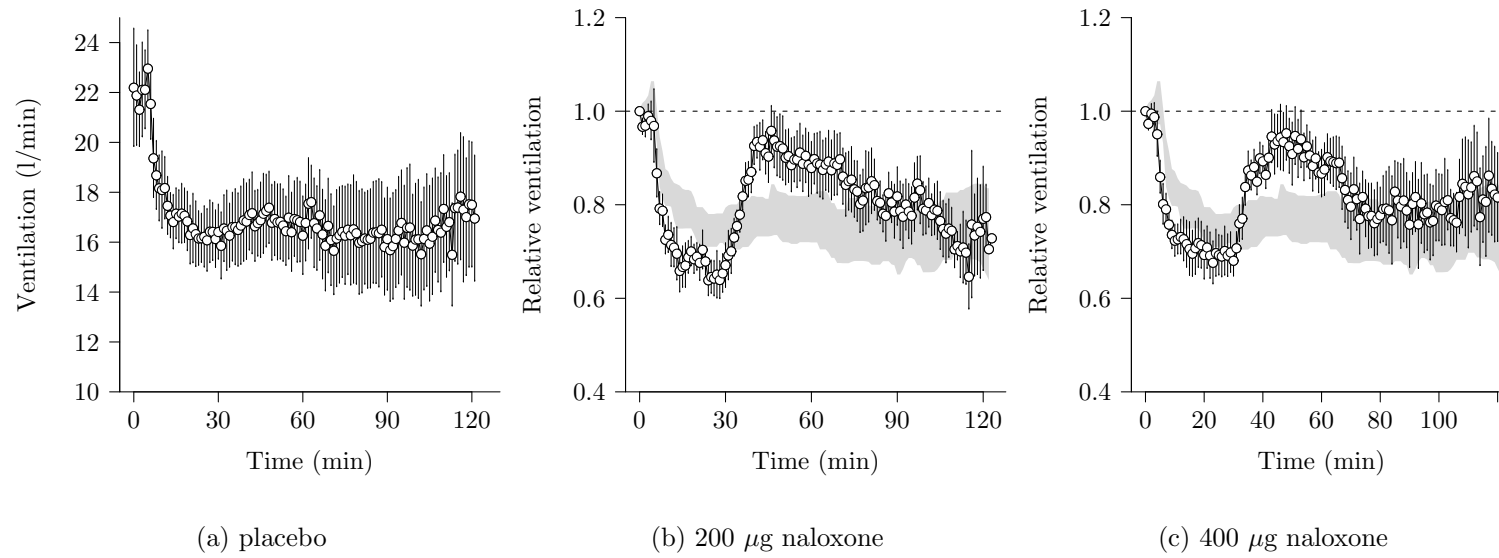


Figure 4.2: Actions of different naloxone doses in study B (morphine). Figure (a) represents the absolute group average $\pm$ SEM for the placebo group. Figures (b) and (c) show separate group means $\pm$ SEM, relative to baseline ventilation. Grey field in the background is the relative mean of the placebo group

	Θ	SE	ω^2	SE
$t_{1/2,k_{e0},M6G}$ (h)	2.72	0.422	0.187	0.0770
$k_{on,M}$ (nM ⁻¹ ·min ⁻¹)	0.0371	0.00825	0.421	0.130
$k_{off,M}$ (min ⁻¹)	0.0327	0.00455	0.0459	0.0456
$t_{1/2,k_{e0},N}$ (min)	5.42	0.532	—	—
$C_{50,N}$ (nM)	0.484	0.102	0.233	0.0622
$\dot{V}_0$ (l·min ⁻¹)	25.3	0.790	0.0180	0.00613
γ	7.42	1.19	1.75	0.869
σ^2 (l·min ⁻¹)	1.83	0.195		

Table 4.4: Pharmacodynamic parameters for study A (M6G). Θ is the population parameter, ω^2 is the variance of Θ across the population in the log domain. ω^2 was not estimable for the $t_{1/2,k_{e0}}$.

	Θ	SE	ω^2	SE
$t_{1/2,k_{e0},M}$ (h)	1.24	0.172	0.160	0.0727
$k_{on,M}$ (nM ⁻¹ ·min ⁻¹)	0.853	0.112	0.192	0.0672
$k_{off,M}$ (min ⁻¹)	0.138	0.0148	0.112	0.0514
$t_{1/2,k_{e0},N}$	11.2	2.66	—	—
$C_{50,N}$ (nM)	1.84	0.181	0.141	0.0463
$\dot{V}_{0,200}$ (l·min ⁻¹)	26.5	1.10	0.0144	0.00342
$\dot{V}_{0,400}$ (l·min ⁻¹)	21.5	1.03	0.0144	0.00342
γ	4.18	0.718	0.938	0.432
σ^2 (l·min ⁻¹)	2.18	0.347		

Table 4.5: Pharmacodynamic parameters for study B (morphine). Θ is the population parameter, ω^2 is the variance of Θ across the population in the log domain. The $\dot{V}_0$ differed significantly between the two groups, and is therefore shown as two separate parameters. The $t_{1/2,k_{e0}}$ for the 400 μ g group was not estimable, so only the $t_{1/2,k_{e0}}$ shown only applies to the 200 μ g. ω^2 was not estimable for the $t_{1/2,k_{e0}}$.

eters, see tables 4.4 and 4.5. In figures 4.3 and 4.4 representative plots of data from individual subjects are shown, together with their model fit.

4.4 Discussion

At the doses chosen, morphine and M6G cause ventilatory depression of similar magnitude with a depression of baseline ventilation of about 35%. The respiratory effects of both opioids can be reversed, but the characteristics of reversal differed. Our data support the clinical observation that reversal of morphine-induced respiratory depression sets in rapidly and lasts about 30 minutes. After that, opioid effect returns and respiratory depression becomes apparent again. For M6G, a different picture emerges from our data: time to maximum reversal is relatively slow (45 minutes for M6G versus 13 minutes for morphine), but reversal does last longer.

From the PK/PD modelling, two main observations were made. First, the C_{50} for naloxone differs between the M6G and morphine groups. This implies that the potency of naloxone after M6G is higher than after morphine, i.e., that M6G's respira-

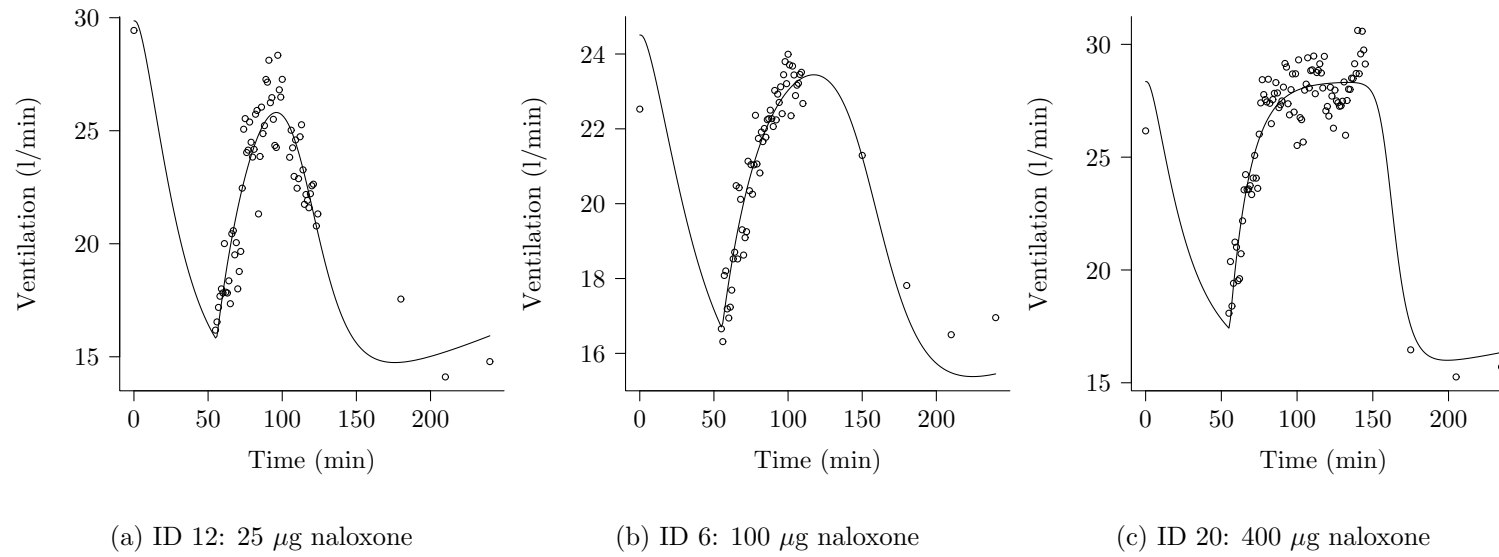


Figure 4.3: Representative examples of individual fits for each subgroup in study A (M6G). R^2 for these fits is 0.83, 0.91 and 0.88 respectively, R^2 for all fits ranges from 0.49 to 0.91, median $R^2 = 0.74$.

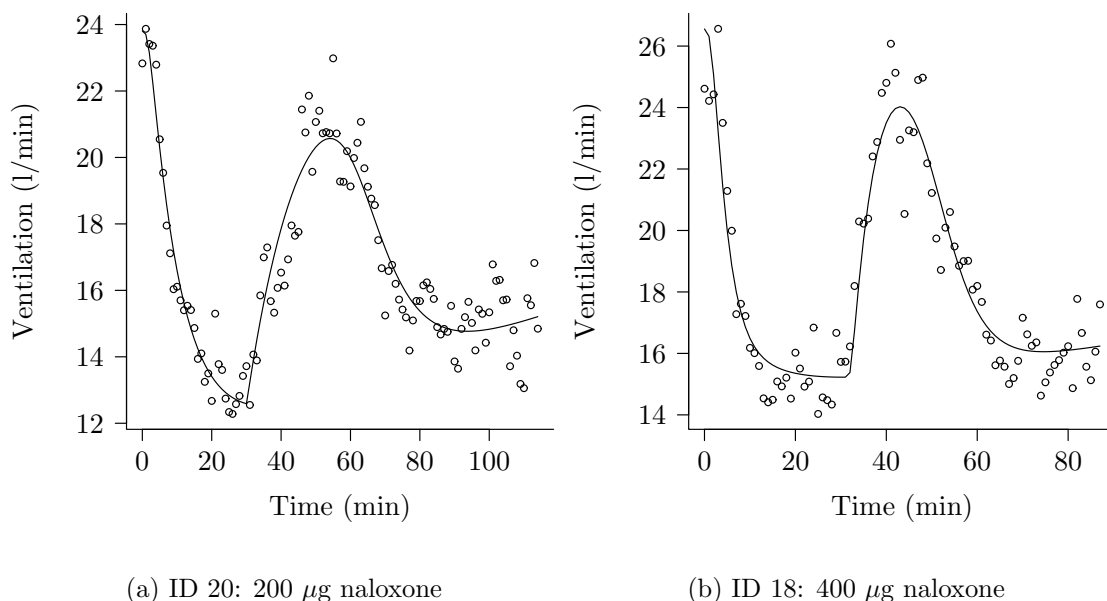


Figure 4.4: Representative examples of individual fits for each subgroup in study B (morphine). R^2 for these fits is 0.89 and 0.88 respectively, R^2 for all fits ranges from 0.38 to 0.90, median $R^2 = 0.78$.

tory effects are reversed at a lower dose of naloxone. A higher potency of naloxone in M6G-induced respiratory depression may be due to a specific kind of interaction between these two drugs at the receptor level, in which one of the two drugs increases the affinity of the other drug at the μ -opioid receptor. This has been described in 1988 by Abbott and Palmour¹⁵, who showed that M6G increased binding of naloxone to μ -opioid receptors at rat brain membrane preparations. They also state that morphine does not have this effect. Furthermore, it could be that M6G changes the state of the G-protein associated with the μ -opioid receptor, thus altering the signalling cascade within the cell. Circumstantial evidence for this is that M6G causes hyperalgesia in specific pain tests, which could be caused by ‘flipping’ the G-protein to a different state.¹⁶ Further, it could be that M6G limits the efflux of naloxone from the brain compartment, and thus increasing the apparent potency of naloxone. Finally, it may well be that morphine and M6G act at a different opioid-receptor (see Chapter 2 and references therein), each with a different naloxone affinity.

A second observation in our PK/PD analysis is that a ‘simple’ competitive interaction model is not sufficient to describe our data: the introduction of a Hill factor ($\gamma \neq 1$) is needed to adequately describe the data. The high γ found for both morphine and M6G would suggest that naloxone reversal of opioid-induced respiratory depression is subject to a threshold phenomenon: if naloxone brain concentration exceeds a certain level, reversal sets in, and if it would fall below this level, reversal wanes. This could be in concordance with the naloxone efflux limitation mentioned above.

The combination of a γ and a difference in C_{50} could also be explained differently. Shafer *et al.* postulate in a recent article that if an antagonist would act at several places in the signalling cascade, this would increase the apparent potency (lower C_{50}) and steepness (γ) parameters.¹⁷ If naloxone would not only act at the μ -opioid receptor, but also at different places in the signalling cascade (a second binding place on the μ -opioid receptor itself, an influence on the G-protein state, a direct influence on the K^+ channels of the neuron, or even a separate receptor), the C_{50} and the introduction of the γ could be explained.

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SECTION III

Analgesia and hyperalgesia

Morphine-6-glucuronide induces
hyperalgesic responses to experimental heat
pain in mice and healthy volunteers

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Anesthesiology 2009; 110

5.1 Introduction

In contemporary clinical medicine, μ -opioids such as morphine are the first choice for treating severe acute and chronic pain.¹ However, opioid use is associated with several unwanted side-effects, including a paradoxical increase in pain sensitivity. This opioid-induced hyperalgesia (OIH) has been reported in pre-clinical studies with rodents and humans and described in the clinical literature.¹⁻⁴ Although it is widely postulated that activating opioid receptors or opioid analgesia are critical initial prerequisites for OIH,⁵⁻⁹ contrary results have been recently reported. For example, infusing the μ -opioids morphine and oxymorphone evoked hyperalgesic responses within 48 hours in opioid receptor triple “knock-out” (TrKO) mice completely devoid of opioid receptors.¹⁰ Hyperalgesia during continuous morphine infusion is also observed in outbred CD-1 mice implanted with pellets containing naltrexone (NTX), a general opioid receptor antagonist.^{11,12} N-methyl-D-aspartate (NMDA) receptor antagonists such as MK-801 reverse morphine hyperalgesia.^{11,12} Since NMDA antagonists also potentiate opioid analgesia, they might attenuate hyperalgesia indirectly, by increasing the latent opioid analgesia obfuscated by the concurrent increased nociception. However, this possibility is precluded by the demonstration that MK-801 reverses morphine hyperalgesia in NTX-pelleted mice.^{11,12}

In humans, morphine undergoes hepatic glucuronidation to more water-soluble compounds, facilitating their renal elimination.¹³ One of these metabolites, morphine-6-glucuronide (M6G), displays affinity at μ -opioid receptors equal to that of morphine, and is a potent opioid analgesic.^{13,14} However, data from some studies suggest that acute M6G doses can cause hyperalgesia. In the first two, a single acute M6G injection reduced tail-withdrawal latencies by up to 40% in mice lacking exons 1 and/or 2 of the μ -opioid receptor.^{15,16} In a third study, low M6G doses (10 and 20 mg/70 kg) progressively increased the time to rescue analgesic medication in patients after orthopedic surgery, while a higher dose (30 mg/70 kg) caused a subsequent decrease in the time to rescue medication, which may be considered a manifestation of hyperalgesia.¹⁷ Finally, we recently demonstrated in an open label study that a single injection of M6G increased pain ratings in healthy volunteers that underwent a cutaneous heat pain assay.¹⁸ Since M6G hyperalgesia was not the specific aim of these studies, several questions remain. Specifically, it is not known whether M6G causes hyperalgesia independently of opioid receptor activity, or whether NMDA receptors contribute to this effect. Furthermore, since only acute doses of M6G were injected in these studies, it is not known what effect longer M6G delivery protocols might have on nociception. These questions can not be addressed by simply extrapolating from studies with morphine, as morphine metabolism in mice does not yield M6G. Furthermore, morphine conjugation in rodents and humans also yields morphine-3-glucuronide (M3G), a pronociceptive metabolite thought to underlie morphine hyperalgesia.^{10-12,19,20} If both morphine metabolites are indeed pronociceptive, it would not be possible to distinguish between their hyperalgesic effects in human subjects treated with morphine.

Here, we addressed these issues by assaying nociceptive sensitivity in mice and human volunteers injected with an acute M6G dose. The contribution of opioid receptors to M6G hyperalgesia was assessed by treating subjects concurrently with an opioid receptor antagonist. Additional evidence was obtained by testing TrKO mice devoid of any opioid receptor type under identical conditions. The long-term consequences of M6G infusion on nociception was also assessed by assaying nociception daily in mice subject to six days of continuous M6G infusion. For both acute and chronic M6G treatment conditions, the ability of the non-competitive NMDA receptor antagonist MK-801 to reverse hyperalgesia in mice was tested. Since MK-801 can potentiate latent M6G analgesia concurrent with hyperalgesia, mice in this treatment condition were also simultaneously treated with NTX.

5.2 Methods

Animal Studies

Subjects and nociceptive assay All procedures were approved by the College of Staten Island/City University of New York Institutional Animal Care and Use Committee and conform to guidelines of the International Association for the Study of Pain. Adult male CD-1 mice were purchased (Charles Rivers, Kingston, NY, USA) whereas TrKO mice (gift of John Pintar, Robert Wood Johnson Medical School, Piscataway, NJ, USA) were derived by cross-breeding mice singly deficient in the genes coding for μ , κ and δ receptors using standard homologous recombination techniques.^{21,22} Accordingly, B6129F1 mice were bred and served as TrKO controls. The combinatorial mice are devoid of brain or spinal cord [³H]-naloxone receptor labelling, indicating the complete absence of any μ , κ and δ opioid receptor subtype, and lack gross behavioural or anatomical alterations.^{21,22} Mice were maintained on a 12:12 hour light/dark cycle in a climate-controlled room with free access to food and tapwater. Each subject was used once and for all studies $n = 6$. The tail-withdrawal test of D'Amour and Smith was chosen for its stability in the context of repeated testing.^{10-12,23} Tails of the mice were immersed in water maintained at 47.3 ± 0.2 °C, which elicits pre-opioid baseline (BL) latencies between 9 and 11 s, minimizing possible floor effects during hyperalgesia. Latency withdrawal was recorded twice at 30 s intervals and averaged. A cutoff latency of 30 s was used to prevent tissue damage. Nociception was tested near mid-photophase to reduce circadian effects on the test-results.²⁴

Drug delivery M6G (NIDA Drug Supply Program, Bethesda, MD, USA) and MK-801 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in saline and injected subcutaneously. Whereas acute doses were injected in a volume of 10 ml/kg, continuous infusion was achieved using osmotic pumps (Alzet Model 2001, Alza, Mountain View, CA, USA).¹⁰⁻¹² The pumps were implanted under O₂/isoflurane anesthesia through a small dorsal midline incision. Osmotic pumps afford continuous opioid infusions and control for hyperalgesia associated with withdrawal in opioid-dependent subjects that

potentially confounds experiments where chronic opioid treatment is accomplished via repeated acute injections.²⁵ Pellets containing 30 mg of the general opioid receptor antagonist naltrexone or a placebo formulation (NIDA Drug Supply Program, Bethesda, MD, USA) were wrapped in nylon mesh and subcutaneously implanted in the nape of the neck twenty-four hours prior to M6G delivery by acute injection or continuous infusion. In rats, 30 mg NTX pellets substantially elevate NTX plasma levels one hour after implant, and sustain pharmacologically active levels of NTX such that there is a greater than fifty-fold rightward shift of the morphine analgesia dose-response curve eight days later.²⁶ In mice, NTX pellets completely abolished the analgesic effect of an acute 10 mg/kg morphine injection starting twenty-four hours after implant (coinciding here with start of M6G infusion) and for a minimum of seven additional days.¹¹

Study design Nociception was assayed before (i.e., baseline or BL) and at 30 minute intervals for 120 minutes after an acute M6G (10 mg/kg) or saline injection in CD-1 mice implanted with NTX or placebo pellets. TrKO mice and their B6129F1 controls were subject to the identical acute injection protocol with the exception that they were not implanted with pellets of any kind. The effect of continuous M6G (1.6 mg·kg⁻¹·24h⁻¹) or saline infusion on nociception was tested for six consecutive infusion days in separate groups of CD-1 mice implanted with NTX or placebo pellets. In these groups, withdrawal latencies were measured before the start of infusion (BL) and on each subsequent day. Finally, the ability of an acute MK-801 (0.05 mg/kg) dose to reverse M6G hyperalgesia was tested in separate groups of CD-1 mice implanted with NTX pellets. The MK-801 dose chosen for study does not increase tail-withdrawal latencies in naive or saline-infused mice.^{10,12,27} For the acute M6G condition, mice were first injected with MK-801 and then an acute M6G dose (10 mg/kg) 30 minutes later. Nociception was assayed immediately before the M6G injection (BL) and at 30 minute intervals for the next 2 hours. Mice subject to continuous M6G infusion (1.6 mg·kg⁻¹·24h⁻¹) were assayed for nociception prior to infusion (BL) and on Day 4 ($t = 0$), at which time all mice were hyperalgesic in agreement with the continuous infusion study above. Immediately after assaying nociception on Day 4, MK-801 was injected and nociception was reassessed at 30 minute intervals for 2 hours. Control mice in both acute and chronic M6G conditions were injected with saline vehicle instead of MK-801.

Data analysis Depending on experimental design, withdrawal latencies were analysed using a two- or three- way analysis of variance followed by Fisher's LSD (protected t-test) for post-hoc comparisons. P-values < 0.05 were considered significant. All animal values are reported as group mean $\pm$ SEM withdrawal latencies.

Human Studies

Subjects Forty human volunteers (aged 18-39 years; twenty women/twenty men; BMI < 30) were recruited to participate in the studies after approval of the protocols

was obtained from the Leiden University Medical Center Human Ethics Committee. All candidates underwent a physical examination and only healthy subjects without a history of illicit drug use or psychiatric illness were allowed in the study. All subjects were advised not to eat or drink for at least eight hours prior to the start of the study. The subjects were randomly allocated to one of four treatment groups:

1. In 10 subjects 0.4 mg/kg iv M6G was injected during a background iv infusion of naloxone (bolus 0.04 mg/kg, followed by $0.04 \text{ l}\cdot\text{min}^{-1}\cdot\text{h}^{-1}$)
2. In 10 subjects 0.4 mg/kg iv M6G was injected during a background iv infusion of normal saline
3. In 10 subjects iv placebo (0.9% NaCl) was injected during a background iv infusion of naloxone (bolus 0.04 mg/kg, followed by $0.04 \text{ l}\cdot\text{min}^{-1}\cdot\text{h}^{-1}$)
4. In 10 subjects iv placebo (0.9% NaCl) was injected during a background iv infusion of normal saline

The naloxone/saline infusion started 30 minutes prior to the M6G/placebo infusion and lasted for 2.5 hours (end of study). Thermal pain measurements were performed just prior to the naloxone bolus infusion ($t = -30$ minutes), just prior to the M6G bolus infusion ($t = 0$ min) and next at 10 minute intervals (first hour of the study) and 20 minute intervals (second hour of the study).

Drugs M6G was donated by CeNeS Ltd (Cambridge, United Kingdom), naloxone was purchased from Orpha-Devel GmbH (Pukersdorf, Austria). Placebo/saline (NaCl 0.9%) was manufactured by the local pharmacy. Randomization (using lists obtained from www.randomization.com) and preparation of the syringes were performed by a physician not involved in the study. M6G bolus was infused over 90 s, naloxone bolus over 120 s.

Pain Measurements Heat pain was induced using the TSA-II device running the WinTSA 5.32 software package (Medoc Ltd, Ramat Yishai, Israel).²⁸ Using a 3 cm^2 Peltier element or thermode, the skin of the volar side of the left forearm was stimulated with a gradually increasing stimulus ($0.5 \text{ }^\circ\text{C/s}$). Baseline temperature was set at $32 \text{ }^\circ\text{C}$. Subjects were asked to verbally rate their pain on a scale from 0 (no pain) to 10 (worst pain imaginable), i.e., a numerical rating scale (NRS). After the subjects were familiarized with the device and NRS scoring, the NRS to three heat stimuli was assessed with the following peak temperatures: 47, 48 and $49 \text{ }^\circ\text{C}$. The lowest stimulus causing a NRS between 5 and 7 was used in the remainder of the study. The test data were discarded. Next baseline values were obtained in triplicate (the averaged value was used in the data analysis). In order to prevent frequent stimulation of just one part of the skin, we divided the volar side of the test arm into six zones and moved thermode from zone to zone (1 to 6 and back) between subsequent stimuli.²⁸

Data Analysis To assess the effect of iv drug infusion over time an analysis of variance using a repeated measures design was performed. To assess the effect of naloxone versus saline treatment, we calculated time adjusted area-under-the Δ effect curves (AUEC, where Δ effect is the effect above the pre-M6G/placebo value) and tested the significance of differences by Student t-test (a separate analysis was performed in M6G treated and placebo treated subjects). P-values < 0.05 were considered significant. Values reported are mean $\pm$ SEM.

5.3 Results

Animal Studies

Nociception after acute M6G injection As illustrated in figure 5.1a, an acute 10 mg/kg M6G dose increased withdrawal latencies for at least 120 minutes in CD-1 mice implanted with placebo pellets ($P < 0.01$). In contrast, this potent analgesia was not evident in NTX pelleted controls. Instead, M6G increased nociception, thereby significantly reducing tail withdrawal latencies from baseline (10.5 ± 0.4 s) at $t = 90$ minutes (8.3 ± 0.2 s, $P < 0.05$) and $t = 120$ minutes (7.8 ± 0.3 s, $P < 0.01$). Similar results were obtained when assaying nociception after acute M6G (10 mg/kg) injection in TrKO mice and their B6129F1 controls (figure 5.1b). Whereas M6G caused maximal analgesia for a minimum of 120 minutes in control mice, it caused only significant hyperalgesia during the same time period in TrKO mice completely lacking opioid receptors. For all strains in both acute M6G conditions, saline injection in either placebo- or naltrexone-pelleted mice did not alter withdrawal latencies from baseline values (data not shown for clarity). This finding is consistent with previous reports.^{10–12,27,29}

Nociception during continuous M6G infusion Continuous subcutaneous M6G infusion ($1.6 \text{ mg} \cdot \text{kg}^{-1} \cdot 24\text{h}^{-1}$) produced no detectable analgesia in either placebo or NTX pelleted mice. Instead, increased nociception was evident starting on infusion Day 1, and continued until the end of study on Day 6 (Figures 5.2a and 5.2b). The magnitude of this hyperalgesia was at a maximum on infusion Day 4, where baseline latencies were reduced from 8.9 ± 0.2 s to 6.1 ± 0.3 s in placebo pelleted mice ($P < 0.01$; figure 5.2a), and from 9.1 ± 0.2 s to 5.3 ± 0.2 s in mice implanted with NTX pellets ($P < 0.01$; figure 5.2b). The magnitude of the latency reductions in placebo and NTX pelleted mice were highly similar throughout the six test days, and significantly differed from each other on Day 3 only. As in previous studies,^{10–12} withdrawal latencies did not differ from baseline values during saline infusion in either placebo- or NTX-pelleted mice (data not shown for clarity).

Effect of NMDA receptor blockade on M6G hyperalgesia Mice injected with saline 30 minutes prior to an acute M6G (10 mg/kg) dose displayed significant reductions in withdrawal latencies relative to baseline at $t = 60$ minutes (figure 5.3a). In contrast, no hyperalgesia was manifest at any time after the identical M6G dose

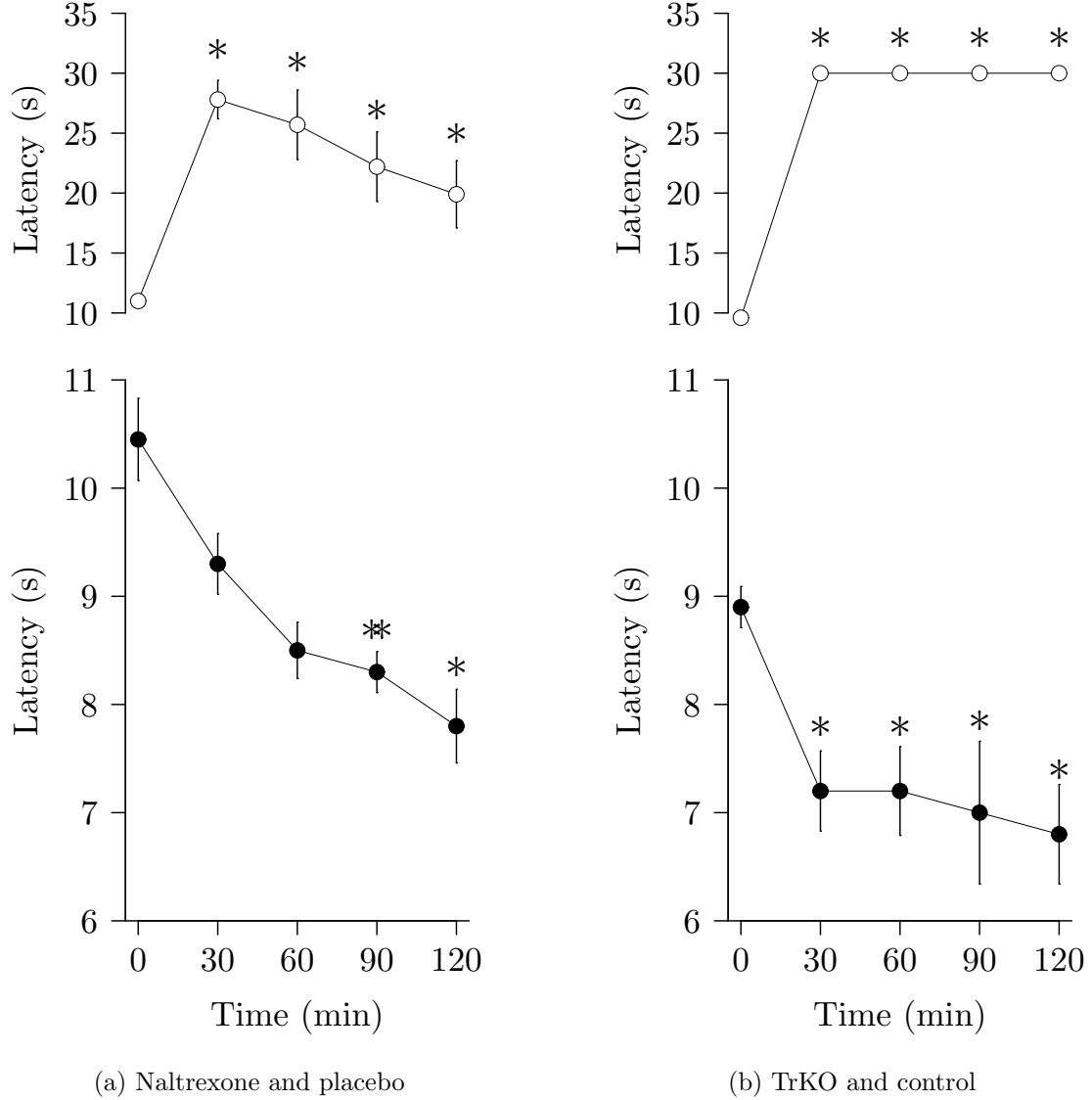


Figure 5.1: Two-hour time course of tail-withdrawal latencies following a single sc injection of 10 mg/kg morphine-6-glucuronide (M6G, given at $t = 0$) in mice. Data are mean $\pm$ SEM latencies obtained prior to M6G infusion (0) and at 30 minute intervals during M6G infusion.

A. CD-1 mice implanted with placebo (open circles, $n = 6$) or naltrexone pellets (closed circles, $n = 11$) 24 hours prior to M6G injection. Data are mean $\pm$ SEM latencies obtained prior to M6G infusion (0) and at 30 minute intervals during M6G infusion. Significant treatment, time and time treatment effects were observed (all $P < 0.001$). Post-hoc comparisons: * $P < 0.01$ and ** $P < 0.05$ versus BL (pre-M6G baseline).

B. Opioid triple knockout mice (TrKO: open circles, $n = 7$) and the B6129F1 control animals (closed circles, $n = 7$). Significant treatment, time and time x treatment effects were observed (all $P < 0.001$). Post-hoc comparisons: * $P < 0.01$ and ** $P < 0.05$ versus BL (pre-M6G baseline).

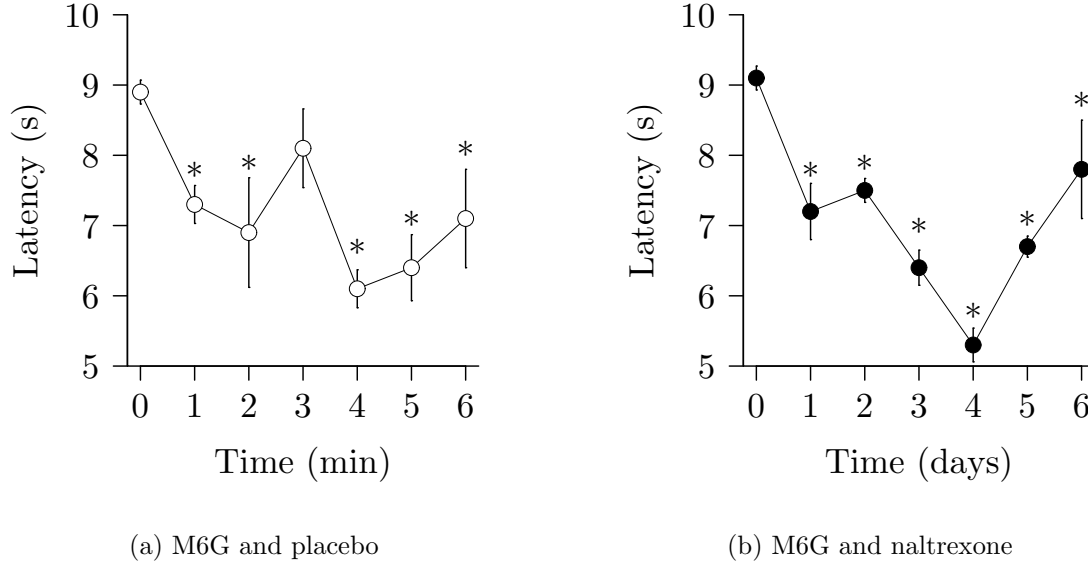


Figure 5.2: Six-day time course of tail-withdrawal latencies in CD1 mice during the continuous subcutaneous infusion of M6G (infusion rate = 1.6 mg/kg per 24 h). Data are mean $\pm$ SEM latencies, obtained prior to M6G infusion (BL) and at daily intervals during M6G infusion. Significant main effects were observed for time ($P < 0.0001$) and time $\times$ treatment ($P < 0.01$), but not for treatment ($P > 0.05$). Post-hoc comparisons: * $P < 0.01$ versus BL.

in subjects injected with MK-801 (0.05 mg/kg) instead of saline. Figure 5.3b illustrates pain responses in mice subject to continuous M6G infusion (infusion rate 1.6 mg $\cdot$ kg $^{-1}\cdot$ 24h $^{-1}$). Whereas latencies were significantly increased relative to pre-infusion BL values (9.6 ± 0.5 s) at $t = 0$ on Day 4 (7.2 ± 0.2 s, $P < 0.01$), a subsequent MK-801 (0.05 mg/kg) injection reversed this hyperalgesia, increasing withdrawal latencies to the BL values obtained prior to the start of the M6G infusion within 30 minutes (9.9 ± 0.1 s, $P < 0.01$ versus $t = 0$ values). Latencies remained elevated for at least 120 minutes after MK-801 injection. In contrast, injecting saline instead of MK-801 did not alter latencies in a separate group of M6G-infused control mice displaying significant hyperalgesia of approximately equal magnitude on Day 4.

Human Studies

The naloxone infusion scheme was designed to achieve a steady state concentration > 10 ng/ml, which is assumed to cause full reversal of μ , κ and δ opioid receptors, even when dealing with high affinity opioids.^{30,31} Subjects receiving M6G (0.4 mg/kg iv) showed increased pain responses irrespective of the naloxone or saline background infusion (figures 5.4A and 5.4C), significantly different from baseline ($t = 0$) from $t = 30$ to $t = 120$ minutes. NRS increased from 6.2 ± 0.2 ($t = 0$) to a maximum of 7.2 ± 0.2 at $t = 60$ minutes in the naloxone group ($P < 0.05$), and from 6.0 ± 0.2 ($t = 0$) to a maximum of 7.1 ± 0.3 at $t = 100$ minutes in the placebo group ($P < 0.05$). AUECs did not differ between groups: 0.76 ± 0.27 mA (saline) versus 0.66 ± 0.24 mA (naloxone). Subjects

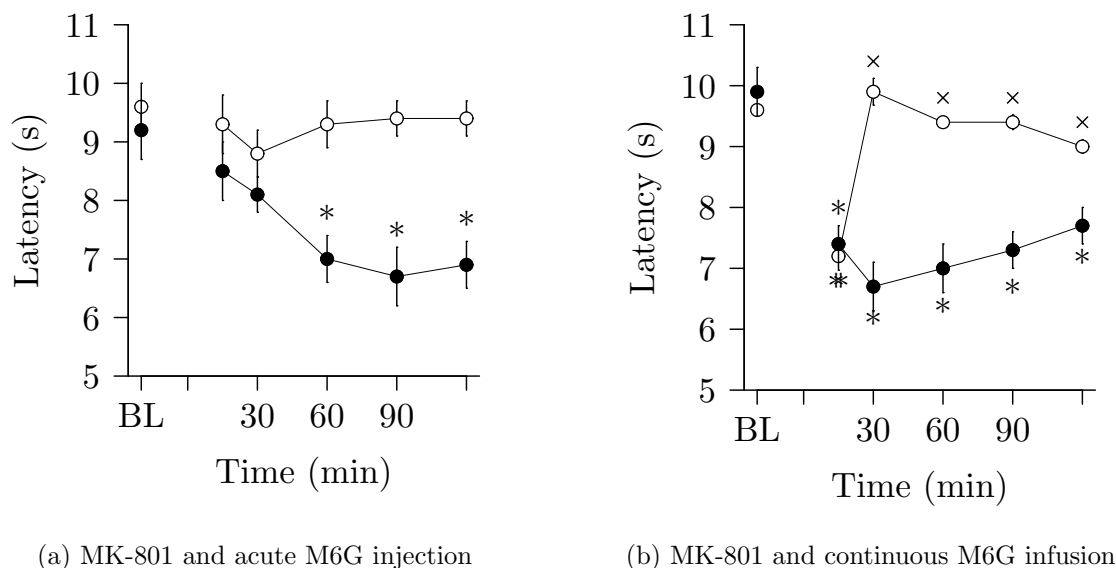


Figure 5.3: Effect of an acute injection of MK-801 on tail withdrawal latencies in CD-1 mice given an acute injection of 10 mg/kg M6G (A) or in CD-1 mice on the fourth day of a continuous infusion of M6G (B). All mice are NTX pelleted. Data are mean $\pm$ SEM values. Post-hoc comparisons: * $P < 0.01$ and ** $P < 0.05$ versus BL (pre-M6G baseline). x $P < 0.01$ versus $t = 0$.

A: Following MK-801 (open circles, $n=6$) or saline (closed circles, $n = 6$) injection, the pain response to an acute injection of 10 mg/kg M6G was tested for two hours. Significant treatment, time and time treatment effects were observed (all $P < 0.01$).

B: During the continuous sc infusion of M6G, 0.05 mg/kg sc MK-801 (open circles, $n = 6$) or saline (closed circles, $n = 6$) was injected just after the latency measurement on day 4 (pre-MK-801 latencies here shown at $t = 0$). Significant treatment, time and time treatment effects were observed (all $P < 0.01$).

receiving placebo/saline (figure 5.4B) and placebo/naloxone (figure 5.4D) showed no systematic changes in NRS. AUECs did not differ between the two placebo groups: 0.10 ± 0.15 mA (saline) versus -0.16 ± 0.13 mA (naloxone).

5.4 Discussion

One of the main findings in mice is that acute M6G injection increases pain sensitivity in mice subject to opioid receptor blockade by naltrexone and in TrKO mice lacking all opioid receptors. In addition, we observed that continuous M6G infusion causes long-lasting (six day minimum) increases in pain sensitivity that start within 24 hours, irrespective of the presence or absence of opioid receptor blockade with naltrexone. The final finding in mice was that NMDA receptor blockade with MK-801 respectively blocks or reverses the increased pain sensitivity induced by the acute injection or continuous infusion of M6G in NTX pelleted mice. In humans, we observed that a single intravenous injection of M6G increased pain sensitivity for at least six hours (figure 5.4). Furthermore, consistent with our above findings in mice, the increased pain sen-

sitivity observed after M6G injection in humans exposed to a noxious thermal stimulus persisted during the simultaneous continuous infusion of naloxone.

An array of mechanisms is proposed to underlie opioid-induced hyperalgesia. For example, opioids can directly activate a subpopulation of opioid receptors coupled to an excitatory (i.e., G_s) effector mechanism, distinct from those (i.e., $G_{i/o}$ -coupled) mediating analgesia, to prolong the action potential of dorsal root ganglion neurons.⁷ Others provide evidence consistent with the hypothesis that hyperalgesia is an adaptive response. In such a scenario, increased nociception is a consequence of an opioid receptor-mediated opponent-process acting as a foil to opioid analgesia.⁷ A series of studies also describe a system-wide mechanism integrating spinopetal projections from the rostro-ventral medulla with spinal alterations that modulate primary afferent activity.⁶ Despite their diversity, these accounts unanimously characterize hyperalgesia as a consequence of opioid receptor activity or analgesia. However, here we report that M6G hyperalgesia is manifest in mice and humans treated concurrently with high enough doses of opioid antagonist so that opioid receptors (and analgesia) are completely blocked. Furthermore, we observed M6G hyperalgesia in TrKO mice where opioid receptors are altogether absent. Importantly, there were no changes in nociception in NTX or placebo-pelleted mice injected with saline, indicating that hyperalgesia was a consequence of M6G exposure. Therefore, the present data indicate that M6G causes hyperalgesia in mice and humans that, like morphine and oxymorphone hyperalgesia in mice,^{10–12} is independent of concurrent opioid receptor activity or analgesia.

We have previously demonstrated that morphine and oxymorphone can cause hyperalgesia via mechanisms unrelated to their opioid activity.^{10–12} To this list of clinically relevant opioids we now include M6G, which is currently undergoing phase III clinical trials.^{17,32} Thus, despite the fact that all three opioids preferentially act via the μ -opioid receptor, their hyperalgesic liability is unrelated to their common opioid receptor pharmacodynamics. We, and others, have previously speculated that OIH might result from the conjugation of the parent opioid compound at the 3'-position into pronociceptive glucuronide metabolites.^{10–12,19,20} M3G, for example, the most abundant morphine metabolite,³³ has no detectable affinity at any opioid receptor subtype or analgesic effect,^{29,34–37} and systemic M3G doses can decrease tail-withdrawal responses in mice and evoke agitation to even innocuous touch in rats that is not diminished by naloxone.^{4,29} M3G accumulation has also long been thought to underlie morphine hyperalgesia in humans. Oxymorphone metabolism also yields oxymorphone-3-glucuronide, a metabolite similar to M3G.³⁸ With regards to M6G, itself a morphine metabolite, we are not aware of any reports showing that M6G metabolism directly yields any neuroexcitatory or pronociceptive fragments. However, M6G injection increases M3G levels in mice within sixty minutes, an effect attributable to the metabolism of morphine that is generated from the enterohepatic circulation of M6G.³⁹ Here, the onset of hyperalgesia after an acute M6G injection in NTX-pelleted mice was generally similar ($t = 90$ minutes). Further implicating the contribution of M3G is our finding that the NMDA

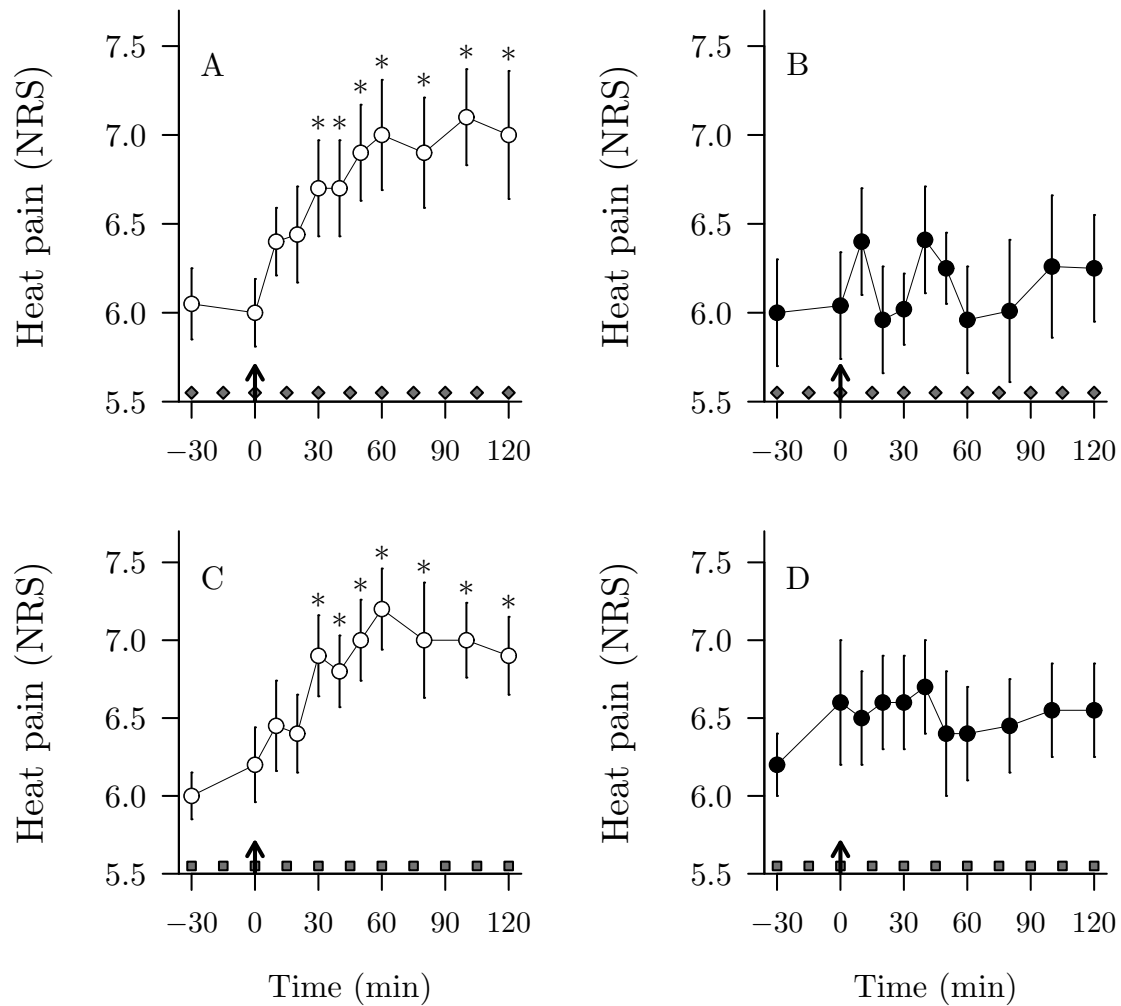


Figure 5.4: Influence of 0.4 mg/kg M6G and placebo on experimental heat pain responses in human volunteers during background exposure to saline (A and B, grey diamonds) and background exposure to naloxone (C and D, grey squares). During saline (A) and naloxone (C) background infusion M6G causes an immediate and persistent hyperalgesic response. In contrast, placebo produces no consistency in response independent of the background infusion (B and D). Values are the mean $\pm$ SEM. Significant main effects: (A) M6G/saline, $P < 0.01$; (C) M6G/naloxone, $P < 0.001$. Post-hoc comparisons: * $P < 0.05$ versus $t = 0$.

receptor antagonist MK-801 blocked or reversed hyperalgesia elicited by an acute injection or the continuous infusion of M6G, respectively. Although the M3G binding site and mechanism of action is not known, the neuroexcitatory effects of M3G are thought to involve NMDA receptor activity and NMDA receptor antagonists dose-dependently reduce M3G symptoms, including enhanced nociception.^{34,40,41} However, based on data from a previous study,³⁹ it is unlikely that the acute M6G dose injected here would result in physiologically relevant concentrations of M3G to cross the blood-brain barrier, although such an accumulation may be possible during continuous M6G infusion. In humans, M3G levels remain undetected after an acute M6G injection, and acute M3G injection in humans was without effect on nociception.^{36,37,42} Therefore, at this time, we can only speculate as to whether M3G might contribute to M6G hyperalgesia. Accordingly, any such contribution may be dependent on the duration of M6G exposure (i.e., acute injection or continuous infusion) and the species studied. These issues will comprise the specific aims of future studies.

Regardless of the mechanism underlying morphine, oxymorphone and M6G hyperalgesia, and whether all three opioids cause hyperalgesia via common mechanisms, the present data suggests that hyperalgesia after M6G has a more rapid onset and is more robust. For example, we previously showed that an acute subcutaneous morphine or oxymorphone injection in TrKO mice at doses identical to M6G doses administered here (10 mg/kg) did not reduce tail-withdrawal latencies even after 120 minutes.¹⁰ In contrast, here we report that opioid receptor blockade significantly reduced withdrawal latencies within 90 minutes in CD-1 mice. Furthermore, whereas hyperalgesia caused by continuous morphine infusion in both placebo- and NTX-pelleted CD-1 mice is delayed until Day 4,^{11,12} significant hyperalgesia is already manifest 24 hours after the start of continuous M6G infusion, regardless of the concurrent pellet treatment. These data suggest that M6G activates pronociceptive mechanisms more rapidly or efficaciously than either morphine or oxymorphone. This might explain why relatively high doses of M6G are required to elicit an adequate analgesic response in experimental and clinical studies with humans.^{13,32,42} That is, the ability of M6G to rapidly evoke significant hyperalgesia in a variety of delivery circumstances may offset any concurrent analgesic effect. At higher M6G doses, there is presumably a greater increase in the analgesic effect relative to hyperalgesia, and analgesia is manifest. This assumption can be directly tested by assaying thermal pain responses in humans subject to morphine. We are embarking on just such a study, and our preliminary data indeed show that a single intravenous injection of morphine does not cause pain ratings on our thermal assay to increase in a manner similar to that observed here with M6G (Van Dorp 2006, unpublished observation), suggesting that M6G hyperalgesia to heat pain is more readily manifest than hyperalgesia wrought by morphine.

Multiple studies show the ability of NMDA receptor antagonists to reverse OIH,^{6,11,12,26} and here the non-competitive NMDA receptor antagonist MK-801 was effective in blocking or reversing hyperalgesia after acute injection and continuous infusion of M6G,

respectively. The present data thus demonstrate that M6G hyperalgesia in mice is dependent on NMDA receptor activity. There is currently no definitive explanation on how NMDA receptor antagonists reverse opioid hyperalgesia. A possible direct interaction of NMDA antagonists with opioid receptors (cf., Sarton *et al.*⁴³) would be a moot point since we show here in NTX-pelleted mice that M6G hyperalgesia is unrelated to opioid receptor activity. For identical reasons, it can also not be the case that MK-801 reversed M6G hyperalgesia only indirectly, by potentiating latent M6G opioid analgesia concurrent with hyperalgesia. Kilpatrick and Smith reported that while M6G was inactive at two binding sites within the NMDA receptor, suggesting the absence of a direct blockade of M6G activity at these sites, it is not yet possible to rule out M6G activity at other sites within the receptor complex.¹⁴ It has also been suggested that NMDA antagonists block or reverse opioid hyperalgesia by antagonizing NMDA receptors localized to central primary afferent terminals that cause spinal sensitization and increased nociceptive input.⁶ To this we add the possible contribution of NMDA receptors at loci up- or down-stream from the site where pronociceptive mechanisms are activated in response to M6G administration. Further studies are needed to address these possibilities.

Although just 5 to 10% of morphine is metabolized to M6G, M6G plasma concentrations increase rapidly after acute morphine administration and reach relatively high values after chronic treatment, particularly when renal function is compromised. Thus, M6G may not only make an important contribution to morphine analgesia but, as we demonstrate here, to hyperalgesia as well. This potential role for M6G as causative factor of morphine hyperalgesia requires further study. M6G is also currently in phase III clinical trials as an opioid analgesic and is thought to possess a pharmacological profile that imbues it with certain advantages relative to other opioids in the management of pain. The addition of another clinically effective opioid is certainly a welcomed addition to the opioid pharmacopeia. However, despite whatever advantages M6G may afford for the treatment of pain, the present results suggest that the absence of hyperalgesia is not one of them.

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SECTION IV

Drug addiction

Naloxone treatment in opioid addiction: the risks and benefits

6.1 Introduction

Although opium has been in use for many centuries, opioid addiction only became a major global problem since the mid 1800s.¹ In the US alone, almost 3 million people aged over 12 years have used heroin, of which 326,000 people received treatment for heroin abuse.² In Europe, 1.2 – 2.1 million people are known to be problematic drug users, most of whom use opioids (often in combination with other (illicit) drugs).^{3,4} Of these drug abusers, 450,000 people receive treatment for their addiction. Besides the fact that addicts are more likely to develop mental illness or exhibit criminal behaviour, they are also at risk for fatal overdose and various infectious diseases, such as hepatitis B and C and HIV. The number of drug-related deaths in EU member states is estimated to be in the range of 7000 – 9000 per year.⁴ Opioid addiction can, therefore, be viewed as a major medical and social problem.

The recent advancements in the understanding of the neurobiology underlying addiction-related behaviour have contributed to the recognition that opioid addiction is a serious complication of chronic opioid intake in some individuals (note that patients receiving opioids for chronic pain do not necessarily develop addiction). Nowadays, addiction is considered a chronic disease of the brain rather than a mental illness carrying a social stigma.⁵ New perspectives in the neurobiology of opioid addiction offer unique opportunities for the development of novel treatment strategies. However, as the disease has a multifactorial etiology, treatment must always be multidisciplinary, combining both pharmacologic and psychological interventions. The pharmacologic interventions are either aimed at detoxification and permanent abstinence from illicit drugs, or at the attenuation of (often protracted) withdrawal symptoms using opioid replacement therapy. Although sometimes complete abstinence is achieved, often lifelong substitution is the chosen therapy mode. Methadone substitution therapy is the main cornerstone in the treatment of opioid addiction, although in some countries a clear shift is seen in treatment approach with buprenorphine rather than methadone being the first-choice substitution therapeutic.⁶

This short review discusses some of the pharmacologic strategies of opioid addiction treatment with special focus on the benefits and risks of the non-selective opioid-receptor antagonist, naloxone.

6.2 Addiction and the μ -opioid receptor

Opioids exert their effects through specific opioid receptors. The existence of three subtypes (μ , κ and δ) is accepted. The μ -opioid receptor subtype, especially, mediates the positive reinforcing effects of heroin and other illicit opioids. This receptor subtype is, therefore, considered crucial in the development of opioid addiction.⁷ Dedicated *in vivo* studies have shown that mice lacking the μ -opioid receptor (exon 2 μ -opioid receptor gene knockout mice) display less self-administration of morphine and reduced

conditioned place preference,⁸ underlining the importance of the μ -opioid receptor in the development of opioid addiction.

Drugs of abuse, in general, overstimulate those neural systems in the brain that are normally reserved for the response to natural reward systems. In this respect, the mesolimbic dopamine system, as well as the nucleus accumbens, is considered a relevant part of the ventral tegmental area in the midbrain.^{9,10} Acute administration of drugs of abuse induces the release of high levels of dopamine in the nucleus accumbens, resulting in an increased feeling of reward. Opioids cause dopamine release by inhibiting the γ -aminobutyric acid-ergic inhibition of dopamine release in the ventral tegmental area, a typical part of the midbrain with a high density of μ -opioid receptors.¹¹ Overstimulation of dopamine results in stronger deregulations of the natural reward pathways (sensitization and tolerance) and learning processes in the brain (reinforcement).⁸

Abrupt abstinence from opioids or the administration of μ -opioid receptor antagonists in opioid-dependent persons will produce the opioid withdrawal syndrome. Signs and symptoms of this syndrome include negative moods, irritability, muscular and abdominal pains, gastrointestinal complaints (nausea, diarrhea), sweating, lacrimation, malaise and insomnia.¹² Symptoms usually start 6 – 12 hours after the last dose of a short-acting opioid and 36 – 48 hours after the last dose of a long-acting opioid, such as methadone. The duration of the syndrome is variable. Some studies report a duration of no more than 7 – 14 days, whereas others also describe a more prolonged withdrawal syndrome lasting from several weeks to a few months. Although the syndrome is not life-threatening, many patients experience difficulties completing this initial phase of the therapy.¹³

6.3 Pharmacologic treatment strategies in opioid addiction

Treatment of opioid addiction should primarily be aimed at the reduction of illicit drug use (next to stabilizing the social functioning of the patient and improving his or her quality of life). This can be done by either gaining control of the patients drug use by drug replacement therapy or by withdrawing the patient from all opioids (detoxification). It is, however, insufficient to regard complete withdrawal as the ultimate therapy; addiction is a chronic disease (reflected in long-term changes in the brain) and should, therefore, be treated as such. Nowadays, most patients receive maintenance therapy consisting of μ -opioid receptor agonists or a combination of μ -opioid receptor agonists and antagonists.

Potent and long-acting opioid agonists with low-intrinsic efficacy are considered good candidates for opioid replacement therapy. Examples of such opioids are methadone and buprenorphine. Methadone is a full agonist at the μ -opioid receptor, buprenor-

phine a partial μ -opioid receptor agonist. This characteristic makes buprenorphine an attractive alternative for methadone, because low-efficacy agonists are associated with a lower abuse potential compared to relatively higher efficacy agonists such as methadone. Furthermore, the partial agonist, buprenorphine, has a better safety profile than full μ -opioid receptor agonists, indicating that it can be more easily titrated to the desired effect even at high doses.¹⁴ In addition, its unique slow receptor association/dissociation characteristic at the μ -opioid receptor contributes to the extended duration of action following single-dose administration.¹⁵

Opioid antagonists, such as naloxone and naltrexone, reverse and prevent opioid effects by blocking the μ -opioid receptor. As discussed in section 6.2, μ -opioid receptor blockade causes the occurrence of acute withdrawal symptoms in opioid-dependent individuals. μ -opioid receptor antagonists are widely used in rapid and ultra-rapid detoxification to facilitate the transition from dependence to abstinence. Antagonists can also be used to prevent relapse, as μ -opioid receptor occupancy by opioid antagonists results in a decreased effectiveness of administered opioids. This diminishes the reinforcing effects of heroin and potentially the association between opioid use and conditioned stimuli.¹²

6.4 Pharmacology of naloxone

For many years, the development of non-addictive opioids, with the beneficial analgesic action of morphine but devoid of any addictive properties, has been considered an important objective. During the twentieth century, various morphine-like substances were synthesized and tested for their non-addictive properties. Nalorphine, a derivative of morphine, was shown to reverse most of morphine's typical effects at a relatively low dose (while inducing analgesia at a high dose). In addition, nalorphine precipitates the abstinence syndrome in opioid addicts. Although nalorphine showed promising blocking properties, the dysphoric effect of this opioid discouraged its widespread clinical use.¹⁶ Additional dedicated structure-activity studies led to the discovery of naloxone. Naloxone, an allyl derivative of noroxymorphone, was first synthesized in 1960. The development of naloxone was encouraged by the need for a real opioid antagonist (in contrast to the partial agonist, nalorphine) devoid of any agonistic activity at the various opioid receptors.¹⁷ Naloxone is a non-selective opioid antagonist at the μ -, δ - and κ -opioid receptors. Naloxone competitively inhibits the pharmacologic effects of opioids and, in line with the classical receptor theory, produces a parallel right shift in the dose-response curves of opioids.¹⁸ When administered to opioid-dependent patients, naloxone induces a severe withdrawal syndrome, as μ -opioid receptor-bound heroin is displaced by naloxone.

Naloxone appears to be readily absorbed after oral administration, but its low bioavailability renders naloxone less suitable for this administration route. Following oral administration, naloxone undergoes extensive hepatic metabolism, indicating high first-

pass effect (>95%). In the liver, naloxone is primarily metabolized into the inactive conjugate naloxone-3-glucuronide. In addition to glucuronidation, naloxone is also metabolized by N-dealkylation and 6-oxo group reduction (note that these metabolism pathways represent only minor fraction of total metabolism). Approximately 30% of the unchanged naloxone dose is excreted in the urine within six hours following intravenous administration; the rest of the dose is recovered as conjugated naloxone metabolites in the urine.¹⁹

In healthy volunteers, the elimination half-life of naloxone in plasma is approximately 30 minutes. Although the elimination half-life is not expected to differ among opioid naive and opioid dependent patients, differences in naloxone distribution in the body may exist. For instance, Handal *et al.* suggest in their review that there may be differences in pharmacokinetics between opioid-dependent and non-dependent persons, reporting a difference in initial plasma concentration of 30%.²⁰

Naloxone is readily transported across the blood-brain barrier and, therefore, has a fast onset of action in reversing opioid effects.¹⁹ However, the ability of naloxone to reverse opioid effects *in vivo* is mainly determined by the pharmacologic characteristics of the interacting opioid agonist (i.e., the opioid that requires antagonism). For example, the onset of reversal of morphine-induced respiratory depression by naloxone can be established within a time frame of one to two minutes. On the other hand, for an opioid with slow μ -opioid receptor association/dissociation kinetics, such as buprenorphine, the interaction with naloxone is rather complex. Not higher doses of naloxone *per se*, but a different mode of naloxone administration (i.e., continuous infusion) is indicated to reverse buprenorphine-induced respiratory depression.^{14,15}

Because naloxone is devoid of agonistic activity at the μ -opioid receptor, it is regarded as a safe drug to use. This notion persists despite earlier clinical experiences showing that naloxone use may (under certain specific circumstances) cause serious and possibly life-threatening side effects, such as pulmonary edema, cardiac arrhythmias, hypertension and cardiac arrest.^{21–23} It is important to note that all of the patients described in these reports were postoperative patients experiencing (severe) pain and stress. In a more recent prospective study²⁴ in comatose patients due to opioid overdose, 453 patients were treated with naloxone. Six patients suffered from severe complications (asystole, pulmonary edema and epileptic seizures), corresponding to 1.3% of the treated population. However, the exact relationship between naloxone treatment and the occurrence of the severe complications was not clear. The possibility that these complications were related to the initial hit (i.e., the opioid overdose) could not be excluded. The primary reason for the development of cardio-respiratory complications after naloxone therapy is the sudden release of central catecholamines.²⁴ Especially when naloxone is administered shortly after the occurrence of opioid-induced vasodilation (this may occur just minutes after the opioid is administered via the intravenous route and is visible as a sudden drop in blood pressure) or the patient is sympathetically unstable (due to pain or stress), high-dose naloxone and/or rapidly infused naloxone (i.e., not titrated) can cause catecholamine-mediated vasoconstriction. This

then may cause cardiac arrhythmias and a fluid shift from the systemic circulation to the pulmonary vascular bed, resulting in pulmonary edema.²¹ Proper monitoring of patients receiving naloxone is therefore mandatory, especially of patients that just recently received an opioid dose via the intravenous route or sympathetically unstable patients. Studies in animals and healthy volunteers confirm the safety of naloxone use in patients^{25,26} even at higher doses up to 10 mg,²⁷ or following constant exposure to intermediate-to-high concentrations of naloxone during one to two hours.²⁸ Taking into account the fact that there are only few reports in the literature on naloxone-related complications (as well as taking into account their own experience), the authors consider naloxone a relatively safe drug with little chance of complications.

As an alternative to naloxone, a second μ -opioid-receptor antagonist, naltrexone, was synthesized with more favourable pharmacokinetic properties than naloxone. Although naltrexone has a relatively low bioavailability (up to 60%), it is two to three times more potent than naloxone.¹⁶ It undergoes extensive hepatic metabolism, but because its metabolite, 6- β -naltrexol, is also highly active, oral administration can be effective. Elimination half-life is approximately 4 hours, with a far longer half-life (up to 13 hours) reported for the active metabolite. Effectively, a 50 mg dose of naltrexone will block the pharmacologic effects of a 25 mg heroin dose for up to 24 hours.²⁹ It is employed in rapid and ultra-rapid detoxification and in abstinence maintenance therapy.³⁰ When compared with methadone maintenance therapy, naltrexone is the less favourable option, as the lack of agonistic effects reduces compliance.²⁹ However, if retention of patients is high enough (for example with highly motivated patients or with patients that cannot be included in a methadone maintenance program), naltrexone maintenance therapy is an effective way of treating opioid addiction.³¹

6.5 Naloxone in the treatment of opioid addiction

Naloxone use in treatment of opioid overdose

The most common use of naloxone is for the treatment of opioid overdose. Heroin overdose is one of the leading causes of death among opioid-dependent patients and non-fatal overdoses are also highly prevalent among these patients.⁴ Overdose often occurs after a drug-free period and is related to a reduction of tolerance and hence a relatively increased opioid potency. Naloxone is effective in the treatment of opioid-overdose and opioid-induced coma in hospital practice. Note, however, that it is vital to take into account the specific opioid that is responsible for causing the overdose. Most opioids used by addicts have relatively long half-lives (of a few hours), whereas naloxone has a half-life of only 30 minutes. As a consequence, respiratory depression, caused by long-acting opioids (methadone, heroin, morphine), returns after the effect of naloxone has worn off.^{17,32} It is, therefore, necessary to adequately dose and monitor the patient.³³ The initial naloxone bolus dose required to reverse opioid overdose should be determined clinically, starting from 0.4 mg given as a slow bolus injection, continuing

until the patient improves. If, after an injection of 4 – 10 mg naloxone, the patient shows no sign of recovery, the cause of the respiratory depression is most likely not opioid-related. After initial recovery, patients should be started on a continuous intravenous naloxone infusion and closely monitored for signs of deteriorating clinical status for at least 24 hours.

It is important to note that the patient may enter an acute withdrawal syndrome after administration of naloxone, with consequent nausea and vomiting. The airway must, therefore, be guarded at all times. Another symptom of acute withdrawal may be patient violence,³⁴ and adequate preparation for this situation (in the form of restraints) is needed. All this taken into account, naloxone remains the first drug of choice in suspected opioid overdose in the hospital setting.

Because an overdose often occurs outside the hospital setting (i.e., at home or on the streets), naloxone may not be readily available and it is therefore difficult to treat the patient timely. Both healthcare professionals and opioid addicts themselves regarded the idea of so-called ‘take-home naloxone’ a good strategy in the prevention of fatal opioid overdose.^{35–37} Several pilot studies investigated this intervention strategy and although the sample sizes in the studies were small, results were promising, with 90 – 100% of naloxone administrations preventing death from heroin overdose.^{38–40}

In the United Kingdom (June 2005), naloxone was added to the list of drugs that ‘may be administered by anyone for the purpose of saving life in an emergency’ (that is, everyone is allowed to administer naloxone to an individual with a suspected opioid overdose).⁴¹ It is important to educate both the patient and his or her caretakers (not necessarily healthcare professionals) in the use of naloxone in case of a suspected overdose. The caretakers should learn how to recognize an overdose, how to perform mouth-to-mouth resuscitation and how to administer naloxone (either subcutaneously, intramuscularly or intravenously).⁴² In addition, they should be made aware of the necessity of always alerting emergency medical services and to provide the monitoring and further treatment needed in case of an overdose. Often, fear of the police and subsequent criminalisation will halt the bystanders (usually fellow addicts) in calling an ambulance — one more reason for distributing take-home naloxone among addicts, thus providing necessary first aid to their peers.³⁸ Providing the family, caretakers and friends of opioid-addicted patients are well instructed in the use of naloxone, take-home naloxone could be a helpful strategy in combating fatal heroin overdose.

Naloxone in detoxification and maintenance

The conventional way of detoxification is treating the patients with tapering doses of opioid agonists (methadone or buprenorphine) and/or with clonidine or lofexidine ($\alpha 2$ -adrenergic-receptor agonists that can relieve the symptoms of withdrawal). The protracted nature of these techniques, however, leads to a high number of initial dropouts (dropout rates in the literature are in the range of 30 – 90%^{31,43}). This was one of the major reasons for the development of new withdrawal strategies, which take less time

Naloxone use	Side effects
Opioid overdose	Acute withdrawal syndrome
Detoxification	Recurrence of respiratory depression
Maintenance (combined with buprenorphine)	Cardiac arrhythmias
	Pulmonary edema

Table 6.1: Use and side effects of naloxone in opioid-dependent individuals.

and may be more comfortable to the patient. Rapid or ultrarapid detoxification under anesthesia or heavy sedation is one such therapy. It consists of the intravenous administration of an opioid antagonist (usually naloxone). The effect of the ensuing acute withdrawal syndrome (lasting 4 – 6 hours) is either treated (or masked) with general anesthesia or heavy sedation (using benzodiazepines), both combined with clonidine and β -adrenergic-receptor blockers (to prevent tachycardia). After this initial phase, patients are introduced on an oral dosing of naltrexone as maintenance therapy, with additional psychologic counselling as support. The effectiveness of this approach has recently been called into question, as there is little evidence of its superiority above ‘ordinary’ opioid maintenance treatment and it appears to have a higher risk of adverse events. In recent years, a few randomized clinical trials were conducted investigating rapid detoxification.^{44,45} All concluded that rapid opioid detoxification had no proven benefits over buprenorphine/clonidine detoxification. As the risk associated with this therapy (e.g., the risk of anesthesia or sedation) is much greater than in the other treatment groups and the costs are significantly higher, it is generally agreed that this form of treatment should not be pursued any further.⁴⁶

Naloxone can also be used to speed up clonidine or lofexidine-assisted opioid detoxification (i.e., rapid detoxification with naloxone/clonidine). These α_2 -adrenergic agonists alleviate withdrawal symptoms in detoxifying patients, and have proven to be as effective as tapering methadone doses in the treatment of opiate dependence.⁴⁷ The addition of an opioid antagonist, such as naloxone, to this form of detoxification therapy leads to a more intense, but less prolonged, withdrawal syndrome. The exact implications for long-term treatment in the form of antagonist maintenance are not yet clear.

In the past, naloxone has been used as an oral abstinence maintenance agent, but its low oral bioavailability and (very) short duration of its action make it unsuitable for this purpose.⁴⁸ However, it can be used as test medication before administering naltrexone to possibly dependent patients. For example, if intravenous naloxone causes no or little withdrawal symptoms in these patients, it is safe to administer the more potent and long-lasting naltrexone in an oral formulation.⁴⁹ Furthermore, it may be used as a diagnostic tool in discriminating between opioid-dependent and non-dependent patients (e.g., occasional abusers or patients behaving like addicts, but ailing from another disorder such as diabetes).

Naloxone in combination with buprenorphine

In 2002, sublingual buprenorphine (SubutexTM, Reckitt Benckiser) and the combination of buprenorphine and naloxone (for sublingual use only, SubuxoneTM, Reckitt Benckiser) was approved by the FDA for use in opioid addiction treatment. Because buprenorphine alone, as a (partial) μ -opioid receptor agonist, is subject to abuse, the combination treatment was intended to minimize the abuse and misuse of the compound.⁵⁰ As this form of therapy gains in popularity, the use of buprenorphine combined with naloxone needs further consideration. When Subuxone is administered sublingually some opioid withdrawal symptoms are only seen in those individuals who are heavily dependent on heroin and/or recently took heroin. Most likely, the bioavailability of naloxone after sublingual administration is too low to cause severe and protracted withdrawal symptoms.

However, when a sublingual dose of Subuxone is administered intravenously, all addicts will experience an immediate opioid withdrawal syndrome.⁵¹ On the basis of the pharmacologic properties of buprenorphine, partial agonism and high affinity at the μ -opioid receptor, one would expect competitive displacement of heroin by buprenorphine rather than by naloxone. Surprisingly, however, there is ample evidence that withdrawal symptoms in this particular population (opioid-dependent patients) are caused by naloxone.^{51,52} This may be related to the fact that several structures in the brain, and more specifically the opioid-receptor system, are subject to changes following chronic exposure to opioids,^{5,9} thereby significantly altering the interaction of buprenorphine with the μ -opioid receptor. One possibility is that chronic exposure to opioids changes the behaviour of intravenous buprenorphine from a partial agonist to a full agonist at the μ -opioid receptor with lesser affinity for the receptor than observed in opioid-naïve volunteers. Further studies are needed to elucidate this matter. Several studies concluded that buprenorphine/naloxone was a good alternative for either methadone or buprenorphine maintenance therapy.⁵²⁻⁵⁵ Not much is known about whether or not the addition of naloxone truly prevents the misuse of the combination. Evidence is only circumstantial, as it is difficult to monitor the amount of misuse.^{56,57}

6.6 Summary and conclusions

Naloxone competitively inhibits the pharmacologic effects of exogenously administered opioids and, in line with the classical receptor theory, produces a parallel right shift in the dose-response curves of opioids. Naloxone is readily transported across the blood-brain barrier and, therefore, has a fast onset of action in reversing opioid effects. Its duration of action is limited due to its short elimination half-life of 30 minutes. The ability of naloxone to reverse opioid effects *in vivo* is mainly determined by the pharmacologic characteristics of the interacting opioid agonist (i.e., the opioid that requires antagonism).

The most common use of naloxone is for the treatment of opioid overdose both in a hospital and out-patient setting. The safety of naloxone in the treatment of opioid overdose is well established in patients and healthy volunteers over a wide dose range (0.4 – 10 mg). There is a special role for intravenous naloxone in rapid detoxification, in which naloxone is combined with the α 2-agonist, clonidine, and β -adrenergic-receptor-blocking agents to treat withdrawal symptoms. The effectiveness of this approach has recently been called into question as there is little evidence of its superiority above ‘ordinary’ opioid maintenance treatment and it appears to have a higher risk of adverse events. Finally, naloxone is used in combination with buprenorphine maintenance therapy. Addition of naloxone minimizes the abuse and misuse of buprenorphine and the buprenorphine/naloxone combination is considered a good alternative for either methadone or buprenorphine maintenance therapy (see also table 6.1).

Although naloxone is relatively safe to use, there are some apparent risks and disadvantages associated with its use. Naloxone induces an acute withdrawal syndrome in opioid-dependent persons. Due to its short half-life, its effect may wear off prematurely when used for treatment of opioid-induced respiratory depression. High-dose or rapidly infused naloxone administered to a patient who is overdosed with an opioid given for the treatment of acute pain may cause catecholamine release and consequently pulmonary edema and cardiac arrhythmias.

6.7 Expert opinion

The non-selective opioid-receptor antagonist, naloxone, is widely used in clinical practice. Anesthesiologists use naloxone for reversal of postoperative respiratory depression induced by potent opioid analgesics, such as fentanyl, sufentail and morphine. Similarly, naloxone may be used to treat opioid overdose in opioid-dependent patients. There are some subtle differences in use between the two patient groups, most importantly there are differences in dosing. In postoperative patients, the initial intravenous dose is 40 – 80 μ g, which can be increased to desired effect using 40 – 80 μ g titration boluses. When respiration has returned to the desired level, an equivalent naloxone dose is administered via the intramuscular route. Reversal is often rapid and the intramuscular depot ensures that reversal lasts for 30 to 45 minutes, a time frame which is often sufficient to overcome the respiratory problems. In opioid dependent patients, the initial dose is 0.4 mg. Depending on the clinical status of the patient, slow titration with doses up to 10 mg of naloxone may be applied. Note, however, that for both patient groups the mode and dose of naloxone administration is dependent on the pharmacological properties of the opioid that induced the overdose. For long acting potent opioids, such as methadone and buprenorphine, a continuous infusion of naloxone rather than multiple bolus injections is indicated.

An interesting new development that deserves support is the use of naloxone outside the hospital setting by non-medically trained people, so called ‘take-home naloxone’. Some

caution is needed though. Acute withdrawal may occur with vomiting, hypertension, tachycardia and delirium. These require acute treatment to prevent further damage (such as aspiration). Training of family and friends of opioid addicts who receive ‘take-home naloxone’ should therefore not be restricted to instructions how to administer naloxone in case of a heroin overdose but also be aimed at the acute treatment of the patient. Often the required measures are very simple: put the patient on one side, remove the vomit and get professional help.

There is some scarce data on the deleterious effects of naloxone on the cardiovascular system. The data is relatively old (1970s) with little new data added since. The data indicate that rapid infusion and high dose naloxone may be dangerous to one specific type of patient: the patient who was treated for acute and severe pain with an opioid. When overdosed and the patient is treated with high dose naloxone (or naloxone is given too rapidly) the abrupt exposure to the underlying problem (pain, stress, sympathetic excitation) may cause a sudden release of catecholamines with consequently pulmonary edema and cardiac arrhythmias. Although there is an absence in recent reports on the cardiovascular side-effects of naloxone, which we relate to the improved care that we give to our patients (for example by careful titration of naloxone), we still recommend that the use of naloxone is performed during adequate cardio-respiratory monitoring. This is especially important for the patient using ‘take-home naloxone’. He or she should immediately be transported to the hospital and monitored for at least 12 hours after being treated with naloxone for near-fatal respiratory depression.

In conclusion, taking into account all relevant data, current opinion is that naloxone is a safe drug to use.

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SECTION V

Conclusions

CHAPTER 7

Summary and conclusions

Opioids still form the gold standard in severe pain therapy, despite their many side-effects. One of the most important side-effects of opioid therapy is respiratory depression, a condition which is easily treatable using opioid antagonists. One such antagonist is naloxone, a short acting opioid antagonist which has high affinity for the μ -opioid receptor, but with lower affinity for the κ - and δ -opioid receptors. In Chapters 2, 3 and 4, we investigated the use of naloxone in respiratory depression that was caused by morphine, morphine-6-glucuronide (M6G) and buprenorphine.

In Chapter 2, we investigated the possible existence of a separate opioid receptor for M6G by using a derivative of naloxone, 3-methoxy-naltrexone (3mNTX). We compared morphine and M6G's respiratory effects in the anesthetized cat, using the dynamic end-tidal forcing (DEF) technique. Using the DEF technique, we can distinguish between carbon dioxide (CO_2) sensitivity of the peripheral and the central chemoreflex loops (G_p and G_c , respectively) which are parameters in a two compartmental model for the relationship between CO_2 and ventilation. We conducted three separate studies. In study 1, we assessed the effect of morphine, 3mNTX and M6G successively, on the ventilatory response to CO_2 . In study 2 we assessed the effect of M6G, 3mNTX and morphine successively. Study 3 assessed the effect of 3mNTX alone on the ventilatory response to CO_2 . With these studies, we showed that both M6G and morphine shifted the ventilatory CO_2 responses to higher end-tidal CO_2 levels. Morphine had a preferential depressant effect within the central chemoreflex loop. In contrast, M6G had a preferential depressant effect within the peripheral chemoreflex loop. Irrespective of the opioid, 3mNTX caused full reversal of, and prevented respiratory depression. The conclusions that could be drawn from these are twofold. We found that in anesthetized cats, the μ -opioids morphine and M6G induce respiratory depression at different sites within the ventilatory control system. Another conclusion drawn from this study is that it is unlikely that a 3mNTX sensitive receptor is the cause of the differential respiratory behaviour of morphine and M6G, as 3mNTX caused full reversal of the respiratory depressant effects of both opioids.

For Chapter 3, we obtained data from an extensive group of healthy volunteers ($n=67$) on buprenorphine's respiratory effects and the reversal of those effects using naloxone. The rationale behind these studies was the worldwide belief that buprenorphine's respiratory effects are supposedly hard to reverse. We combined data from three separate studies in this Chapter. In all studies, respiration was measured against a constant, increased end-tidal CO_2 -level. In the first study, we investigated the effect of an intravenous bolus dose of 0.8 mg naloxone on 0.2 mg buprenorphine-induced respiratory depression versus the effects of placebo. As this turned out to be insufficient to cause reversal of the respiratory effects, we decided to test a dose range of naloxone (0.5 – 7 mg), given in a 30 minute infusion. Using the information from this study, the third step was to test the effect of a combination of a bolus dose of naloxone and a longer continuous naloxone infusion (lasting two hours) on 0.2 and 0.4 mg buprenorphine induced respiratory depression.

A bolus dose of naloxone turned out to be ineffective for the reversal of respiratory depression from buprenorphine, as was observed in study 1. From study 2, we found out that in the dose range between 2 – 4 mg, naloxone was able to cause full reversal of respiratory depression. A higher dose (above 5 mg) caused a decline in reversal activity. In the third study, we saw that it is possible to reverse both 0.2 and 0.4 mg buprenorphine’s respiratory effects by using a combination of a bolus dose (2 – 3 mg) and a subsequent continuous infusion of naloxone of 4 mg/h. The main conclusions from this study were that reversal of buprenorphine’s respiratory effect is possible, but that it depends on doses of both naloxone and buprenorphine. There seems to be an inverse U-shaped curve for naloxone reversal of buprenorphine’s effects (see figure 3.3), meaning that a higher naloxone dose does not necessarily cause more reversal of respiratory depression. Next to that, the respiratory effects may outlast short lasting infusions of naloxone, so it is important to use a continuous infusion of naloxone for the reversal of buprenorphine induced respiratory depression.

In Chapter 4, we modelled the effects of naloxone on M6G- and morphine-induced respiratory depression. We conducted a study in 56 healthy volunteers. First we compared the effects of 400 μ g naloxone and placebo on the respiratory effects of morphine and M6G. Next, we investigated the effects of different naloxone doses (200 μ g in the morphine group, 25 and 100 μ g in the M6G group) in both opioids. All studies were performed under constant end-tidal CO₂ pressures. We found that morphine’s effects were quickly reversed and that this reversal was shortlasted, whilst in the M6G group, the time to maximum effect was longer (45 minutes versus 13 minutes in the morphine group), and the reversal lasted longer (up to 90 minutes). We fitted the data to a PK/PD model, from which we were able to conclude two things. The first was that a Hill factor (γ) needed to be introduced to our model for an appropriate fit and the second was that there were differences in naloxone C_{50} in the morphine and M6G studies. This means that naloxone has a different potency in M6G and morphine (less naloxone is needed to reverse M6G induced respiratory depression than in morphine induced respiratory depression). The conclusion from these studies is that morphine’s and M6G’s effects are differently reversed by naloxone. We can still only speculate as to how these differences are caused, but most likely is that naloxone does not only act at the μ -opioid receptor itself, but also at a different site of action in the signalling cascade.

Reversal of respiratory depression is not the only application of naloxone, however. Chapters 5 and 6 elaborate on different uses of naloxone, i.e., in hyperalgesia (Chapter 5) and in opioid addiction (Chapter 6).

Opioid induced hyperalgesia has recently gained the interest of researchers. It is probably caused by activation of the NMDA-receptor, which in turn could be caused by μ -opioid receptor-activation. In Chapter 5 we tested this hypothesis specifically for M6G in mice (outbred CD-1 and opioid receptor triple knockout mice) and men (healthy vol-

unteers). In mice, we studied the effect of chronic and acute infusions of M6G against a background of naltrexone or normal saline. In human volunteers, we tested the effect of a bolus dose of M6G to a heat pain stimulus, in the presence of a continuous high dose naloxone infusion or saline infusion. The results from the mice studies show that acute and chronic injections of M6G cause hyperalgesia, both in naltrexone and saline treated mice. Injection of NMDA-receptor antagonist MK-801 blocked and reversed hyperalgesia in the chronic and the acute M6G treatment. The data from the human volunteers indicate that M6G causes hyperalgesia after an acute injection, lasting more than six hours. We can conclude that M6G induced hyperalgesia is independent of opioid receptor activation and that a causal role for the NMDA receptor is indicated in mice.

In Chapter 6, we undertook a literature search for all uses of naloxone in opioid addiction. Naloxone is a non-selective, short-acting opioid receptor antagonist that has a long clinical history of successful use and is presently considered a safe drug, even at high doses (up to 10 mg). In opioid-dependent patients, naloxone is used in the treatment of opioid-overdose induced respiratory depression, in (ultra)rapid detoxification and in combination with buprenorphine for maintenance therapy (to prevent intravenous abuse). There are several risks related to naloxone use in opioid-dependent patients. The induction of an acute withdrawal syndrome is a potentially life threatening one, due to possible occurrence of vomiting and aspiration. When used in the treatment of opioid-induced respiratory depression, the effect of naloxone may wear off prematurely and cause renarcotization and subsequent coma. The final risk is that in patients treated for severe pain with an opioid, high-dose naloxone and/or rapidly infused naloxone may cause catecholamine release and consequently pulmonary edema and cardiac arrhythmias. These risks warrant the cautious use of naloxone, together with adequate monitoring of the cardiorespiratory status of the patient during naloxone administration.

CONCLUSIONS

The data collected in this thesis show

- There is probably no separate M6G receptor in the respiratory system of the cat.
- M6G has its respiratory effects mainly on the central chemoreceptor and not on the peripheral chemoreceptor in the respiratory system of the cat.
- Buprenorphine induced respiratory depression can be reversed using a continuous, high dose infusion of naloxone.
- The reversal of buprenorphine's respiratory effects by naloxone has an inverse U-shaped dose-response curve.
- M6G and morphine's respiratory effects are differently reversed by naloxone, in both time of maximum reversal and duration of reversal.
- M6G induced hyperalgesia is independent of opioid receptor activation.
- In opioid dependence, naloxone has its use mostly in reversing opioid overdose.

HOOFDSTUK 8

Samenvatting en conclusies

Al vele decennia vormen opioïden de gouden standaard in behandeling van acute en chronische pijn, ondanks de grote hoeveelheid bijwerkingen die ze veroorzaken. De belangrijkste (want levensbedreigende) bijwerking is ademdepressie – een toestand die gemakkelijk te verhelpen is door het gebruik van opioïd-antagonisten. Naloxon is zo’n middel – een kortwerkende opioïd-antagonist met een hoge affiniteit voor de μ -opioïd receptor en een lagere affiniteit voor de κ - en δ -opioïd receptoren. In hoofdstuk 2, 3 en 4 onderzochten we het gebruik van naloxon in door verschillende opioïden veroorzaakte ademdepressie.

In hoofdstuk 2 onderzochten we het bestaan van een specifieke morfine-6-glucuronide (M6G) receptor door middel van 3-methoxy-naltrexon (3mNTX), een van naloxon afgeleid geneesmiddel dat specifiek is voor de M6G-receptor. We vergeleken de ademhalingseffecten van morfine en M6G in katten onder narcose door middel van de zogeheten ‘dynamic end-tidal forcing technique’ (DEF). Met deze techniek is het mogelijk om onderscheid te maken tussen de CO₂ gevoeligheid van de perifere en de centrale chemoreflexbogen (G_p en G_c , respectievelijk). Deze variabelen zijn parameters in een twee-compartimenten model voor het verband tussen CO₂ en ademhaling. Hiertoe werden drie verschillende studies uitgevoerd. In studie 1 werd het effect van achtereenvolgens morfine, 3mNTX en M6G op de ademhaling gemeten. In studie 2 werd het effect van achtereenvolgens M6G, 3mNTX en morfine bekeken. Tenslotte werd in studie 3 het ademhalingseffect van 3mNTX alleen bekeken. Met deze studies werd aangetoond dat zowel M6G als morfine de ademrespons op CO₂ naar rechts verschuiven – er is een hogere CO₂ concentratie nodig om hetzelfde ademminuutvolume te bereiken. Uit de studies bleek verder dat morfine met name de centrale chemoreflexboog remde, terwijl M6G juist de perifere chemoreflexboog remde. Met 3mNTX was het mogelijk om de ademdeprimerende effecten van beide opioïden om te keren. Uit deze studies vallen twee dingen te concluderen. Ten eerste hebben we gevonden dat in katten onder narcose, de opioïden M6G en morfine hun ademdeprimerende effect op verschillende plaatsen binnen het ademhalingsregulatie systeem uitoefenen. Daarnaast kan geconcludeerd worden dat de verschillen in ademhalingseffect van morfine en M6G veroorzaakt worden door een 3mNTX-sensitieve receptor, omdat 3mNTX de ademhalingseffecten van beide opioïden volledig omkeerde.

In hoofdstuk 3 onderzochten we in een grote groep gezonde vrijwilligers (n=67) de ademhalingseffecten van buprenorfine, en de mogelijkheid om deze om te keren door middel van naloxon. Het idee achter deze studie was dat de ademhalingseffecten van buprenorfine schijnbaar moeilijk omkeerbaar zijn door naloxon. In dit hoofdstuk is data uit drie verschillende onderzoeken gecombineerd. In al deze studies werd de ademhaling gemeten tegen een constante, verhoogde eind-expiratoire CO₂-concentratie. In het eerste onderzoek onderzochten we het effect van een intraveneuze bolus van 0.8 mg naloxon of van placebo (0.9% NaCl) op de ademhalingseffecten die door 0.2 mg buprenorfine werden geïnduceerd. Deze dosis bleek onvoldoende om de ademdepressie door buprenorfine om te keren. Daarom besloten we hierna een reeks van verschillende

naloxon doseringen te testen (0.5 – 7 mg) die gegeven werden door middel van een continu-infusie van 30 minuten. Met de informatie uit dit onderzoek onderzochten we vervolgens het effect van een bolus naloxon in combinatie met een twee uur durende continu-infusie op de ademhalingseffecten van 0.2 en 0.4 mg buprenorfine.

Een bolus naloxon is niet voldoende om de ademhalingseffecten van buprenorfine om te keren, zo bleek uit het eerste onderzoek. In het tweede onderzoek ontdekten we dat een dosis naloxon tussen de 2 – 4 mg wel volledige omkering van de ademhalingseffecten kan geven. Een dosis hoger dan 5 mg bleek echter weer minder omkering te geven. In de derde studie zagen we dat ademdepressie door buprenorfine omgekeerd kan worden door een bolus naloxon, gevolgd door een continu-infusie naloxon van twee uur. De belangrijkste conclusie uit dit onderzoek is dat omkering van door buprenorfine veroorzaakte ademdepressie mogelijk is, maar dat het wel afhangt van de naloxon en buprenorfine doseringen. Er lijkt een klokvormige curve ('bell-shaped curve') te zijn voor omkering van buprenorfines ademhalingseffecten door naloxon (zie figuur 3.3), dus een hogere naloxon dosering wil niet altijd zeggen dat er meer omkering is. Daarnaast is het belangrijk om te beseffen dat de ademhalingseffecten van buprenorfine wellicht langer aanwezig kunnen zijn dan dat de werking van naloxon aanhoudt, dus is een continu-infusie van naloxon nodig voor het omkeren van de ademhalingseffecten van buprenorfine.

In hoofdstuk 4 hebben we de effecten van naloxon op door M6G en morfine geïnduceerde ademdepressie gemodelleerd. Het betrof een studie in 56 gezonde vrijwilligers. Eerst vergeleken we de effecten van 400 μg naloxon en placebo (0.9% NaCl) op de ademhalingseffecten van morfine en M6G. Vervolgens onderzochten we verschillende andere doseringen (200 μg in de morphine groep, 25 and 100 μg in de M6G groep) in beide opioïden. Het effect op de ademhaling werd steeds getest bij een constante eind-expiratoire CO_2 concentratie. De effecten van morfine bleken snel om te keren en kortdurend, terwijl de effecten van naloxon in de M6G-groep langer op zich lieten wachten: de tijd tot het maximale effect was bereikt was veel langer in de M6G groep (45 minuten vergeleken met 13 minuten in de morfine groep) en als maximale omkering was bereikt, hield deze ook veel langer aan (tot wel 90 minuten). We fitten een farmacokinetisch/farmacodynamisch model op de data en hierin vallen twee dingen op. Ten eerste was de invoering van een extra parameter, γ , nodig voor een adequaat model van onze data. Daarnaast vonden we een verschil in de naloxon C_{50} tussen de morfine en de M6G groepen. Dit betekent dat naloxon een verschillende potentie heeft voor de omkering van de ademhalingseffecten van beide opioïden (er is minder naloxon nodig om de ademhalingseffecten van M6G om te keren dan voor de omkering van de ademhalingseffecten van morfine). De conclusie die vervolgens te trekken valt is dat de ademhalingseffecten van morfine en M6G verschillend worden omgekeerd door naloxon. Het blijft speculatie, maar het meest waarschijnlijk is dat deze verschillen worden veroorzaakt doordat naloxon niet alleen effecten heeft op de μ -opioïd receptor zelf, maar ook verder in de signaleringscascade van de ademhalingsregulatie.

De omkering van opioïd-geïnduceerde ademdepressie is echter niet de enige toepassing van naloxon. Hoofdstukken 5 en 6 gaan in op ander gebruik van naloxon, te weten in hyperalgesie (Hoofdstuk 5) en in opioïd-verslaving (Hoofdstuk 6).

Hyperalgesie veroorzaakt door opioïden is pas recent onder de aandacht van onderzoekers gekomen. Het wordt waarschijnlijk veroorzaakt door activatie van de N-methyl-D-aspartaat receptor of NMDA-receptor, die op zijn beurt weer geactiveerd zou kunnen worden door μ -opioïd receptor activatie. In hoofdstuk 5 hebben we deze hypothese onderzocht in muizen en in gezonde vrijwilligers. In muizen bestudeerden we het effect van korte en langdurige M6G infusies tegen een achtergrond van naltrexon of 0.9% NaCl. In gezonde vrijwilligers onderzochten we het effect van een bolus dosis M6G op een hittepijntest, ook weer tegen een achtergrond van naloxon of 0.9% NaCl. De resultaten van de muizenstudies laten zien dat zowel korte als langdurige infusies van M6G hyperalgesie veroorzaken, en dat het niet uitmaakt of er naltrexon of zoutoplossing op de achtergrond werd gegeven. Toediening van de NMDA receptor antagonist MK-801 verhinderde het ontstaan van hyperalgesie en zorgde voor een omkering van hyperalgesie in zowel de korte als de langdurige M6G toediening. De resultaten van de vrijwilligers laten zien dat M6G ook in mensen een hyperalgetisch effect heeft, dat in ieder geval zes uur duurt. Ook hier is er geen invloed van het al dan niet toevoegen van naloxon. We kunnen daarom concluderen dat M6G hyperalgesie veroorzaakt en dat dit onafhankelijk is van opioïd-receptor activatie. In de muizenstudie werd aangetoond dat er een belangrijke rol is voor de NMDA receptor in het ontstaan van hyperalgesie na opioïd-toediening.

In hoofdstuk 6 tenslotte zijn de resultaten van een literatuuronderzoek naar het gebruik van naloxon in opioïd-verslaving samengevoegd. Naloxon is een niet-selectieve, kortwerkende opioïd-antagonist die reeds lang met succes in de klinische praktijk toegepast wordt. Het wordt beschouwd als een veilig middel, zelfs bij hoge doseringen (tot en met 10 mg). In opioïd-verslaafde patiënten wordt naloxon gebruikt in de behandeling van ademhalingsdepressie als gevolg van een overdosis opioïden, in snelle ontwenning en in combinatie met buprenorfine voor langdurige onderhoudstherapie (waarbij de naloxon intraveneus misbruik moet voorkomen). Er zijn verschillende risico's verbonden aan het gebruik van naloxon in opioïd-verslaafde patiënten, waarbij de belangrijkste het ontstaan van een acuut ontwenningssyndroom is. Hierbij is met name het optreden van braken en het daarmee gepaard gaande risico op aspiratie van maaginhoud gevaarlijk. Als naloxon gebruikt wordt voor de behandeling van ademdepressie door een overdosis bestaat de kans dat de effecten van naloxon te snel afnemen, waardoor er opnieuw sedatie en zelfs coma kan ontstaan. Ten slotte is bekend dat in patiënten die voor ernstige pijn met opioïden behandeld werden, een te snelle of te hoge dosering van naloxon een catecholamine-release kan veroorzaken, die vervolgens weer kan leiden tot longoedeem en hartritmestoornissen. Gezien deze risico's is het belangrijk om naloxon voorzichtig te gebruiken en waar nodig de cardiale en pulmonale toestand van de patiënt te bewaken.

CONCLUSIES

Uit de gegevens in dit proefschrift is het volgende te concluderen:

- Er is waarschijnlijk geen aparte M6G receptor in het ademhalingscentrum van de kat.
- M6G oefent zijn ademhalingseffecten voornamelijk uit binnen de centrale chemoreflexboog, en niet op de perifere chemoreflexboog, in het ademhalingsregulatiesysteem van de kat.
- Door buprenorfine geïnduceerde ademhalingsdepressie kan worden omgekeerd door een hoge dosering naloxon in een continu infusie.
- Er is sprake van een klokvormige curve bij de omkering van de ademhalingseffecten van buprenorfine door naloxon.
- De omkering van ademhalingseffecten van M6G and morfine door naloxon verschillen zowel in de tijdsduur tot het maximale omkeringseffect is bereikt, als in de duur van de omkering.
- Door M6G veroorzaakte hyperalgesie is onafhankelijk van opioïd-receptor activatie.
- De belangrijkste toepassing van naloxon in opioïd-verslaafde patiënten is de omkering van opioïd-overdoseringen.

Curriculum Vitae

Eveline Louise Arianna van Dorp was born on January 7th, 1982 in Leiden. She obtained her VWO (gymnasium) diploma at the Stedelijk Gymnasium Leiden in June 2000. Later that year she entered Medical School at the University of Utrecht. During her early clinical rotations, she became interested in anesthesiology and found a place as a data-management assistant for the peri-operative complication registration in the Leiden University Medical Center in 2003. In 2005, she became a PhD student at the Department of Anesthesiology in the Leiden University Medical Center and started the investigations described in this thesis. She transferred to Leiden University for the final year of Medical School and in June 2006, she received her Medical Degree from Leiden University. In June 2009, she hopes to start her residency in Anesthesiology in the Department of Anesthesiology, Leiden University Medical Center.

List of Publications

1. Van Dorp E and Smit R: ‘Softwarematige straaljagerpiloten.’ *VPRO-Gids* 30, 1999.
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