Opioid Therapy: A Trade-Off between Opioid-Analgesia and Opioid-induced Respiratory Depression - M.C.A. Boom

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Section I

INTRODUCTION

CHAPTER 1
INTRODUCTION:

1.1 INTRODUCTION

Opioids are the first choice in the treatment of severe acute and chronic pain. Apart from their intended effect (analgesia), opioids come with a variety of side effects with respiratory depression as potentially life-threatening. Opioid-Induced Respiratory Depression (OIRD) is often moderate to mild from which the patient recovers spontaneously or is rescued by other means such as stimulation of the patient to take a deep breath or chin lift. In severe cases of OIRD, breathing becomes initially irregular, followed by cyclic breathing (breathing shows an on/off pattern alike Cheyne-Stokes breathing) and apnea. Rescue is by resuscitation, intubation and assisted ventilation and reversal of opioid effect by naloxone. The incidence of respiratory depression from opioid treatment, acute or chronic, is poorly documented. A recent systematic review of the literature on post-operative OIRD estimates an average incidence of 0.5% with a range of 0.2-2%.[1] This suggests that only 1 in 200 patients develops a respiratory event from opioids that requires an intervention. There are indications that this number is an underestimation with an incidence of OIRD many times higher than 1:200. For example, following patient controlled analgesia with morphine hypoventilation (defined by a respiratory rate < 8 breaths.min^{-1}) occurs in one in three patients.[2] No valid data are available on the occurrence of OIRD in chronic pain patients.[1] Accidental deaths from OIRD in this patient population are often attributed to the progression of underlying disease (for example cancer), old age or co-existing diseases. Although a recent systematic review of case reports on OIRD in chronic pain patients does not provide quantitative data on the occurrence of OIRD, it does provide important information on its development:[3] (i) Since the year 2000, methadone, fentanyl and oxycodone are predominantly involved in OIRD while before 2000 morphine was the most prevalent cause of OIRD, (ii) the incidence of OIRD increased sharply in non-cancer chronic pain patients over the last 10 years and (iii) co-medication affecting the opioid's pharmacokinetics and dynamics is an important cause for opioid toxicity. Observation #2 is probably related to an increased awareness to treat severe chronic pain and also due to the aggressive promotion of opioids by the pharmaceutical industry. Observation #3 is important in chronic pain patients but also important in perioperative and acute settings when opioids are combined with additional drugs that depress ventilation (such as midazolam, propofol, muscle relaxants).

1.2 MECHANISMS OF ACTION

The drive to breathe is generated in multiple respiratory centers in the brainstem.[1] Respiratory neurons receive inputs from various sites in the CNS (cortex, limbic system, hypothalamus, spinal cord), a set of receptors located in the brainstem (central chemoreceptors), and in the carotid bodies (peripheral chemoreceptors). These chemosensors send information, changes in pH, PCO_2 and PO_2 of the CSF and arterial blood, to the brainstem respiratory centers, which appropriately adjust breathing rate and pulmonary tidal volume. For example, acidosis, hypercapnia and hypoxia will cause

hyperventilation, while hypocapnia and alkalosis will reduce minute ventilation. Opioid effects on μ-opioid receptors (MOR), expressed on respiratory neurons, are the main cause for the reduction of the drive to breathe. When an opioid is administered to a patient and the injection rate is sufficiently slow (over minutes) or the passage of the opioid across the blood-brain barrier is slow, depression of the respiratory neurons in the brainstem coincides with the accumulation of arterial CO_2. The stimulatory effect of the increased CO_2 at the peripheral and central chemoreceptors will offset the decrease in tidal volume and reduced respiratory rate. OIRD is then observed as an increase in arterial (and end-tidal PCO2) with little effect on ventilation. When the opioid is infused rapidly and passage across the blood-brain barrier is fast, the depression of the respiratory neurons in the brainstem will dominate as there is insufficient time for CO_2 to accumulate in the body. This then will cause severe OIRD with hypoventilation and initially near normal PCO_2 values. See also Chapters 2 and 3 of this thesis.

Although the incidence of opioid-induced respiratory depression is low, fatalities do regularly occur. For example, Lötsch et al.[4] describe a young female (BMI 19 kg/m2) who had peripheral orthopaedic surgery under general anesthesia (sevoflurane 2-3% with 200 μg fentanyl). In the recovery room the patient received four doses of morphine over 2 hours, reaching a total of 35 mg or 0.7 $mg.kg^{-1}$. Forty minutes after the last dose she developed OIRD followed by a fatal cardiac arrest. At that time estimated brain concentrations were about 150 nM, which is above the toxic range for morphine. This case understates the need for a close understanding of the pharmacokinetics and dynamics of any opioid that is used in any patient. The physicians involved in this case did not take into account the very slow passage of morphine across the blood-brain barrier which caused a peak in central effect hours following peak plasma concentration. And while the onset of analgesic occurred relatively rapidly following the last dose, the fatal respiratory depression occurred 40 min later. This report demonstrates further the need to view OIRD in light of the opioid's wanted effect, analgesia. There are few studies that address the composite effects of opioids. One way to compare the different effects of one opioid is to construct a safety or utility function. These functions, originally used in economics and first applied in pharmacology by Sheiner and Melmon in 1978,[5] are constructed by estimating the difference in probability of analgesia and respiratory depression as derived from experimental pharmacokinetic/pharmacodynamic modelling studies. While application of these functions is currently difficult to envision in a clinical setting, they are useful in the development of novel opioids aimed at maximizing analgesia while simultaneously minimizing OIRD, for example when making a choice for a drug dose to test in a phase III trial from data obtained in phase II studies. For further elaboration see also Chapter 4 of this thesis.

1.3 DEVELOPMENT

Development of an opioid that is without any respiratory depression or other dangerous side effects (the holy grail of opioid pharmacology) seems not possible. At least no such drugs have been developed so far. Fortunately, the pharmaceutical industry still does attempt to develop opioids with restricted respiratory depressant properties. Various companies are focussing on single chemical molecules that activate multiple receptor system including the MOR and a secondary system that possibly counteracts the side effects of the activated MOR. Again the properties of such opioids should always be viewed in terms of their wanted effects. For example, the opioid buprenorphine is known to have a ceiling in its respiratory depressant effects. Such a phenomenon is only advantageous when respiratory ceiling does not coincide with ceiling in analgesia. Indeed ceiling in analgesia seems to occur at higher (supra clinical) doses than respiratory ceiling.[6] This is further adressed in Chapter 5 of this thesis.

Opioid effect is extremely variable with effects that may vary a factor 20-40 between patients. The cause of variety is multiple and has pharmacokinetic and pharmacodynamic origins. Known causes of variability include genetics (for example due to polymorphic enzymes involved in drug metabolism),[3] sex (women are more sensitive to opioids than men),[7] underlying disease (in children repetitive hypoxic events from obstructive sleep apnea are associated with increased opioid sensitivity; in Alzheimer's disease opioid sensitivity seems reduced due to the loss of descending inhibition), age (the elderly have an increased opioid sensitivity),[8,9] co-medication and smoking (smoke contains polycyclic aromatic hydrocarbons that interact with metabolizing enzymes in an often unpredictable fashion).[4] Other factors are less well known and poorly studied, such as for example the nutritional state of the patient and the circadian rhythm, factors that play an important role in the pharmacodynamics of other drugs such as inhalational anesthetics and opioids.[10] See also Chapter 6 of this thesis.

1.4 AIM OF THIS THESIS

The aim of this thesis is to investigate the influence of strong opioids on the control of breathing, taking into account their analgesic properties.

In **Chapter 2** the respiratory pharmacokinetics and dynamics of opioids are discussed. Furthermore, an overview is given of agents that are able to reverse OIRD. Apart from agents that antagonize the MOR, other agents are discussed that stimulate breathing via other receptor systems and theoretically restore breathing without compromising analgesia.

In **Chapter 3** the respiratory depressant effects of the potent opioid remifentanil are modelled using a mathematical model that incorporates the depressant effect of

remifentanil on respiratory neurons in the brainstem and the stimulatory effects of carbon dioxide on the chemoreceptors. This model enables us to predict the behaviour of the ventilatory control system under various remifentanil administration paradigms.

In **Chapter 4** a safety function or utility function is described for the strong opioid fentanyl. As discussed above, the function describes the opioid's behaviour in light of its benefit (analgesia) and harm (respiratory depression).

In **Chapter 5** the respiratory behaviour of an experimental opioid (MR30365/07) is studied and compared to placebo and fentanyl. By performing dose-response studies for respiratory depression and analgesia it is shown that this opioid behaves differently from fentanyl. Possible mechanisms are discussed.

In **Chapter 6** the influence of the circadian rhythm on pharmacodynamic effect of fentanyl is studied. In a first approach, fentanyl's analgesic effects are studied at 4 time points: 02 AM, 08 AM, 2PM and 8PM.

All experiments were performed in healthy young volunteers in the Anesthesia & Pain Research Unit of the Department of Anesthesiology of the Leiden University Medical Centre (LUMC).

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Section II

REVIEW

CHAPTER 2

NON ANALGESIC EFFECTS OF OPIOIDS: OPIOID-INDUCED RESPIRATORY DEPRESSION

Review

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2.1 INTRODUCTION

Opioids induce respiratory depression.[1] Morphine, however, remains the gold standard for the treatment of postoperative pain and opioids such as the fentanyl derivatives are widely used as part of anesthetic procedures. Although the overall risk of postoperative respiratory depression is relatively low with an estimated total incidence of respiratory events in the range of 0.5 to 2%,[1] it is nevertheless wise to remember that the first 24 hours after surgery represent a high risk period for a respiratory event as a result of opioid use,[2] that fatal outcomes to respiratory depression may occur[3] and that this risk is exacerbated in some patient groups.[1-4] Similarly, opioid-induced respiratory depression in chronic opioid treatment remains rarely reported and is most probably grossly underreported.[4] Effective rescue treatment, usually with naloxone, is available to reverse opioid-induced respiratory events, particularly in an emergency setting. However, naloxone antagonizes both opioid-induced respiratory depression and analgesia. Consequently, pain management during crisis recovery of hypoventilation/apnea will be compromised. There are clear advantages, therefore, in the design and availability of drugs that antagonize the respiratory depressant effects of opioids without decreasing their analgesic actions.

Opioid-induced analgesia and respiratory depression arise from stimulation of μ-opioid receptors (MORs). MORs are expressed on neurons involved in the control of breathing, primarily located in the brainstem. Opioid-induced breathing alterations are complex, but may be characterized by an increase in arterial carbon dioxide concentration (hypercapnia) and reduction in tidal and minute volume. Respiration becomes slow, irregular (with hypercapnia and hypoxia,[5] and eventually fatal apnea may occur. The respiratory centers responsible for these complex events are many and varied.[6] The main drive for respiration is located in the brainstem, particularly in the respiratory rhythm generating areas such as the pre-Bötzinger complex (although this area has not been identified in humans), which is active during inspiration and is opioid-sensitive,[7] in association with the retro-trapezoid and parafacial respiratory groups (which are active in expiration and are insensitive to opioids), together with input from other brain areas and tonic drive from multiple chemoreceptor areas in the lower brainstem.[6] Opioid-receptors responsible for respiratory depression are abundant at a number of anatomical loci within the respiratory centers, particularly at the pre-Bötzinger complex.[6,7]

The current review will address opioid receptor-mediated respiratory depression, its reversal by naloxone and, in particular, some possibilities into mechanisms and drug prospects that may inhibit opioid-induced respiratory depressant effects, without reducing analgesia.

2.2 THE ROLE OF OPIOID RECEPTORS IN RESPIRATORY DEPRESSION

Opioids exert their pharmacological effects through interactions with multiple opioid receptors, initially classified as μ-, κ- and δ-opioid receptors,[8] to which the non-classical nociceptin receptor (nociceptin/orphanin FQ peptide receptor) may be added.[9] Opioid

receptors are members of a large seven trans-membrane superfamily of G-protein coupled receptors (GPCRs). Opioid ligand attachment to the receptor results in binding of the $G_{i/o}$ protein and formation of a $G\alpha_i$-guanosine tri-phosphate (GTP) complex, which is primarily responsible for perhaps the best known opioid intracellular pathway, inhibition of adenylyl cyclase and reduction of intracellular cyclic adenosine mono-phosphate (cAMP) levels, resulting in changes in membrane currents and inhibition of transmitter release. Various other intracellular signaling pathways may also be activated by opioid binding (such as the MAPK/ERK (microtubule-associated protein kinase/extracellular signal-regulated kinase) and Akt (Protein Kinase B)), which may result in activation or inhibition of many cellular proteins and signaling mechanisms that lead to different biological outcomes.[10,11]

Pharmacodynamic responses to opioid stimulation depend on the nature of the receptor and the affinity and efficacy of the opioid for that receptor. The common properties of morphine, for example, are attributable to binding and activation of the MOR[12] and result in morphine-induced actions such as analgesia, respiratory depression, sedation, euphoria, constipation, vomiting and nausea. MORs are located in the central nervous system in centers associated with pain[13] and stimulation induces strong analgesia. With regard to respiration, MORs are found in abundance in respiratory control centers in the pons and brainstem (see above and [6,7]).

Using the more recent techniques of gene knockout studies, a link between MOR-induced analgesia and respiratory depression has been demonstrated clearly; in MOR knockout mice the administration of morphine or other MOR agonists, failed to induce both antinociception and respiratory depression.[14,15] Hence all known MOR agonists, such as morphine, fentanyl, hydromorphone etc., induce potentially both analgesia and respiratory depression. To investigate whether opioid analgesic drugs without respiratory depressant effects may be developed, there has been interest over many years in opioid agonist ligands specific for κ- and δ-opioid receptors.[16,17] However, the development of analgesic agents acting at these opioid receptors has been limited by the association of such ligands with serious adverse events other than respiratory depression. For example ligands at the κ-opioid receptor may cause psychotomimetic and dysphoric effects,[18,19] ligands at the δ-opioid receptor may cause convulsions.[25] Further development of therapeutically useful drugs acting at these opioid receptors still may be possible, for example (dimeric) peptides to act as multiple opioid ligands (e.g., δ-κ ligands).[21,22]

Although MOR-induced analgesia invariably has the potential to also induce respiratory depression, there has been much speculation whether different opioids, dose regimens, routes of administration and other measures may separate these μ-opioid properties. Although naloxone is a competitive antagonist at the MOR and its administration may normally reverse both respiratory depression and analgesia, in clinical practice, the judicial use of naloxone titration may be used to selectively reverse respiratory effects, rather than analgesic effects, since respiratory depression may occur at a higher degree of

receptor occupancy than necessary for some degree of analgesia.[1,23]
The possibility that MOR subtypes may exist and hence that subtype selective opioid ligands could allow separation of respiratory depression and analgesia, was supported by studies in which separation of these morphine-induced actions in rats was achieved with the use of the antagonist naloxonazine.[24] In these studies, two receptor subtypes, μ1- and μ2-opioid receptors, were described; the μ1-opioid receptor was held responsible for the analgesic effects and the μ2-opioid receptor for respiratory depressant effects.[19] Naloxonazine showed selective antagonism of μ1-opioid receptor sites.[24] Some separation of μ-opioid-induced analgesic and respiratory depressant effects has also been shown with the metabolite of morphine, morphine-6-glucuronide (M6G), which like its parent molecule morphine is a potent MOR agonist.[25] In experimental human and clinical studies M6G induces less respiratory depression than morphine at equianalgesic doses.[26-28] This selectivity of action has been attributed to selective effects on MOR subtypes, since some receptor binding studies have shown M6G to have a lower affinity for the μ2-opioid receptor compared to the μ1-opioid receptor.[29, 25] An affinity profile for M6G of $\mu 1 > \mu 2$, therefore, may be expected to demonstrate a profile of analgesia with less respiratory effect than morphine. A note of caution is necessary, however, μ1- and μ2-opioid receptor subtypes have not been formally recognized;[30] sufficiently selective ligands to define the μ1- and μ2 receptor subtypes, correlation with identified MOR splice variants and adequate experiments in humans are lacking so far.
Apart from opioid receptor selectivity, the pharmacokinetics (PK) and pharmacodynamics (PD) of different opioids will also play a significant role in determining the actions of an opioid in vivo. Indeed, PK-PD human studies and modeling is proving of great value in further understanding and subsequently choosing of opioids for clinical use.[1,28,31-32]

2.2.1 FULL OPIOID AGONISTS

Many of the common opioids in therapeutic use for pain management, such as morphine and its derivatives, and fentanyl and its congeners, are full agonists at the MOR. Acting at the MOR, all opioid agonists may induce respiratory depression over some part of their dose-response range, particularly at high doses. In vitro and in vivo studies show that full agonists at the MOR display relatively fast association and dissociation to and from the receptor.[33-34] For rapidly acting drugs like fentanyl, the in vivo rate constants for receptor association and dissociation are fast relative to their PK in brain and plasma, indicating that in vivo binding to, and dissociation from, the MOR occurs essentially instantaneously.[34] When considering the time courses of opioid-induced biological effects, including respiratory effects, association/dissociation from the receptor is not the rate determining factor and neither are the rates of drug removal from the plasma, since these opioids undergo rapid metabolism. The most important factor for opioid agonists in determining the link between plasma concentration and respiratory depressant outcome, as demonstrated by mechanism-based PK-PD modeling in humans, is the rate of transfer

of a drug from the plasma to an effect (*i.e.* a receptor containing) compartment.[28,31,34] A schematic diagram of the relationship between biophase kinetics, receptor kinetics and outcome is shown in Fig 1. For opioid agonists with a fast rate of transfer, such as remifentanil, alfentanil and fentanyl, the half-life for the transfer of drug from plasma to receptor site ($t_{½}k_{e0}$) is short. For example, $t_{½}k_{e0}$ estimates from opioid-induced changes in electroencephalographic activity range from 0.8 to 1.3 min for remifentanil, 0.6 to 1.3 min for alfentanil and 4.7 to 6.6 min for the fentanyl.[34] As a result of the fast rate of transfer, biological effects will commence rapidly, whilst they may be more delayed for opioid agonists like morphine with slower rates of drug transfer to the effect compartment (e.g., $t_{½}k_{e0}$ estimates from morphine-induced miosis: 2.8– 3.9 h).[31] A consequence of the rapid transfer and immediate association of potent opioids (like fentanyl) to the MOR is that infusion of a high intravenous dose may lead to a rapid onset of dangerous levels of respiratory depression and apnea before arterial carbon dioxide concentration may rise sufficiently to stimulate breathing.[35] Such rapid respiratory effects may be less evident if the $t_{½}k_{e0}$ of the agonist is slower. Hence for the induction of rapid respiratory depression at equianalgesic doses, a hierarchy of opioid agonists may be deduced from fastest to slowest: alfentanil > fentanyl > morphine > M6G.[28,31,34] For opioid agonists of markedly less lipophilicity, such as morphine and M6G,[20] transfer to the effect site compartment is very slow (M6G $t_{½}k_{e0}$ ranges from 1.4 to 6.4 h), hence biological effects such as respiratory depression are considerably slower in onset.[28,36] For compounds like M6G, slow transfer rates to different effect compartments may result in marked differences in the $t_{½}k_{e0}$ estimates for the induction and time courses of various M6G-induced biological effects. With the complex hydrophilic nature of M6G,[32] these variations in $t_{½}k_{e0}$ may be related to a number of factors, including transfer between brain compartments.[37]

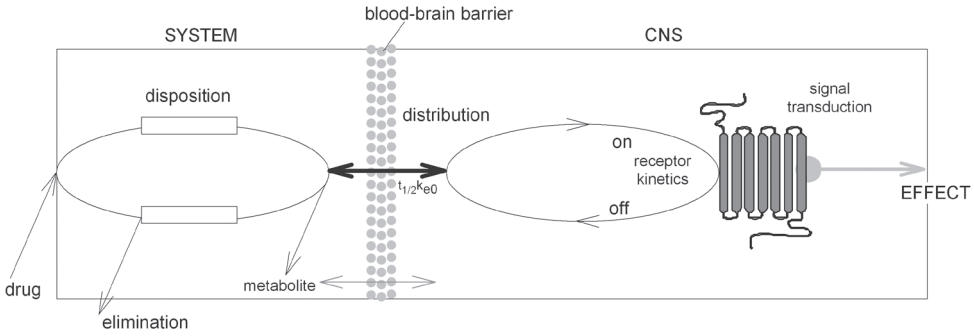

Figure 1. Schematic diagram of the pharmacokinetics of an opioid analgesic in the body (system) and central nervous system (CNS) compartments. After disposition part of the drug reaches the brain compartment after passage across the blood-brain barrier (with half-life t½ke0). Next the drug will distribute to the receptor site and attach to the receptor with association (on) and dissociation (off) kinetics.

2.2.2 OPIOID PARTIAL/MIXED AGONISTS: BUPRENORPHINE

One approach to the development of opioid analgesic drugs with lower risk factors for respiratory depression has been the development of partial agonists at the MOR, such as buprenorphine.[38] Partial agonists typically display a less than maximum or ceiling effect in their dose-response relationships, as has been described for buprenorphine-induced respiratory effects.[35,39] At a very high dose (80% of its LD_{50}), buprenorphine has minimal respiratory responses compared to fentanyl or morphine.[40] A reduced maximum respiratory depressant effect has obvious advantages for the safety profile of buprenorphine. Unlike respiratory events, buprenorphine-induced analgesia, at least over clinical dose ranges, does not typically show a ceiling effect in postoperative pain.[41] In detailed animal studies, buprenorphine has been shown to achieve maximum antinociceptive effects in some, although not all, animal models[42] and, when antinociceptive ceiling effects are observed, these may be reached only at high doses, higher than those required for the maximal respiratory effects and outside of the 'clinical dose range'.[35,38,43] A recent review of the clinical use of buprenorphine by a consensus group of experts, reiterated that, consistent with receptor theory, buprenorphine behaves as a full μ-opioid receptor agonist for analgesia, with no ceiling effect, whilst there is a ceiling effect for respiratory depression with buprenorphine, clearly reducing the likelihood of potentially fatal adverse events.[45]

When considering onset and offset of buprenorphine effect, it is necessary, in addition to the biophase distribution kinetics ($t_{½}k_{e0}$, that was the sole determinant parameter for biological on/offset effects of fentanyl) to add the rate of the association and dissociation of buprenorphine to and from the MOR.[34] A characteristic feature of buprenorphine is its slow receptor binding kinetics; buprenorphine displays slow association kinetics and even slower dissociation kinetics.[33,46] For comparison in respiratory studies, the $t_{½}k_{e0}$ for fentanyl and buprenorphine were 16.4 and 75.3 min respectively, and whilst dissociation of fentanyl from the receptor was essentially immediate, the half time of receptor dissociation for buprenorphine was 68 min.[35] PK-PD models need to reflect these differences and, for buprenorphine, only the combined expressions for biophase equilibrium and receptor kinetics describe accurately buprenorphine's effects on respiratory function [34].

Buprenorphine has high affinity at δ- and κ-receptors, but efficacy at these receptors is highly variable. Although dependent upon the system tested, buprenorphine is described usually as a partial agonist at the MOR, an antagonist at the δ-receptor and a low efficacy agonist or antagonist at the κ-receptor.[47] Interaction with these other receptor types may reflect buprenorphine's actions on some pain systems, such as neuropathic systems,[48-49] but are unlikely to have a major influence on respiratory function.

2.2.3 OPIOID PARTIAL/MIXED AGONISTS: TRAMADOL

Tramadol is a racemic opioid widely used for relief of acute and chronic pain[50] and is considered to have a low potential for respiratory depression.[51,52] At opioid receptors, tramadol is an opioid agonist, selective for MORs, but with a low affinity for these

receptor sites, approximately 6000 times less than morphine.[53,54] The major metabolite of tramadol, O-desmethyltramadol (ODT) is a more potent agonist at the MOR, with an affinity approximately 200 times greater than that of the parent drug.[51] Both parent and metabolite may participate in the overall actions of tramadol. With tramadol, however, the low incidence of respiratory effects compared to analgesic effects, is due to additional non-opioid mediated actions that contribute to the analgesia. Tramadol is a monoamine re-uptake inhibitor. Both (+)- and (-)-tramadol inhibit the synaptic reuptake of 5-HT and noradrenaline. This leads to an increased stimulation of monoamine spinal descending inhibitory pathways and possibly of brainstem and thalamic analgesia-inducing sites.[55] The analgesic effects of tramadol, therefore, are thought to result from the sum of opioid receptor and monoamine reuptake activities[55] as evidenced by studies in animals with pharmacological antagonists, which show that naloxone, yohimbine (an α2-receptor antagonist) and ritanserin (a 5-HT_2 receptor antagonist) may all partially block the antinociceptive effects of tramadol.[53,56,57] Still, patients receiving tramadol may experience respiratory depression, particularly after a considerable time lag from dosing, since elimination of its more potent metabolite ODT is slow (elimination half-life 5.6 h). However, since this MOR agonist displays a slow transfer from plasma to the effect compartment (and back),[58] carbon dioxide accumulation will restrict exaggerated respiratory effects. In contrast to pure opioid agonists, therefore, drugs like tramadol with mixed actions may provide effective and safer analgesic regimens for the management of postoperative pain in modern clinical practice.[59]

2.3 INVOLVMENT OF MICROGLIA IN OPIOID-INDUCED RESPIRATORY DEPRESSION: ROLE OF TOLL-LIKE RECEPTOR 4 (TLR4)

There may be possibilities for using non-neuronal opioid effects for the development of novel therapeutic analgesics with reduced risk of respiratory depression. One such possibility comes from expanding knowledge on the role of immune cells in opioid-related mechanisms. Glial cells and inflammatory mechanisms, along with neuronal responses, are important mechanisms in some types of pain[58] and opioid interaction with glial cells through interactions with non-opioid receptors, for example the toll-like receptor 4 (TLR4) protein, may offer further targets for the novel therapeutic development of analgesics.[1] Opioids may interact with microglia via activation of MORs,[59] but recent studies on opioid-induced glial activation through non-opioid receptor mechanisms have revealed new insights into opioid-induced centrally mediated effects such as analgesia and respiratory depression.

Recognition of the role of microglia (and astrocytes) in pain mechanisms originated in the 1990s and glial activation has become accepted as an important mechanism contributing to neuropathic and chronic pain.[63] In various animal models following nociceptive peripheral tissue or nerve injury microglia are activated by a variety of factors. The activated state is characterized by glial release of a diverse range of proinflammatory mediators, such

as cytokines, chemokines and prostaglandins that produce a battery of effects, not least the enhancement of neuronal excitability and pain. Several activation signals have been described between neurons and glia, including fractalkine acting through glial CX3CR1 sites [64] and ATP acting through P2X-type receptors.[65] A further mechanism of glial activation is via stimulation of toll-like receptors and this receptor type may have particular relevance to opioid mechanisms. Morphine stimulates proinflammatory mediator release from glia and elevated proinflammatory mediators and the TLR4 receptor appear to play a key role in this process.[66,68] TLR4s are distributed on microglia, but not on neurons[69] and glial TLR4s are activated by opioids to release proinflammatory mediators.[68] The importance of TLR4 in opioid-induced activation of glial cells and subsequent release of neuroexcitatory mediators has been demonstrated, for example, by knockout mice lacking TLR4, which show markedly less proinflammatory cytokine release after a peripheral nerve lesion and less neuropathic pain-like behaviour.[70] Other toll-like receptors, TLR2 and TLR3, may also play related roles.[71,72] An opioid-induced effect that may arise from opioid stimulation of these TLR4 and subsequent release of proinflammatory mediators is hyperalgesia.[67,68] Hyperalgesia, an effect directly counter to the analgesic actions of opioids, is a well recognized feature of opioids in animals and humans and may be an issue in patient analgesic care [see reviews[73-75]]. Opioid-induced stimulation of TLR4, however, is not dependent on classical opioid receptors. This may be illustrated by the lack of stereoselectivity requirements in opioids for stimulation of TLR4, hence the (+)-morphine isomer has been shown to induce glial activation and hyperalgesia,[76] as has the opioid receptor inactive morphine metabolite morphine-3-glucuronide (M3G).[77]

If glial cells and TRL4 receptors are implicated in playing a part in the overall analgesic actions of opioids, are they also involved in other opioid-induced effects such as respiratory depression? Although this has been little researched, there is some evidence in favor of this concept. Glial cells have been implicated in respiratory mechanisms and, in brain slices, selective blockade of the glial Krebs cycle has been shown to inhibit rhythmic respiratory burst activity.[78] More specifically, glial cells have been shown recently to contribute to the purinergic excitation of the respiratory rhythm generating pre-Bötzinger complex.[79] Studies on glial and astrocyte function are more complex to carry out in vivo, but again recently, their role modulating central CO_2 chemosensitivity and ventilation has been described.[80] Similarly, the result of glial activation, namely proinflammatory mediator release, may also influence respiratory function. Interleukin 6, for example, has been implicated in respiratory diseases and may be considered as a biomarker for the risk assessment of asthma or chronic obstructive lung disease[81] and may alter respiratory mechanics.[82] It is possible, therefore, that opioids may interact via TRL4 with glial cells in the respiratory centers to influence respiratory control. Further evidence of this has been obtained with the use of minocycline to block morphine-induced TLR4 stimulation and glial activation and this will be considered below.

2.4 NALOXONE-REVERSAL OF OPIOID-INDUCED RESPIRATORY DEPRESSION

Since the consequences of opioid-induced respiratory depression may be serious, even fatal, it is a clear clinical priority that rapid and effective reversal of respiratory depression is available. As discussed previously, opioid-induced respiratory depression is largely mediated through MORs, hence antagonists at this receptor site are key agents for its reversal. Two opioid antagonists are available clinically as rescue medications, but the one approved for this therapeutic indication and by far the most commonly used is naloxone. In the clinic, many studies have shown that naloxone may be used effectively for the rapid treatment of opioid-related events, although the extent and duration of naloxone-induced reversal of opioid-induced respiratory effects is highly variable.[83-85] When considering effective reversal regimens for naloxone, the PK and PD characteristics of the opioid analgesic (*i.e.*, rate of metabolism, receptor kinetics and efficacy at the MOR) and of the antagonist itself must be taken into account. We have reviewed these agonist and antagonist PK-PD interactions extensively elsewhere[1] and here present a brief summary.

Naloxone is a competitive antagonist at the MOR with fast receptor association and dissociation kinetics.[86] Naloxone is also lipophilic and therefore has rapid access to the brain ($t_{½}k_{e0}$ is short, 6.5 min). Consequently, transfer and equilibrium of naloxone from the plasma to brainstem MORs is rapid.[87] As for full MOR agonists, for naloxone receptor kinetics is not the rate-limiting factor governing its ability to reverse opioid agonist actions. For full agonists such as fentanyl, single bolus administrations of naloxone are expected to completely reverse opioid-induced respiratory depression but this effect is critically dependent on the dose and mode of administration of naloxone and its PK. The elimination half-life of naloxone is about 33 min[87]; hence, with any opioid agonist that has a longer elimination half-life than naloxone (*e.g.*, morphine, M6G, methadone), care must be taken since renarcotization may occur with time, particularly after a bolus administration of naloxone. Care must also be taken if rapidly metabolized opioid agonists are administered by continuous infusion, rather than by bolus injection, since following a bolus administration of naloxone, reversal of any respiratory depression will initially be rapid and complete, but respiratory depressant effects may reassert themselves as the agonist infusion continues and naloxone plasma levels decline.[88] Even rapidly metabolized agonists, such as fentanyl, will show renarcotization if they are administered in high doses or by multiple injections (as occurs during anesthesia), as fentanyl plasma levels will remain high, whilst naloxone levels decline after a single bolus administration. However, a continuous infusion of naloxone may be used successfully to reverse even high doses of fentanyl.[89] The viability of the use of naloxone for opioid reversal reaches some limits with opioid agonists exhibiting ultra-short duration of action (e.g. remifentanil, $t_{½}k_{e0}$= 1 min, plasma elimination $t_{½}$ = 3min),[33] where simply stopping the infusion is more effective than attempting to achieve naloxone-induced reversal of effects.

More complex interactions, however, exist between long lasting partial agonists, like buprenorphine, and naloxone. Respiratory effects observed with rising doses of

buprenorphine exhibit an apparent maximum or ceiling effect.[35] Naloxone showed a complex and bell-shaped dose-response curve for reversal of buprenorphine-induced respiratory effects:[85] bolus doses of naloxone that successfully reverse opioid agonist depressant effects (up to 0.8 mg) failed to reverse the respiratory depressant effects of buprenorphine, higher doses (2 - 4 mg) showed reversal of the effects, but even higher doses (5 - 7 mg) showed a decline in reversal activity.[85] As discussed previously, the biological actions of buprenorphine is governed not only by plasma half-life and drug transfer to the effects compartment(s), but also by slow receptor kinetics. The slow association and dissociation of buprenorphine with the MOR is at the basis of the difficulties observed for the reversal of buprenorphine-induced respiratory depression by naloxone.[36,90] A PK-PD model of the reversal of buprenorphine-induced respiratory depression by naloxone predicts that continuous infusions of naloxone are required for reversal of buprenorphine's respiratory effects (doses of 4 - 8 mg.h^{-1}).[85,87] This was experimentally verified.[85] The cause of the bell-shaped naloxone dose-response curve, however, remains unknown, although it may involve buprenorphine effects at receptor sites other than the μ-opioid receptors.[38] PK-PD modeling, therefore, may be used to design adequate naloxone regimens for reversal of opioid-induced respiratory depressant effects of opioid agonists. As shown in Fig. 2, PK-PD modeling demonstrates that an opioid with slower dissociation kinetics is more difficult to reverse with naloxone and consequently reversal develops more slowly and may require continuous infusion of the antagonist while the duration and magnitude of the reversal is dependent on the naloxone dose. The estimated order of difficulty (in terms of speed of reversal, Fig. 2), therefore, for naloxone to reverse a series of opioid agonists

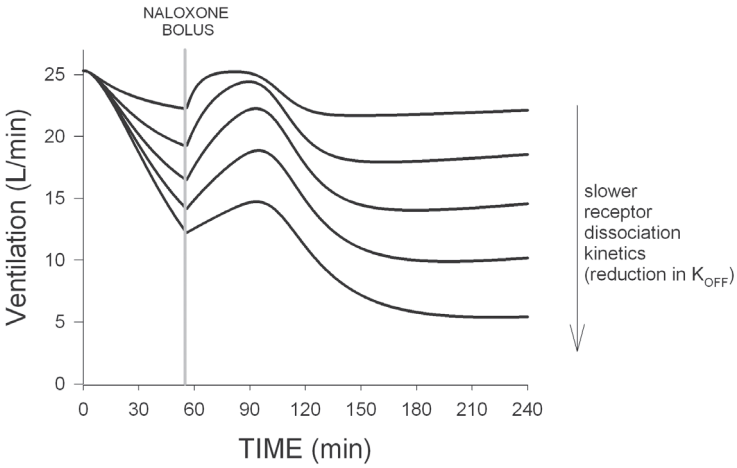

Figure 2. Effect of slowing of receptor dissociation kinetics on the rate of reversal of opioid-induced respiratory depression by a single dose of naloxone. Simulation data adapted from ref [32]. With slower the dissociation, the rate of reversal slows. At t = 0 an opioid dose is given; at t = 55 min a naloxone dose is given.

taking into account the range of μ-opioid receptor kinetics is (from most difficult to least difficult): buprenorphine > M6G > morphine > fentanyl.[32] For full MOR agonists with rapid receptor kinetics, the PK of the opioid agonist and naloxone are evenly important in determining the administration regimen of naloxone required.[88]

2.5 ALTERNATIVE STRATEGIES TO REVERSE OPIOID-INDUCED RESPIRATORY DEPRESSION

Naloxone is a MOR competitive antagonist with high affinity and low efficacy at the receptor site and shifts MOR agonist dose-response curves to the right in a parallel manner.[91] In receptor model terms, naloxone fulfills the concept of a classical competitive antagonist that is able to displace the agonist at a single conformational active site of the receptor and then, with its low efficacy, is unable to produce the conformational changes that are required to induce intracellular responses leading to biological effects. Naloxone will antagonize the whole range of opioid effects: opioid-induced analgesia, respiratory depression, sedation, gastrointestinal effects, etc. Naloxone, therefore, may reverse respiratory depression very effectively, particularly in an emergency situation, but will also reverse opioid-induced analgesia presenting real clinical difficulties. To try to design MOR antagonists to selectively reverse some opioid-induced biological actions, but not others, *e.g*. to antagonize respiratory depression, but not analgesia, is theoretically and practically difficult with competitive antagonists, and resides in trying to introduce different PK and PD characteristics, or selectivity for MOR subtypes, if they exist. PK-PD modeling shows that it may be possible to titrate naloxone to reverse respiratory depressant effects and not compromise analgesia in some situations where respiratory depression may occur at higher receptor occupancy than some degree of analgesia.[1] However, it is difficult to see a 'naloxone-based' approach to this problem being able to achieve complete reversal of opioid-induced respiratory depression whilst leaving opioid-induced analgesia unaffected.

It may also be valid to mention at this point, that more recent experimental studies on ligand interactions with receptor conformations have shown that the 'one size fits all' type of activation (as described above for naloxone) is not adequate to describe the observations in all systems. There appears to be diversity in receptor activation states, whereby ligands of varied efficacies produce different receptor conformational changes, thereby stabilizing conformationally distinct active states of the receptor, and not a single active conformational state. Ligands of different efficacies, therefore, may effect stimulation of different active conformational states of the same receptor leading to activation of separate intracellular signaling pathways and varied biological outcomes, *i.e.*, functional selectivity [see review [92]]. On this model, allosteric antagonist ligands ('biased' antagonism) may be able to selectively antagonize certain conformational active states of the receptor resulting in selective reversal of some biological effects. An example of the effect of allosteric receptor sites is given for AMPA receptors later in this review. These concepts have been

discussed for opioid receptors.[93,94] As members of the GPCR superfamily, opioid receptor activation by different opioid ligands may stimulate different Gα subunits.[90] At least one hypothetical possibility arising from ligand specificity for Gα activation may be the separation of analgesic effects from respiratory depression and other unwanted side effects of opioids.[93] However, for opioid receptors at present there remains uncertainty whether different opioid-induced effects may result from effects at different receptor types rather than different active site conformations of one receptor, or if indeed selective stimulation of Gα subunits does occur (i.e., that analgesia and respiratory depression may be induced by different Gα subtypes). Nevertheless, the design of selective allosteric opioid ligands with varied efficacies for the μ-opioid receptor with functional selectivity remains an interesting concept for improvements over naloxone.

Alternative approaches, separate to any based directly on interactions with μ-opioid receptors, may be required for the design of selective compounds that will reverse opioid-induced respiratory depression, without compromising opioid-induced analgesia, for use outside of emergency settings. We will consider two such potential approaches involving inhibitors of glial activation and antagonists of other transmitter systems present in respiratory centers.

2.5.1 INHIBITION OF GLIAL ACTIVATION

The toll-like receptor family, particularly TLR4, have been demonstrated to be involved in opioid-induced glial activation.[66-68] However, the TLR4 requirements for opioid ligand binding are very different from those for classical opioid-receptors and we have previously made reference in this review to the lack of stereoselectivity requirements in opioid agonists for stimulation of TLR4. Hence the (+)-morphine isomer has been shown to induce glial activation and hyperalgesia,[76] as has the opioid receptor inactive morphine metabolite M3G.[77] This lack of stereospecific requirements by TLR4 is also shown by antagonists and both (+)- and (-)-naloxone have been demonstrated to block TLR4 mediated signalling and cytokine production in HEK-293 cells in vitro.[94] In vivo, both isomers of naloxone also suppress neuropathic pain following sciatic nerve chronic constriction injury in rats.[95] The lack of stereoselective requirements in antagonists on TLR4, therefore, may enable development of opioid antagonist isomers that are inactive at neuronal μ-opioid receptor-mediated analgesic effects, but may reverse glial TLR4-mediated respiratory depressant effects and hence separate these opioid-induced events.

Antagonists at the TLR4 site, however, do not have to be based structurally on classical opioid antagonists as a diverse range of molecules may block opioid-induced TLR4-mediated events. Minocycline is a semi-synthetic, second generation tetracycline antibiotic, first introduced in 1972, that has long been known to possess anti-inflammatory and neuroprotective effects unrelated to its antibacterial activity [see review[96]]. Although there is considerable experience with minocycline clinically, it is associated with a range of adverse side-effects, some of which, such as vestibular symptoms, may be serious.[97]

Minocycline exerts anti-inflammatory actions by modulating microglia and the subsequent release of cytokines, chemokines and other inflammatory mediators.[91] Recently, PET scan imaging has been used to demonstrate activation of rat brain microglia by zymosan and its inhibition by minocycline.[98] Minocycline, as an inhibitor of microglial activation, has been investigated in animal models of opioid-induced respiratory depression and analgesia.[99] Minocycline suppressed various measures of morphine-induced respiratory depression, such as tidal volume and minute ventilation, inspiratory and expiratory force, although, at the doses used, it did not block changes in respiratory rate. In contrast, the same doses of minocycline enhanced the antinociceptive effects of morphine. These contrasting effects of minocycline on respiration and antinonciception may both be due to inhibition of the release of proinflammatory agents from glia that, as discussed previously, may induce centrally mediated respiratory depression and hyperalgesia. In these studies, the lack of observed effects of minocycline on respiratory rate, compared for example with minute volume, is discussed in relation to the different dorsal and pontine central respiratory sites from which tidal volume and respiratory rate originate (e.g., ventrolateral division of the nucleus tractus solitarius and pre-Bötzinger complex respectively) and possible glial heterogeneity and/or site specific effects of opioid mediated glial events.[99] Various further recent studies continue to explore minocycline-induced microglial inhibition of proinflammatory mediators and their varied effects, for example, reversal of hyperalgesia through minocycline-induced inhibition of cytokines from microglia and a subsequent reduction in potassium chloride co-transporter 2 in the spinal cord.[100]

A brief further illustration of the potential for the separation of opioid-induced effects by inhibition of glial activation may be made with the drug AV411 (ibudilast), although with the differentiation of analgesia and opioid withdrawal rather than with respiratory depression. AV411 is a relatively non-selective phosphodiesterase (PDE) inhibitor and has been marketed in some countries for bronchial asthma and post-stroke dizziness.[101-103] However, AV411 is also a microglial inhibitor with anti-inflammatory effects. Its widespread effect on PDEs and on bronchial smooth muscle do not make the drug a target for exploring opioid-induced respiratory depression, but AV411 has successfully been shown, through actions on microglia, to significantly reduce opioid withdrawal whilst enhancing analgesia.[101-103] Hence, both minocycline and AV411 have been demonstrated to inhibit glial activation and release of proinflammatory mediators resulting in enhanced analgesia, but with reduced other opioid-mediated events, respiratory depression and opioid withdrawal, respectively. Together with the non-stereoselective isomers of naloxone, these materials illustrate a potential direction for the development of selective glial inhibitors, perhaps particularly TRL4, to be targeted towards the effective separation of opioid-induced analgesic from respiratory depression.

2.5.2 STIMULATION OF NEUROTRANSMITTER SYSTEMS IN THE PRE-BÖTZINGER COMPLEX

Respiration is under the control of central neural networks and one of the most important

Table 1

Target for interference with opioid-induced respiratory depression
Opioid-receptors (on respiratory neurons in the brainstem)
Toll-like receptor 4 (TOLL on glia cells in the spinal and possibly supraspinal sites)
5 hydroxytryptamine receptors in respiratory centers in the brainstem
AMPA receptors in respiratory centers in the brainstem
Phosphodiesterase-4 in respiratory neurons

is located in the pre-Bötzinger complex. The pre-Bötzinger complex plays a major role in the modulation and generation of respiratory drive, particularly respiratory rhythms underlying the active inspiratory phase of breathing.[6,7] MORs are located in this area and opioid-depressant effects, especially life-threatening apnea, may originate from within the complex. Many other neurotransmitter systems and receptors related to their transmitters are also located in this neural network, some being co-expressed with MORs. Several important questions now arise. If neurotransmitters in the pre-Bötzinger complex operate to stimulate respiratory function, would stimulation of these systems induce ventilatory stimulation even in the ongoing presence of opioid-induced respiratory depression? Would activation of stimulatory respiratory systems functionally reverse opioid-induced respiratory depressant effects, but without reduction of opioid-induced analgesia for which the mechanistic centers are located elsewhere in the brain? If so, would selective ligands for the respective relevant neurotransmitter receptors form the basis of new therapeutic agents to enhance the safety margins of opioid analgesics? Several possible receptor targets arise from the identification of respiratory stimulatory systems in the pre-Bötzinger complex that suggest ventilatory drive may be increased in the presence of opioid respiratory depression. Three ventilatory stimulatory systems, 5-hydroxytryptamine (5HT), α-amino-3-hydroxy-5-methyl-D-aspatate (AMPA) and phosphodiesterase-4 (PDE4) will be reviewed briefly [see also review[1]]. (Table 1).

2.5.3 5-HYDROXYTRIPTAMINE (5-HT)

5HT receptor types and subtypes are expressed both centrally and peripherally and are involved in mediating many diverse clinical states including mood, memory and learning, aggression, sleep and pain, but are also present in the pre-Bötzinger complex and related neural networks. In this region, 5HT is a respiratory stimulatory neurotransmitter and a number of 5HT receptors subtypes have been identified with high density. 5HT receptors are members of the GPCR superfamily (except $5HT_3$ receptors, which are cation channels). 5HT enhances activity in respiratory neurons in this network through an action on $5HT_{1A}$, $5HT_4$ and $5HT_7$ receptors:[104]

a) One of the earliest $5HT_{1A}$ agonists to point toward a potential stimulation of respiration without affecting antinociception was the tetralin derivative, 8-OH-DPAT. In studies in

rats, for example, 8-OH-DPAT was demonstrated to reverse opioid-induced depression of ventilation without antagonizing antinociception.[105,106] In the most recent studies, 8-OH-DPAT was demonstrated to recover breathing following fentanyl-induced respiratory depression in the rat in vivo.[107] The partial agonist buspirone has also been investigated, which unlike 8-OH-DPAT, was available for studies in man and was shown in clinical cases to improve rhythmical respiration where damage to the brainstem caused apneustic breathing.[108,109] However, buspirone failed to counteract opioid-induced respiratory depression induced in healthy volunteers.[110] Buspirone is probably a poor tool to explore the capacity of 5-HT_{1A} ligands to induce respiratory stimulation since it is a partial agonist and full agonists at this receptor site may be required to effect strong ventilatory improvements.[111] The most recent 5-HT_{1A} full agonist to be studied in rats for reversal of opioid-induced respiratory depression is repinotan, which has been shown to be effective in stimulating ventilation without impairing antinociception.[112] It is worth noting, however, that repinotan-induced stimulation of spontaneous breathing after morphine-induced respiratory depression displayed a 'bell-shaped' dose-response curve, hence reversal of the opioid-induced respiratory effects by repinotan was dose dependent and declined at higher doses. Bell-shaped dose response relationships are known for other 5-HT_{1A}-induced responses, e.g. neuroprotection, and have been suggested to arise from dose dependent effects eliciting opposing stress responses.[113] Hence, studies with repinotan support the earlier findings with 8-OH-DPAT that full agonists at $5HT_{1A}$ receptors may induce stimulation of respiration without reducing opioid-induced analgesia. Recent important mechanistic studies on $5HT_{1A}$-induced respiratory stimulation have demonstrated that $5HT_{1A}$ receptors located on inhibitory glycinergic interneurons of the pre-Bötzinger respiratory networks appear to be critical for this function. Stimulation of these receptors leads to modulation of glycine receptors, network reorganization and disinhibition that restores breathing rhythms after opioid-induced depression.[114,115] Although each of the examples of 5-HT_{1A} ligands cited above has limitations, selective 5-HT_{1A} agonists may provide interesting leads for potential therapeutic agents to stimulate spontaneous breathing even in the presence of opioid-induced depression of respiratory function.

b) $5HT_{4(a)}$ and μ-opioid receptors are co-localized on respiratory neurons in the pre-Bötzinger complex, but display opposing actions resulting in $5HT_{4(a)}$-mediated increase in cAMP and increased inspiratory drive, compared to μ-opioid receptor-induced decrease in cAMP and decreased inspiratory drive.[116,117] Hence, in animal studies, the $5HT_{4(a)}$ receptor agonist BIMU8 was shown to overcome fentanyl-induced respiratory depression and apnoea, without affecting antinonciception[116] and zacopride was demonstrated to reverse etorphine-induced respiratory depression.[118] In the former of these studies, the respiratory stimulating effect was believed to be specific to $5HT_{4(a)}$ receptors because the effect of BIMU8 was antagonized by the $5HT_{4(a)}$ receptor antagonist GR113808.[104,116] However, the $5HT_{4(a)}$ receptor agonist RS67333 has more recently

been shown to fail to recover breathing in opioid-induced respiratory depression[107] and, in human healthy volunteers, another $5HT_{4(a)}$ receptor agonist, mosapride, also demonstrated no effect on respiratory depression induced by morphine.[119] The negative results with mosapride and RS67333 may be attributable to low potency or PK considerations with insufficient effect-site concentrations being achieved, as suggested by PK-PD modeling for maosapride.[119] or from different pharmacological profiles of the many $5HT_4$ splice variants,[120] but an explanation remains a conjecture a present.

c) Although some selective antagonists, SB-269970-A and SB-656104-A, for the $5HT_7$ receptor have been reported,[121] selective receptor agonists are not available for this receptor. A number of 5HT ligands will stimulate $5HT_7$ nonselectively, including 8-OH-DPAT,[122] which has raised questions as to whether the respiratory stimulatory actions and reversal of opioid-induced respiratory depressant effects of 8-OH-DPAT are mediated by $5\text{-}HT_{1A}$ or $5HT_7$ receptors.[104,118] There is a need for better selective ligands at $5HT_7$ receptors to become available before a role for these receptors within the respiratory networks can be defined.

2.5.4 AMPAKINES

Another respiratory stimulatory mechanism within the pre-Bötzinger complex is glutaminergic transmission through AMPA receptors. AMPA receptor modulators do not interact with the receptor site directly as agonists (or antagonists), but bind to an allosteric site within the glutamate receptor complex. Allosteric binding on the AMPA receptor modulates the kinetics of deactivation (channel closing and transmitter dissociation) and desensitization[123] and AMPA modulators increase the duration of glutamate-induced AMPA receptor-gated inward currents.[124] Within the pre-Bötzinger complex, activation of AMPA receptors is important for rhythmogenesis and induction of increased respiratory frequency through an increase in glutamate-mediated excitatory inspiratory drive.[125] Several distinct classes of AMPA receptor ligands have been described, with much interest centered upon the benzamides, a group collectively called ampakines. Several examples of ampakines that interact in different ways with the allosteric binding site have been studied in respiratory systems, e.g., CX516, CX546, CX614, CX717,[1] but, for the current review, the focus will be on CX717 as this ampakine has been the most studied, including with early investigations in humans. Treatment of rats with CX717 markedly protected the animals from fentanyl-induced respiratory depression, mechanistically described as due to accentuation of the AMPA-receptor mediated glutaminergic excitation that counteracts the μ-opioid receptor-mediated suppression of the pre-Bötzinger complex neuronal excitability.[126,127] Pretreatment with CX717 did not significantly alter fentanyl-induced antinociception.[126,127] One importance of these finding with CX717 is that this material is available for use in humans (CX717 has been tested for safety and efficacy in the treatment of human ADHD [see references in[126-128]]). A proof of concept study has been carried out in healthy human volunteers to test the hypothesis that opioid-induced ventilatory

depression may be selectively reduced by prior administration of CX717.[128] In this study, a single oral dose of CX717 (1500 mg) successfully reduced all measures of respiratory depression induced by a subsequent intravenous infusion of alfentanil administered to reach a target plasma concentration of 100 $ng.ml^{-1}$. At this plasma concentration, alfentanil was shown to induce analgesia in the subjects when tested with two models of experimental pain, effects that were not apparently compromised by prior administration of CX717, but could be revered by naloxone.[128] Hence, ampakines like CX717 may offer a therapeutic potential for the suppression of opioid-induced postoperative respiratory depression, and hence an increase in safety, without negating their analgesic effects.

2.5.5 PHOSPHODIESTERASE-4-INHIBITORS

The methylxanthines caffeine and theophylline have been used to counter apneas and stabilize breathing in preterm infants, although both drugs are associated with adverse events.[129] Methylxanthines block adenosine receptors and, although this has been suggested for the action of methylxanthines in neonates, adenosine receptor antagonists do not inhibit inspiratory neurons[130] and alternative mechanisms for the methylxanthines have been sought. At concentrations of methylxanthines that have demonstrated respiratory effects in vitro, these agents also inhibit phosphodiesterase-4 (PDE4), which results in elevated cAMP and stimulation of phosphokinase A.[131] As described previously, $5HT_{4(a)}$ receptor agonist also enhance cAMP levels and stimulate inspiratory drive and this may be a mechanism of action for methylxanthines. More recent studies have supported this hypothesis and methylxanthines have been shown to reverse opioid-induced depression of the respiratory rhythm in the pre-Bötzinger slices in the newborn independently of adenosine receptors and apparently associated with PDE4 inhibition.[132] In further support of this, the specific PDE4 antagonist rolipram, alone and combined with theophylline, was able to reactivate respiratory rhythm after severe depression with the μ-agonist DAMGO alone.[132] Whilst all these agents are limited currently by adverse effects, particularly rolipram, new specifically targeted PDE4 inhibitors (particularly of PDE4 subtypes) may allow improved treatment for breathing control in the premature and newborn infants.

2.6 CONCLUSIONS

Opioid-induced respiratory depression remains a potentially life-threatening side effect of opioid treatment of severe acute and chronic pain. Whilst data on the incidence of opioid-related morbidity remain difficult to unearth from the literature, estimates on respiratory events in the perioperative setting range from 0.5 to 2%. Data on respiratory events in chronic pain patients on potent opioid therapy are even scarcer. We assume that this is partly because of the unwillingness to report fatal complications and partly because respiratory-depression related death in chronic cancer-pain patients is an accepted fact in the course of the disease or is assumed to be due to the 'natural' progression of

the underlying disease. Irrespective, we consider fatal (or near-fatal) opioid-induced respiratory depression an avoidable complication. Strong opioids should be titrated to effect, or even better, to the multiple effects, analgesia and respiratory depression. While insufficient analgesia requires further dosing the occurrence of breathing irregularities or cyclic breathing is an immediate sign to stop further dosing. Evidently, knowledge on the pharmacokinetics and (mechanism-based) pharmacodynamics of the MOR agonist in relation to patient specifics (e.g., disease-state, age, fluid status, cardiac, liver and renal function) is at the basis of sensible titration. Separation of opioid-induced respiratory depression and analgesia seems improbable taken the fact that the MOR is the molecular target of both effects and the absence (so far) of proof in humans for distinct opioid-receptor subtypes involved in analgesia versus respiratory depression or selective stimulation of Gα subunits. Development of opioids with effect-selectivity will rely on these distinctions. Current practice of reversal of opioid-induced respiratory depression is based on antagonism of the MOR with naloxone, a non-selective antagonist that will antagonize not only respiratory depression but the whole range of opioid effects including opioid-induced analgesia. Because of this loss of analgesia, the rapid onset/offset of naloxone (and consequently high chance of renarcotization) and naloxone's relative difficulty with reversal of opioids with slow receptor dissociation alternative strategies are being developed. These alternative modes are aimed at reversal and prevention of opioid-induced respiratory depression without compromising analgesia. Agents that are being studied include 5HT agonists, ampakines, phosphodiesterase inhibitors and drugs that stabilize activated glia cells in the pons and spinal cord. Especially the latter group of therapeutics is of interest as animal data indicate that reduction in opioid side effects coincides with improved analgesic efficacy.

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Section III

TRADE-OFF: PK-PD MODELING

OF

EFFECTS AND SIDE EFFECTS

CHAPTER 3

MODELING THE NON-STEADY STATE RESPIRATORY EFFECTS OF REMIFENTANIL IN AWAKE AND PROPOFOL SEDATED HEALTHY VOLUNTEERS

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3.1 INTRODUCTION

Opioids affect breathing by activation of μ-opioid receptors expressed on respiratory neurons in the brainstem.[1,2] As a consequence ventilation is depressed and arterial carbon dioxide increases. Since carbon dioxide activates chemoreceptors in the neck and brainstem (peripheral and central chemoreceptors), part of the opioid-induced respiratory depression is concealed by carbon dioxide-induced respiratory stimulation (the so-called carbon dioxide chemoreflex).[3] Especially, when the opioid slowly passes into the brain, the subsequent slow increase in carbon dioxide will offset major respiratory depression. On the other hand, when the opioid rapidly passes the blood-brain barrier or the opioid is overdosed, depression of the respiratory neurons is faster and more noticeable than the respiratory stimulation from the carbon dioxide increase.[3] Then the opioid's effect is most dangerous. In general, opioids are considered safe with about 0.5% of patients receiving opioids for treatment of acute pain requiring immediate treatment for sometimes life-threatening respiratory depression.[1] However, in specific patient groups this number is certainly much greater (e.g., patients with sleep-related apnea, obesity, muscle weakness, pulmonary disease). Furthermore, even in patients considered not at risk opioid-induced mortality still occurs.[4,5]

The number of studies on the effect of potent opioid analgesics on breathing is still limited. Even sparser are studies that quantify intravenous opioid effect on breathing using meaningfully parameterized pharmacodynamic models. The latter models are important as they allow, apart from the reliable description of opioid effect, the comparison among opioids (e.g. on potency), the study of drug-drug interaction, and prediction of specific breathing-related idiosyncrasies, such as the occurrence and duration of apnea. Current available models may be divided into two groups: 1. Steady-state models, where the end-tidal carbon dioxide concentration (PCO_2) is kept constant (by breath-to-breath manipulation of the inspired carbon dioxide concentration) and just the drug's effect on ventilation is measured (these models are also called open-loop models as the feedback loop between ventilation and arterial PCO_2 is broken –the loop is now open);[6-10] and 2. Non-steady-state models, in which the effect of the drug on arterial (or end-tidal) carbon dioxide and ventilation are both measured (these models are also called closed-loop models as the feedback loop between ventilation and arterial carbon dioxide remains active).[11-13] In contrast to steady-state models, non-steady-state models need to take into account the depressant effect that the opioid has on respiratory neurons in the brain causing the reduction of breathing and consequently the increase in arterial PCO_2, but also and equally important, these models need to take into account the stimulatory effect of carbon dioxide on breathing. Only when both components are properly incorporated in the model reliable estimates of the drug's respiratory potency are obtained and a prediction of its respiratory behavior can be made.[3]

We previously performed steady-state experiments and applied steady-state models to describe opioid-induced respiratory effects and their interaction with anesthetics

(sevoflurane, propofol).[9,10] While it allowed for the accurate description of the synergistic opioid-anesthetic interaction on breathing, this was unable to predict 'real-life' non-steady-state conditions such as occur when drug concentrations rapidly change. In the current study we performed non-steady-state experiments by applying increases in remifentanil concentration of different rates of rise. Experiments were performed in healthy volunteers in the awake condition and at the background of a low-dose propofol infusion. Next, we developed a non-steady-state pharmacokinetic-pharmacodynamic model of opioid-induced respiratory depression.

The control of breathing is a complex system using both feedback and feed forward control tools to maintain cellular homeostasis.[14] Hence, it is important to make choices when considering the site of action of opioid effect within the control system. We constructed a relatively simple model with drug concentration and end-tidal PCO_2 as input and measured inspired ventilation as output. Basic characteristics of the model are (i) it assumes that drug and carbon dioxide have opposing effects on breathing;[3] (ii) it is based upon the linear and well described relationship between carbon dioxide and ventilation, $\dot{V} = G\,(PCO_2 - B)$, where $\dot{V}$ is inspired minute ventilation, G is the gain of the ventilatory control system and B the extrapolated end-tidal PCO_2 at which apnea occurs (apneic threshold);[14-16] (iii) the opioid effect is on B, while anesthetic effect is on G (Fig. 1).[10] In our study, the model's behavior is tested to assess whether it accurately predicts apnea at finite opioid drug concentrations and whether all important model parameters are estimable (using a sensitivity analysis).

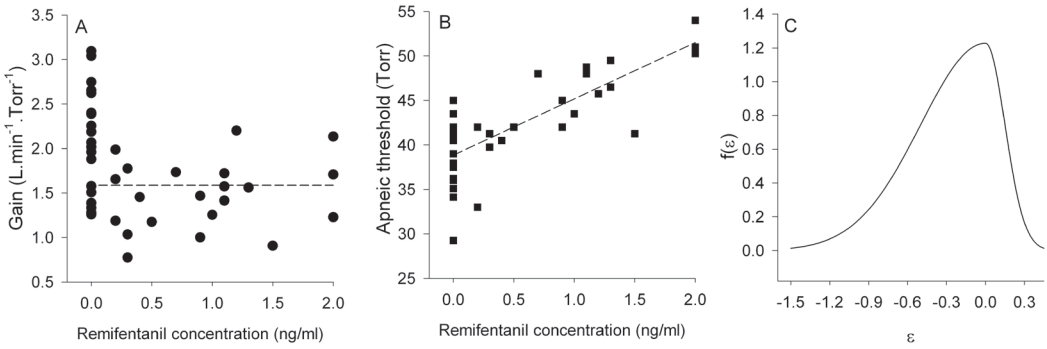

Figure 1. **A**. Effect of remifentanil on the central gain of the respiratory controller as measured by Nieuwenhuijs et al.[6] in steady-state experiments. At remifentanil concentrations > 0 the gain remains constant at 1.6 ± 0.1 $L.min^{-1}.Torr^{-1}$. **B**. Effect of remifentanil on the apneic threshold (B) of the respiratory controller as measured by Nieuwenhuijs et al.[5] B increases linearly with increasing remifentanil concentrations according to the function $B(C_{REM}) = 39*(1 + C_{REM}/6.14)$. **C.** Probability density function for end-tidal carbon dioxide concentration with $\sigma_A^2 = 0.0225$ and $\sigma_B^2 = 0.25$ (eqn. (9)).

3.2 MATERIALS AND METHODS

3.2.1 SUBJECTS

Ten healthy male volunteers (age 18-30 years; body mass index < 28 $kg.m^{-2}$) were recruited to participate in the study after approval of the protocol by the local Human Ethics Committee (Commissie Medische Ethiek, LUMC, Leiden, The Netherlands). Written and oral informed consent was obtained prior to inclusion in the study. All subjects were instructed not to eat or drink for at least 6 h before the study.

After arrival in the laboratory, an arterial line for blood sampling was placed in the left or right radial artery. In the contralateral arm an intravenous line was inserted for drug infusion. Each subject participated in three remifentanil infusions separated by 120 min washout-intervals. The first two were without a background infusion of propofol; the last with a propofol infusion aimed at a target bispectral index value of 80 (average target plasma concentration = 1 $\mu g.ml^{-1}$). We randomly assigned one of the two initial experimental runs (*i.e.*, without propofol infusion) to be performed without blood sampling. In the other two runs 3-6 arterial blood samples were obtained for remifentanil measurements at arbitrary time points.

Remifentanil was administered using a target controlled infusion system. For remifentanil we used a custom-built infusion pump that was programmed with a pharmacokinetic data set (Remifusor, University of Glasgow, Glasgow, UK).[17] We applied different remifentanil infusion schemes among the ten subjects as is described in table 1. We aimed at obtaining different rates of increases of the remifentanil plasma concentration (ranging from slow to fast). This was obtained by applying step increases in remifentanil plasma concentration (in 7 of 10 subjects we used steps of 1 $ng.ml^{-1}$) with varying step durations (0.5, 1, 1.5, 2, 3, 4 or 6 min) and with varying numbers of steps (2, 4, 5 or 6). In two subjects we performed a single 1-min step with step sizes of 6 and 9 $ng.ml^{-1}$. In the appropriate runs, one to three blood samples were randomly obtained during remifentanil infusion (but always just prior to a change in target remifentanil concentration); following infusion two to three blood samples were obtained, again at random times. Each volunteer was subjected to three identical target remifentanil infusion schemes. In case of irregular breathing with periods of apnea (no breathing for periods > 10 s) and/or significant oxygen desaturations (SpO2< 90%) the subject was initially stimulated to take a deep breath. If this had no effect the subject was artificially ventilated by bag via the mask and pneumotachograph for 20-30 s. The investigators could terminate or adapt the infusion at any time during the experiment when they felt that this was required to alleviate apnea and/or hypoxemia.

3.2.2 MEASUREMENTS

A face mask was applied over mouth and nose. Inspired and expired gas flows were measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal (Hans Rudolph, Myandotta, MI).

During the studies the subjects inhaled 100% oxygen. The oxygen and carbon dioxide concentrations of the in- and expired gases and the arterial hemoglobin-oxygen saturation were measured with a Datex Multicap gas monitor (Datex-Engstrom, Helsinki, Finland). The electroencephalogram was recorded using an A-2000 monitor with software version 3.3 (Aspect Medical Systems, Norwood, MA). The monitor computed the bispectral index (BIS) over 2-s epochs. We averaged the BIS-values over 1-min. End-tidal PCO_2 and inspired minute ventilation were stored on disc for further analysis. We further report the measured SpO_2 and BIS during the remifentanil infusions.
Samples for the determination of blood remifentanil concentrations were collected into tubes containing sodium heparin and immediately transferred to tubes containing 50% citric acid (to inactivate esterases) before freezing at –20°C. The assay method is based on tandem mass spectrometry detection.[10]

3.2.3 DATA ANALYSIS

A population pharmacokinetic-pharmacodynamic analysis was performed on the data. The analysis was performed in two steps. In step 1 a population remifentanil pharmacokinetic analysis was performed. Next using the individual Bayesian pharmacokinetic estimates a population pharmacodynamic analysis was performed.

Remifentanil pharmacokinetics. The description of remifentanil pharmacokinetics was aimed at obtaining individualized drug input functions to the pharmacological model. The actual infusion rates from the log file of the target controlled infusion device were used. The distributions of the structural parameters were fixed to the values of the three-compartmental model reported by Minto *et al.*[17] (*i.e.*, the typical values and interindividual variabilities), and individualized by adjusting for age and lean body mass. A population analysis was performed which allowed for Bayesian individualization of the structural parameters (albeit within the constrained distribution).[18] A remifentanil effect-site was postulated where the concentration lags with respect to the central compartment concentration as quantified by the equilibration half-life parameter $t_{½}k_{e0}$.

Carbon dioxide pharmacokinetics (figure 2). The relationship between carbon dioxide content (C) and its partial pressure (P) was assumed to be linear, so that $P = \lambda_0 \cdot C$, where $\lambda_0 = 0.863$ Torr.(ml CO_2 in 100 ml blood)$^{-1}$.[19] The following mass balance equations were used for the lungs and body (approximating the body by one compartment):

$$V_{AL} \cdot \frac{dP_A}{dt} = -\dot{V} \cdot P_A + \lambda_1 \cdot \dot{Q} \cdot (P_V - P_A)$$

$$V_{TS} \cdot \frac{dP_V}{dt} = \dot{Q} \cdot (P_A - P_V) + \lambda_2 \cdot \dot{V}_{CO_2}$$

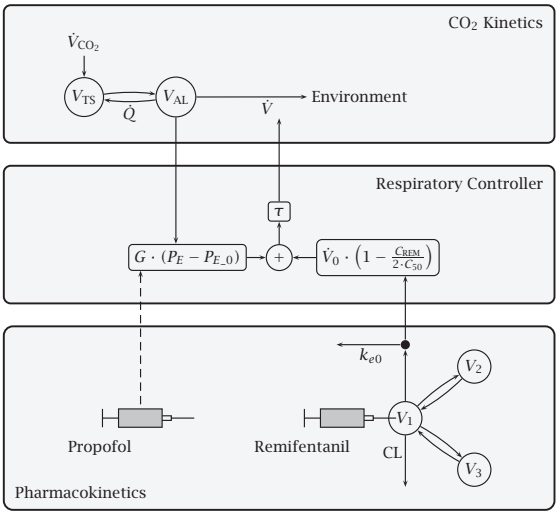

Figure 2. Schematic representation of the pharmacokinetic-pharmacodynamic model. The model has three distinct parts. I. The remifentanil pharmacokinetic part, consisting of the distribution of remifentanil though the body, including the effect-site (i.e., the respiratory controller in the brainstem) (via a rate constant, k_{e0}). Part II is the respiratory controller in the brainstem. Remifentanil's effect on the ventilatory control system results in a reduction of ventilation (via a delay, τ). Part III is the part that describes carbon dioxide kinetics. Carbon dioxide production determines together with ventilation the arterial carbon dioxide concentration. Since remifentanil causes the reduction of ventilation and carbon dioxide production is minimally affected, the ability of the system to clear carbon dioxide has diminished and arterial carbon dioxide concentrations will rise. This will have a stimulatory effect on the respiratory controller (part II). The main effect of adding propofol on top of remifentanil is shown as an effect on the gain factor G.

CL = clearance; C_{REM} = remifentanil concentration in plasma; C_{50} = concentration remifentanil causing 50% respiratory depression; G = gain of the ventilatory control system or slope of the hypercapnic ventilatory response; P_E = end-tidal carbon dioxide partial pressure; P_{E_0} = baseline (predrug) end-tidal carbon partial pressure; Q = cardiac output; k_{e0} = blood-effect site rate constant; τ = time constant of the ventilatory control system; $\dot{V}$= inspired minute ventilation; $\dot{V}_0$ = baseline (predrug) inspired minute ventilation; $\dot{V}_{CO2}$= carbon dioxide production; V_{ALV} = alveolar volume; V_{TS} = tissue volume; $V_{1\text{-}3}$ = volumes of compartments 1 to 3 of the kinetic remifentanil model.

where V_{AL} is alveolar volume, P_A arterial carbon dioxide pressure (which is assumed to equal alveolar pressure), $\dot{V}$ inspired minute ventilation, Q cardiac output, P_V venous carbon dioxide pressure, V_{TS} apparent tissue volume, and $\dot{V}_{CO2}$ is carbon dioxide production. Since ventilation enters the model of carbon dioxide kinetics directly (see eqn. 2) no correction was made for dead space ventilation. Furthermore, $\lambda_1 = k \cdot P_{BW}.\lambda_0^{-1} .100^{-1} \approx 10$ and $\lambda_2 = 100 \cdot \lambda_0$,, where k is the volume conversion factor from standard temperature and pressure, dry to body temperature and air saturated with water and P_{BW} the barometric pressure minus the pressure of air saturated with water. In the data analysis we fixed V_{AL} to 3L.[19] $V_{CO2}$was estimated from the baseline end-tidal carbon dioxide concentration and V. These baseline values, Q and V_{TS} were parameters to be estimated.

Pharmacodynamic analysis: Modeling the effect of remifentanil on the ventilatory control system (Fig. 2). The effect of end-tidal PCO_2 (P_E) on ventilation under hyperoxic conditions can be modeled as follows:[14-16]

$$\tau \frac{d\dot{V}}{dt} = G \cdot (P_E - B) - \dot{V} \qquad \text{eqn. (2)}$$

where G is the central gain, B the apneic threshold and τ a time constant. Nieuwenhuijs *et al*.[10] characterized the remifentanil-propofol interaction on the ventilatory control system using a response modeling approach. For the present study we re-analyzed those earlier data to characterize the effect of remifentanil on B and G (see Figs. 1A and B). From these analyses we estimated that at remifentanil concentration (C_{REM}) > 0, G remained constant while B increased linearly (the concentration remifentanil that doubles B is 6.14 ± 0.77 $ng.ml^{-1}$). Hence we assumed that in the current study remifentanil changed B but not G.

In the steady-state we have:

$$\dot{V}(C_{rem}) = G \cdot (P - B(C_{rem})) \qquad \text{eqn. (3)}$$

with

$$B(C_{rem}) = B_0 \cdot \left(1 + \frac{C_{rem}}{C_{100}}\right) \qquad \text{eqn. (4)}$$

where B_0 is the apneic threshold when $C_{REM} = 0$ and C_{100} the concentration remifentanil that causes a doubling of B. Rewriting equation 3 and defining baseline or resting end-tidal carbon dioxide concentration (*i.e.*, end-tidal PCO_2 before the remifentanil infusion) as P_{E_0} we get:

$$\dot{V}(C_{rem}) = G \cdot (P_{E\_0} - B(C_{rem})) + G \cdot (P_E - P_{E\_0}) \quad \text{eqn. (5)}$$

Baseline ventilation ($\dot{V}_0$) = $G \cdot (P_{E\_0} - B_0)$ and concentration remifentanil causing 50% respiratory depression, $C_{50,} = 0.5 \cdot C_{100} \cdot \frac{P_{E\_0} - B_0}{B_0}$. We then rewrite equation 6 into

$$\dot{V}(C_{\text{rem}}) = \dot{V}_0 \cdot \left(1 - \frac{C_{\text{rem}}}{2 \cdot C_{50}}\right) + G \cdot (P_E - P_{E\_0}) \qquad \text{eqn. (6)}$$

.

Note that C_{50} causes 50% depression of ventilation when $G.[P - P_{E\_0}] = 0$, which may occur when $P_E = P_{E\_0}$ (*e.g.*, after an acute remifentanil infusion when carbon dioxide did not rise as yet) or when G = 0 (as may occur when combining remifentanil with high propofol concentrations). This equation allows for the possibility of apnea at finite drug concentrations which is appealing from a clinical point of view. Finally, in eqn. (2) τ was fixed to 2.5 min.[15]

Modeling the end-tidal carbon dioxide pressure. End-tidal PCO_2 is (under 'normal' circumstances) an accurate indicator of alveolar PCO_2. However, close to apnea, the breathing pattern is such that end-tidal PCO_2 as measured by the gas monitor is likely to be inaccurate and possibly measured too low. So, if we write for the residual error:

$$P_E = \hat{P}_E + \epsilon \qquad \text{eqn. (7)}$$

where P_E is the measured value and $\hat{P}_E$ the predicted value. The variance of ε should be smaller (σ_A^2) when $P_E > \hat{P}_E$ and larger when (σ_B^2) when $P_E < \hat{P}_E$. Therefore the probability density of ε was written as:

$$f(\epsilon; \sigma_A, \sigma_B) = \begin{cases} \frac{2}{(\sigma_a + \sigma_B)\sqrt{2\pi}} \exp\left(\frac{-\epsilon^2}{2\sigma_A^2}\right) \\ \frac{2}{(\sigma_a + \sigma_B)\sqrt{2\pi}} \exp\left(\frac{-\epsilon^2}{2\sigma_B^2}\right) \end{cases} \qquad \text{eqn. (8)}$$

which is a continuously differentiable function and integrates to 1. Since the distribution of ε is asymmetric and the mean of $\varepsilon \neq 0$, $\hat{P}_E$ displayed in the figures is the mode. An example of the asymmetric probability density function with $\sigma_A^2 = 0.0225$ and $\sigma_B 2 = 0.25$ is given in figure 1C.

Furthermore, measured end-tidal carbon dioxide concentrations were determined to be missing values if they were lower than 37.5 Torr or when the corresponding measured ventilation was below 1 $L.min^{-1}$, because in those cases it can be expected that end-tidal PCO_2 is inaccurate.

Modeling minute ventilation. Minute ventilation (V) was assumed to be normally distributed with variance σ_v^2. However, during apnea manual ventilation (by mask) was applied. In that case the measured ventilation values were determined to be missing values but used for the uptake and distribution model of carbon dioxide.

Statistical Analysis. The models as described above were implemented in NONMEM VII (ICON Development Solutions, Ellicott City, MD).[18] The differential equations were solved with NONMEM's routine ADVAN6; the probability density functions (eqn. 8) were used with NONMEM'S LIKELIHOOD option. NONMEM VII's Markov Chain Monte Carlo Bayesian analysis method was used for parameter estimation. This method yields probability distributions of the model parameters from which means, standard errors and 95% confidence intervals can be obtained. Uninformative priors were used for the interindividual variability terms for the pharmacodynamic analysis (in the pharmacokinetic analysis these were fixed); no priors were required for the structural parameters because of the highly informative data. An interoccasion variability term was incorporated for both parameters, $\dot{V}_0$, and P_{E_0} (as their product is related to carbon dioxide production). Interindividual variability terms with a large standard error (larger than the estimate) were removed from the model. The burn-in samples were tested for convergence (all parameters and objective functions over 20 iterations, each 50 iterations apart; $P < 0.05$); 1000 iterations were used to obtain parameter distributions. Significance of factors representing a deviance of pharmacokinetic parameters from those obtained by Minto *et al.*,[17] and significance of factors representing a decrease in the pharmacodynamic model parameters G and C_{50} were tested by checking whether their 95% confidence intervals excluded 1.

3.2.4 SENSITIVITY ANALYSIS

A sensitivity analysis of a proposed model will indicate whether the parameter values of the model can be estimated with finite precision from the measured data.[20] Parameters may not be estimable for various reasons: because of the model structure, dependence on other parameters, or the specific input function chosen. We performed a sensitivity analysis of simulated data in which C_{REM} increases to 5 $ng.ml^{-1}$ in 5 steps using three distinct input functions: A. step size = 1 $ng.L^{-1}$, duration of step = 1 min; B. step size = 1 $ng.ml^{-1}$, duration of step = 5 min; C. step size = 1 $ng.ml^{-1}$, duration of step = 0.1 min. The analysis was performed by fixing one parameter *(i.e.* not allowing it to be estimated) at a time to a series of values (from 50% to 150%) of the 'best' value of the parameter. Next, the other parameters were estimated and the -2log likelihood (–2LL) values were determined. This so-called likelihood profile method will show whether any of the parameters are or are not estimable. If not, the curve of -2LL *versus* the fixed parameter values (the 'cost' function) will be flat.

3.2.5 SIMULATION STUDY

To get an indication of the effect of slowing the rate of rise of remifentanil on the nadir in ventilation or duration of apnea, we performed thirty simulations on remifentanil effect in the absence and presence of propofol. We simulated linear increases in remifentanil concentration at the effect site with rates of rise of 5 to 0.17 $ng.ml^{-1}.min^{-1}$. The infusion was terminated when the effect-site concentration had reached 5 $ng.ml^{-1}$.

3.3 RESULTS

All subjects completed the study without unintended effects. An example of one experimental run is given in Fig. 3; it is the data from one subject (id003) on the effect of a staircase increase in remifentanil concentration during a constant propofol infusion (run #3). The top panel shows the target increase in remifentanil plasma concentration to 4 $ng.ml^{-1}$. Note that the infusion was aborted early (due to the occurrence of apnea, panel C). Panel B shows the measured BIS values, panel C inspired minute ventilation per breath. BIS values were on average 80 indicating moderate sedation, in agreement with the observation that the subject was unresponsive to verbal command. Breathing reduced rapidly upon exposure to remifentanil and apnea occurred after 3 min. Apnea was followed by irregular and cyclic breathing which continued for 15 to 20 min, well after the remifentanil infusion was stopped. Note in panel D that during irregular breathing with low tidal volumes or apnea an accurate measurement of end-tidal carbon dioxide was not possible .

The infusion schemes applied in the studies are given in table 1. The estimated plasma concentration rates of rise varied from 0.17 to 9.0 $ng.ml^{-1}.min^{-1}$. BIS values were 93.2 ± 4.6 (mean ± SD) in remifentanil runs and 82.2 ± 4.8 ($P < 0.05$) in runs where remifentanil was given on top of propofol. The lowest values for SpO2 were 93.8 ± 7.2% in the remifentanil runs and 87.5 ± 8.4 % in the remifentanil-propofol runs ($P < 0.05$). Saturation values < 90% occurred on average 30 ± 41 s in the remifentanil runs and 110 ± 86 s in the remifentanil-propofol runs ($P = 0.05$). Apnea did not occur in remifentanil runs but in 8 remifentanil-propofol runs (averaged duration = 4.4 min, range 1 to 7 min). In runs of subjects 007 and 009 (remifentanil rates of rise 0.17 and 0.22 $ng.ml^{-1}.min^{-1}$) the duration of apnea was at its lowest range, 1 and 2 min respectively.

3.3.1 PHARMACOKINETIC ANALYSIS

To get an indication of the goodness of the pharmacokinetic data fits, we plotted the measured concentrations versus the individual and population predicted remifentanil concentrations (Fig. 4A and B). The plots indicate that the pharmacokinetic model adequately described the data. Two examples of pharmacokinetic data fits given in figure 4 are in agreement with this statement. The pharmacokinetic parameter estimates were not significantly different from those of Minto *et al.*[17] except for parameter V_2 (volume of compartment 2) which was a factor of 0.522 ± 0.125 (95% confidence interval 0.323-0.808)

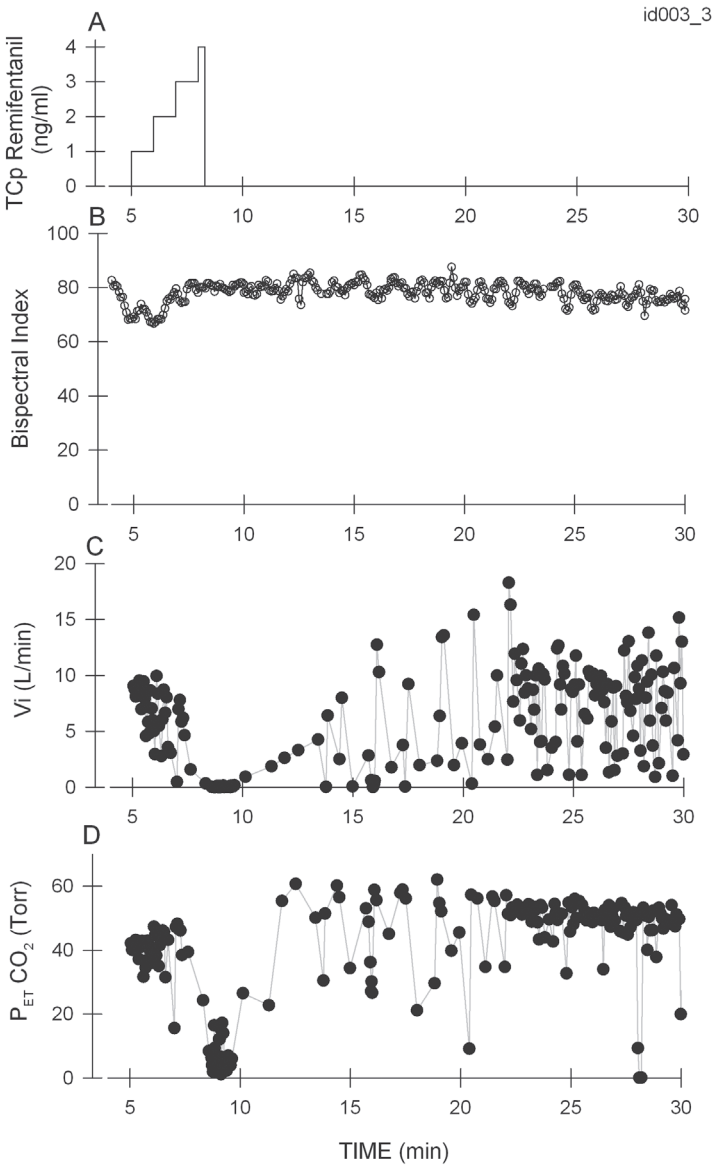

Figure 3. Example of the effect of remifentanil staircase infusion against the background of a constant propofol infusion. **A.** Target plasma remifentanil concentration (T_{CP}). **B.** Bispectral index (BIS). **C.** Measured inspired ventilation. Note that ventilation quickly reaches apneic values after the initiation of the remifentanil infusion. Next breathing remains irregular with a reduced breathing frequency and periods of cyclic breathing. To get an indication of the sequential breathing pattern the breaths are connected by a grey line. **D.** Measured end-tidal carbon dioxide concentration.

Table 1. Remifentanil infusion schemes

Subject #	Step size ($ng.ml^{-1}$)	Number of steps	Duration of step (min)	Total duration of infusion (min)	Max. target conc. ($ng.ml^{-1}$)	Remifentanil increase ($ng.ml^{-1}.min^{-1}$)
001	2.0	2	2	4	4.0	1.0
002	1.0	2	1.5	3	4.0	0.75
003	1.0	5	1	5	5.0	1.0
004	1.0	4	4	16	4.0	0.25
005	6.0	1	1	1	6.0	6.0
006	1.0	6	0.5	3.0	6.0	2.0
007	1.0	4	2	8	4.0	0.50
008	1.0	4	6	24	4.0	0.17
009	1.0	4	3	12	4.0	0.33
010	9.0	1	1	1	9.0	9.0

Table 2. Pharmacodynamic parameter estimates

	Estimate	SE	95% c.i.	ω2	SE	95% c.i.
$\dot{V}$ (L/min)	7.2	1.2	5.0-9.9	0.20	0.14	0.07-0.54
P_0 (Torr)	42.3	6.3	30.0-56.3	1.4	0.9	0.5-3.9
IOV				0.24	0.13	0.11-0.53
V_{TS} (L)	9.5	0.2	9.0-9.9	*	*	*
$t_{½}k_{e0}$ (min)	0.53	0.02	0.49-0.58	*	*	*
G ($L.min^{-1}.Torr^{-1}$)	0.42	0.01	0.40-0.44	*	*	*
C_{50} ($ng.ml^{-1}$)	1.6	0.03	1.5-1.67	0.14	0.10	0.05-0.34
Q ($L.min^{-1}$)	5.5	0.35	4.9-6.2	*	*	*
σ_A^2	0.044	0.0026	0.04-0.05			
σ_B^2	0.22	0.0069	0.21-0.23			
σ_v^2	5.55	0.11	5.4-5.8			
Propofol effect on G	0.46	0.015	0.43-0.49			
Propofol effect on C_{50}	0.84	0.030	0.79-0.90			

$\dot{V}_0$ is baseline ventilation (*i.e.*, ventilation prior to remifentanil infusion);

P_0 is baseline end-tidal PCO_2 (*i.e.*, end-tidal PCO_2 prior to remifentanil infusion);

IOV is the interoccasion variability (each subject participated in three distinct runs) based on the variability in $\dot{V}_{CO2}$, which was incorporated for V_0 and P_0;

V_{TS} is tissue volume (see eqn. 2);

$t_{½}k_{e0}$ is the blood-effect-site equilibration half-life for remifentanil; G is the central gain of the respiratory controller;

C_{50} is given the (effect-site) concentration remifentanil causing 50% depression of ventilation; Q is cardiac output;

σ_A^2 and σ_B^2 are variances of the residual error of PCO_2 (see eqn. (9)); σ_v^2 is the variance of ventilation;

Propofol effect: A significant effect (at the $P < 0.05$ level) of propofol was observed on G and C_{50}. The factor by which propofol was affected is the estimate given: Gc during the combined infusion of propofol and remifentanil was 0.46*[Gc observed during just remifentanil] = 0.46*0.42 = 0.19 $L.min^{-1}.Torr^{-1}$. Similarly for C_{50}, during the combinded remifentanil/propofol infusion C_{50} = 0.84*1.6 = 1.3 $ng.ml^{-1}$.

* not estimable.

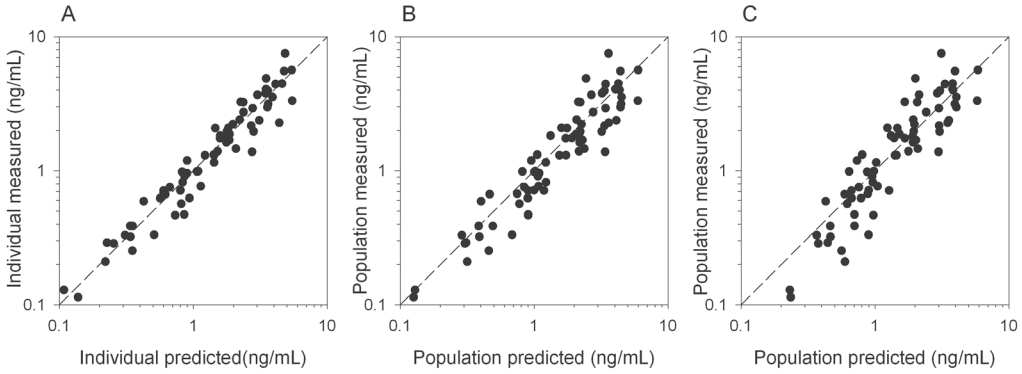

Figure 4. Goodness of fit plots for the pharmacokinetic model. **A** and **B** are the measured concentrations (y-axis) versus the individual (A) and population (B) predicted values. **C.** The pharmacokinetic analysis performed according to the parameter estimates of Minto *et al*.[17] (that is without a factor for V_2).

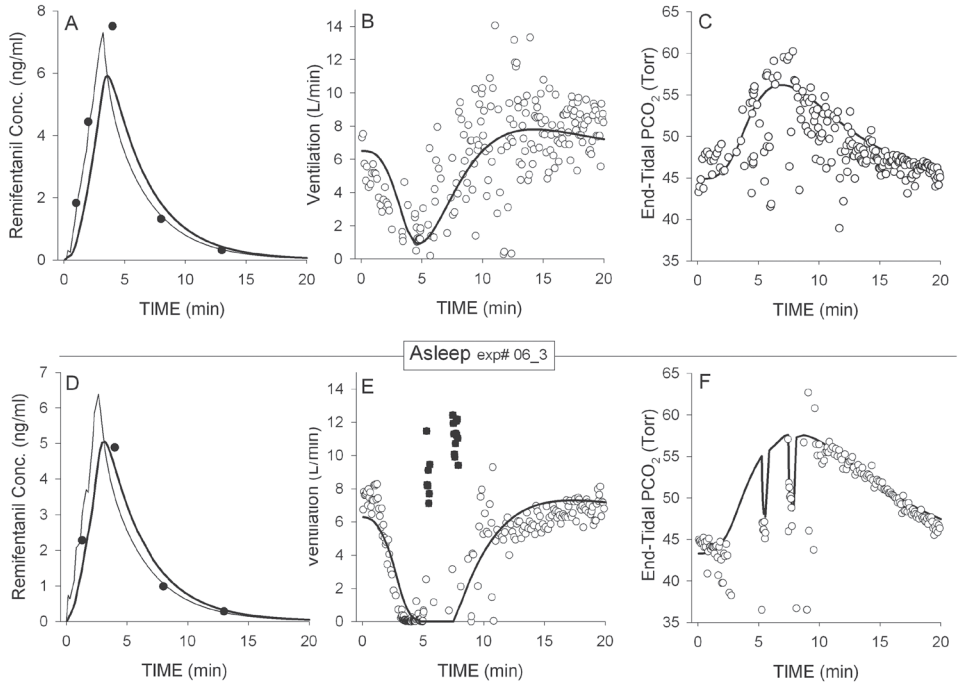

Figure 5. Examples of data fits of the effect of remifentanil on ventilation in one subject in the awake state (panels A-C) and asleep with propofol (panels D-F). **A** and **D**: Measured remifentanil concentration (closed circles), pharmacokinetic data fits (thin line), and estimated effect-site (thick line) concentrations. **B** and **E**: Minute ventilation with each spontaneous breath an open circle. Artificial breaths are depicted by closed squares. The lines through the data are the data fits. During sleep (panel E) the subject is apneic and requires artificial breathing assistance. **C** and **F**: End-tidal carbon dioxide values (open circles) and data fits. In panel F the effect of the artificial ventilation is clearly visible in the data fits.

of that of Minto *et al*. The factor in V_2 causes population measured versus population predicted concentrations to lie more closely on the line of identity. Without the factor, concentrations at the high end are underestimated and vice versa (figure 4C).

3.3.2 PHARMACODYNAMIC ANALYSIS

The pharmacodynamic model adequately described the data. Examples of data fits are given in Fig. 5. The data are from one subject (id006) and are fits of ventilation and end-tidal carbon dioxide concentrations under awake (panels A-C) and sedated (panels D-F) conditions. The effect of artificial ventilation by mask is clearly visible on end-tidal carbon dioxide in panel F as these periods of artificial ventilation were incorporated in the pharmacodynamic model. Since no spontaneous ventilation was observable during the period of artificial ventilation the fit through the ventilation data still (correctly) predicts apnea (Fig. 5E).

In table 2 the population pharmacodynamic model parameters are given. The concentration remifentanil causing 50% respiratory depression is 1.6 ± 0.03 ng.ml^{-1}. A notable observation is the low value for G in the remifentanil runs (0.42 L.min^{-1}.Torr^{-1}). Low-dose propofol significantly decreased parameters G by more than 50% to 0.19 L.min^{-1}.Torr^{-1}, and parameter C_{50} by about 20% to 1.3 ng.ml^{-1}. Propofol had no effect on baseline ventilation, baseline end-tidal carbon dioxide concentration, V_{TS}, Q and remifentanil $t_{½}k_{e0}$. All of these latter values were within the expected ranges.

3.3.3 SENSITIVITY ANALYSIS

The results of the likelihood profile method of the pharmacodynamic model are shown in figure 6. The Δ-2log likelihood (or the 'cost' function) indicates that the estimated model parameters (G, C_{50}, $t_{½}k_{e0}$, V_{TS}, Q) when applying relatively slow remifentanil input functions (that is, step durations of 1 and 5 min) were estimated with acceptable accuracy (± 10% of the actual value). Due to noise on the simulated data, deviations from optimal parameter values were sometimes observed (i.e., lower values of -2LL at 'optimal' parameter values that were different from those used in the simulation). Much faster infusion (step duration is 0.1 min) yielded a significant estimation bias. Since we applied mostly slow input functions (step duration 1 min or larger in 9 out of 10 subjects) we may assume that all important model parameters were estimable without any bias. Furthermore, visual inspection of the sensitivity analysis indicates that combining fast and slow input functions (as performed in our study) yields a reliable estimation without bias for all estimated parameters (intersection of all three in lines is around the x-value of 1, the simulated parameter value). Interestingly, Q was estimable at acceptable accuracy (which is an argument for the two-compartment carbon dioxide model that we used). Of the fixed parameters, estimation of parameters τ and V_{ALV} was poor (τ) or impossible (V_{ALV}). We relate this to the specific design of the study. A different experiment design with periods of artificial ventilation will result in estimability of V_{ALV}, while steps in carbon dioxide would have resulted in the accurate estimation of τ.

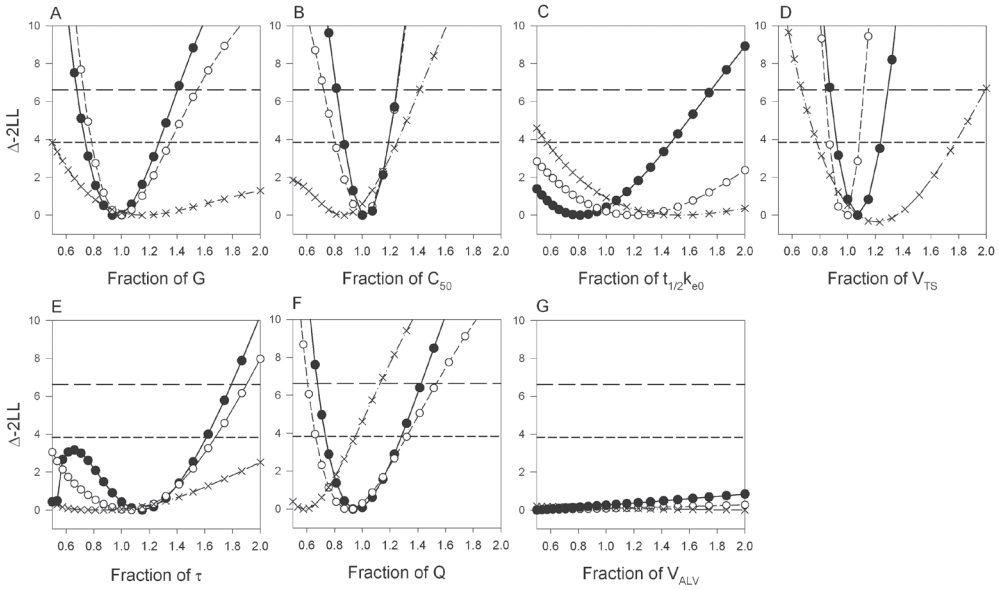

Figure 6. Sensitivity analysis for the model parameters. **A**-**D** and **F**: estimated parameters, **E** and **G**: fixed parameters. Δ–2LL is the difference in –2log likelihood between the simulated data and the best fit with one of the parameters held constant. The x-axis of each plot shows the parameter value as fraction of the value observed or fixed in the analysis of the experimental data set. The short-dashed line indicates the $P = 0.01$ level, the long-dashed line, the $P = 0.05$ level. •-• input function: step increase in plasma concentration of 1 ng.ml^{-1}, duration of step is 1 min (rate of rise = 1 ng.ml^{-1}. min^{-1}), number of steps is 5. ∘-∘ input function: step increase in remifentanil concentration is 1 ng.ml^{-1}, duration of step is 5 min (rate of rise = 0.2 ng.ml^{-1}.min^{-1}), number of steps is 5. x-x input function: step increase in plasma concentration of 1 ng.ml^{-1}, duration of step is 0.1 min (rate of rise = 10 ng.ml^{-1}.min^{-1}), number of steps is 5.

3.3.4 SIMULATION STUDY

Results of six simulations are given in Fig. 7. Linear remifentanil infusions with a rate of rise of 5 ng.ml^{-1}.min^{-1} (panels A-C), 0.5 ng.ml^{-1}.min^{-1} (panels D-F) and 0.2 ng.ml^{-1}.min^{-1} (panels G-I) are shown for the awake condition (thin line) and at the background of propofol (thick lines). The effects of the rate of rise on the nadir in ventilation (in the awake studies) and duration of apnea (in the propofol studies) are given in figure 8 for all 30 simulations. For both end-points the effect of slowing the rate-of-rise is biphasic. The nadir in ventilation decreased going from 5 to 1 ng.ml^{-1}.min^{-1} (from 2.3 to 1.6 L.min^{-1}, figure 5), after which it increased (5 ng.ml^{-1}.min^{-1}= linear infusion of 1 min; 1 ng.ml^{-1}.min^{-1} = linear infusion of 5 min). The duration of apnea increased going from 2.5 to 0.6 ng.ml^{-1}. min^{-1} (2 min and 9 min, respectively) after which it decreased rapidly. At infusion rates of 0.31 ng.ml^{-1}.min^{-1} (16 min) and slower no apnea occurred. During the two most rapid, short-term infusions (5 and 2.5 ng.ml^{-1}.min^{-1} or 1 and 2 min infusions) the remifentanil exposure was insufficient to cause apnea.

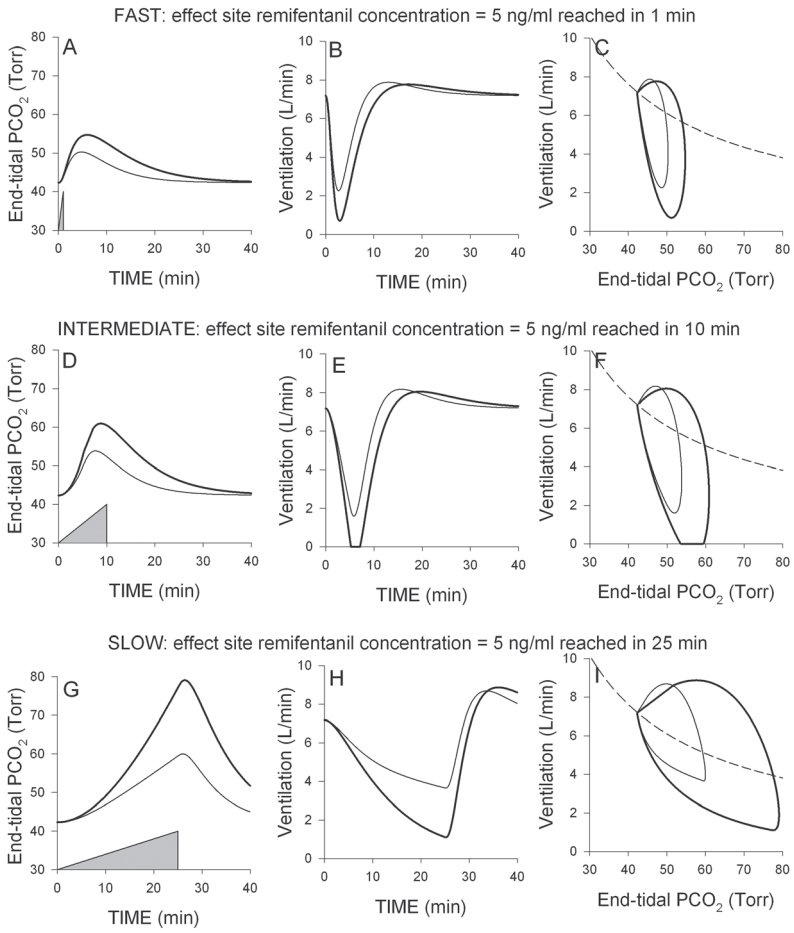

Figure 7. Simulation study on the effect of changes in the rate of rise of the effect-site remifentanil concentration on breathing (panels **A**, **D** and **G**) and end-tidal carbon dioxide concentration (panels **B**, **E** and **H**) in awake state (no propofol present, thin lines) and during sleep (due to a low-dose propofol background infusion, thick line). The remifentanil rates of rise are linear and vary from 5 $ng.ml^{-1}.min^{-1}$ given for 1 min (panels A-C) to 0.5 $ng.ml^{-1}.min^{-1}$ given for 10 min (panels D-F) and 0.2 $ng.ml^{-1}.min^{-1}$ given for 25 min (panels G-I), so that peak effect-site remifentanil concentration was 5 $ng.ml^{-1}$ in all simulations. Panels **C**, **F** and **I** depict the counter clockwise end-tidal carbon dioxide – ventilation loops (continuous lines) and metabolic hyperbola (dashed line). The gray triangles depict the linear remifentanil infusion schemes.

The carbon dioxide concentration – ventilation (counter clockwise) loops shown in Fig. 7 (panels C, F and I) are graphical representations of the link between the two parameters in areas below and above the metabolic hyperbola (dashed lines). In the simulation studies we assumed no effect of adding low dose propofol on metabolic rate (which was experimentally verified, as resting PCO_2 and minute ventilation were similar between the awake and propofol sedates states). The graphs indicate that loops in the horizontal plane (such as observed in panel I) are desirable when aiming at and maintaining spontaneous breathing. The graphs show further that independent of the infusion rate hyperventilation in the recovery phase (upswing of the loop) is greater when ventilatory depression is more pronounced and carbon dioxide accumulates in the body.
Finally, the simulations are in close agreement with the observed data; for example compare panels 7B with 5B, and 7E with 5E and 3C.

3.4 DISCUSSION

Using a 'simple' non-steady-state pharmacokinetic-pharmacodynamic model of opioid-induced respiratory depression (eqn. 6), we described the ventilatory behavior of varying remifentanil infusion schemes in awake and propofol sedated volunteers. The model is based on the linear relationship between carbon dioxide and ventilation. Most important parts of the model parameters were identifiable and estimable (*e.g.*, the drug concentration causing 50% respiratory depression, the gain factor of the respiratory controller, the remifentanil effect-site equilibration half-life), while others were fixed to values obtained from previous studies from our laboratory or obtained from the literature (the time constant for carbon dioxide of the ventilatory control system and alveolar volume). As we assumed that ventilation was dependent not only on the remifentanil concentration at its effect-site (the brainstem) but also on the metabolic product carbon dioxide, our model may be described as an indirect response model. The first (and only) previous indirect response model of opioid-induced respiratory depression was developed by Bouillon *et al*.[11,12] While their model differs at important points from ours it even so shares important characteristics. We will discuss the similarities and differences between the two models in the section 'Model Comparisons' below.

3.4.1 THE MODEL: HOW IT WORKS AND PARAMETER ESTIMATES

A schematic description of the model is given in Fig. 2. The model has three parts. The remifentanil pharmacokinetic part (part I), consisting of the distribution of remifentanil throughout the body. Part of the remifentanil passes (with a delay described by rate constant k_{e0}) to the effect site, the brainstem, where it affects the control of breathing (part II of the model via term 1 of eqn. 6: $[1 - C_{REM}/2C_{50}]$). Respiration is diminished (with time constant τ) due to activation of μ-opioid receptors expressed on key-parts of the ventilatory control system (for example, premotor neurons of the ventral respiratory group (especially within the pre-Bötzinger complex) and the pontine respiratory group).[1,2]

Part III of the model, the carbon dioxide kinetics, is affected by the diminished breathing as it reduces the efficacy of carbon dioxide output and as a consequence arterial PCO_2 increases. This again has an effect on the respiratory control system (part 2 of the model) as it stimulates breathing (via term 2 of eqn. 6: $G \cdot [P_E - P_{E\_0}]$). So, two opposing additive effects influence breathing after remifentanil infusion: the direct depressant effect *via* depression of respiratory neurons and a stimulatory effect of the increasing arterial PCO_2. In figure 9A, the effect of just term 1 of eqn. 6 is plotted (lines 1 for awake and line 3 for asleep subjects). The effect of combining terms 1 and 2 is represented by lines 2 (awake) and 3 (asleep). It is apparent from the graphs that adding term 2 has a stimulatory effect on the remifentanil-ventilation data.

An important assumption on which our model is based is that opioids (in our case remifentanil) cause a linear increase in the position of the ventilatory carbon dioxide response curve (in our model parameter B) with little change in the value of the response slope (in our model parameter G). There is ample evidence that opioids indeed cause a parallel shift of the steady-state Ventilatie-PCO_2 response slope.[10,21,22] However, some studies indicate that there is some sex dependency with a reduction in slope in women (we exclusively performed studies in men), while others showed that the opioid effect is dependent on the technique used to measure the response slope.[23] As discussed previously, we consider the parallel shift of the Ventilatie - PCO_2 response slope a typical opioid effect and reduction of the slope is in our opinion due to a lowered arousal state (from sleep or sedatives/anesthetics).[10]

The value of $t_{½}k_{e0}$ that we observed (0.5 min) is in the same range as values from Babenco *et al.* using the isohypercapnic method (2 min) and studies on electroenecephalographic end-points (1.6 min).[8] The low $t_{½}k_{e0}$ value is related to remifentanil's rapid passage across the blood-brain barrier. This rapid movement of remifentanil into the brain compartment is a potential danger, as it may produce depression of respiratory neurons (via term 1 of eqn. 6, fig 9A) before any stimulatory effect of the accumulation of carbon dioxide is generated (*via* term 2 of eqn. 6, Fig. 9A). Other opioids with a much slower passage across the blood-brain barrier (such as morphine and its active metabolite morphine-6-glucuronide with a slow passage ($t_{½}k_{e0}$ in the range of hours), but also drugs as buprenorphine (1 hour), and fentanyl (5 min)), do allow for at least some accumulation of carbon dioxide while the respiratory neurons are being depressed, hence with a lesser chance of apnea.[24-26] However, this is only true when the drug is not overdosed. Otherwise, severe respiratory depression with apnea should always be in mind.[27] Our data further indicate that the chances of apnea are increased when the subject is asleep (BIS values = 80) with propofol. When term 2 of equation 6 equals zero the remifentanil concentration causing 50% depression of ventilation is by definition C_{50} (now only term 1 of eqn. 6 is operative). This occurs in situation in which PCO_2 remains constant at baseline levels (e.g., in isohypercapnic experiments or when carbon dioxide has not yet risen above baseline values due to the rapid action of the opioid). Our C_{50} (1.6 $ng.ml^{-1}$) is therefore well

comparable to values observed in isohypercapnic studies (*cf.* Babenco *et al*.[8] who estimated a value of 1.4 ng.ml^{-1}). In a previous isohypercapnic study from our laboratory studying the remifentanil propofol interaction the C_{50} value 0.7 ng.ml^{-1} for ventilation measured at a fixed end-tidal PCO_2 of 55 Torr.[10] The observed C_{50} is a low value, probably related to a differences in parameterization (see eqn. (4) of ref. [10]) and the fact that we analyzed the whole remifentanil-propofol surface rather than just the remifentanil-ventilation relationship.

The value of G (table 2) is smaller than observed in a previous study on the effect of combining remifentanil and propofol on the ventilatory carbon dioxide response curve (value of G at $C_{REM} > 0$ = 1.6 L.min^{-1}.Torr^{-1}, see figure 1).[10] The reason for the smaller estimated value of G may be the fact that we previously tested our subjects in normoxia versus hyperoxia in the current study. Hyperoxia significantly blunts the peripheral chemoreceptors at the carotid bodies causing a 30-40% reduction of G.[15] Furthermore, we believe that the current study was performed on the dog-leg of the V - PCO_2 response slope. There is ample proof that the linear response curve flattens around resting carbon dioxide values.[14] The value observed by us is in agreement with the value presented by Babenco *et al*. following a remifentanil bolus infusion.[8] Reassuring is the observation of a 50% reduction of G by low-dose propofol, which is in agreement with earlier findings.[8]

The likelihood profile method is a tool to assess whether the parameters may be estimated from the data and also yield their 95% confidence intervals. We observed that the ability to obtain accurate parameter estimates is dependent on the specific input function chosen. Slower remifentanil infusion rates (duration 1 min or longer) resulted in more precise estimates than a rapid bolus infusion (see figure 6). This may be related to the fact that we applied a rapid infusion in just one subject while the nine others received the slower rates. However, the analysis also indicates that when applying fast and slow infusion rates in one study and analyzing the data set using a population approach, the estimation precision is acceptable and that the fast infusions do not affect the outcome of the estimates negatively.

3.4.2 SUMMARY

In summary, the estimated parameter values are in close agreement to previous reported values in the literature. This together with the results of the sensitivity analysis gives us confidence that our model adequately describes the effect of remifentanil on breathing. Furthermore, we were well able to describe and predict the occurrence of apnea.

Apnea. To the best of our knowledge there are no earlier studies that assessed remifentanil effect on apnea in healthy volunteers. The study of Egan *et al*.[28] comes closest to ours. They assessed the effect of various bolus doses of remifentanil and used a respiratory intervention scale, based primarily on SpO_2, as endpoint. Low SpO_2 values (< 85%) did occur at the higher dose range (100 μg and greater), and predominantly in a population of older subjects (60 – 75 years). The low SpO_2 values are most probably related to the

occurrence of apnea. We simulated the dosing schedule of Egan *et al.* and observed apnea at doses of 100 μg and greater (data not shown). The observation of hypoxemia is of interest. Similarly to Egan *et al.*[28] we observed hypoxia although it was mild and occurred late in the experiment (3-5 min after the start of apnea). We relate this to the inspiration of 100% oxygen in our study causing an oxygen store sufficient for 4-5 min before serious desaturation (SpO2 < 90%) sets in. Low arterial oxygen has a stimulatory effect on breathing through activation of the peripheral chemoreceptors at the carotid bodies and increases the value of G.[15,16] This may have occurred in our experiments and may have had an effect on the duration of apnea. We did not take this into account in our current model but a G dependent on SpO_2 may be introduced in future models.

Remifentanil-propofol interaction. We previously assessed the interactive effects of remifentanil (0 – 2 $ng.ml^{-1}$) and propofol (0 – 2 $\mu g.ml^{-1}$) on breathing using steady-state isohypercapnic response surface modeling techniques.[10] We observed a synergistic interaction of the two drugs on resting ventilation, resting end-tidal PCO_2, ventilation at a fixed PCO_2 of 55 Torr and the V - PCO_2 response slope. Comparison with the current data analysis is difficult due to the differences in experimental set up and modeling approach. In contrast to our previous study, we currently studied just one low propofol plasma concentration (target on average 1 $\mu g.ml^{-1}$). However, we believe (in retrospect) that the observation of large periods of apnea (1 – 7 min) during infusion of remifentanil against the background of low-dose propofol precludes testing of higher propofol doses in volunteers. The large difference in responses to remifentanil in awake and propofol-sedated states on apnea occurrence is in agreement with a synergistic interaction between the two drugs. Whether the propofol effect is related to γ-amino-butyric acidergic depression of respiratory neurons or secondary to the change in arousal-state remains unknown.[2] In agreement with the latter possibility is the finding that sleep induces a substantial enhancement of the depressant effect of morphine on ventilatory control.[29]

Speed of remifentanil infusion. It has been suggested that by slowing the opioid infusion rate the degree of respiratory depression diminishes due to the stimulatory effect of the accumulation of carbon dioxide.[3,12] We performed a simulation study to test this suggestion and observed a more complex interaction between remifentanil infusion rate and degree of depression of ventilation as measured by the nadir in ventilation (awake studies) and duration of apnea (propofol sedated studies; Fig. 7 and 8). Going from a rapid (linear) infusion (5 $ng.ml^{-1}.min^{-1}$) to a slow (linear) infusion (0.17 $ng.ml^{-1}.min^{-1}$) in target effect-site concentration (peak concentration = 5 $ng.ml^{-1}$), both endpoints showed a worsening of ventilatory depression with a lower nadir in ventilation going from 5 to 1 $ng.ml^{-1}.min^{-1}$ (infusion duration: from 1 to 5 min) and an increase in apnea duration going from 2.5 to 0.55 $ng.ml^{-1}.min^{-1}$ (infusion duration: from 2 to 9 min). Thereafter respiratory depression decreased with slowing of the infusion rate. Apnea disappeared at infusion

rates ≤ 0.31 $ng.ml^{-1}.min^{-1}$ (infusion duration = 16 min). Our analysis indicates that the degree of respiratory depression (as defined by the nadir in ventilation and duration of apnea) is related to the opioid's pharmacokinetics, the speed of opioid infusion, the total amount opioid given (at infusion durations of 1 and 2 min the total amount of remifentanil given is insufficient to cause apnea, Fig. 8), and the target plasma concentration, all relative to the carbon dioxide kinetics and dynamics. In order to prevent overt respiratory depression and apnea one should be considering all of these variables. As the simulations performed by us are not to be extrapolated to other scenarios than those applied here, a simulation for each new circumstance should be performed.

Respiratory variability. We observed great variability in the respiratory data during exposure to low-dose remifentanil (see Fig. 3 and 5). Mitsis *et al.* modeled variability of spontaneous respiration during low-dose remifentanil administration and concluded that the increase in variability due to the opioid was related to a decrease in the strength of the controller part of the ventilatory loop.[30] Our observation of a low value of G is in agreement with this statement and indeed may be the cause for the inability to strictly perform a breath-to-breath feedback control based upon the also quite varying carbon

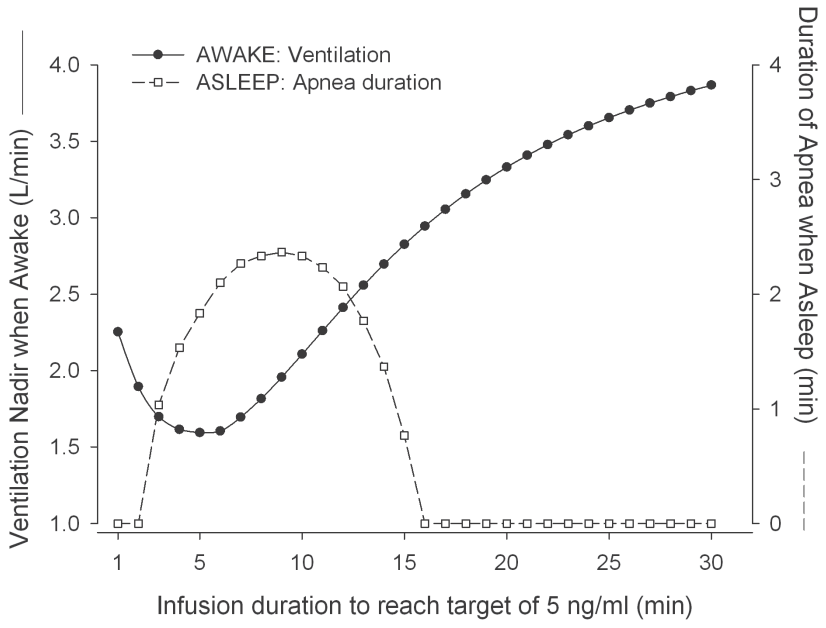

Figure 8. Simulation study on the effect of varying linear rates of rise of remifentanil effect-site concentration on the nadir in ventilation (simulations performed in the absence of propofol, closed circles) and on the duration of apnea (simulations performed in the presence of propofol, open squares).

dioxide input.

Model Comparisons. Bouillon *et al*. were the first to study and model the respiratory effects of opioids (alfentanil and remifentanil) in the non-steady state.[11,12] Their initial attempts are well appreciated and our modeling work should be considered an extension to the original ideas postulated by Bouillon *et al*.

Bouillon *et al*.[11-13] used an indirect response model consisting of two multiplicative terms, a sigmoidal Emax function and a power function of the form (eqn. 11 in ref.[12]):

$$\dot{V} = \dot{V}_0 \cdot \left[1 - \frac{C_{REM}^{\gamma}}{C_{50}^{\gamma} + C_{REM}^{\gamma}}\right] \cdot \left[\frac{P}{P_0}\right]^{F}$$

where F is the slope of the ventilatory carbon dioxide response curve (equivalent to our parameter G) and γ a shape parameter. We compared the model to our model in Fig. 9 (panel B) by plotting the acute (term 1, the sigmoid-Emax function: line 3 in Fig. 9B), and

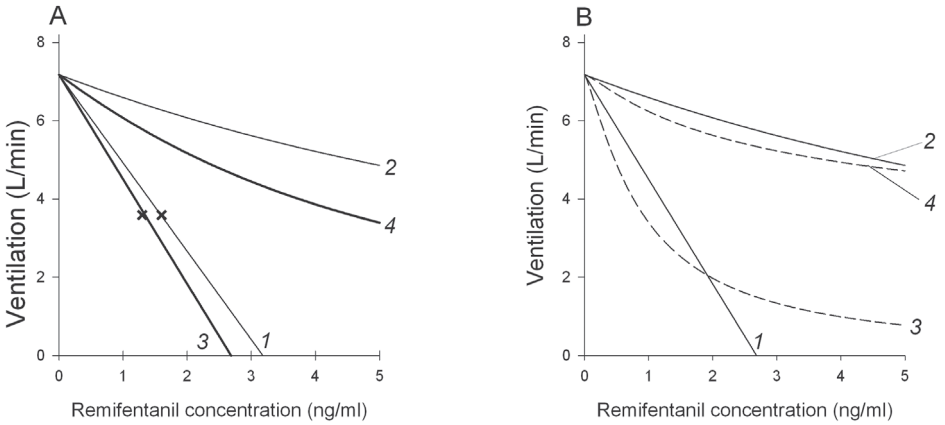

Figure 9. A. The relationship between remifentanil concentration and ventilation is made up of two additive linear terms (eqn. 6). The term that describes the effect of remifentanil on ventilation when carbon dioxide has not accumulated (the first term of eqn. 6) is given by the linear line 1 for the awake state and line 3 for the propofol sedated state. A steady state in ventilation occurs when carbon dioxide accumulates: for the awake state this is reflected by the non-linear line 2, and for the sedated state by the non-linear line 4. Now both terms of eqn. 6 are active. x are C50 values.

B. Comparison of the remifentanil – ventilation relationships derived from the model advanced by Bouillon *et al*.[12] and our model. Lines 1 and 2 are the acute (line 1) and steady-state (line 2) relationships derived from eqn. 6. Equivalent lines for the Bouillon model are lines 3 (acute relationship) and 4 (steady-state relationship) as derived from equation 14 of ref. [12.] The large difference between models is the inability to predict apnea in the latter model (line 3). The steady-state relationships are much alike (compare lines 3 and 4).

the combined (term 1 + term 2, line 4) steady-state relationships. The largest difference between the two models is the difference in the acute relationship as reflected by lines 1 and 3. Due to the sigmoidal Emax nature of term 1 and the multiplicative nature of the model, no apnea may be described or predicted. At 2 times the C_{50} the Bouillon model predicts ventilation levels > 2 $L.min^{-1}$,[12] while in our approach $2C_{50}$ equals the concentration at which apnea occurs (C_{APNEA} = 3.2 $ng.ml^{-1}$; Fig. 9). The overall steady-state relationships (lines 3 and 4) are comparable between models. In order to get an impression of the ability of the model of Bouillon *et al*.[10] to describe our data, we analyzed our data with their model. In contrast to Bouillon *et al*. we added a delay between remifentanil concentration and effect-site (parameter k_{e0}). Without this parameter no meaningful data fits were obtained. Two main observations were made: as expected, the occurrence of apnea could not be modeled but yielded systematic misfits; and the effect of manual ventilation on PCO_2 yielded misfits as well. The latter is probably related to the single carbon dioxide compartment in the Bouillon *et al*. model versus two compartments in our model. In the study of Bouillon *et al*. no systematic misfits were reported. However, this may partly be related to the remifentanil function applied (one to four 15 min steps of varying magnitudes of C_{REM}, which yielded changes towards the steady state in both PCO_2 and ventilation) together with a relatively small number of arterial carbon dioxide samples. This may have yielded little contribution of the first term of the model to the overall effect but a predominant contribution of the power function, which depends on the accumulation of carbon dioxide. Possibly at different input functions (such as bolus infusions) or during sedation, the misspecifications of the model would become apparent. Bouillon *et al*.[12] suggest to address the issue of apnea by using a logistic probability model. Our current model indicates that this is not required as apnea is accurately predicted at realistic drug concentrations. Our model has two linear terms (eqn. 6). In order to assess whether non-linear functions would improve the data fits we analyzed the data with a model consisting of power functions. No systematic improvements were observed (data not shown).

In conclusion, we developed a novel pharmacokinetic-pharmacodynamic model that is able to describe the non-steady-state effects of remifentanil on breathing under a variety of circumstances ranging from fast to slow drug infusions, under awake and sleeping conditions. Furthermore and possibly most important, the model allowed description and prediction of an important idiosyncrasy of the ventilatory control system, the development of opioid-induced apnea.

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CHAPTER 4

UTILITY FUNCTION: A RISK-BENEFIT COMPOSITE OF PAIN RELIEF AND BREATHING RESPONSES

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4.1 INTRODUCTION

It is well known that opioids are able to produce life-threatening respiratory depression.[1] A recent editorial brought to attention the sharp rise, since the early 1990s, of unintentional drug overdose and consequently loss of life due to ingestion of prescription painkillers in the United States.[2] As discussed, this is related to a 10-fold increase in the medical use of synthetic opioids, marketing tactics and the proactive identification of patients with chronic pain.[2] Also in the perioperative setting opioid-induced respiratory depression is a common observation with regular reports of fatalities.[3-5] Overall there is the ongoing need for vigilance when potent opioids are administered to spontaneously breathing and opioid-naïve patients.[1,2] Opioid risk (in the acute setting: respiratory depression) is best viewed in context of its beneficial effect, ie. analgesia. Recently, Katz proposed the construction of a risk benefit composite for opioid use in chronic pain patients. This composite is based on days of chronic pain treatment causing pain relief ≥30% and no or mild adverse effects.[1] One method to quantify opioid-induced respiratory depression relative to analgesia is the construction of a Utility (or safety) Function (UF). Cullberg first introduced the concept of UFs when characterizing the outcome of a thrombin inhibitor.[6] He defined the UF as the probability of drug-induced thrombus regression minus the probability of drug-induced bleeding related event. In the current study we constructed the UF for the opioid analgesic fentanyl. The fentanyl UF is constructed based on the results of a population pharmacokinetic-pharmacodynamic (PK-PD) study on the effect of fentanyl on respiration and antinociception. Next, a simulation study is performed, using the PK-PD parameter estimates and their variances, in which 9,999 simulated subjects received fentanyl. The resultant distributions are used to calculate the UF. In the current approach we calculated the probability of an increase in pain tolerance of 50% or more minus the probability of respiratory depression of 50% or more.

4.2 METHODS EN MATERIALS

4.2.1 SUBJECTS

After approval of the protocol by the local Human Ethics Committee (Commissie Medische Ethiek, LUMC, Leiden) and the Central Committee on Research involving Human Subjects (Commissie Mensgebonden Onderzoek, The Hague, The Netherlands) and informed consent was obtained according to the Declaration of Helsinki, 12 male volunteers, aged 18-45 years, were initially recruited to participate in the study. One subject dropped out and was replaced by a 13th subject. All recruits were subjected to a medical history, physical examination, 12-leads electrocardiogram and blood screening before inclusion. Only healthy subjects, without a history of alcohol or illicit drug abuse and a body mass index between 20 and 28, were included in the study.

4.2.2 STUDY DESIGN

All subjects were studied twice with at least two weeks between sessions (the sequence of sessions was random). On one occasion the effect of a single intravenous infusion of fentanyl (3.5 $\mu g.kg^{-1}$ infusion over 90-s) on respiration was tested, on the other occasion the effects of fentanyl (3.5 $\mu g.kg^{-1}$ infusion over 90-s) pain responses to a cutaneous noxious electrical stimulus were tested.

Respiratory measurements. During the respiratory studies, subjects breathed through a facemask (fitted over nose and mouth). The airway gas flow was measured with a pneumotachograph (#4813, Hans Rudolph, Kansas City, MO) connected to a pressure transducer, which yielded a volume signal. The pneumotachograph was heated (37 ^{o}C) throughout the study period. This signal was calibrated with a 1 L calibration syringe (Hans Rudolph). 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-Tec, Veenendaal, The Netherlands) via which the flow oxygen, nitrogen and carbon dioxide could be set individually at any desired level. A computer provided control signals to the mass-flow controllers allowing adjustment of the inspired gas mixture to force the end-tidal gas concentrations of oxygen and carbon dioxide to follow a specific pattern in time. Gas concentrations were measured with a gas analyzer (Datex Multicap, Helsinki, Finland); arterial hemoglobin oxygen saturation was measured via a finger probe with a Masimo pulse oximeter (Irvine, CA).
Respiration was measured at a clamped elevated end-tidal carbon dioxide tension (PCO_2) using the 'Dynamic End-Tidal Forcing' (DEF) technique.[7-10] In each subject, the end-tidal PCO2 was elevated in steps of 2-3 mmHg until ventilation reached a value 20 - 24 $L.min^{-1}$. The end-tidal PCO_2 was kept constant at this level throughout the study (baseline end-tidal PCO_2 level). This procedure was performed in a 'training' session 30 to 45 min prior to dosing. The end-tidal oxygen concentration (PO_2) was kept constant at 110 mmHg throughout the study. After a steady state in ventilation was obtained, fentanyl was infused and continuous breath-to-breath ventilatory measurements were obtained for the next 90 min. Thereafter, 3-min measurements were obtained at 30-min intervals until t = 4 h following administration and at 1-h intervals until t = 6 h. The following variables were collected on a breath-to-breath basis on a computer disc for further analysis: inspiratory minute ventilation, end-tidal PCO_2, end-tidal PO_2 and oxygen saturation.

Pain responses. Acute pain was induced by an electrical current through two surface electrodes (Red Dot, 3M, London, Ontario, Canada) placed on the skin overlaying the left tibial bone (transcutaneous electrical stimulation).[10-12] The electrodes were attached to a computer interfaced current stimulation (Leiden University Medical Center, Leiden, The Netherlands). The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 $mA.2\ s^{-1}$. The stimulus train consisted of a square-wave pulse of 0.2 ms duration

applied at 10 Hz and had a cutoff of 128 mA. The subjects were instructed to press a control button when they felt pain (pain threshold) and when no further increase in stimulus intensity was acceptable (pain tolerance; this ends the stimulus train). Pain responses were obtained at baseline and at t = 10, 25, 40, 55, 70, 90, 110, 130, 160, 190, 220, 250, 310 and 370 min. after dosing. We analyzed the pain tolerance data for the current report.

Blood sampling and plasma drug concentrations measurements. Blood samples (3 mL) were obtained from an arterial line placed in the left or right radial artery (opposite of the arm through which the drug was infused) for determination of the plasma concentrations of fentanyl. Blood samples were obtained at t = 2, 5, 10, 15, 30, 60, 90, 150, 210, 270, 330, 390 and 480 min after dosing. Plasma was separated within 30 min of blood collection and stored at -20^{o}C until analysis.

Samples were prepared by liquid/liquid extraction of the plasma samples with t-butyl methyl ether. Samples with fentanyl concentrations above the calibration range were diluted prior to the measurement using human blank plasma. Chromatographic separation was performed using an Agilent 1200 liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA) and a PAL HTC-xt (Axel Semrau GmbH & Co., Sprockhövel, Germany) autosampler. A Pursuit 3μ C18 30 x 2 (30 mm x 2 mm) column (Varian, Walnut Creek, CA) and MetaGuard Pursuit 3μ C18 10 x 2 mm guard column (Varian, Walnut Creek, CA) were used as stationary phases. The analytes were eluted using gradient elution with a mobile phase consisting of formic acid (0.1%) and acetornitril (containing 0.1% formic acid) at 30°C with a flow rate of 0.7 ml.min^{-1}. Fentanyl was detected in the MRM mode using an electrospray ion source on a triple quadrupole mass spectrometer API-5500 QTrap equipped with a Turbo-Ionspray™ Interface (Applied Biosystems, Framingham, MA) operating in positive ionization mode. The MS/MS transitions 337/188 for fentanyl and 342/188 for D5-fentanyl were monitored for quantitation of the compound. The calibration range of the bioanalytical assay was 0.005 – 5.00 ng.ml^{-1}. Calibration samples were prepared at 0.005, 0.010, 0.050, 0.200, 1.00, 2.00, 3.75 and 5.00 ng fentanyl.mL^{-1} plasma. Qualtity control samples were prepared at concentrations of 0.015, 2.00 and 5.00 ng fentanyl.ml^{-1} plasma. The values for the overall accuracy and the overall precision for quality controls assayed during analysis of study samples were 97.2% – 106.2% and 1.7% – 9.5%, respectively. The samples were analyzed in six batches with interbatches accuracy of 95.9% – 104.9% and coefficient of variation of 1.9 – 6.9% over the calibration range of 0.005 to 5.0 ng.ml^{-1}.

4.2.3 PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS

Multiple compartment models were fitted to the fentanyl pharmacokinetic data. Model selection (the number of compartments) was based on the Goodness of Fit criterion. Only the 'best' models will be described here.

To eliminate a possible hysteresis between plasma concentration and effect, an effect

compartment was postulated that equilibrates with the plasma compartment with a half-life $t_{½}k_{e0}$ (i.e., the blood-effect-site equilibration half-life).
The ventilation data were modeled as:[7-10]

$$\text{Effect}(t) = \text{Emax} + [\text{Emin} - \text{Emax}] \cdot [A/(1+A)] \text{ and } A = [Ce(t)/C_{50}]^{\gamma} \quad \text{eqn.(1)}$$

where effect is the effect at time t (minute ventilation), Emax maximum or predrug effect (baseline ventilation), Emin minimum effect (an Emin of zero indicates that apnea may be reached), Ce(t) effect-site concentration at time t, and C_{50} the effect-site or steady-state concentration causing 50% depression of ventilation.

Transcutaneous electrical pain responses were modeled as:[10-12]

$$\text{Pain Response}(t) = \text{Baseline} \cdot [1 + 0.25 \cdot B] \text{ and } B = [Ce(t)/C25]^{\gamma} \quad \text{eqn.(2)}$$

where Pain Response(t) is the stimulus intensity at which a pain tolerance response occurs at time t, Baseline the predrug stimulus intensity at which a pain threshold or pain tolerance response occurs, Ce(t) the effect-site concentration at time t, and C_{25} the effect-site or steady-state concentration causing an increase of 25% stimulus intensity for a response. Estimation of C_{25} rather than C_{50} was performed as the 25% increase in stimulus intensity was midpoint of the observed responses.
The pharmacokinetic-pharmacodynamic data were analyzed with the mixed-effects modeling software NONMEM VII (ICON Development Solutions, Ellicott City, MD)13 The PK/PD analysis was performed in two stages. From the first stage (pharmacokinetic analysis), empirical Bayesian estimates of the pharmacokinetic parameters were obtained. In the second stage (pharmacodynamic analysis), the pharmacokinetic parameters were fixed to those obtained in the first stage. An integrated data analysis was performed in NONMEM, *i.e.* combining all pharmacodynamic data (respiratory and pain data) in one analysis. Model parameters were assumed to be log-normally distributed. Residual error was assumed to have both an additive and a relative error for concentrations and only an additive error for all effect parameters. Covariance between random effects (ηs) for the pharmacodynamic end-points were explored using $OMEGA BLOCKs.
The number of compartments in the pharmacokinetic analysis was determined by the magnitude of the decrease in the minimum objective function value (MOFV; X^2-test; $P < 0.01$ was considered significant). In the pharmacokinetic analysis weight and height were considered as covariates. *P*-values less than 0.01 were considered significant.

4.2.4 VISUAL PREDICTIVE CHECK

Visual predictive checks were performed to assess the adequacy of the description of both fixed and random effects by simulating data using the models and calculating their 2.5th,

50^{th} and 97.5^{th} percentile at all sampling times.

4.2.5 UTILITY FUNCTION

The utility of drug effect, U, was defined as the probability of obtaining the desired effect minus the probability of obtaining a side effect.[6,14] If we define the desired effect as an increase in pain tolerance of 50% or more and the side effect as respiratory depression of 50% or more, and the probability of obtaining these as $P(A) \geq 0.5$ and $P(R) \geq 0.5$, respectively, then $U = P(A \geq 0.5) - P(R \geq 0.5)$. The utility of fentanyl's effect was calculated as function of effect site concentration (U_1) and of time (U_2) after administration of 3.5 $\mu g.kg^{-1}$:

$$U_1(Ce) = P(A(C_e) \geq 0.5) - P(R(C_e) \geq 0.5) \qquad \text{eqn.(3)}$$

and

$$U_2(t) = P(A(t) \geq 0.5) - P(R(t) \geq 0.5) \qquad \text{eqn.(4),}$$

where P is probability, A analgesia and R respiratory depression.

The UF was calculated from the population pharmacokinetic and pharmacodynamic model with established values for the population and their inter-individual variability parameters (ω^2). To this end 2 × 9,999 simulations were performed using NONMEM's simulation step (with SUBPROBLEMS = 9999). The occurrence of desired and side effects were counted and divided by 9999 to estimate probabilities (note that these are uncorrelated because no correlations between the ω^2 were identified in the pharmacodynamic analysis). U-values range from –1 to +1; $U > 0$ indicate that the chance for a desired effect exceeds the chance for an unwanted effect (here respiratory depression) while $U < 0$ indicates that the chance for the unwanted effect exceeds the chance for analgesia. U-values between –0.2 and 0.2 are small effects, U-values between –0.2 and –0.4, and 0.2 and 0.4 are moderate effects and U-values < –0.4 and > 0.4 are large effects. Small effects indicate absence of selectivity of action (*i.e.*, no clinically relevant greater chance for analgesia than for respiratory depression and *vice versa*).
Sensitivity analyses were performed to assess the effect of changes in context (numerical response thresholds in P(A) and P(R)) and effect of changes in pain parameter estimates $t_{½}k_{e0}$ and C_{50} on the form of the utility functions.

4.3 RESULTS

One subject dropped out and was replaced by another. The pharmacodynamic data of the dropout were incomplete and therefore discarded. His pharmacokinetic data were used in the population analysis. Consequently the number of subjects was 13 for the pharmacokinetic data and 12 for the pharmacodynamic data. In one subject just one set

of pharmacokinetic data was available due to failure of arterial line insertion on one occasion. No unexpected or major side effects occurred during the studies. All subjects (n = 13) were white males with a mean age of 22.1 years (range 19-27 years), height 186 cm (176-200 cm), weight 80.5 kg (64-111 kg) and body mass index of 23.2 $kg.m^{-2}$ (21-28 $kg.m^{-2}$).

Mean fentanyl plasma concentrations, respiration and pain responses are shown in Figure 1. In Figure 2, the individual pain responses are plotted showing the variability in the data. The peak increase in pain tolerance averaged to 21.9 mA, which (apart from one outlier of 91 mA) was normally distributed (range 5.5 to 33.5 mA, n = 11). Similar response variabilities have been observed in other studies on opioid analgesic efficacy using this same pain model.[10-12]

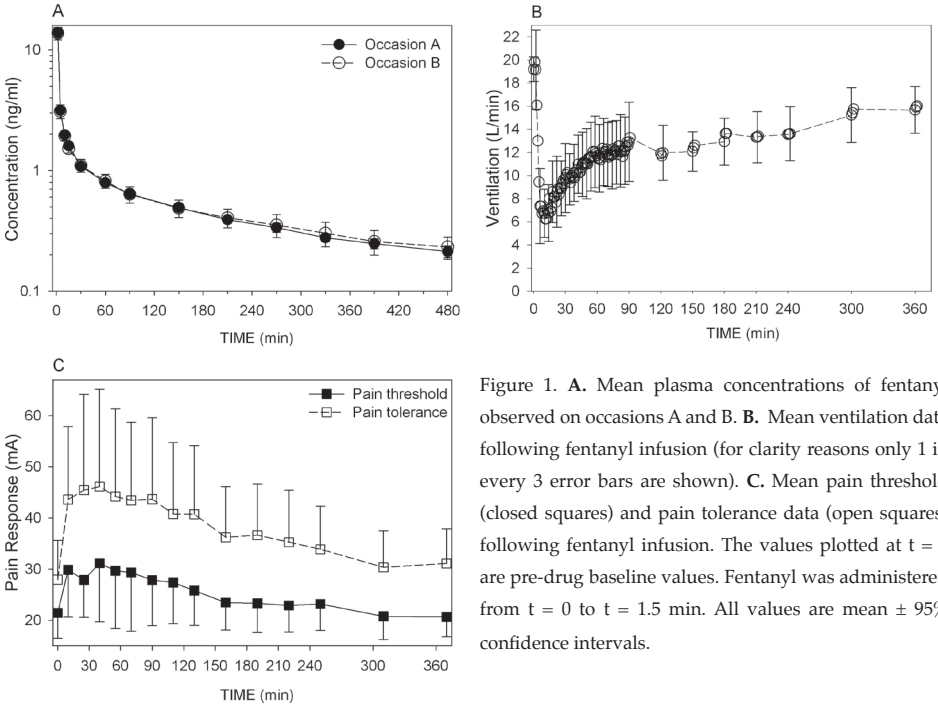

Figure 1. **A.** Mean plasma concentrations of fentanyl observed on occasions A and B. **B.** Mean ventilation data following fentanyl infusion (for clarity reasons only 1 in every 3 error bars are shown). **C.** Mean pain threshold (closed squares) and pain tolerance data (open squares) following fentanyl infusion. The values plotted at t = 0 are pre-drug baseline values. Fentanyl was administered from t = 0 to t = 1.5 min. All values are mean ± 95% confidence intervals.

4.3.1 PHARMACOKINETIC ANALSIS

The mean plasma fentanyl concentrations are given in Figure 1A. The variability in fentanyl concentrations obtained on the two distinct study occasions was small as observed in Figure 1A. The final pharmacokinetic model consisted of a three-compartment model with one central (V_1) and two peripheral compartments (V_2 and V_3). The pharmacokinetic

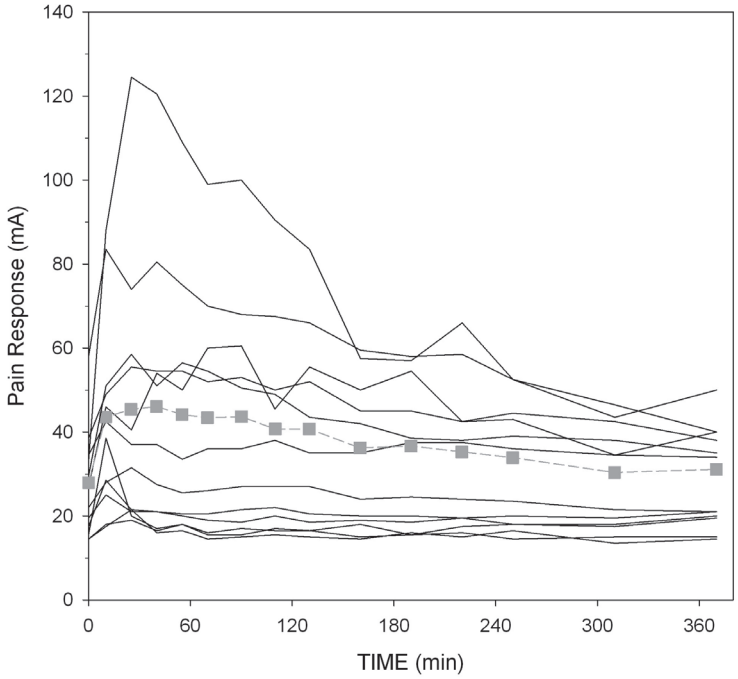

Figure 2. Spaghetti-plot of the pain tolerance responses during and following fentanyl infusion. The lines are the individual responses, the oranges squares are the mean data.

Table 1. Pharmacokinetic model parameter estimates

Parameter	Typical value	SEE (%)	ω^2	SEE	η-shrinkage[&]
V_1 (L)	8.867	0.652 (7)	0.065*	0.021 (32)	1%
V_2 (L)	42.056	3.243 (8)	0.042	0.016 (38)	4%
V_3 (L)	191.709	9.529 (5)	0.027	0.011 (41)	4%
CL_1 ($L.h^{-1}$)	0.751	0.0538 (7)	0.027	0.016 (59)	4%
CL_2 ($L.h^{-1}$)	4.291	0.322 (8)	0.065*	0.021 (32)	6%
CL_3 ($L.h^{-1}$)	1.932	0.157 (8)	0.065*	0.021 (32)	6%
IOV on CL_1	0.004	0.002 (50)			77%
σ^2	0.109	0.005 (5)			8%[#]

* one η was used for V_1, CL_2 and CL_3.

SEE is standard error of the estimate in the column to the left (in brackets the SEE as %).

V_1, V_2 and V_3 are the volumes of compartments 1, 2 and 3; CL_1 is elimination clearance; CL_2 and CL_3 are the clearances between compartment 1 and compartment 2 and 3, respectively.

IOV is inter-occasion variability.

ω^2 is the inter-subject variability (in the log-domain).

η^2 is the variance of the residual (relative) error.

&: η shrinkage of empirical Bayesian estimates to the population values.

#: ε shrinkage of model output to the observations.

parameter estimates are given in Table 1. Covariate weight (WT in kg) had a significant effect on parameters V_1, V_2, CL_1 and CL_2 with the volumes scaled by (WT/70) and the clearances scaled by $(WT/70)^{0.75}$ ($P < 0.01$). No effect of WT was observed for V_3 and CL_3. Covariate height had no effect on any of the model parameters. Goodness of fit plots (measured versus individual predicted concentrations and individual weighted residuals versus time) are given in Fig. 3. Examples of data fits are given in Figures 4 and 5. The goodness of fit plots and inspection of the individual data fits indicate that the pharmacokinetic model adequately describes the pharmacokinetic data.

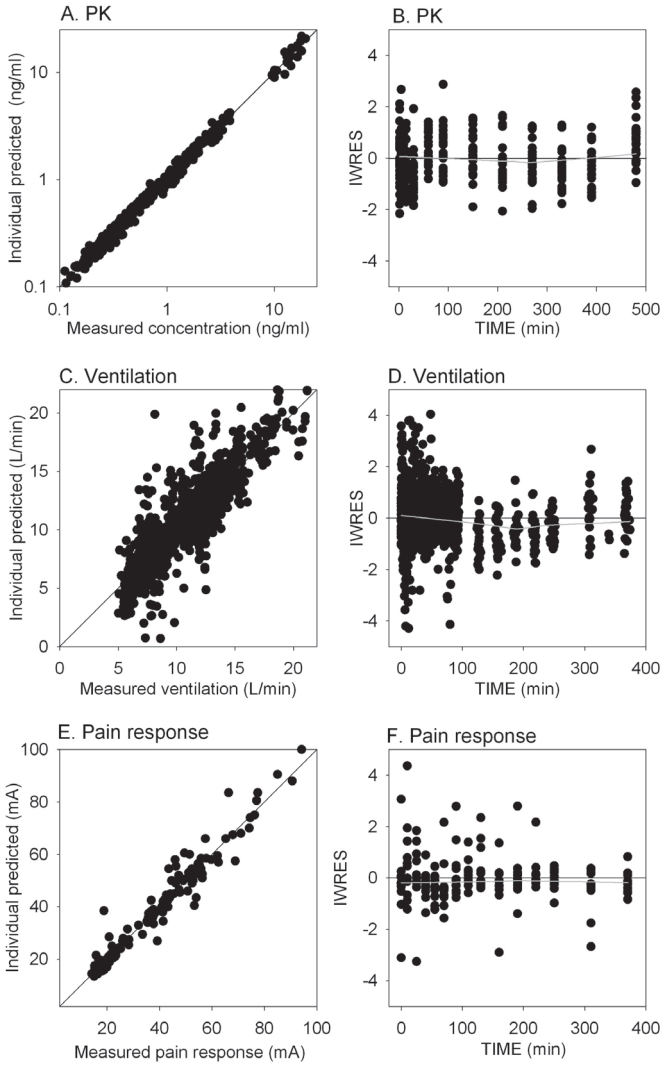

Figure 3. Goodness of fit plots.
A. Individual predicted concentrations versus measured concentration.
B. Individual weighted residual (IWRES) of concentration versus time.
C. Measured ventilation versus individual predicted ventilation.
D. Individual weighted residuals (IWRES) of ventilation versus time.
E. Measured versus individual predicted pain response.
F. IWERS of pain tolerance responses versus time. In panels B, D and F Loess smoothers are given in grey. PK is pharmacokinetics.

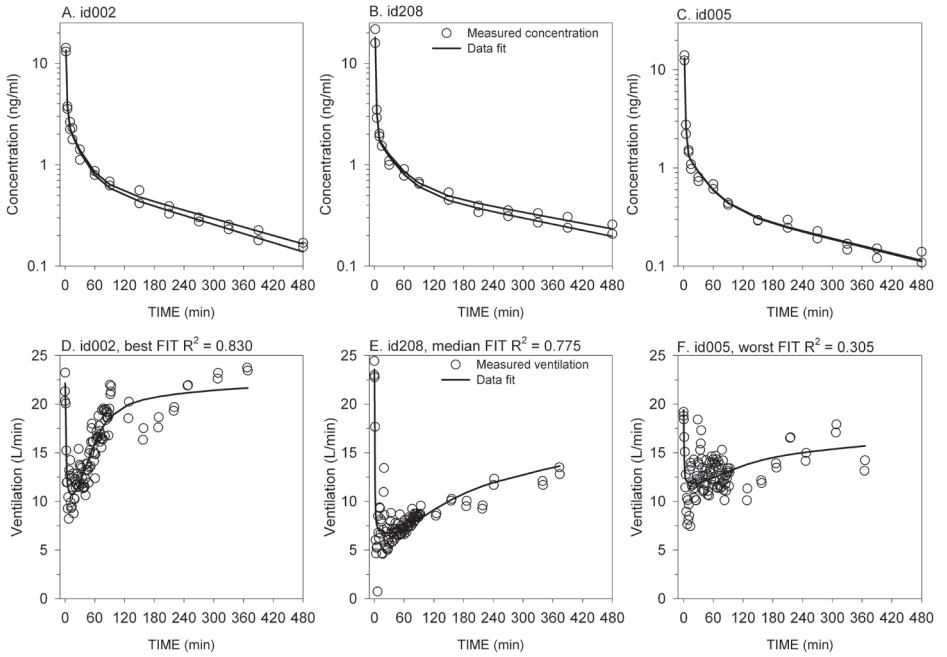

Figure 4. Effect of fentanyl on ventilation. Best, median and worst fits (as determined by the coefficient of determination, R^2) are given together with the corresponding pharmacokinetic data fits. Open symbols are the measured values; the continuous lines are the data fits. **A** and **D**. subject id 002, best pharmacodynamic fit; **B** and **E**. subject id 208, median pharmacodynamic fit; **C** and **F.** subject id 005, worst pharmacodynamic fit. Since fentanyl was given on two occasions both pharmacokinetic fits are given (panels A-C).

4.3.2 PHARMACODYNAMIC ANALYSIS

Mean ventilatory and pain responses are given in Figures 1 and 2. These figures show that fentanyl had an appreciable effect on both end-points. Best, median and worst PD data fits (and corresponding pharmacokinetic fits; the pharmacokinetic fits include the data from both occasions) for the three end-points are given in Fig. 4 and 5. The goodness of fit plots are given in Figure 3C to F. These plots and inspection of the individual data fits indicate that the pharmacodynamic models adequately describe the pharmacodynamic data. Model parameter values are given in Table 2. The ventilation Emin value was not significantly different from 0 $L.min^{-1}$ ($P > 0.05$), which indicates that at a sufficiently high fentanyl dose apnea will occur. A critical ventilation level (arbitrarily defined as < 4 $L.min^{-1}$ or 20% of baseline) is attained at steady-state plasma fentanyl concentrations of 4 $ng.ml^{-1}$ or greater. Potency estimates for respiratory depression and pain responses were 1.0 $ng.ml^{-1}$ (C_{50}) and 0.9 $ng.ml^{-1}$ (C_{25}), respectively ($P < 0.01$). The intra-individual variabilities (ηs) were not correlated indicating that the parameter values ($t_{½}k_{e0}$, C_{50} and γ) of the three end-points were uncorrelated. Peak respiratory depression ranged among subjects from 7 to 12 min following the start of the 90-s infusion.

The results of the Visual Predictive Checks (VPC) for pharmacokinetic and pharmacodynamic data are plotted in Figure 6, showing the median predicted values (continuous lines) and 95% intervals (dashed lines).

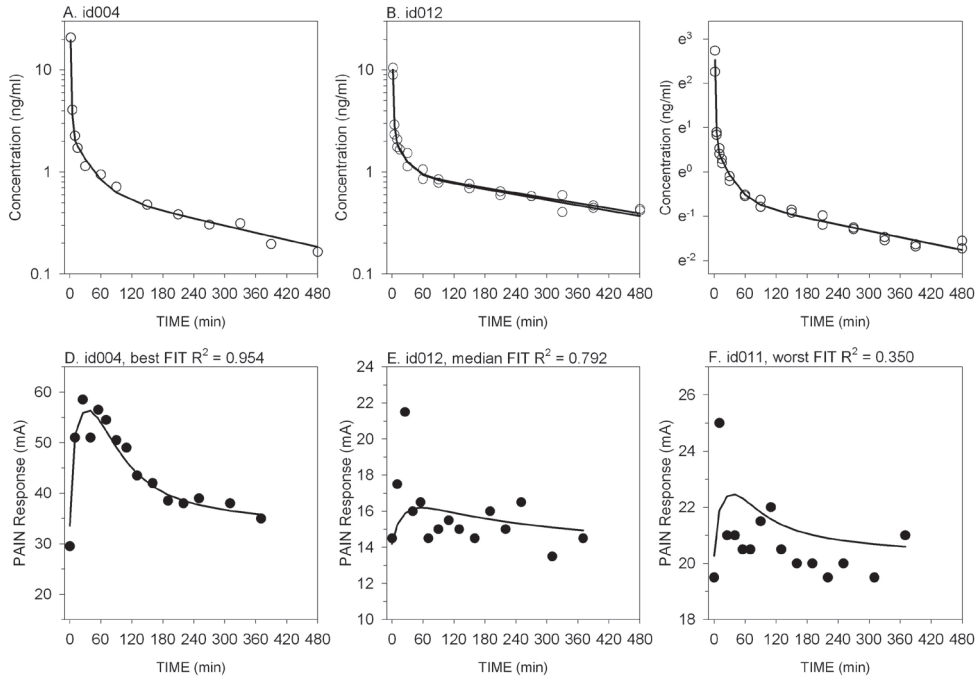

Figure 5. Effect of fentanyl on pain tolerance. Best, median and worst fits (as determined by the coefficient of determination, R^2) are given, together with the corresponding pharmacokinetic data fits. **A** and **D**. subject id 004, best pharmacodynamic fit; **B** and **E**. subject id 012, median pharmacodynamic fit; **C** and **F.** subject id 011, worst pharmacodynamic fit. Since fentanyl was given on two occasions both pharmacokinetic fits are given if available (panels A-C; subject id004 has PK data from just one occasion).

Table 2. Fentanyl pharmacodynamic model parameters

	Typical value	SEE (%)	ω^2	SEE	η-shrinkage[&]
Ventilation					
$t_{½}k_{e0}$ (min)	17.10	3.44 (20)	0.49	0.12 (24)	–2%
Emax ($L.min^{-1}$)	19.90	0.92 (5)	0.02	0.01 (50)	1%
Emin ($L.min^{-1}$)	0 (fixed)	*	0 (fixed)	*	-
C_{50} ($ng.ml^{-1}$)	1.02	0.18 (18)	0.31	0.07 (23)	–2%
γ	1 (fixed)	*	0.41	0.26 (63)	*
Σ^2	3.71	0.59 (16)			1%#
Pain Relief Response					
$t_{½}k_{e0}$ (min)	41.6	9.27 (22)	0.15	0.07 (47)	20%
Baseline tolerance (mA)	26.0	2.87 (7)	0.14	0.04 (29)	–5%
C_{25} ($ng.ml^{-1}$)	0.91	0.29 (32)	0.85	0.35 (41)	16%
γ	1.59	0.05 (3)	0 (fixed)	*	-
σ^2	10.9	3.84 (35)			8%#

*: not estimable.

ω^2 is the inter-subject variability (in the log-domain), σ^2 is the variance of the residual error.

$t_{½}k_{e0}$ is the blood-effect-site equilibration constant; C_{50} and C_{25} are potency parameters or steady-state concentrations at which 50% or 25% of the effect op occurred; Emax is baseline ventilation level; Emin is the minimum ventilation level; γ is a shape parameter.

– Not included in the statistical model.

SEE is standard error of the estimate in the column to the left (in brackets the SEE as %).

&: η shrinkage of empirical Bayesian estimates to the population values.

#: ε shrinkage of model output to the observations

4.3.3 UTILITY FUNCTION

The calculated UFs are shown in Fig. 7A and B. In the concentration domain (U_1, eqn. 3), the utility is positive for low fentanyl effect-site or steady-state concentrations (< 0.7 $ng.ml^{-1}$, fig. 7B). At high concentrations the function is negative indicating that the chance for respiratory depression of 50% or greater exceeds the chance for analgesia (increase in pain tolerance by 50% or more). At a concentration of 1.74 $ng.ml^{-1}$ the UF was most negative (U_1 = –0.23) with a chance for analgesia and respiratory depression of 60% and 83%, respectively.

In the time domain (U_2, eqn. 4, Fig. 7A), the UF after a fentanyl dose of 3.5 $\mu g.kg^{-1}$ is initially negative due to the high fentanyl concentrations from the bolus infusion (at t = 6.6 min the value of U_2 = –0.44 with changes for analgesia and respiratory depression of 21% and 65%). With decreasing concentrations the function becomes positive at t > 100 min. This corresponds with plasma fentanyl concentrations < 0.6 $ng.ml^{-1}$. At t = 240 min the value of U = 0.06 with changes for analgesia and respiratory depression of 10% and 4% respectively.

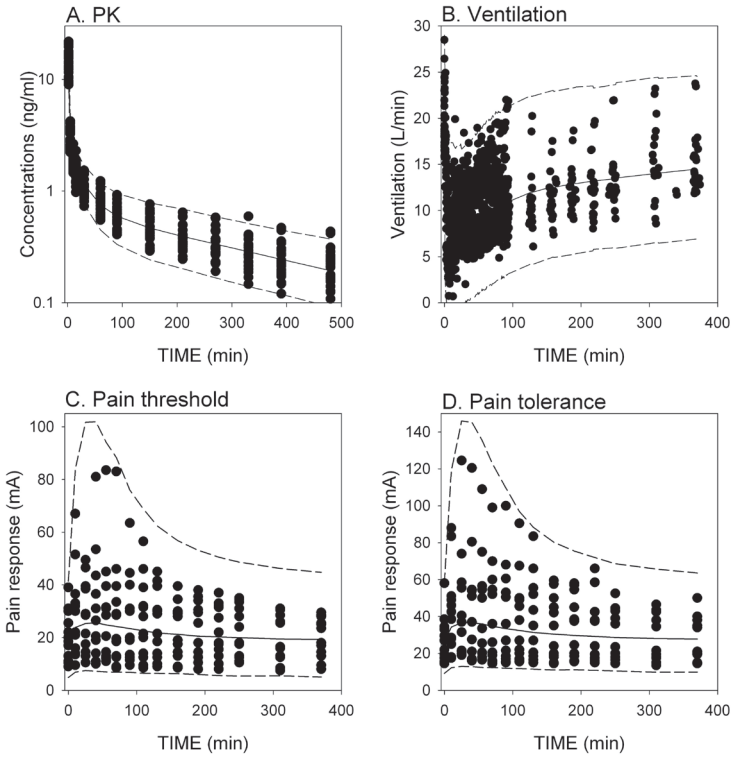

Figure 6. Visual Predictive Checks of the fentanyl pharmacokinetic and pharmacodynamic model outcomes. Simulations performed with an input function similar to the current study.

A. Fentanyl concentration. **B.** Ventilation. **C.** Pupil diameter. **D.** Pain tolerance. **E.** Pain threshold. Continuous lines: the median data fit; broken lines ± 95% confidence intervals.

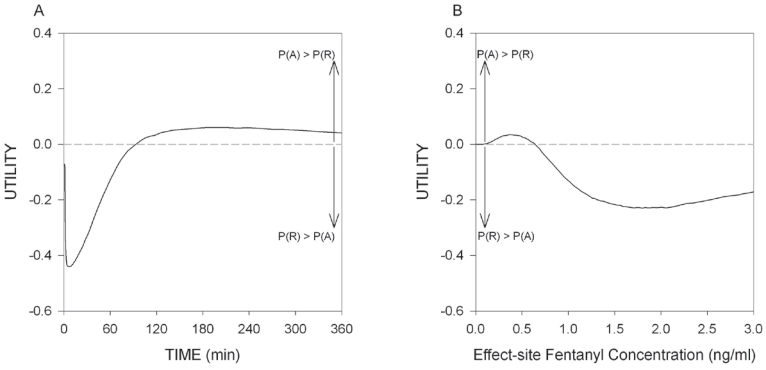

Figure 7. Utility functions (P(A ≥ 50%) – P(R ≥ 50%)) for respiratory responses and pain relief in the time (**A**, following a 3.5 $\mu g.kg^{-1}$ injection) and concentration domains (**B**), where P(A ≥ 50%) is the probability for an increase in pain tolerance of 50% or greater and P(R ≥ 50%) is the probability for respiratory depression of 50% or greater. P(A) > P(R) indicates that the probability for positive effects (analgesia) exceeds the probability for negative effects (respiratory depression); P(R) > (P(A) indicates that the probability for negative effects (respiratory depression) exceeds the probability for positive effects (analgesia).

4.3.4 SENSITIVITY ANALYSIS

Effect of variations in P(A) and P(R) response thresholds. The results of the sensitivity analyses are given in Fig. 8A and B. Fig. 8A shows the results of the analyses in the concentration domain, while Fig. 8B gives the analyses in the time domain. Each set of lines with identical lines represents a set of fixed probabilities for respiratory depression, while within these sets the probability for analgesia increases from top to bottom. For analgesia, the probability for greater analgesic effects (for example P(A > 75%) or an increase in pain tolerance of at least 75%) occurs at the lower end of the set as the greater opioid concentrations that are required to reach such analgesic levels coincide with a greater probability for respiratory depression. For respiratory depression, going from P(R > 75%) to P(R > 25%) the probability for respiratory depression increases irrespective of the P(A) thresholds. For example, the probability of respiratory depression of at least 25%, P(R > 25%), exceeds that observed for the other effect threshold values, and consequently the function P(A) – P(R) is more negative than the other functions.

Effect of variations in (pain model) parameter estimates $t_{½}k_{e0}$ and C_{50}. The results are given in figure 9 and show that an increase in analgesic potency and, although to a lesser extent, a smaller value for $t_{½}k_{e0}$ causes an upward shift of the utility function. The reverse is true in case of a lesser analgesic potency and a slower analgesic onset/offset.

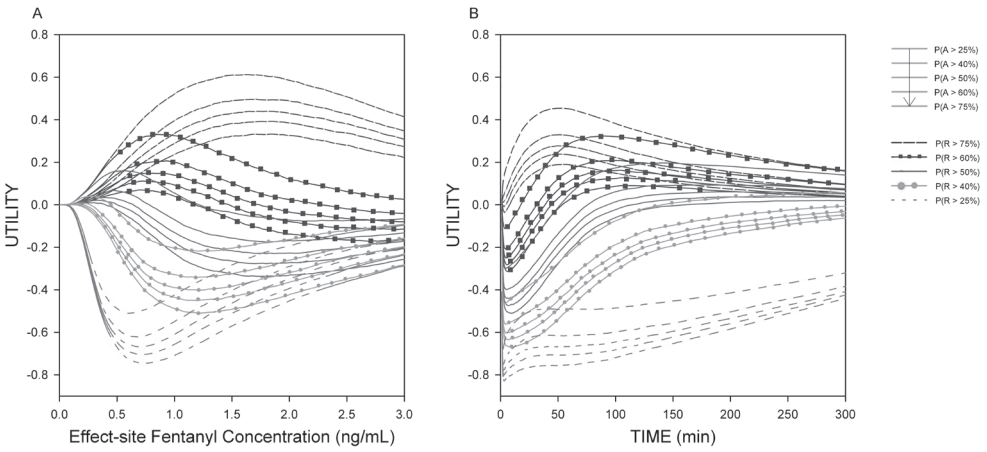

Figure 8. Sensitivity analysis of the effect of different numerical response threholds in P(A) and P(R) on the shape of the utility functions. Each set of lines with identical lines represents a set of fixed probabilities for respiratory depression, while within these sets the probability for analgesia increases from top to bottom (See legend). **A.** UFs in the concentration domain. **B.** Utility functions in the time domain.

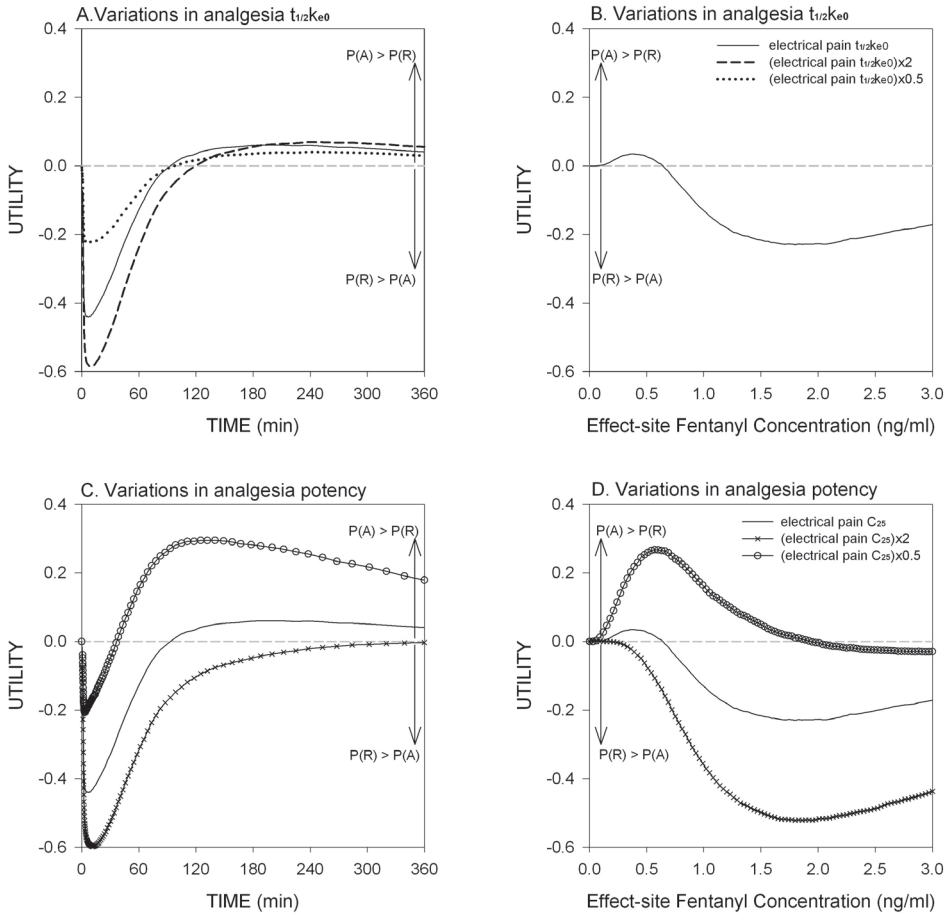

Figure 9. Influence of variations in blood-effect-site equilibration half-life, $t_{½}k_{e0}$ (**A** and **B**), and potency parameter, C50 (**C** and **D**), on the shape of the utility functions (UFs). Continuous lines are the data estimated from the current study (UF in time domain are constructed for a 3.5 μg/kg fentanyl injection). **A** and **B**. Dashed line is the UF with analgesia's $t_{½}k_{e0} \times 2$, the dotted line is the UF with analgesia's $t_{½}k_{e0} \times 0.5$ (Note that in the concentration domain changes in $t_{½}k_{e0}$ do not affect the shape of the UF). **C** and **D**. x-x- are the UFs with a lower potency for analgesia ($C_{25} \times 2$), o-o- are the UFs with a higher potency for analgesia ($C_{25} \times 0.5$).

4.4 DISCUSSION

The risk of opioid toxicity is best considered in the context of the opioid's benefit (*i.e.* analgesia). Integrating opioid risk and benefit is important in acute and chronic pain treatment as it allows the comparison of net efficacy among opioids. For example, Katz showed in 946 patients with chronic lower back pain that the risk-benefit composite (defined as the proportion of days where the patient is an opioid responder (ie. experiencing ≥30% pain relief) with no or just moderate adverse events) is significantly greater for the opioid tapentadol than for oxycodone (30 vs. 25%).* This may help in the choice of opioid treatment for that specific patient population. In the current study, we assessed the risk-benefit composite of fentanyl in an acute administration paradigm by construction of a Utility Function as previously described by Cullberg,[6] *i.e.* the difference between the probability of a wanted effect (antinociception, a positive effect) minus the probability of a side effect (respiratory depression, a negative effect).

The UF is context sensitive. The context is the chosen probability for effect and side effect. We chose $P(A \geq 0.5) - P(R \geq 0.5)$ which is the probability for an increase in pain tolerance of at least 50% minus the probability for 50% respiratory depression or more. We choose 50% as it compares to other 50% response values used in anesthesia such as minimum alveolar concentration (which gives the anesthetic concentration causing a response in 50% of patient) and C_{50} (which is the drug concentration causing 50% effect). Evidently other probabilities will result in UFs that deviate in shape from the current functions. A sensitivity analysis using different numerical response threholds in P(A) and P(R) does indeed show that the UF is context sensitive (Fig. 8). The response threshold of $P(A \geq 0.5) - P(R \geq 0.5)$ is well positioned in the middle of the observed functions and reflects the "mid"-response comparable to the minimum alveolar concentration or C_{50}. How these results may be interpreted in a clinical setting and which of the response thresholds is most appropriate requires further studies.

The UFs were constructed from 2 × 9,999 simulations using the PK-PD parameter estimates. The sometimes-large variance of some parameters is therefore taken into account in the UF. We determined the utility as function of effect-site or steady-state concentration (U_1, eqn. 3) and time (U_2, eqn. 4) following a 3.5 $\mu g.kg^{-1}$ fentanyl injection. Following the injection, U2 values are negative for the first 90 min with large negative values (< –0.4) from t = 2 to t = 17 min (Fig. 6A). At t > 90 min, when plasma fentanyl concentrations were < 0.6 $ng.mL^{-1}$, U_2 becomes positive, but the positive values are small. The U_1 (concentration) function shows a variation of 0.25, a rather modest variation ranging from +0.05 to –0.20, but still predominantly negative (Fig. 6B). Overall the UFs show what also clinically is apparent: (1) following a fentanyl bolus dose the probability of respiratory depression (relative to analgesia) is highest in the first hour following the injection, with a diminishing (but certainly not vanishing) probability of respiratory depression at later times; and (2) only at low doses of fentanyl (up to 50 μg) does the probability for analgesia exceed that of respiratory depression.

The UFs we constructed are based on specific pharmacodynamic end-points commonly used in our laboratory when studying opioid effect and therefore allow comparison among opioids. Ventilation was measured with the dynamic end-tidal forcing technique in which the end-tidal PCO_2 is clamped at a fixed elevated level such that pre-drug baseline ventilation increased to 20 - 24 $L.min^{-1}$.[7-10,15-17] This approach has various advantages including the assessment of respiratory effect at constant carbon dioxide stimulation. Under 'non-clamped' conditions (i) the arterial carbon dioxide level changes over time following the administration of an opioid resulting in confounding variations in the stimulation of peripheral and central chemoreceptors, and (ii) the speed of opioid injection influences the time profile of respiratory depression.[18] The estimated model parameters of the respiratory effects of fentanyl (C_{50} = 1 $ng.ml^{-1}$, $t_{½}k_{e0}$ = 17 min) are in close agreement with earlier observations in healthy volunteers.[9,19] In the current study, C_{50} is the fentanyl concentration at the effect site (or the steady-state plasma concentration) causing 50% depression of ventilation under conditions of a clamped and elevated end-tidal carbon dioxide concentration. Under non-clamped conditions the estimated C_{50} value will result in an increase in arterial PCO_2 by 30 to 40% combined with a reduction in ventilation by 30-40%.

The nociceptive model used by us is transcutaneous electrical stimulation. This model is used frequently in pain research as it allows repetitive testing without confounders such as sensitization or adaptation.[10-12,19] The opioid response to electrical stimulation is slower than expected from other end-points including respiratory depression and changes in electroencephalographic parameters.[11,19-22] The slower response has been observed for other opioids as well and is further discussed by Olofsen *et al.*,[11] It is most probably related to slow neuronal dynamics and activation of short-term potentiation at central sites involved in the processing of electrical pain.[11] Irrespective, the fentanyl potency value (C_{25} = 0.9 $ng.mL^{-1}$; extrapolated C_{50} = 1.4 $ng.mL^{-1}$) is well within the expected clinical concentration range and corresponds with the concentration causing a 40% reduction in isoflurane minimum alveolar concentration and potent analgesia in clinical settings.[23] To get an indication of the importance of variations in $t_{½}k_{e0}$ and potency on the shape of the UFs we performed an additional set of simulations (Fig. 9) with variations in the analgesia model parameters. Variation in C_{25} was the more dominant factor with larger changes in the shape of the UF compared to changes in $t_{½}k_{e0}$. At a lower analgesic potency the UF becomes more negative, both in time and concentration domains. A smaller value for $t_{½}k_{e0}$ (ie. a more rapid onset/offset of effect) results in an upward shift of the UF (Fig. 9A). Variations in $t_{½}k_{e0}$ have no effect on the steady-state UF (Fig. 9B).

The UF in drug research is still experimental and our study is exploratory. It may already be argued that the UF may be useful for comparing the safety profile of specific drugs and for choosing a specific opioid dose with an optimal UF. Traditionally the behavior of opioids is presented in terms of concentration-effect relationships (Fig. 10). However, in contrast to the UF, such static relationships do not take into account the dynamics of

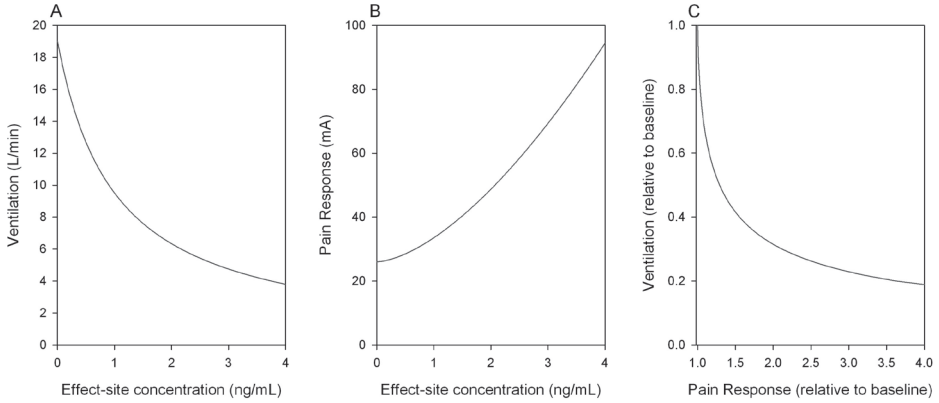

Figure 10. Static fentanyl concentration-effect relationships (**A** and **B**) and static pharmacodynamic-pharmacodynamic analysis (**C**). **A.** Fentanyl effect-site concentration versus respiratory depression. **B.** Fentanyl effect-site concentration versus pain response (pain toleranace). **C.** Ventilation versus pain response (values are aligned at identical effect-site concentration).

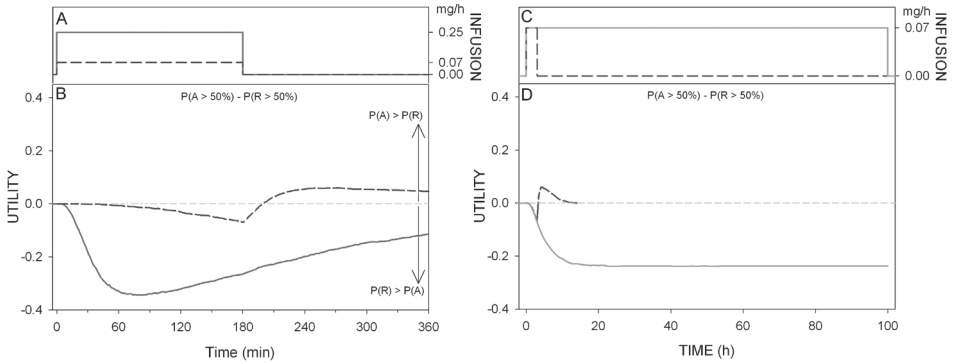

Figure 11. Effect of a continuous fentanyl infusion (**A** and **C**) on the utility function ($P(A \geq 50\%) - P(R \geq 50\%)$) for respiratory responses and pain relief over time (**B** and **D**). **A** and **B.** Infusion duration is 3 h. Continuous line: fentanyl dose = 0.25 $mg.h^{-1}$ in a 70 kg patient; dashed line: fentanyl dose = 0.07 $mg.h^{-1}$ in a 70 kg patient. **C** and **D.** Infusion duration is 100 h (continuous line) and 3 h (dashed line).

the response (as represented in our model by parameter $t_{½}k_{e0}$) and most importantly do not address the large inter-individual variability in the measured responses. While the static relationships are important in understanding the steady-state behavior of the opioid (for example, such analyses enable the direct comparison of multiple effects, Fig. 10C), the UF gives an insightful and dynamic presentation of possible drug effects in time and concentration domains in terms of probability of occurrence of multiple effects. For example, in Fig.11, UFs are created for two 3-h continuous infusions of fentanyl (one at relative high dose: 0.25 $mg.h^{-1}$ for a 70 kg patient; and one at a dose commonly used

for treatment of chronic pain in transdermal patch formulations: 0.07 $mg.h^{-1}$) and one 100 h infusion (dose 0.07 $mg.h^{-1}$). As expected the UF is initially more negative for the high-dose infusion (minimum value –0.34; Fig. 11 A and B). Subsequently this UF slowly increases due the increase in the probability for analgesia. At the low-dose infusion (Fig. 11C and D), the UF value slowly decreases in value (*i.e*. it becomes more negative over time) as the probability for respiratory depression increases more than the probability for analgesia. The UF reaches a steady state after 17-18 h (steady-state value –0.23, Fig. 11D). This suggests that patients on a fentanyl patch have a predominant (moderate) negative UF. Ending the 0.07 $mg.h^{-1}$ infusion after 3 h causes an increase in UF to a positive value (maximum value +0.06, Fig. 11B). Such behaviors could not be predicted from static concentration-effect relationships. Apart from respiratory depression other end-points may be used in the construction of UFs, including sedation, dizziness, nausea/vomiting, and psychomimetic side effects.

In conclusion, we performed an explorative study on the construction of utility functions in which the probability for respiratory depression is subtracted from the probability for analgesia as part of a risk-benefit analysis of opioid effect. The shape of the utility function is dependent on the context, that is the numerical response thresholds for P(A) and P(R) as well as the administration history (*i.e.* the change in effect-site concentration over time). In the current study probabilities for P(A) and P(R) of ≥ 50% were used that reflect an increase in pain tolerance of at least 50% and respiratory depression of at least 50%. These functions are useful in the comparison of the safety profile among drugs relative to their analgesic efficacy, and consequently may become an important tool in drug development. Further studies are required to compare our experimental data with clinical observations and assess whether the UF is valuable in clinical practice as well.

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CHAPTER 5

COMPARISON OF RESPIRATORY AND ANALGESIC EFFECTS OF MR30365/07 AND FENTANYL IN MEN

A double blind placebo controlled randomized phase 1 study

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Submitted

5.1 INTRODUCTION

Opioid analgesics form the cornerstone of contemporary treatment of moderate to severe (acute and chronic) pain. Opioids are associated with a series of side effects including opioid-induced respiratory depression (OIRD), which is potentially life threatening.[1] In recent years the number of lethal opioid-related respiratory complications has increased significantly,[2,3] mainly due to the misuse or abuse of legally prescribed opioids for moderate to severe chronic pain (most importantly lower back pain). Opioids produce respiratory depression via activation of μ-opioid receptors (MORs) expressed on pontine neurons involved in the respiratory control.[4] Full MOR agonists produce a dose dependent respiratory depression with apnea at high doses.[5] Few studies address the (positive or negative) contribution of the other opioid receptors on respiratory depression. We recently showed both in rodents and humans that buprenorphine (a partial agonist at the MOR, antagonist at the κ-opioid receptor (KOR), and with activity at the opioid-receptor-like (ORL1) receptor) produces a ceiling in respiratory depression; ceiling is defined as an apparent maximum effect regardless of drug dose tested.[5,6] This is a major advantage over other opioids and implicates some protective effect at high doses. The molecular mechanism underlying the ceiling in respiratory effect has not yet been elucidated. In mice, Lutfy *et al*. showed that the ceiling in analgesia is related to the activation of the ORL1 receptor.[7] Extrapolation of these findings to the respiratory system would suggest that some of the opioid receptors may counteract, at least in part, the respiratory depression induced by the activation of the MOR.[5,6]

In the current phase 1 study, we assessed the respiratory and analgesic effects of the experimental drug MR30365/07, an opioid with high affinity for the MOR, δ-opioid receptor (DOR), KOR and lower affinity for the ORL1 receptor (confidential data, Mundipharma Research Ltd.). We compared MR30365/07 to fentanyl, a selective and high affinity MOR agonist that produces dose-dependent respiratory depression and apnea at high doses (2-3 $\mu g.kg^{-1}$ and greater).[5,6,8] Experiments were performed in healthy male volunteers. The study consisted of two parts. Part 1 was a single blinded, placebo controlled pilot study on the respiratory effects of a range of MR30365/07 doses, designed to select the MR30365/07 dose most suitable for use in the main phase of the study (Part 2). Part 2 was a double-blind, randomized, placebo-controlled study, which compared the respiratory and analgesic effects of MR30365/07 and fentanyl.

5.2 METHODS

This phase 1 study had two parts. Initially a MR30365/07 dose-ascending, cohort group, single-blinded pilot study (part 1) was performed for dose-finding. After the pilot study was completed, the MR30365/07 doses were selected for the main study (part 2), a randomized, double-blind, placebo- and active comparator (fentanyl)-controlled parallel group study was performed.

5.2.1 SUBJECTS

One hundred and two healthy male volunteers (10 in the pilot study and 92 in the main study) participated in the study after approval of the protocol was obtained from the Leiden University Medical Center (LUMC) Human Ethics Committee and the Central Committee on Research Involving Human Subjects (CCMO, The Hague). Written and oral informed consent was obtained prior to enrolment into the study. All volunteers provided a medical history, and a physical examination, 12-lead ECG and blood screening was conducted before enrollment. The eligible volunteers were between the ages of 18 and 45 years, weighed between 60 and 100 kg, had a body mass index between 18 and 30 $kg.m^{-2}$, and a forced expired lung volume in 1 s of > 85% of predicted. Study subjects were healthy with no history of major medical disease, alcohol abuse, illicit drug use or heavy smoking. Volunteers could not have used medication (including vitamins, herbal and/or mineral supplements) in the seven days preceding dosing, or during the course of the study, or opioids or opioid antagonists in the 90 days prior to dosing. Finally, participants had to fast for 6 hours prior to the administration of study medication.

5.2.2 STUDY DESIGN

Pilot study. The respiratory effects of 3 escalating doses of MR30365/07 and 1 infusion of placebo were tested on 4 separate days with at least 1 week for wash-out between test sessions. Three subjects received 0.025, 0.05 and 0.1 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 1), three others 0.0125, 0.075 and 0.1 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 2) and the last three subjects 0.05, 0.125 and 0.15 $\mu g.kg^{-1}$ MR30365/07 and placebo (cohort 3). From the results of this study the doses of the main study were determined. After infusion of the drug was completed, ventilation was continuously measured breath-to-breath for 1 h under iso-hypercapnic conditions (see below).

Main study. In this double-blind randomized study 92 volunteers participated. None of them had been part of the pilot study and all were dosed only once. 46 subjects participated in the respiratory part of the study, 46 others in the analgesia part. In both parts, placebo (n = 6), 0.0125 $\mu g.kg^{-1}$ MR30365/07 (n = 4), 0.075 $\mu g.kg^{-1}$ MR30365/07 (n = 6), 0.125 $\mu g.kg^{-1}$ MR30365/07 (n = 6), 0.15 $\mu g.kg^{-1}$ MR30365/07 (n = 4), 0.5 $\mu g.kg^{-1}$ fentanyl (n = 4), 1 $\mu g.kg^{-1}$ fentanyl (n = 6), 2 $\mu g.kg^{-1}$ fentanyl (n = 6) and 3 $\mu g.kg^{-1}$ fentanyl (n = 4) were administered by intravenous infusion over 10 min. The randomization list was prepared by the sponsor of the study and sent to the local pharmacy where blinded syringes were prepared based on the weight of the subject. Each syringe was identical in size, drug volume and color and was unmarked. The randomization list was available to the sponsor, the pharmacy and an independent data safety monitoring committee.

Study medications. Citrate buffer, fentanyl and MR30365/07 were obtained from Mundipharma Research Limited (Cambridge, UK). All drugs were infused intravenously

(in the arm or hand) using a syringe pump (Beckton Dickinson, St. Etienne, France).

5.2.3 MEASUREMENTS

Respiratory measurements. Following infusion, ventilation was continuously measured on a breath-to-breath basis for 1 hour under iso-hypercapnic conditions. End-tidal gas forcing and data acquisition were performed using the dynamic end-tidal forcing technique (see Dahan *et al*.[9,10] for an explanation of the technique). In brief: Subjects breathed through a facemask connected to a pneumotachograph and differential pressure transducer (#4813, Hans Rudolph, Myandotta, MI). The pneumotachograph was connected to a custom-made gas mixing system attached to three mass-flow controllers (Bronkhorst, Veenendaal, The Netherlands). A computer delivered signal to the mass flow controllers so that the composition of the inspired gas could be adapted to steer the end-tidal oxygen and carbon dioxide concentrations according to a pre-set pattern over time. The inspired and expired oxygen and carbon dioxide concentrations and the arterial hemoglobin–oxygen saturation were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). End-tidal concentrations of oxygen and carbon dioxide, inspired minute ventilation (Vi), and oxygen saturation were collected for further analysis. Ventilation levels and end-tidal concentrations were observed in real time on a breath-to-breath basis on a computer screen.
In the current study the end-tidal oxygen level was clamped to a value of 110 mmHg, while the end-tidal carbon dioxide concentration was slowly increased to a value that caused ventilation levels of 20 ± 2 $L.min^{-1}$. This end-tidal carbon dioxide value was maintained throughout the study. Respiratory measurements started when the inspired minute ventilation had reached a steady state; 4-5 min later drug infusion started. Respiratory measurements ended 60 min after the end of drug infusion (t = 70 min).

Pain measurements. Pain was induced using a transcutaneous electrical stimulus to the skin over the left tibial bone (10 cm above the ankle).[11] A 20 Hz (pulse duration 0.1 ms) stimulus train was delivered to the subject causing activation of cutaneous nociceptors. The stimulus train starts at 0 mA and was increased at a rate of 0.5 mA per 2 s (with a cutoff value of 128 mA). The delivery of the current is controlled by a computer via a current stimulator, which is connected to a control box with two buttons. The subject was instructed to press the first button when pain was felt (*i.e.* pain threshold) and the second button when the subject wanted the stimulus train to stop (*i.e.* pain tolerance). These respective currents were collected on disc for further analysis. The subject was familiarized with the system prior to the study to obtain reliable baseline values. In this study, the pain threshold values were used in the analysis. Four pain threshold values were obtained in the 30 minutes prior to drug infusion. These values were averaged and served as a baseline estimate. Following drug infusion, pain measurements were obtained

at the following time points (t = 0 is the start of drug infusion): 10 (end of infusion), 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min.

5.2.4 SAMPLE SIZE AND STATISTICAL ANALYSIS

The pilot study was designed to determine which doses of MR30365/07 were to be tested in the main study. Four doses were chosen for the main study: 0.0125, 0.125, 0.075 and 0.15 $\mu g.kg^{-1}$.

For the main study, sample size selection was achieved by performing a power analysis in NONMEM,[12] using estimated data on the effect of opioids on respiratory depression.[8] We assumed inter-subject variability in effect of 50% ($\omega^2 = 0.25$) and a 10% residual error for effect ($\sigma^2 = 0.01$) and aimed to detect a value of $\rho < 0.5$ or > 2.0 (where $C_{50A,\ MR30365/07}/C_{50R,MR30365/07}$) $= \rho \times C_{50A,FENTANYL}/C_{50R,FENTANYL}$ and C_{50A} and C_{50R} are the concentrations causing 50% analgesia and respiratory depression for drugs MR30365/07 and fentanyl, respectively), with $\alpha < 0.05$ and $\beta = 0.8$. In the analysis we assumed that $C_{50A,MR30365/07} = C_{50A,FENTANYL}$ (i.e. concentrations are equianalgesic). Values of $\rho < 0.5$ indicate that fentanyl produces respiratory depression at concentrations at least twice as low as MR30365/07 and vice versa for $\rho > 2$. It was assumed that the logarithm of the C_{50} ratio has a normal distribution with variance = 1. The sample size selection was next verified by simulations in NONMEM with 1,000 simulated data sets. The analysis resulted in a sample size of 34, which was rounded upwards to 40 (20 subjects per opioid treatment). Six additional subjects were added to receive placebo. The subject number chosen for the analgesia part of the study was identical to that calculated for the respiratory part of the study, as we, somewhat arbitrarily, assumed similar drug effects and variability.

Average respiratory drug effect. The breath-to-breath data were averaged over 1-min episodes. In order to get an impression of the average drug effect on respiration, we calculated the area below the respiration curve (AUC) from t = 0 to t = 70.[5,13] The AUC was subtracted from the area obtained by taking baseline ventilation forward (BASELINE AREA, from t = 0 to t = 70 min, see Fig. 1). Next the data were normalized by the baseline area giving an average % of respiratory depression (average drug effect = [baseline area AUC – AUC]/baseline area AUC). An average drug effect of 40 indicates an average of 40% respiratory depression over the measured time period (0-70 min). The average drug effect and time to peak effect were analyzed using a one-way analysis of variance (factor dose). The MR30365/07 and fentanyl data were analyzed separately in Sigmaplot v12.3 (Systat Software GmbH, Ekrath, Germany). *P*-values < 0.05 were considered significant. Values given are mean ± SD.

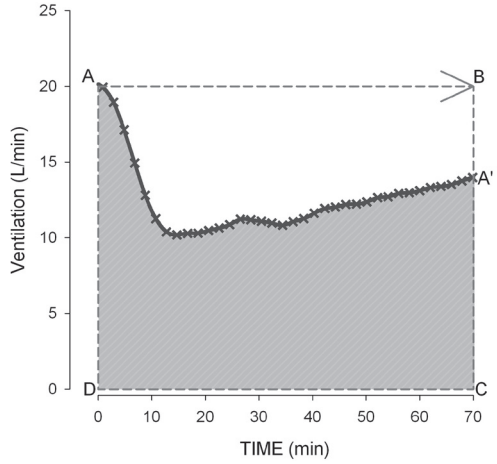

Figure 1. Calculation of the average respiratory drug effect. First the area-under-the curve (AUC) is calculated for the respiration curve (line from A to A'). This AUC (grey field) was subtracted from the area obtained by taking baseline ventilation (A) forward (the arrow from point A to B; the baseline area AUC is the box depicted by the red broken lines ABCD). Next the data were normalized by the baseline area giving an average % of respiratory depression (average drug effect = [baseline area AUC – AUC]/baseline area AUC).

Peak respiratory depression. For each subject peak respiratory depression was calculated as the nadir in ventilation and presented as ratio relative to baseline (*e.g.* a value of 0.5 indicates a nadir in ventilation in magnitude 50% of baseline ventilation). Using the statistical package *R* (version 8.2; www.r-project.org), a sigmoid EMAX function was fitted through the MR30365/07 and fentanyl dose-effect data (effect = peak respiratory depression) using a model of the form:

$$\text{Peak effect(dose)} = 100 + [E_{MIN} - 100] \times [\text{dose}^{\gamma} + ED50^{\gamma}] \qquad \text{eqn. (1)}$$

where ED_{50} is the dose causing a 50% effect (ventilation in the middle of baseline ventilation and E_{MIN}), E_{MIN} the asymptotic minimum in ventilation, and γ a shape parameter. *P*-values < 0.01 were considered significant. The data analysis was performed on the complete data set (fentanyl data and MR30365/07 data from pilot and main studies). The data are presented as mean ± SD.

Analgesic effect. Two measures of analgesic effect were calculated in each experiment: peak analgesia (defined as the highest value of pain threshold in mA) and average analgesic effect (as defined as the area under the pain threshold curve from t = 0 to t = 8 h normalized by the baseline area, see above). Peak and average analgesic effects were analyzed using a one-way analysis of variance (factor 'dose'). The MR30365/07 and fentanyl data were analyzed separately using SigmaPlot v. 12.3. *P*-values < 0.05 were considered significant. Values given are mean ± SD.

5.3 RESULTS

Pilot study. Nine volunteers completed the study without unexpected side effects. One subject developed ECG changes that, although not clinically relevant, precluded proper assessment of the effect of the study medication on the ECG. As a precautionary measure,

another subject replaced this subject after having completed a placebo and 0.05 μg.kg^{-1} MR30365/07 experiment. The clamped end-tidal PCO_2 was 6.6 ± 0.5 kPa (49.5 ± 4.5 mmHg) and baseline (pre-drug) ventilation was 21.5 ± 1.7 L.min^{-1}. The mean respiratory responses to MR30365/07 are given in Fig. 2A. At all dosages, the drug displayed a nadir in ventilation, which occurred at t = 17.1 ± 3.8 min following the start of drug infusion. The respiratory responses to MR30365/07 dosages of 0.075, 0.10, 0.125 and 0.15 μg.kg^{-1} overlap. The dose-response curves (peak respiratory depression and average drug effect) are given in Figs. 2B and C showing that the dose-response levels off at dosages of 0.075 μg.kg^{-1} and greater (MR30365/07 at 0.075, 0.125 and 0.15 μg.kg^{-1}: $P > 0.05$).

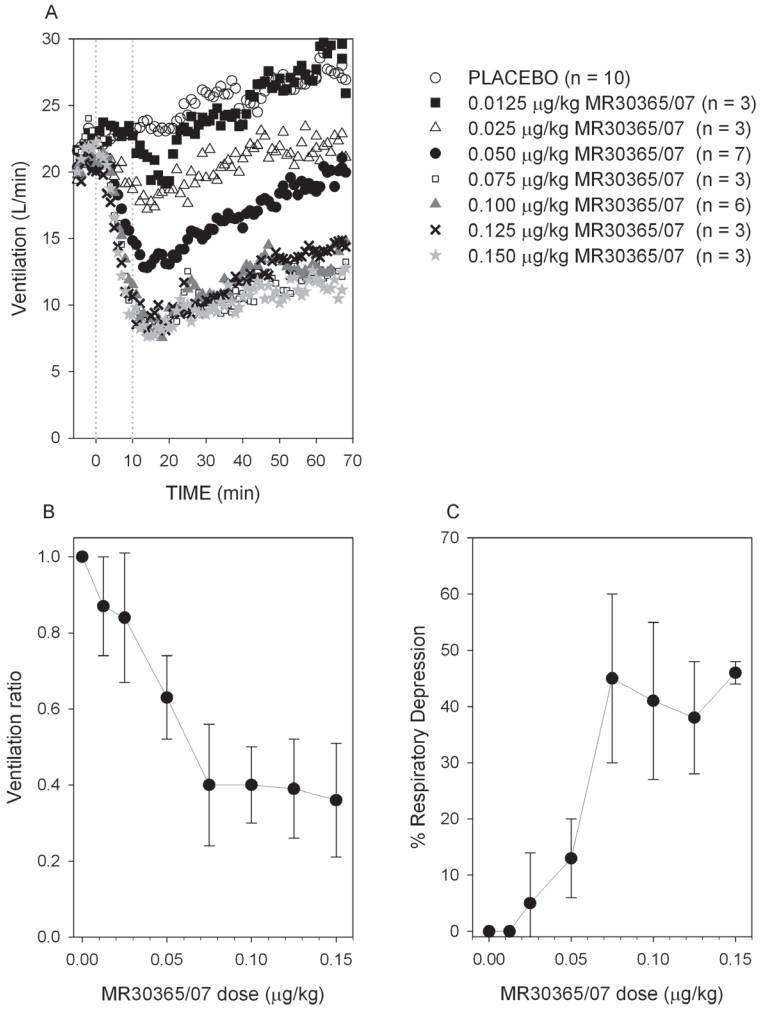

Figure 2. Results of the pilot study. **A**. Mean respiratory responses to placebo and MR30365/07. **B.** Dose-response data: Peak respiratory depression. **C**. Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

A small positive trend was observed in the ventilation data as was best observed in the placebo responses (Figs. 2-4). The magnitude of the trend ranged from 30-60 ml.min^{-2} (about 1.5-3% of total ventilation) and corresponds with the presence of a slow component (time constant about 1 h) in the ventilatory response to CO_2.[9,14]

Main study: Respiration. All 46 subjects completed the study without unexpected side effects. In the MR30365/07 experiments, the end-tidal PCO_2 was clamped at 6.8 ± 0.2 kPa (51.0 ± 1.5 mmHg) and baseline (pre-drug) ventilation was 19.3 ± 1.4 L.min^{-1}. The

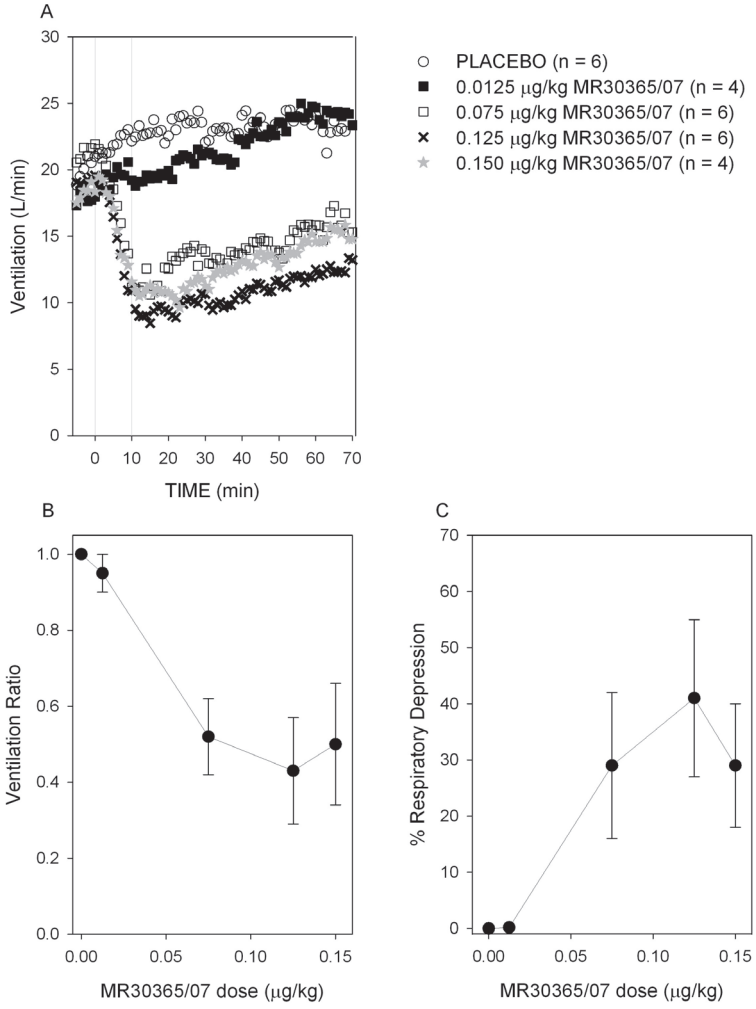

Figure 3. Results of the main study: Effect of MR30365/07 on respiration. **A.** Mean respiratory responses to placebo and MR30365/07. **B.** Dose-response data: Peak respiratory depression. **C.** Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

mean respiratory responses to MR30365/07 are given in Fig. 3A. No nadir in ventilation was observed in the placebo data and the lowest MR30365/07 dose tested. The time to peak effect was dose-independent and occurred at 17.3 ± 5.5 min. The dose-response curves for peak respiratory depression and average drug effect are given in Figs. 3B and C, respectively, showing that the dose-response levels off at a ventilation level of approximately 50% of baseline. None of the subjects that received MR30365/07 developed irregular breathing or apnea.

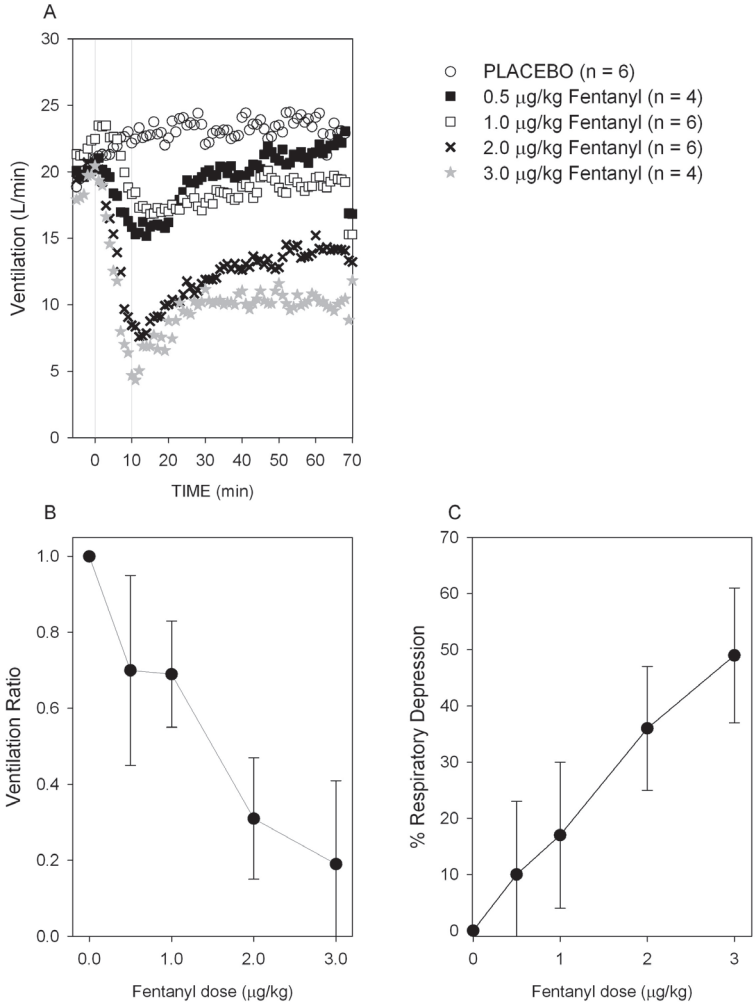

Figure 4. Results of the main study: Effect of fentanyl on respiration. **A**. Mean respiratory responses to placebo and the four fentanyl dosages. **B.** Dose-response data: Peak respiratory depression. **C**. Dose-response data: Average drug effect. The data in panels B and C are mean ± SD.

In the fentanyl experiments, the end-tidal PCO_2 was clamped at 6.6 ± 0.1 kPa (49.5 ± 0.8 mmHg) and baseline (pre-drug) ventilation was 20.2 ± 0.9 $L.min^{-1}$. A nadir in respiratory response was observed for all doses tested (Fig. 4A). The time to peak effect was dose-independent and occurred on average at 12.8 ± 2.1 min. The dose-response curves for peak respiratory depression and average drug effect are given in Figs. 4B and C, respectively. Dose-dependent respiratory depression was apparent in peak ventilation ($P < 0.001$) and average drug effect ($P < 0.001$). The maximum observed respiratory depression was observed at the highest fentanyl dose tested (3 $\mu g.kg^{-1}$; peak effect = 19% of baseline). Two subjects developed irregular breathing after the highest dose of fentanyl, one of which developed apnea (defined by the absence of breathing activity > 20 s), just after ending the 10-min fentanyl infusion.

The parameter estimates of the model analysis of peak respiratory depression are given in Table 1. The model fits are given in Figs. 5A (MR30365/07) and B (fentanyl). Two parameters were significantly different between treatments ($P < 0.01$): ED_{50} and E_{MIN}. An apparent 30-fold difference in potency was observed with ED_{50} values of 0.04 $\mu g.kg^{-1}$ for MR30365/07 and 1.27 $\mu g.kg^{-1}$ for fentanyl. For fentanyl the value of E_{MIN} or the asymptotic minimum ventilation was not different from zero, but greater than zero for MR30365/07: 32.8% of baseline ventilation or 6.6 $L.min^{-1}$ ($P < 0.01$). The shape parameter γ and residual error variance (σ^2) did not differ between treatments.

Main study: Analgesia. All 46 subjects completed the study without unexpected side effects. Baseline pain thresholds were 11.8 ± 0.9 mA (MR30365/07), 12.7 ± 0.4 mA (fentanyl) and 11.0 ± 0.6 mA (placebo). The effect of placebo was limited with an effect no greater than 10% of baseline. Both MR30365/07 and fentanyl produced dose-dependent effects in terms of peak analgesic effect and average drug effect (Figs. 6 A and B; drug-effect: $P < 0.01$) with no indication of reaching a ceiling.

5.4 DISCUSSION

Development of novel opioid analgesics with limited respiratory depression at high doses is highly relevant as OIRD is a major cause of opioid morbidity and mortality.[1-3] In this experimental phase 1 study we showed that in contrast to fentanyl, MR30365/07 displayed a ceiling in respiratory depression in the tested dose range starting at 0.075 $\mu g.kg^{-1}$. The respiratory effects of MR30365/07 0.075, 0.10, 0.125 and 0.15 $\mu g.kg^{-1}$ overlap with peak respiratory depression at about 40% of baseline ventilation. Fentanyl showed dose-dependent respiratory depression with, at high dose, irregular breathing and apnea. Neither fentanyl, nor MR30365/07, produced a ceiling in analgesia over the dose range tested.

We used the computer-steered 'dynamic end-tidal forcing' or DEF technique to clamp end-tidal PCO_2 to a fixed ventilation level of 20 ± 2 $L.min^{-1}$.[9,10] On average this was achieved by increasing end-tidal PCO_2 to 6.65 kPa (50 mmHg). The advantages of the DEF technique

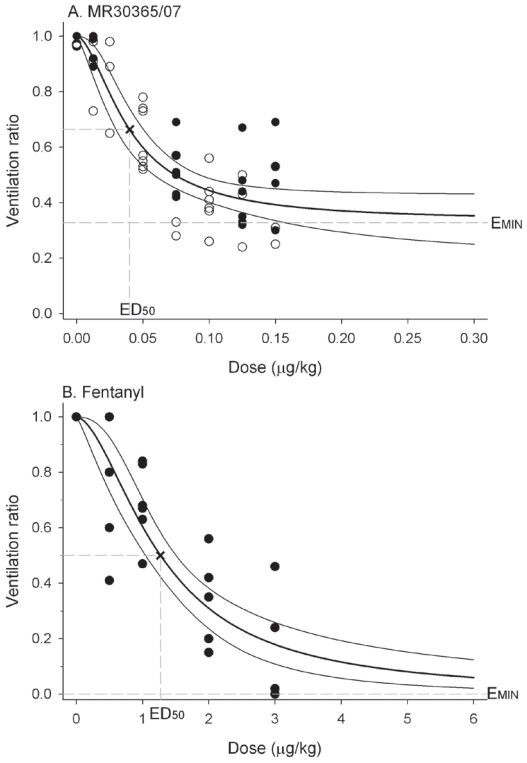

Figure 5. Model fits of peak respiratory depression versus dose for MR30365/07 **(A)** and fentanyl **(B)**. On the y-axis, ventilation relative to pre-drug baseline ventilation. The continuous thick lines are the model fits and the thin lines are the 2.5% and 97.5% percentiles. The curves are extrapolated to 0.3 $\mu g.kg^{-1}$ MR30365/07 and 6 $\mu g.kg^{-1}$ fentanyl. In panel **A,** the closed circles are data from the main study, the open circles are data from the pilot study. In panels **A** and **B,** the respective ED_{50} and E_{MIN} values are depicted by the symbol × and broken grey lines. For both drugs the ED_{50} is the dose half-way between baseline ventilation and E_{MIN}; for fentanyl this is at 50% respiratory depression, for MR30365 at 33.6%.

Table 1. Parameter estimates of the model analysis of peak respiratory depression

Parameter	Mean	SD	2.5% percentile	97.5% percentile
ED_{50} MR30365/07 ($\mu g.kg^{-1}$)	0.04	0.009	0.026	0.06
ED_{50} Fentanyl ($\mu g.kg^{-1}$)	1.27	0.116	1.04	1.50
γ	1.80	0.32	1.23	2.51
E_{MIN} MR30365/07 (% of baseline)*	32.8	0.06	16.7	42.6
E_{MIN} Fentanyl (% of baseline)*	0	-	-	-
σ^2	0.014	0.002	0.010	0.019

ED_{50} is the dose causing a 50% reduction in ventilation, E_{MIN} the asymptotic minimum in ventilation, γ a shape parameter, and σ^2 the variance of the residual error.

* baseline ventilation = 100%.

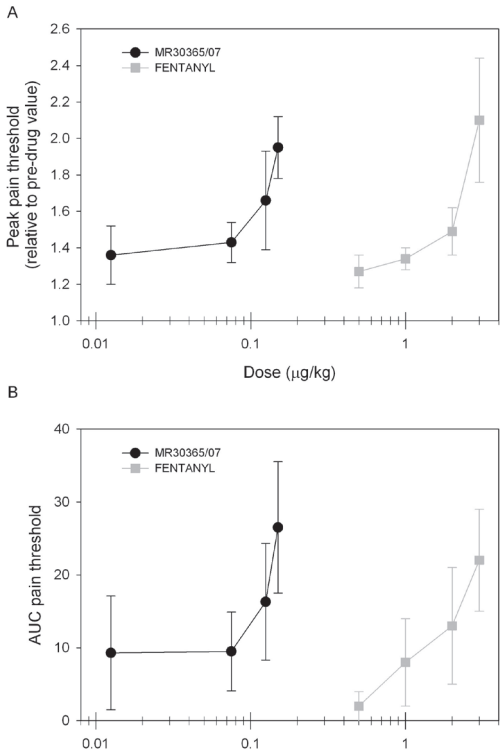

Figure 6. Results of the main study: Effect of MR30365/07 and fentanyl on pain threshold. **A.** Peak analgesia, defined as the highest value of pain threshold in mA). **B.** Average analgesic effect, defined as the area under the pain threshold curve (AUC) from t = 0 to t = 8 h normalized by the baseline area. Note that the logarithmic x-axis scales.

over more conventional techniques are that respiratory response of the test drug is (i) independent of the confounding effects of changes in arterial CO_2 and (ii) independent of the speed of administration of the drug.[5,6,15] These two items are interconnected. For example, we showed previously that administration of remifentanil aimed at a target plasma concentration of 5 ng.ml^{-1} will cause severe respiratory depression when the drug target is reached after 5 min.[14] This is due to the rapid transport of the drug to the brain causing immediate depression of brainstem respiratory neurons (no CO_2 having accumulated at this time). Respiratory depression is less likely when the remifentanil target is reached after 30 min. Part of the respiratory depression is offset by the accumulation of CO_2 in the brain compartment. Performing studies under poikilocapnic conditions would therefore lead to an underestimation of respiratory potency of a drug as is exemplified by the study of Mildh *et al*. for fentanyl.[16] They estimated the fentanyl plasma concentration that caused a 50% depression in respiration at 6.1 ng.ml^{-1}. Considering the changes in arterial CO_2 (for example, by modeling the stimulatory effects of CO_2 on the respiratory control system)

or by using the DEF technique, a value of 1.1 $ng.ml^{-1}$ is estimated;[8] 6-fold lower than estimated from poikilocapnic studies and in agreement with clinical observations. The DEF technique allows reliable comparison of drug effect on the respiratory control system. In our studies, the differences observed for MR30365/07 and fentanyl in dose-response relationship (ceiling in respiratory depression for MR30365/07 but not fentanyl), the more rapid onset of effect for fentanyl (peak effect occurred at 2.8 min after the end of the 10 min infusion versus 7.3 min for MR30365/07) and the longer-lasting respiratory depression are due to CO_2-independent differences in pharmacokinetics and pharmacodynamics.

From a clinical perspective, the existence of ceiling in respiratory depression is advantageous only when no ceiling in analgesic efficacy exists or when ceiling occurs at much higher (supra clinical) drug concentrations. Indeed, in our study we observed that over the dose range tested, both fentanyl and MR30365/07 displayed a dose-dependent increase in peak pain response and average analgesic effect (Figs. 6A and B). These data give reasonable evidence that for MR30365/07, in contrast to respiration, pain relief does not display ceiling over the dose range tested. At the highest dose tested both drugs produced an increase in pain threshold of about 100% (MR30365/07 0.15 $\mu g.kg^{-1}$ response = 1.95 × pre-drug response; fentanyl 3.0 $\mu g.kg^{-1}$ response = 2.1 × pre-drug response). This indicates a MR30365/07-fentanyl difference in potency of 18.5. This difference is smaller than the apparent potency difference observed for respiratory depression (factor = 30). However, since the ED_{50} is an estimation of ventilation in the middle of baseline ventilation and E_{MIN}, a better comparison than ED_{50} would be the dose causing 50% depression of ventilation (in absolute values). For fentanyl this is identical to ED_{50} (1.27 $\mu g.kg^{-1}$), and for MR30365/07 this is 0.075 $\mu g.kg^{-1}$. This then suggests a potency difference of 17 very similar to the value observed for antinociception. Taking all data together, our data suggests that MR30365/07 has a better therapeutic window than fentanyl.

Three issues remain unresolved by our studies. The first is the cause of the ceiling in MR30365/07 respiratory effect. To the best of our knowledge, ceiling in respiratory depression has been demonstrated in humans for only one other opioid, buprenorphine.[5,6] The partial agonism (*i.e.* partial effect despite full receptor occupancy) of buprenorphine at the MOR is considered responsible for its ceiling effect. This mechanism is not relevant for our studies as MR30365/07 is a full agonist at the MOR. Lutfy *et al.*,[7] however, propose a different mechanism for development of ceiling. They showed lack of buprenorphine-induced antinociception in MOR knockout mice but enhancement of antinociception in ORL1 receptor knockout mice with loss of ceiling in analgesia. In that study, it was concluded that the concurrent activation of ORL1-receptors severely compromised the MOR-mediated antinociceptive effect of buprenorphine in mice.[7] In contrast to these data, a recent study in primates shows that ORL1 receptor agonists enhance buprenorphine-induced antinociception, however, without causing additional respiratory depression.[17] These data suggest that ORL1 activation may be implicated in the mechanism of ceiling in respiratory effect as observed in our studies. However, although MR30365/07 has affinity

for the ORL1 receptor, its Ki is several orders of magnitude higher than for the MOR (confidential data, Mundipharma Research Limited). Whether such low affinity for the ORL1 receptor is sufficient to cause the profound ceiling we observed is questionable. Another possible mechanism may be related to MR30365/07's high affinity for the KOR, which is approximately 1 order of magnitude lower than for the MOR (confidential data, Mundipharma Research Limited). There is evidence that KOR agonists may selectively antagonize MOR agonist effects, including respiratory depression.[18,19] For example, the KOR agonist U50,488H antagonized MOR agonist-induced respiratory depression in the rat, a KOR mediated effect.[18] It may well be that at high doses the MR30365/07-induced and MOR-mediated respiratory depression is antagonized by the effect of MR30365/07 at the KOR. KOR agonists have been associated with dysphoria.[20] Interestingly, in our study, over a 24-h observation period no significant differences in dysphoria were observed between MR30365/07 and fentanyl.

A final proposed mechanism involves the intra neuronal regulatory protein β-arrestin.[6] Opioid receptors belong to the 7-transmembrane G-protein-coupled receptors that, upon activation, bind to intracellular G-proteins and β-arrestin 1 and/or β-arrestin 2 proteins. Recent studies show that absence of β-arrestin 2 protein causes the attenuation of morphine-induced respiratory depression with maintained antinociception.[21,22] It was hypothesized that (G-protein independent) activation of β-arrestin 2 is involved in MOR signal transduction of respiratory neurons but not in neurons involved in modulation of pain pathways.[21] It may well be that extent of G protein and of β-arrestin 2 activation is ligand specific. Such ligand-specific, *i.e.* biased differences in G protein and β-arrestin activation, has been first described for the angiotensin1A receptor.[23] Extrapolating these findings to our study would suggest that our results are then well explained by a lesser ability of MR30365/07 to activate β-arrestin 2. Further work is required to elucidate the mechanism by which ceiling of respiratory effect is observed at the high MR30365/07 doses as observed in our study.

A second issue is the mechanism of the differential MR30365/07 effect on respiration and analgesia. We previously proposed and discussed that this may be due to a difference in receptor density at brain sites involved in analgesia versus brain sites involved in respiratory depression.[6] This has for example been demonstrated experimentally by performing a progressive MOR knockdown by administration of the MOR antagonist β-funaltrexamine.[24] Reduction of opioid bindings sites transformed the MOR agonist alfentanil into a partial agonist. Another mechanism involved may be the lesser ability of MR30365/07 to engage the transduction protein β-arrestin 2, as discussed above. This latter mechanism explains both the observed ceiling effect in MR30365/07-mediated respiratory depression and the selectivity of the ceiling effect.

A third issue is whether the experimental observation of ceiling in the respiratory effects of MR30365/07 may be extrapolated to the clinical setting, and whether such a phenomenon

indeed leads to less respiratory events in patients. This is a highly relevant topic as there has been a recent increase in the number of fatalities from misuse or abuse of legally prescribed opioids.[1-3,25] An apparent ceiling in respiratory depression at higher doses of MR30365/07 is certainly an advantage over other opioids that lack such a profile. However, whether this behavior persists in patients with their own complexities (co-medication, underlying disease, genetics, overdosing, etc.), needs to be further investigated.

In conclusion, we showed in this phase 1 study that in contrast to fentanyl, MR30365/07 shows ceiling in respiratory depression, but not analgesia over the dose range tested (0.0125-0.15 $\mu g.kg^{-1}$ MR30365/07).

ACKNOWLEDGEMENT

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CHAPTER 6

OPIOID CHRONOPHARMACOLOGY: INFLUENCE OF TIMING OF INFUSION ON FENTANYL'S ANALGESIC EFFICACY

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6.1 INTRODUCTION

Chronopharmacology studies the effect of the timing of drug administration (in terms of the hour in a 24 h period, the day in a 1 month or 1 year period, or the year in a life-time) on the drug's pharmacokinetics and/or pharmacodynamics.[1,2] When applied to the 24 h circadian rhythm, it is now known that numerous drugs exhibit a differential response depending on the time of administration. This also applies to drugs used in anesthesia such as local anesthetics, barbiturates, muscle relaxants and opioids.[1-2] For opioids, circadian effects have been observed for drug disposition (*e.g.*, meperidine and morphine) and therapeutic sensitivity (*e.g.*, tramadol and codeine).[2,4] However, the number of studies on opioid pharmacology is restricted and hence knowledge on the influence of the circadian rhythm on opioid analgesic efficacy remains poor.[2] Evidently further understanding and application of a chronotherapeutic approach to opioid treatment of acute and chronic pain would increase opioid efficacy and possibly improve the efficacy-safety balance.

To scrutinize the hypothesis that opioids display a diurnal antinociceptive effect, we performed a study on the influence of four distinct timing moments on fentanyl-induced analgesia in healthy volunteers. The analgesic effect of intravenous fentanyl, administered at 8 AM, 2 PM, 8 PM or 2 AM, was examined using an experimental heat pain model.

6.2 METHODS

6.2.1 SUBJECTS

Following approval of the protocol by the Leiden University Human Ethics Committee sixteen healthy volunteers (age 18-30, BMI < 28 $kg.m^{-2}$, 12 women, 4 men) were enrolled in the study. The study was registered at trialregister.nl (#NTR1254). Written and oral informed consent were obtained prior to the inclusion in the study. Exclusion criteria were: age < 18 years, body mass index > 30 $kg.m^{-2}$, presence of underlying disease, history of drug allergy, history of psychiatric disease, history of illicit substance abuse. All female subjects were taking oral contraceptives. The subjects were instructed not to eat or drink for at least 6 h before the study.

6.2.2 DESIGN

The subjects were randomly divided into two experimental groups. The first group received fentanyl at 2 PM and 2 AM; the second group at 8 AM and 8 PM. The experimental days were separated by a two week washout period. We studied two distinct groups in order to reduce the number of occasions at which the healthy volunteers received a potent opioid. At the appropriate time 2.1 $\mu g.kg^{-1}$ intravenous fentanyl was administrated intravenously over 90 s. Subsequently, heat pain measurements were taken every 10 minutes for 3 hours (first pain test at10 min after the start of the fentanyl infusion). Additionally, at each testing interval a Verbal Rating Score of sedation using a scale ranging from 0 to10 (0 = fully alert – 10 = severely sedated and sleepy) and end-tidal carbon dioxide measurements were

obtained via a face mask connected to a gas monitor (Multicap, Datex, Helsinki). Arterial hemoglobin oxygen saturation was measured via a finger probe (SpO_2) with a Masimo pulse oximeter (Irvine, CA). The study was powered to observe a 1 cm difference in visual analogue score (VAS) of a 10 cm scale ranging from 0 (= no pain) to 10 (= most intense pain imaginable) between two study groups (power = 90%, alpha = 0.05).

6.2.3 MESUREMENTS

Heat pain was induced using the TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). Using a 3 cm^2 probe, the skin on the volar side of the left or right forearm was stimulated with a gradually increasing stimulus (0.5 $^{o}C.s^{-1}$; baseline temperature 32 ^{o}C). Following heat stimulation, the subjects scored their VAS pain intensity on a 10 cm long scorecard. The thermode peak temperature depended on an initial trial phase in which the subject rated the pain to three peak temperatures: 46, 48 and 49 ^{o}C. The lowest stimulus causing a VAS > 5 cm was used in the remainder of the study. The test data were discarded. Next, baseline values (i.e., pre drug VAS) were obtained. The volar side of the arm was divided into six zones and marked as previously described.[5] The thermode was moved from zone to zone between stimuli to avoid sensitization to heat stimulus.

6.2.4 PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS

A linear mixed model was used to compare the baseline parameter values (thermode temperature to reach a VAS > 5, sedation score and end-tidal CO_2) using SPSS 16.0. (Chicago, IL). *P*-values < 0.05 were considered significant. In order to quantify the effect of fentanyl on pain relief we initially assessed the effect relative to baseline (*i.e.* ΔVAS, by subtraction of baseline VAS at each time point), and subsequently we calculated the area between the VAS data points and the zero-line (area between the effect-time curves, AEC). Consequently, a more negative the AEC means a higher analgesic the response. We present the AEC data as mean change in VAS over time (*i.e.*, AEC/180 min, unit = cm). Next, to get an indication of the presence of a circadian effect on fentanyl analgesia, the data were modeled using a sinusoid function:

$$AEC(t) = offset + A.\sin(2\pi ft + \varphi) \qquad \text{(eqn. 1)}$$

where the A = amplitude, f = frequency (occurrence of the sinus per 24 h) and φ = a phase shift. To obtain the 95% confidence interval of the sinusoid a bootstrap analysis was performed using 1000 reiterations with replacement. Data analysis was performed using the statistical package NONMEM version VI (ICON Development Solutions, Ellicott City, MD).[6] Similar procedures were performed for sedation and end-tidal CO_2.

6.3 RESULTS

No differences in baseline parameters were observed with-in group or between groups (see table 1). In each group there were 6 women and 2 men. All subjects completed the study without major side effects. Incidental occurrences of low SpO_2 (< 95%) were treated by prompting the subject to take a deep breath.

All injections were performed at the planned time of day ± 4.3 min (maximal range; not significant different between groups). After injection, all volunteers reached maximal analgesia within 20 min and returned to within 10% of their baseline pain sensitivity levels by the end of the experiment. The influence of the time of infusion on ΔVAS is shown in Fig. 1 and 2. Time-related variations are observed for peak analgesic effect (with the least effect at 2 AM), duration of effect (with the shortest duration at 8 AM) and the occurrence of a small hyperalgesic response (most pronounced at 2 AM). Individual AEC values (all divided over 180 min giving the mean change in VAS over 180 min) versus study time are given in Fig. 3 together with the data fit (± 95% confidence interval). A significant sinus wave was present in the data (the wave was significantly different from a linear response line, $P < 0.01$). Parameter values are: offset = –0.63 ± 0.25 cm, A = 0.65 ± 0.20 cm, φ = 27 ± 21 degrees (all parameters $P < 0.01$, values are typical value ± SE).

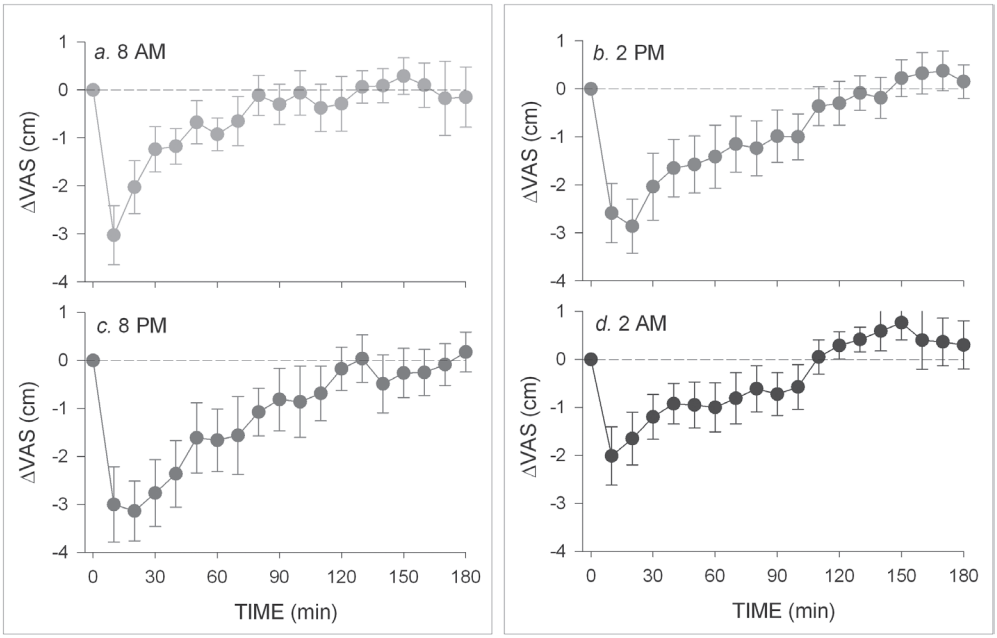

Figure 1. Effect of fentanyl on heat pain scores in two groups of subjects. One group received intravenous 2.1 $\mu g.kg^{-1}$ fentanyl at 8 AM and 8PM (**A** and **C**), the other group at 2 PM and 2 AM (**B** and **C**). Values are mean ± SD.

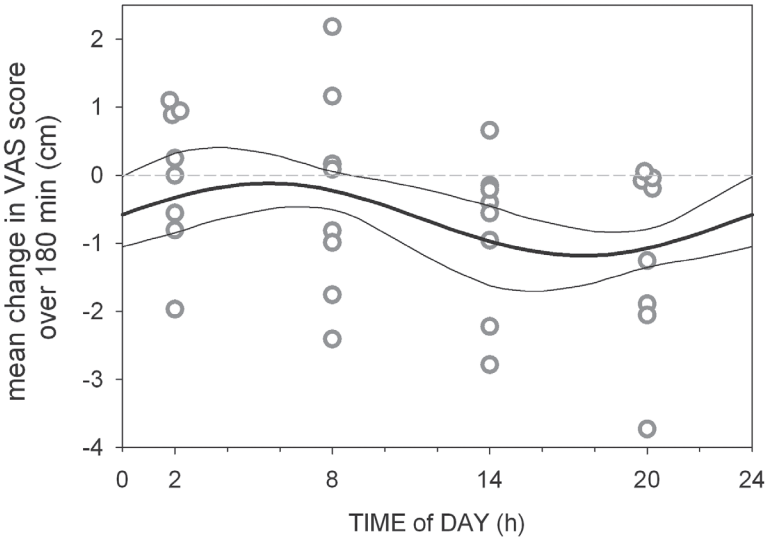

Figure 2. Mean pain scores after injection of 2.1 $\mu g.kg^{-1}$ fentanyl observed at 2 AM, 8AM, 2 PM and 8 PM.

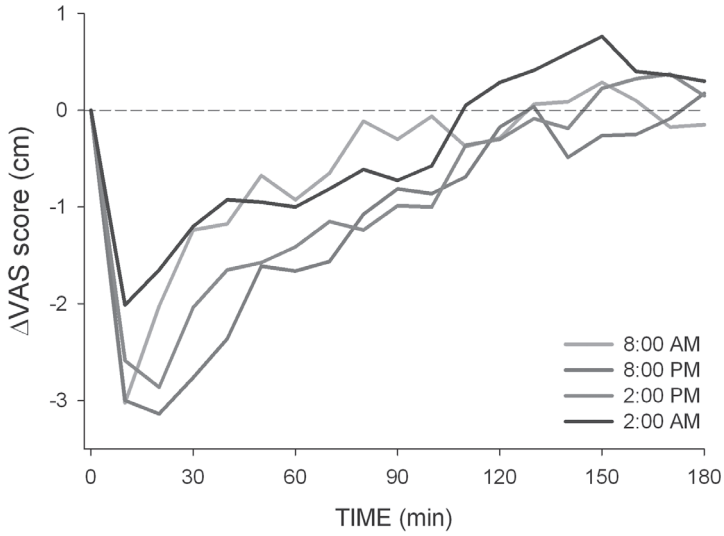

Figure 3. Data fit of analgesic effect from 2.1 $\mu g.kg^{-1}$ iv fentanyl versus time of day at which the drug was injected. Analgesic effect is defined as the mean change in VAS over the 180 min study period. Each circle represents the analgesic effect of one subject. The fit is a sinusoidal curve (thick continuous line) ± 95% confidence interval (thin continuous lines). The broken line denotes a separation between mean analgesic responses (data below the broken line) and hyperalgesic responses (above the broken line).

The negative value of the offset indicates that on average at all times an analgesic response occurred. An amplitude of 0.65 means that the average VAS varied by 1.3 cm over time (recalculation for just the first 90 min of the experiment would yield a variation in VAS of 2 cm; Note that these variations are model predictions). The value of φ of 27 degrees indicates that at midnight (0 h in figure 3) the sinus was shifted by 27 degrees. The frequency value f was fixed to 1 as we assumed that the sinus occurred once every 24 h. Fentanyl was most analgesic in the late afternoon and early evening hours (between 2 PM and 8 PM, minimum of the sinus occurred at 5 AM), while it was least analgesic in the early morning hours (from 2 AM to 8 AM, maximum of the sinus occurred at 3 PM).
Side effects showed much less of a variation over time than analgesia with no differences among observations within and between groups (anova: $P > 0.05$; table 1). A significant sinus could not be demonstrated for end-tidal PCO_2 or sedation.

Table 1. Baseline parameter values and 3-hour area-under-the-time-effect curve for end-tidal CO_2 and sedation

	8 AM – 8 PM	**2 AM – 2 PM**
Baseline values		
Temperature of thermode (°C)	48.5 ± 0.8 – 48.6 ± 0.8	48.2 ± 1.5 – 48.7 ± 1.4
Baseline pain VAS (cm)	7.8 ± 0.4 – 7.7 ± 0.4	6.6 ± 0.9 – 6.8 ± 0.7
Baseline CO_2 (vol. %)	4.7 ± 0.6 – 5.1 ± 0.5	4.7 ± 0.8 – 4.6 ± 0.5
Baseline sedation NRS (cm)	3.1 ± 0.7 – 1.9 ± 0.8	2.0 ± 0.4 – 1.0 ± 0.3
3 h AUEC's		
CO_2 (time.vol. %)	47 ± 22 – 41 ± 21	33 ± 40 – 48 ± 14
Sedation (time*cm)	125 ± 131 – 198 ± 153	188 ± 110 – 272 ± 190

No significant differences in parameter values were obtained among the study times (analysis of variance: $P > 0.05$)

6.4 DISCUSSION

We observed a circadian sinusoidal rhythm in the analgesic effect of fentanyl. Variations were observed for peak analgesic effect, duration of effect and the occurrence of hyperalgesia. When using AEC as end-point, we observed a peak in pain relief late in the afternoon (5:30 PM) and a trough in the early morning hours (5:30 AM). The difference between the peak and trough in pain relief corresponds to a difference in VAS of 1.3 to 2 cm. This indicates that the magnitude of the diurnal variation of fentanyl analgesia is significant albeit relatively small with increased sensitivity to fentanyl in the late afternoon and evening hours (1 to11 PM).

6.4.1. OPIOID EFFECT ON THE CIRCADIAN RHYTHM

In mammals the suprachiasmatic nucleus (SCN) in the hypothalamus is the site that controls circadian behavioral rhythmicity (*i.e.*, the master clock).[7,8] The SCN is

synchronized by external stimuli of which the light/dark cycle is the most important (the retina is directly linked to the SCN via the retinohypothalamic tract). Other synchronizers include locomotor activity, drugs (*e.g.*, benzodiazepines, opioids, serotonin agonists) and social interaction. The SCN controls many cyclic events in the mammalian body including the synthesis and release of hormones such as melatonin and cortisol and body temperature. Generation of rhythmicity in the SCN is genetically determined and based on feedback loop that involves several genes, including *Per1*, *Per2* and Clock.[7,8] The SCN and its afferent and efferent pathways contain various neurotransmitters including neuropeptide Y, γ-amino butyric acid (GABA) and enkephalins. The role of enkephalins in the circadian system has received increasing attention as δ-opioid receptors were identified in the hamster SCN and the μ-opioid receptor agonist fentanyl induces a phase shift in the circadian rhythm of hamsters independent of any behavioral effects of the opioid.[9,10] We showed previously that fentanyl modifies the circadian pacemaker possibly via direct effects on SCN electrical activity and regulation of *Per* genes.[9] This suggests that pathways regulating the circadian clock intersect directly or indirectly with pathways that express opioid receptors. Our current study, in which a diurnal variation in fentanyl's analgesic behavior is observed (*i.e.*, an effect opposite to fentanyl's influence on the clock), similarly suggests involvement of the opioid system in the circadian rhythm.

We refrained from measuring plasma fentanyl concentrations in our observational study. We argued that frequent blood sampling could interfere with the subjects rating of heat pain possibly causing stress-induced analgesia that encompasses strong circadian variations.[11] Consequently, the variation in fentanyl's effect may be due to a true increase in the opioid's antinociceptive efficacy (a pharmacodynamic effect), as suggested above, but we cannot exclude a diurnal variation in fentanyl's pharmacokinetics. An increase in plasma fentanyl concentrations in the late afternoon and early evening may well explain our findings. Variations in plasma morphine concentrations following oral administration in patients with cancer pain have been observed due to variations in absorption and/or changes in the volume of distribution over a 24 h period.[12] Similarly, intramuscular meperidine injections in patients with sickle cell anemia were associated with circadian changes in drug disposition and elimination over the day.[2] In contrast, oral codeine and tramadol given to healthy volunteers in the morning or evening did not show any differences in pharmacokinetics.[4] Similarly, and of importance to our study, in two separate studies in volunteers receiving intravenous fentanyl, the plasma fentanyl concentration-time profiles were independent of the time of infusion.[13] This then suggests that our findings are related to a circadian effect on fentanyl's pharmacodynamics and not to its pharmacokinetics.

6.4.2 CIRCADIAN VARIATIONS IN PAIN RESPONSE

Several animal studies showed that the response to noxious stimuli is not constant over a 24 h period.[1,2,11,14] The results of human experimental and clinical studies are less clear

with some studies finding no difference in pain over time, while other found more pain in the morning or evening.[1-3,15,16] Experimental pain studies indicate that variations in pain sensitivity depend on the tissue tested and the nociceptive assay employed.[1-3,15,16] Using a similar thermode as we did, Strian *et al*.[15] did observe a variation in pain threshold values to warm and cold stimuli but these variations were small and had no consistent pattern among subjects. Our study was not designed or powered to study variations in pain sensitivity and, as expected, we did not observe significant differences in temperature to induce VAS > 5 cm. However, at this point we cannot exclude some effect of variations in pain sensitivity on the antinociceptive responses that we observed with less pain reporting between 1 and 11 PM (and hence a greater analgesic response at these times). Indeed, skin sensitivity to heat is minimal at 6 PM and maximal at 6 AM and also painful stimulation of the nasal mucosa with carbon dioxide is increased during evening test sessions.[3,17] Further studies are needed to investigate the complex interaction between variations in pain sensitivity and opioid treatment. An important question in this respect is, for example, whether the pain and analgesic rhythms display antagonistic or synergistic interactions.

6.4.3 MECHANISMS OF OPIOID CIRCADIAN RHYTHM

The mechanism though which the circadian rhythm affects opioid analgesic efficacy remains unknown. Variations in hormones (*e.g*., cortisol, melatonin) and endogenous opioid peptides (meta-enkephalin and β-endorphins) could play an important role interacting with the nociceptive pathways and opioid system.[18-20] For example, the analgesic effect of melatonin is more pronounced at night.[21] An interesting observation in mice is that μ-opioid receptor expression displays a 24-h rhythm.[22] Down regulation of the brain μ-opioid receptor was associated with a decrease in morphine analgesia. Extrapolation of these animal data to ours in humans then suggests that during the late evening, morning and early afternoon, human μ-opioid receptors are down regulated via a direct or indirect (e.g. hormonal) influence of the SCN. Our finding of enhanced analgesic fentanyl efficacy from 1 to 11 PM is in agreement with other human studies showing similar patterns of opioid effect. Non-lethal opioid overdose (*i.e*., increased opioid sensitivity causing respiratory depression) shows a significant peak in the afternoon and early evening, an effect that was independent of the opioid plasma concentrations.[23] Oral codeine and tramadol display greater analgesic sensitivity when administered in the early evening.[4]

6.4.4 HYPERALGESIA

A somewhat surprising observation in our study was the occurrence of a moderate hyperalgesic response (pain sensitivity greater than baseline) following analgesia in subjects receiving fentanyl at 2 AM (figure 1). This phenomenon was outspoken in 5 subjects tested at 2 AM and occurred on 11 occasions in the whole study (figure 3). Hyperalgesia in response to opioids has been observed in various species, including

humans. Recent data indicate that opioid-induced hyperalgesia is not related to activation of opioid receptors but possibly due to activation of N-methyl-D-aspartic acid receptors within pain pathways.[24,25] Animal studies showed that hyperalgesia induced by opioid-receptor blockade by naloxone (*i.e.*, a non opioid receptor phenomenon) follows a diurnal rhythm.[26] This then suggests that our results may have been influence by three separate rhythms: an inherent pain rhythm, a fentanyl analgesic and anti-analgesic rhythm.

6.4.5 CRITIQUE OF METHODS

It is possible that the observed rhythm is entirely due to the use of two distinct subject groups, one of which was studied at 8 AM and 8 PM, the other at 2 PM and 2 AM. This could, for example, occur when the two groups would differ in their AEC's without a within-group difference between measurement points (for example: [AEC(group 1) at 2 PM = 2 AM] > [AEC(group2) at 8 AM = 8 PM]). However, this was not the case (figures 1 and 2). In both groups the data collected in the morning hours (2 AM or 8 AM) displayed a peak effect and AEC of lesser magnitude than the data collected in the afternoon or early evening hours (2 PM or 8 PM). This suggests that the observed rhythm was inherently present in the two study groups and not related to the design of the study.

We modeled the data with a symmetrical sinusoid function. This function was significantly better than a linear function. We did assess also non-symmetrical sinusoid functions by allowing the four parts of the sinusoid to vary independently in amplitude (with factor FAC). However, no significant improvements in minimum objective function were observed in comparison to FAC values of 1. Furthermore, assessing the residuals of the symmetrical sinusoid functions showed the absence of any bias (means residuals per test period not different from zero). This indicates that the sinusoid chosen adequately described the data.

We subtracted the baseline pain score from the VAS-time data to allow objective assessment of the change in VAS over time (AEC). This was possible in our data set as we observed little variation in the baseline VAS (*i.e.*, predrug) score. We cannot exclude, however, that some error in baseline values may erroneously propagate to the estimates of the model parameters. However, in our analysis, the error only propagates to the inter-individual variability of the model parameter offset. We tested the variance in offset and observed that it was not different from zero, suggesting that subtraction of baseline pain scores did not affect our study outcome. In some studies the analysis of circadian effects is sensitive to 'edge effects' or the moment in time defined as the start of day or start of analysis (this is often related to the use of a smoothing function).[27] We chose midnight as starting point of our analysis. Our NONMEM analysis of the data with a non-smoothed sinusoid does not have any edge effects.

Recent studies on chronopharmacology of labor analgesia with intrathecal bupivacaine indicate that one has to be careful with the interpretation of rhythmic patterns in the duration of analgesia.[27] This concerns patient studies in which daily routines (external

rhythms such nursing and anesthesia provider shifts) produce artifacts (suggesting a biological rhythm in intrathecal analgesia duration) that have little to do with biological rhythms.[27,28] We were aware of these pitfalls and designed our study to prevent influences from external rhythms. However, despite our efforts we cannot exclude some albeit small effect from external sources on our study outcome.

6.5 CONCLUSIONS

We observed a circadian rhythm in the analgesic effect of fentanyl in human volunteers using an experimental heat pain model. Our data indicate an increase in analgesic efficacy in the late afternoon and early evening hours. We argue that the most probable cause for our findings is chrono-pharmacodynamic effect regulated by the circadian clock in the hypothalamus. This may be a direct effect through shared pathways of the circadian system and the opioid system or an indirect effect via diurnal variations in hormones or endogenous opioid peptides that rhythmically change the pain response and/or analgesic response to fentanyl.

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Section IV

SUMMARY AND CONCLUSIONS

CHAPTER 7

SUMMARY & CONCLUSIONS

7.1 SUMMARY

Despite many side effects opioids remain the first choice in the treatment of severe acute and chronic pain in contemporary medicine. In this thesis, the influence of strong opioids, used in the treatment of severe acute and chronic pain, on the control of breathing are studied and discussed relative to their wanted effect, analgesia. The issue of opioid-induced respiratory depression is highly relevant as accidental casualties due to OIRD do regularly occur. In some areas, such as the US and Canada, the number of accidental deaths from legally prescribed drugs (predominantly opioids prescribed for chronic non-cancer pain and sedatives voor anxiety and sleeping disorders) is high with a frightening 70 deaths per day.[1,2]

In **Chapter 2** the detrimental respiratory effects of opioids and their relevant pharmacokinetics and dynamics are discussed in the first part of the chapter. Opioids induce respiratory depression via activation of μ-opioid receptors (MORs) at specific sites in the central nervous system including the pre-Bötzinger complex, a respiratory rhythm generating area in the pons. A distinction is made between full and partial MOR agonists. Full opioid agonists like morphine and fentanyl affect breathing with onset and offset profiles that are primarily determined by opioid transfer to the receptor site, while the effects of partial MOR agonists such as buprenorphine are governed by transfer to the receptor site together with receptor kinetics, in particular dissociation kinetics. In the second part of the chapter respiratory depression reversal options are discussed. Opioid-induced respiratory depression (OIRD) may be reversed by the opioid receptor antagonist naloxone, an agent with a short elimination half-life (30 min). The rate-limiting factor in naloxone-reversal of opioid effect is the receptor kinetics of the opioid agonists that requires reversal. Agents with slow dissociation kinetics (buprenorphine) require a continuous naloxone infusion while agents with rapid kinetics (fentanyl) will show complete reversal upon a single naloxone dose. Since naloxone is non-selective and reverses analgesia as well, efforts are made by several pharmaceutical companies on the development of compounds that reverse OIRD without affecting analgesic efficacy. Such agents include ampakines and 5HT-receptor agonists which are aimed at selectively enhancing central respiratory drive. A novel approach is aimed at the reduction of respiratory depression from opioid-activation of (micro-)glia cells in the pons and brainstem using microglia cell stabilizers. Since this approach simultaneously enhances opioid analgesic efficacy it seems an attractive alternative to the classical reversal strategies with naloxone.

In **Chapter 3** the dynamic effects of the potent opioid remifentanil on respiration are described and a mathematical model of respiratory depression is developed. Studies as described here are rarely performed possibly due to the complexity of the respiratory control system. We show here that a model with intact feedback control of carbon dioxide on ventilation (non-steady-state models) that correctly incorporates the complex interaction between drug concentration, PCO_2 and ventilation yields reliable descriptions and predictions of the opioid's behavior. We measured the respiratory effect of remifentanil

with and without a background infusion of propofol.
Ten male healthy volunteers received remifentanil infusions with different infusion speeds (target concentrations of 4 to 9 ng.ml^{-1} at infusion rates of 0.17 to 9 ng.ml^{-1}.min^{-1}) while awake and at the background of low-dose propofol. The data were analyzed with a non-linear model consisting of two additive linear parts, one describing the depressant effect of remifentanil and one the stimulatory effect of carbon dioxide on ventilation.
The model adequately described the data including the occurrence of apnea. Most important model parameters were: C_{50} for respiratory depression with an estimated value of 1.6 ± 0.03 ng.ml^{-1} (median ± SE), the gain of the respiratory controller (G) with an estimated value of 0.42 ± 0.1 L.min^{-1}.Torr^{-1} and the remifentanil blood effect-site equilibration half-life ($t_{½}k_{e0}$) with a value 0.5 3± 0.2 min. Propofol caused a 20-50% reduction of C_{50} and G but had no effect on $t_{½}k_{e0}$. Apnea occurred during propofol infusion only. A simulation study revealed an increase in apnea duration at infusion rates of 2.5 to 0.5 ng.ml^{-1}.min^{-1} followed by a reduction in duration. At speeds ≤ 0.31 ng.ml^{-1}.min^{-1} no apnea was present. This is related to the slow accumulation of CO_2. This study shows that the mathematical description of the respiratory depressant effects of remifentanil together with the respiratory stimulating effects of carbon dioxide is possible. Furthermore it allows for the prediction of the opioid's respiratory behavior and as such may be used in the development of infusion regimens aimed at the sustainment of spontaneous breathing activity even at high remifentanil infusion dosages. With the presented model we were able to remove some of the limitations of models derived from earlier studies. Further studies are required to incorporate a fourth and fifth factor, next to drug concentration, PCO_2 and ventilation, in the model, namely pain and upper airway obstruction. These factors have excitatory (pain) and disturbing (upper airway obstruction) influences on the control of breathing. Both factors are present in our (often obese) patient population treated with potent opioids for a variety of reasons (such as postoperative pain relief, chronic pain relief, sedation for diagnostic procedures, dyspnea, palliation), although their presence is often episodic rather than continuous, a fact that makes incorporation in a predictive model difficult but hopefully not impossible.
Integrating opioid risk and benefit is important as it allows for the comparison of net efficacy among opioids. In **Chapter 4** an explorative study on the effects of fentanyl on analgesia and respiratory depression was performed to construct fentanyl risk-benefit or utility functions, a new concept in opioid pharmacology. Twelve volunteers received a 3.5 μg.kg^{-1} fentanyl intravenous injection on two separate study days. On one occasion ventilation at a clamped elevated carbon dioxide was measured, on another the pain tolerance to electrical stimulation. In both sessions arterial plasma samples were obtained. The data were analyzed with a population pharmacokinetic-pharmacodynamic (PKPD) model. Two-times 9,999 simulations were performed, using the PK-PD parameter estimates and their variabilities, in which simulated subjects received 3.5 μg.kg^{-1} fentanyl. The resultant distributions were used to calculate the utility functions, defined as the

probability of at least 50% analgesia (an increase in pain tolerance by at least 50%) minus the probability of at least 50% respiratory depression. Utility functions were constructed in concentration (UFc) and time domains (UFt). The PKPD analysis showed that fentanyl had an approximate two-fold faster onset/offset and two-fold greater potency with respect to respiratory depression compared to analgesia. The constructed utility functions were successful with negative UFc values at effect-site concentrations > 0.5 $ng.mL^{-1}$ and negative UFt values in the first 90 min following the 3.5 $\mu g.kg^{-1}$ bolus infusion. From these results it may be concluded that successful construction of clinically relevant utility functions is possible. UF of other opiods may be constructed from previous studies performed in our laboratory. One such opioid, morphine, displays UFc and UFt values more positive than fentanyl. While this suggest that morphine has a lower probability than fentanyl in producing respiratory depression for a given amount of analgesia it is important to be vigilant as severe respiratory depression in an individual patient remains possible, even at low dose morphine. Further studies should address the issue of applicability of the UF under clinical circumstances and assess the influence of pain of the function. For now it can be concluded that the UF is useful in drug selection in drug development programs and dose selection for experimental Phase III studies.

In **Chapter 5** a phase 1 study is presented, in which the effect of an experimental opioid from Mundipharma Research Ltd (Cambridge, UK), MR30365/07, with high affinity for the three classical opioid receptors (MOR, delta-opioid receptor, (DOR), kappa-opioid receptor, (KOR)) and low affinity for the recently discovered opioid-receptor-like (ORL1) receptor, on respiration and analgesia was compared to fentanyl, a selective, high affinity MOR agonist that, at high doses, produces dose dependent respiratory depression and apnea. In this double-blind, randomized controlled study 46 healthy male volunteers participated in respiratory studies, 46 others in analgesia studies. In each group, six subjects received placebo, twenty received MR30365/07 (four received 0.0125, six 0.075, six 0.125 and four 0.15 $\mu g.kg^{-1}$) and twenty received fentanyl (four received 0.5, six 1.0, six 2.0 and four 3.0 $\mu g.kg^{-1}$). Active and placebo treatment was given intravenously over 10 min. Breathing was measured on a breath-to-breath basis at a fixed elevated end-tidal PCO2. Analgesic responses to pain detection (pain threshold) were measured using transcutaneous electrical stimulation.

Fentanyl displayed typical dose-dependent effects in respiratory depression and analgesia. MR30365/07 showed dose-dependent respiratory depression with ceiling starting at a dose of 0.075 $\mu g.kg^{-1}$, with a minimum ventilation of 32.8% of baseline. No ceiling was observed in the analgesic effects of MR30365/07 over the dose range tested. MR30365/07 was about 18 times more potent than fentanyl in producing analgesia.

These data are promising in that this is an opioid with limited respiratory effect (at least as observed over the dose range tested) retaining full analgesia efficacy. In contrast to buprenorphine which shows similar behavior, this drug is a full agonist for the MOR. Possibly the favorable behavior of MR30365/07 is due to its agonist effect at the KOR

although other mechanisms are not excluded (including a differential effect at recruitment of second messengers and intracellular peptides). Further studies are required to assess the behavior of this opioid at higher doses, in clinical settings and in the combination with other drugs (such as sedatives) to assess whether the ceiling in respiratory depression is sustained.

Chronopharmacology studies the effect of the timing of drug administration on drug effect and may have important effects in clinical practice and is possibly an important cause of opioid variability. In **Chapter 6**, the influence of four timing moments on fentanyl-induced analgesia was evaluated. Eight healthy volunteers received 2.1 $\mu g.kg^{-1}$ intravenous fentanyl at 2 PM and 2 AM, with at least 2 weeks between occasions, eight others at 8 AM and 8 PM. Heat pain measurements using a thermode placed on the skin were taken at regular intervals for 3 h and Verbal Analogue Scores (VAS) were then obtained. The data were modeled with a sinusoid function using the statistical package NONMEM. A significant circadian sinusoidal rhythm in the antinociceptive effect of fentanyl was observed. Variations were observed for peak analgesic effect, duration of effect and the occurrence of hyperalgesia. A peak in pain relief occurred late in the afternoon (5:30 PM) and a trough in the early morning hours (5:30 AM). The difference between the peak and trough in pain relief corresponds to a difference in VAS of 1.3 to 2 cm. Only when given at 2 AM did fentanyl cause a small but significant period of hyperalgesia following analgesia. No significant changes were observed for baseline pain, sedation or the increase in end-tidal CO_2. The observed possible influence of the circadian rhythm may be a direct effect through shared pathways of the circadian and opioid systems or an indirect effect via diurnal variations in hormones or endogenous opioid peptides that rhythmically change the pain response and/or the analgesic response to fentanyl.

These data show significant but small effects of the circadian clock on fentanyl-induced analgesia. It remains questionable whether such effects may be unearthed from the noise (related to a multitude of other factors, including sex, age, underlying disease, comedication, anxiety, and genetics) in a clinical setting.

7.2 CONCLUSIONS

The following conclusions may be drawn from the data presented in this thesis:

1. The results from this thesis indicate that currently-used opioids may produce life-threating respiratory depression. Despite our efforts to understand OIRD, individual prediction of development of OIRD is limited and therefore titration to effect is the best option when treating patients with potent opioid analgesics. This is true for all patients receiving opioids, irrespective of the indication.
2. The ideal drug for antagonism of respiratory depression has not yet been found. At present naloxone seems the most appropriate drug although reversal of OIRD coincides with loss of analgesia. New reversal agents acting via non-opioidergic pathways are under investigation and are aimed at reversal of OIRD without compromising analgesia.

3. Mathematical modeling of the non-steady state effects of respiratory depression by opioids is not only possible, but also yields comprehensible results. Still despite adequate prediction of a drug's respiratory behavior on a population level, the model does not allow individual prediction.
4. Utility functions may serve as a composite function to describe the effect-side effect profile of a drug. For example, the utility function of fentanyl is predominantly negative except at low dose, indicating that for the dose tested the probability of respiratory depression exceeds the probability for analgesia. While this function seems applicable in experimental and phase I/II/III settings, its clinical use requires further validation.
5. The Anesthesia & Pain research Unit is especially appropriate for studying the effect of experimental drugs on respiration and analgesia. An example of such a drug is MR30365/07, an opioid acting at all three classical opioid receptors. In contrast to fentanyl this agents produces ceiling in respiratory depression but not analgesia over the dose range tested.
6. Fentanyl-induced pain relief is influenced by the circadian clock with increased efficacy during the later hours of the afternoon. Whether such effects are sustained in a clinical setting remains unknown.

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CHAPTER 8

SAMENVATTING & CONCLUSIES

8.1 SAMENVATTING

Ondanks veel bijwerkingen blijven opioiden de eerste keus in de behandeling van ernstige acute pijn in de huidige tijd.
In dit proefschrift wordt de invloed van sterke opioïden, gebruikt in de behandeling van ernstige acute en chronische pijn, op de controle van de ademhaling bediscussieerd. Dit effect op de ademhaling, een bijwerking, wordt ook gerelateerd aan het gewenste effect, analgesie.
Het onderwerp van de opioïd-geinduceerde ademhalingsdepressie is zeer relevant, aangezien er regelmatig slachtoffers vallen als gevolg van deze ademhalingsdepressie.
In sommige gebieden, zoals de US en Canada, is het aantal slachtoffers als gevolg van legaal voorgeschreven geneesmiddelen (voornamelijk opioïden voorgeschreven voor chronische niet-kanker pijn en slaapmiddelen voor anst en slaapstoornissen) met een beangstigend aantal van 70 doden per dag.[1,2]

In **hoofdstuk 2** worden de ernstige ademhalingsbijwerkingen van opioïden en hun relevante farmacokinetiek en -dynamiek bediscussieerd in het eerste gedeelte van het hoofdstuk. Opioïden induceren ademhalingsdepressie via activatie van de μ-opioïd recptoren (MORs) op specifieke lokaties in het centrale zenuwstelsel, waaronder het pre-Bötzinger complex, een gebied in de pons wat het ademhalingsritme genereert.
Een onderscheid wordt gemaakt tussen volledige en partiële MOR-agonisten. Volledige opioïd-agonisten, zoals morfine en fentanyl, hebben invloed op de ademhaling met in- en uitwerkingsprofielen ('onset and offset profiles') die in eerste instantie vooral bepaald worden door de overgang van het opioïd naar de lokatie van de receptor, tewijl de effecten van partiële MOR-agonisten, zoals buprenorphine, veroorzaakt worden door overgang naar de lokatie van de receptor , in combinatie met receptor-kinetiek, in het bijzonder de dissociatiekinetiek. In het tweede gedeelte van het hoofdstuk worden mogelijkheden voor antagoneren van ademhalingsdepressie besproken. Opioïd-geinduceerde ademhalingsdepressie (OIRD) kan geantagoneerd worden door de opioïd receptor antagonist naloxon, een middel met een korte eliminatie-halfwaardetijd (30 min). De snelheidsbeperkende factor bij antagonisme van een opioïd-effect door naloxon is de receptorkinetiek van de opioïd-agonist die antagonering behoeft. Middelen met een langzame dissociatiekinetiek (buprenorphine) hebben een continue naloxon-infusie nodig, terwijl middelen met een snelle kinetiek (fentanyl) volledige omkering van het effect zullen laten zien na een enkele dosering naloxon. Aangezien naloxon niet-selectief is en analgesie ook teniet doet, wordt er door diverse farmaceutische industrieën gewerkt aan het ontwikkelen van middelen die ademhalingsdepressie opheffen, maar het analgetisch effect niet. Zulke middelen zijn onder andere ampakines en 5HT receptor agonisten, welke gericht zijn op het selectief doen toenemen van de ademhalingsprikkel. Een nieuw aangrijpingspunt is gericht op het verminderen van ademhalingsdepressie door opioïd activatie van (micro-)glia cellen in de pons en de hersenstam, gebruik makend

van microglia-cel stabiliserende middelen. Aangezien deze benadering tegelijkertijd een positieve invloed heeft op het analgetisch effect van opioiden, lijkt dit een aantrekkelijke mogelijkheid ten opzicht van de klassieke antagoneringsmethoden.

In **hoofdstuk drie** worden de dynamische effecten van het potente opioïd remifentanil op de ademhaling beschreven en wordt er een wiskundig model van ademhalingsdepressie ontwikkeld. Studies zoals hier beschreven worden zelden verricht vanwege de complexiteit van het ademhalingssysteem. We laten hier zien dat een model met een intact feedback systeem van koolstofdioxide op ademhaling (zogenaamde non-steady-state modellen), wat op juiste wijze de complexe interactie tussen geneesmiddelenconcentratie, PCO_2 en ademhaling incorporeert, een bruikbare beschrijving en voorspelling van geneesmiddeleneffect geeft. We onderzochten het effect van remifentanil op ademhaling zowel met als zonder een achtergrondinfusie van propofol.
Tien gezonde mannelijke vrijwilligers ontvingen remifentanil infusies met verschillende toedieningssnelheden (doelconcentraties in plasma 4 tot 9 $ng.ml^{-1}$ bij infusiesnelheden van 0.17 to 9 $ng.ml^{-1}.min^{-1}$), zowel 'wakker' als met een achtergrondinfusie van laaggedoseerd propofol. De data werden geanalyseerd met een non-lineair model, bestaande uit twee additieve lineaire gedeelten, waarvan één het deprimerende effect van remifentanil op ademhaling beschrijft, en één het stimulerende effect van koolstofdioxide op diezelfde ademhaling.
Het model beschreef de gevonden waarden adequaat, inclusief het optreden van apneu. De belangrijkste modelparameters waren: C_{50} voor ademhalingsdepressie met een geschatte waarde van 1.6 +/- 0.03 $ng.ml^{-1}$ (median ± SE), de responsecurve (*ie.* de 'gain') van het ademhalingscentrum (G) met een geschatte waarde van 0.42 +/- 0.1 $L.min^{-1}.Torr^{-1}$ en de remifentanil bloed effect-site equilibrilatie halfwaarde tijd ($t_{½}k_{e0}$) met een waarde van 0.53 ± 0.2 min. Propofol veroorzaakte een 20 tot 50% afname van de C_{50} en G maar had geen effect op de $T_{½}k_{e0}$. Apneu trad alleen op tijdens propofol-infusie. Een simulatie-studie liet een toename in apneu-duur zien bij infusie snelheden van 2.5 tot 0.5 $ng.ml^{-1}.min^{-1}$ gevolgd door een afname van deze duur. Bij snelheden < 0.31 $ng.ml^{-1}.min^{-1}$ werd er geen apneu gezien. Dit is gerelateerd aan de langzamere toename van CO_2 bij een langzamere infusieduur. Deze studie laat zien dat de wiskundige beschrijving van het ademhalingsdeprimerende effect van remifentanil in combinatie met het stimulerende effect van koolstofdioxide mogelijk is. Verder staat het de voorspelling van het gedrag van het opioïd toe, en kan het gebruikt worden om infusie-regimes te ontwikkelen gericht op het behoud van spontane ademhaling, zelfs bij hoge doseringen remifentanil. Met het gepresenteerde model zijn we in staat geweest enige beperkingen van de modellen van eerdere studies op te heffen.
Verder onderzoek is noodzakelijk om nog een vierde en vijfde factor aan het model te kunnen toevoegen, naast geneesmiddelenconcentratie, ademhaling en CO_2, namelijk pijn en bovensteluchtwegobstructie. Deze factoren hebben respectievelijk een stimulerend (pijn)

en verstorend (bovenste luchtwegopstructie) effect op de regulatie van de ademhaling. Beide factoren zijn aanwezig in onze (vaak obese) patiëntenpopulatie die behandeld wordt met opioïden om uiteenlopende redenen (zoals postoperatieve pijnstilling, chronische pijnstilling, sedatie voor diagnostische procedures, dyspnoe, palliatie). Vaak is hun aanwezigheid echter meer intermitterend dan continue, wat implementatie in een voorspellend model zeer moeilijk, maar hopelijk niet onmogelijk maakt.

Het integreren van zowel werking als bijwerking van opioiden is belangrijk, aangezien het vergelijking van netto-effectiviteit van opioiden mogelijk maakt. In **hoofdstuk 4** wordt een verkennende studie van het effect van fentanyl op analgetisch effect en ademhalingsdepressie gepresenteerd. Deze is verricht teneinde voor fentanyl 'risk-benefit' (*ie.* de verhouding werking-bijwerking) of 'Utility Functions' te construeren, een nieuw concept binnen het beschrijven van effecten van opioïden. Twaalf vrijwilligers ontvingen een 3.5 $mcg.kg^{-1}$ fentanyl-injectie op twee verschillende studiedagen. Bij de ene gelegenheid werd de ademhaling gemeten bij een gecontroleerde verhoogde uitademings-koolstofdioxide-concentratie, op de andere dag werd pijnstilling gemeten middels electrische stimulatie. Tijdens beide sessies werd arterieel plasma afgenomen. De data werden geanalyseerd middels een farmacokinetisch-farmacodynamisch (PK-PD) model op populatieniveau. Tweemaal 9.999 simulaties werden verricht, gebruikmakend van de PK-PD-parameter schattingen en hun variabiliteit, waarbij gesimuleerde proefpersonen 3.5 $mcg.kg^{-1}$ fentanyl toegediend kregen. De uiteindelijke uitkomsten werden gebruikt om 'Utility Functions', gedefinieerd als de waarschijnlijkheid om 50% pijnstilling te verkrijgen (gemeten als een toename van 50% tolerantie ten aanzien van de electrische pijntest) minus de waarschijnlijkheid om 50% ademhalingsdepressie te verkrijgen. 'Utility functions' werden zowel voor het concentratiedomein (UFc) als het tijddomein (UFt) geconstrueerd. De PK-PD analyse liet zien dat fentanyl een ongeveer tweemaal grotere 'onset/offset' en een tweemaal grotere potentie met betrekking tot ademhalingsdepressie - in vergelijking met de analgesie - liet zien. De geconstrueerde Utility Functions lieten negatieve UFc waarden bij effect-site-concentraties > 0.5 $ng.ml^{-1}$ en negatieve UFt waarden in de eerste 90 min volgend op de 3.5 $mcg.kg^{-1}$ bolus-infusie. Uit deze resultaten kan geconcludeerd worden dat een succesvolle constructie van klinisch relevante utility functions mogelijk is. UF van andere opioiden kunnen geconstrueerd worden vanuit eerder onderzoek uit ons laboratorium. Eén van deze opioïden, morfine, laat UFc -en UFt-waarden zien die positiever zijn dan die van fentanyl. Terwijl dit suggereert dat morfine een lagere kans heeft dan fentanyl op het produceren van ademhalingsdepressie voor een gegeven hoeveelheid analgesie, is het belangrijk alert te blijven, aangezien ernstige ademhalingsdepressie in een individuele patient mogelijk blijft, zelfs bij een lage dosering morfine. Verdere studies zouden het vraagstuk van de toepasbaarheid van UF onder klinische omstandigheden moeten beoordelen, evenals de invloed van pijn op de uitkomsten. Vooralsnog kan geconcludeerd worden dat de Utility Function zinvol is in geneesmiddelenselectie in

geneesmiddelenontwikkelingsprogramma's en doseringenselectie voor experimentele fase III studies.

In **hoofdstuk 5** wordt een fase I studie gepresenteerd, waarin het effect van een experimenteel opioïd van Mundipharma Research Ltd (Cambridge, UK), te weten MR30365/07, met een hoge affiniteit voor de drie klassieke opioïdreceptoren (MOR, de δ-opioidreceptor, DOR, en de κ-opioidreceptor, KOR) en een lage affiniteit voor de recent ontdekte opioïd-receptor-like (ORL1) receptor, op ademhaling en analgesie werd vergeleken met fentanyl, een selectieve MOR-agonist met een hoge affiniteit die bij hoge doseringen, dosisafhankelijke ademhalingsdepressie en apneu laat zien. Binnen deze dubbelblinde gerandomiseerde en gecontroleerde studie namen 46 gezonde mannelijke proefpersonen deel aan een ademhalings-studie, en 46 anderen aan een analgesie-studie. In elke groep ontvingen zes personen placebo, twintig ontvingen MR30365/07 (vier ontvingen 0.0125, zes 0.075, zes 0.125 en vier 0.15 $mcg.kg^{-1}$) en twintig ontvingen fentanyl (vier ontvingen 0.5, zes 1.0, zes 2.0 en vier 3.0 $mcg.kg^{-1}$). Zowel de actieve als de placebobehandeling werd toegediend in 10 minuten. Ademhaling werd gemeten op een breath-to-breath basis (i.e. iedere ademhalingsteug werd gemeten) bij een op een vast niveau gefixeerde verhoogde uitademingskoolstofdioxide. Pijnstilling werd gemeten door bepaling van de pijnwaarnemingsdrempel op een electrische pijntest.
Fentanyl liet een typisch dosis-afhankelijk effect zien in ademhalingsdepressie en analgesie. MR30365/07 liet een dosis-afhankelijke ademhalingsdepressie zien met een plafond effect beginnend bij dosering 0.075 $mcg.kg^{-1}$, met een minimum-ademhaling van 32.8% van de baseline. Er werd geen plafondeffect werd gezien bij de analgetische effecten van MR30365/07 over het geteste doseringsinterval. MR30365/07 was ongeveer 18 maal potenter dan fentanyl in het produceren van analgesie. Deze data zijn veelbelovend in de zin dat dit een opioïd lijkt met een beperkt effect op ademhaling (tenminste over het gemeten doseringsinterval) met behoud van analgetische effectiviteit. In tegenstelling tot buprenorphine, wat een soortgelijk gedrag laat zien, is dit middel een volledige agonist van MOR. Mogelijk is het gunstige gedrag van MR30365/07 te danken aan een agonistisch effect op de KOR, alhoewel andere mechanismen niet uitgesloten kunnen worden (inclusief een wisselend effect op de activering van 'second messengers' en intracellulaire signaaleiwitten). Verdere studies zijn nodig om het effect van dit opioid bij hogere doseringen te beoordelen, zowel in klinische omstandigheden als in combinatie met andere middelen (zoals sederende middelen) om te zien of het plafond-effect in ademhalingsdepressie aanhoudt.

Chonopharmacologie bestudeert het effect van het tijdstip van toediening van een geneesmiddel op het gemeten effect. Dit kan grote invloeden hebben op de klinische praktijk en is mogelijk een belangrijke oorzaak van variabiliteit in effect van opioiden. In hoofdstuk 6 wordt de invloed van moment van toediening op fentanyl-geinduceerde

analgesie geëvalueerd. Acht gezonde vrijwilligers ontvingen 2.1 mcg.kg^{-1} fentanyl intraveneus om 2 PM en 2 AM, met tenminste twee weken tussen de verschillende gelegenheden, acht anderen ontvingen deze infusie om 8 AM en 8 PM. Hittepijn-metingen, gebruikmakend van een thermode geplaatst op de huid, werden op gezete tijden verricht gedurende 3 uur, en Visual Analogue Scores (VAS) werden afgenomen om de pijnstilling te evalueren. De data werden gemodelleerd met een sinusoïdvormige functie – met behulp van het statistiekprogramma NONMEM. Er werd een significant circadiaan ritme in het analgetisch effect van fentanyl gevonden. Variaties werden gezien in piek-analgetisch effect, duur van het effect en het optreden van hyperalgesie. Een piek in pijnstilling trad laat in de middag op (5:30 PM) en een dal vroeg in de ochtend (5:30 AM). Het verschil tussen de piek en het dal in pijnstillingseffect was een VAS-score van 1.3 tot 2 cm. Slechts om 2 AM gaf fentanyl een kleine maar significante periode van hyperalgesie volgend op analgesie. Er werden geen significante verschillen gevonden voor uitgangsscore in pijn, sedatie of toename van uitademings-CO_2 concentratie. De mogelijke invloed van het circadiane ritme kan een direct effect zijn van gedeelde signaalpaden van het circadiane systeem en het opioïd-respons systeem of een indirect effect via 24-uurs variaties in hormonen of endogene opioïd-eiwitten die de pijnrespons en/of het analgetisch effect van fentanyl beïnvloeden.
Deze data laten een klein maar significant effect zien van het circadiane ritme op fentanyl geïnduceerde analgesie. Het blijft de vraag of zulke effecten van belangrijke invloed zullen zijn temidden van alle andere effecten (zoals bijvoorbeeld geslacht, leeftijd, onderliggende aandoening, comedicatie, angst en genetische effecten) die een rol spelen in klinische omstandigheden.

8.2 CONCLUSIES

De volgende conclusies kunnen getrokken worden uit de data gepresenteerd in dit proefschrift:
1. De resultaten van dit proefschrift laten zien dat de huidige gebruikte opioiden levensbedreigende effecten kunnen hebben op de ademhaling. Ondanks onze inspanningen om OIRD te begrijpen, is een individuele voorspelling van het ontwikkelen van OIRD slechts beperkt mogelijk, en is titreren naar effect de veiligste optie indien patienten met potente opioïden behandeld worden. Dit geldt voor alle patienten die opioïden krijgen, onafhankelijk voor welke indicatie.
2. Het ideale middel voor het antagoneren van ademhalingsdepressie is nog niet gevonden. Op dit moment lijkt naloxon het juiste middel, alhoewel omkering van OIRD samengaat met verlies van analgetisch effect. Nieuwe antagonisten, werkend via niet-opioïdreceptor gemedieerde wegen, worden op dit moment onderzocht en zijn gericht op omkering van OIRD zonder remming van het analgetisch effect.
3. Wiskundig modelleren van non-steady state effecten van ademhalingsdepressie door opioiden is niet alleen mogelijk, maar levert ook klinisch bruikbare resultaten op.

Ondanks adequate voorspellingen van een middel op populatie-niveau, levert het model geen goede resultaten bij voorspellingen voor het individu.
4. 'Utility functions' kunnen dienen als wiskundige beschrijving van de ratio werking-bijwerking van een geneesmiddel. De 'utility function' van fentanyl is voornamelijk negatief, behoudens bij lage doseringen, implicerend dat voor de geteste doseringsintervallen de kans op een bepaalde mate van ademhalingsdepressie de kans op eenzelfde mate van analgesie overtreft. Alhoewel deze beschrijving toepasbaar lijkt in experimentele en fase I/II/III settings, heeft de toepassing in de kliniek nader onderzoek nodig.
5. De Anesthesia & Pain Research Unit is speciaal geschikt voor het bestuderen van het effect van experimentele geneesmiddelen op ademhaling en analgesie. Een voorbeeld van een dergelijk middel is MR30365/07, een opioïd werkend op alle drie klassieke opioid-receptoren. In tegenstelling tot fentanyl produceert dit middel een plafond-effect in ademhalingsdepressie maar niet in analgesie over het gemeten doseringsinterval.
6. Fentanyl-geinduceerde pijnstilling wordt beinvloed door het circadiane ritme met een toegenomen effectiviteit tijdens de late uren van de middag. Of zulke effecten van belang zijn in klinische omstandigheden blijft vooralsnog onduidelijk.

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CURRICULUM VITAE

Merel (Maria Catharina Anna) Boom werd geboren op 25 januari 1983 te Alkmaar. In 2001 behaalde zij in diezelfde stad haar gymnasiumdiploma aan het Murmellius-gymnasium. Direct daarna begon zij aan een studie Geneeskunde aan het Leids Universitair Medisch Centrum (LUMC). Zij onderbrak haar studie in 2004-2005 om zich een jaar lang in te zetten als bestuurslid van de Medische Faculteit der Leidse Studenten (M.F.L.S.) en om de Leiden International Medical Student Conference 2005 (LIMSC) te organiseren. In 2009 rondde zij haar co-schappen en daarmee haar studie Geneeskunde af. Zij was als student-assistent betrokken bij diverse onderzoeken van de afdeling Anesthesiologie. Zo werd haar belangstelling voor deze richting binnen de geneeskunde en het onderzoek al vroeg gewekt. Later liep zij eveneens haar wetenschappelijke stage op de afdeling Anesthesiologie, alwaar zij daarna een vierjarig promotietraject startte onder supervisie van prof. dr. Albert Dahan. Dit onderzoek heeft zij eind 2012 grotendeels afgerond. Zij startte in oktober 2012 met de opleiding tot anesthesioloog aan het LUMC.

SYMBOLS AND ABBREVIATIONS

A	Amplitude
ADHD	Attention Deficit Hyperactivity Disorder
AEC	Area between the Effect-time Curve
Akt	Protein Kinase B
AMPA	α-amino-3-hydroxy-5-methyl-D-aspatate
ATP	Adenosinetriphosphaat
AUC	Area Under the Curve
B	Apneic treshold
BIS	Bispectral Index Monitoring
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
C	Content/Concentration
°C	Degrees of Celsius
C_{APNEA}	Concentration at which apnea occurs
C_{drug}	Concentration of certain drug
CCMO	Committee on Research Involving Human Subjects
Ce(t)	Effect-site concentration at time t
CL	Clearance
CNS	Central Nervous System
CO_2	Carbon dioxide
C_{50}	Concentration causing 50% of drug effect
DAMGO	D-Ala2, N-MePhe4, Gly-ol]-enkephalin
DEF	Dynamic End-tidal Forcing
DOR	δ-opioid receptor
ED_{50}	Dose causing 50% of effect
Emin/Emax	Minimum effect/Maximum effect
f	Frequency
G	Gain
GPCR	G-protein coupled receptor
GTP	Gai-guanosine tri-phosphate
h	Hour
HEK293	Human Embryonic Kidney (293) cells
5-HT	5-hydroxytrptamine
k	Constante
k_{eo}	Blood effect site rate constant
Ki	Concentration of drug necessary to occupy 50% of the receptors
KOR	κ-opioid receptor
LD50	Lethal dose for 50 % of subjects

LUMC	Leiden University Medical Centre
mA	Milli Ampere
MAP	Microtubule-associated protein kinase
ERK	Signal-regulated kinase
min	Minute
MOR	μ-opioid receptor
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
O_2	Oxygen
ODT	O-desmethyltramadol
OIRD	Opioid Induced Respiratory Depression
P	Partial pressure
P	Probability
PCO_2	Carbon dioxide concentration
PO_2	Concentration of oxygen
PET	Positron Emission Tomography
PD	Pharmacodynamics
PDE	Phosphodiesterase
PK	Pharmacokinetics
Q	Cardiac Output
R^2	Coefficient of determination
s	Second
SCN	Suprachiasmatic nucleus
SEE	Standard Error of the Estimate
SpO2	Saturation
t½ke0	Blood-effect-site equilibration half-life
Tcp	Target Plasma Concentration
TLR	Toll-like receptor
t	Time
t½	Half life
U	Utility of drug effect
$\dot{V}$	Ventilation
V	Volume
V_{alv}	Alveolair volume
$\dot{V}_{ts}$	Tissue volume
V_{CO2}	Carbon dioxide production
VAS	Visual Analogue Scale
VPC	Visual Predictive Check
WT	Weight

γ	Shape parameter
λ	Solubility coefficient
φ	Phase shift
ρ	Measure of potency
σ^2	Variance of the residual error
τ	Delay / time constant
ω^2	Inter-subject variability

&:η	Shrinkage of empirical Bayesian estimates to the population values
#:ε	Shrinkage of model output to the observations
(+) or (-)	Stereo-isomers
$\in$	Residual error