Opioid therapy : a trade-off between opioid-analgesia and opioid-induced respiratory depression

Boom, M.C.A.

CHAPTER 2

NON ANALGESIC EFFECTS OF OPIOIDS: OPIOID-INDUCED RESPIRATORY DEPRESSION

Review

Merel Boom MD, Marieke Niesters MD MSc, Elise Sarton MD PhD, Leon Aarts MD PhD, Terry W. Smith PhD Albert Dahan MD PhD

Current Pharmaceutical Design 2012; 18(37): 5994-6004

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]

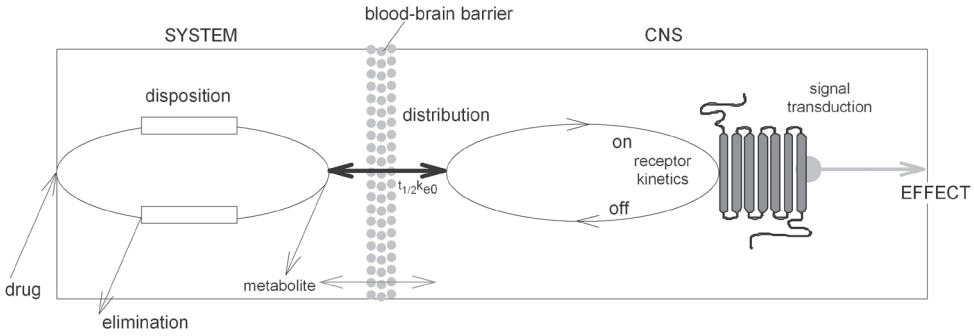

Figure 1. Schematic diagram of the pharmacokinetics of an opioid analgesic in the body (system) and central nervous system (CNS) compartments. After disposition part of the drug reaches the brain compartment after passage across the blood-brain barrier (with half-life t½ke0). Next the drug will distribute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

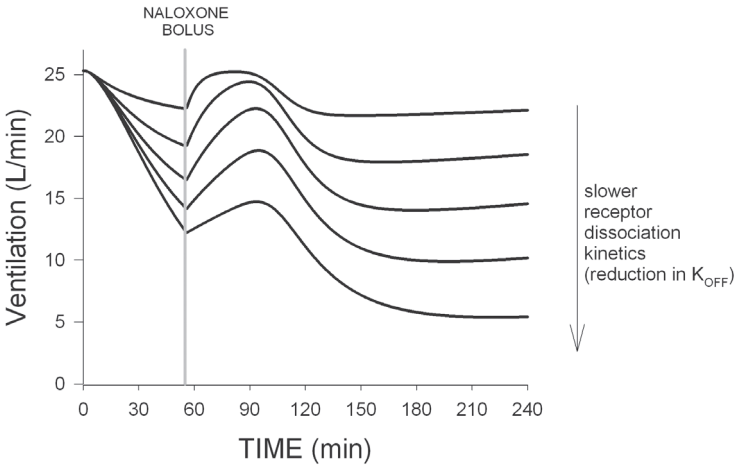

Figure 2. Effect of slowing of receptor dissociation kinetics on the rate of reversal of opioid-induced respiratory depression by a single dose of naloxone. Simulation data adapted from ref [32]. With slower the dissociation, the rate of reversal slows. At t = 0 an opioid dose is given; at t = 55 min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

REFERENCES

1.. Dahan A, Aarts L, Smith TW. Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 2010; 112: 226-38.
2. Taylor S, Kirton OC, Staff I, Kozol RA. Postoperative day one: a high risk period for respiratory events. *Am J Surg* 2005; 190: 752-6.
3. [3] Lötsch J, Dudziak R, Freynhagen R, Marschner J, Geisslinger G. Fatal respiratory depression after multiple intravenous morphine injections. *Clin Pharmacokinet* 2006; 45: 1051-60.
4. Overdyk FJ. Postoperative opioids remain a serious patient safety threat. *Anesthesiology* 2010; 113: 259-60.
5. Leino K, Mildh L, Lertola K, Seppälä T, Kirvelä O. Time course of changes in breathing pattern in morphine- and oxycodone-induced respiratory depression. *Anaesthesia* 1999; 54: 835-840.
6. Pattinson KTS. Opioids and the control of respiration. *Br J Anaesth* 2008; 100: 747-58.
7. Montandon G, Qin W, Ren J, Greer JJ, Horner RL. PreBötzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression. J Neurosci 2011; 31: 1291-301.
8. Martin WR. Opioid antagonists. *Pharmacol Rev* 1967; 19: 463-521.
9. Meunier JC, Mollereau C, Toll L, et al. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* 1995; 377:532-5.
10. Tegeder I, Geisslinger G. Opioids as modulators of

cell death and survival – unraveling mechanisms and revealing new indications. *Pharmacol Rev* 2004; 56: 351-69.

11. Chen YL, Law P-Y, Loh HH. The other side of the opioid story: modulation of cell growth and survival signaling. *Curr Med Chem* 2008; 15: 772-8.
12. Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. *Ann Rev Biochem* 2004; 73: 953-90.
13. Jessell TM, Kelly DD. In: Kandel ER, Schwartz JH, Jessell TM, Eds. *Principles of Neural Science*. 3rd Edition Prentice-Hall International Inc. 1991; pp. 385-99.
14. Dahan A, Sarton E, Teppema L, et al, Anesthetic potency and influence of morphine and sevoflurane on respiration in μ-opioid receptor knockout mice. *Anesthesiology* 2001; 94: 824-32.
15. Romberg R, Sarton E, Teppema L, Matthes H, Kieffer B, Dahan A. No difference between morphine and morphine-6-glucuronide on respiration in μ-opioid receptor- deficient mice. *Br J Anaesth* 2003; 91: 862-70.
16. Aldrich JV, Vigil-Cruz SC. Narcotic analgesics. In: Abraham DJ Ed. Burger's Medicinal Chemistry and *Drug Discovery* Vol 6. Hoboken: Wiley, 2003: 329-481.
17. Corbett AD, Henderson G, McKnight AT, Paterson SJ. 75 years of opioid research: the exciting but vain conquest for the Holy Grail. *Br J Pharmacol* 2006; 147 Suppl 1: S153-62.
18. Pfeiffer A, Brantl V, Herz A, Emrich HM. Pyschotomimesis mediated by kappa opiate receptors. *Science* 1986; 233: 774-6.
19. Barber A, Gottschlich R. Novel developments with selective, non-peptidic kappa-opioid receptor agonists. *Exp Opin Investig Drugs* 1997; 6: 1351-68.
20. Coop A, Rice KC. Role of δ-opioid receptors in biological processes. *Drug News Perspect* 2000; 13: 481-7.
21. Aldrich JV, McLaughlin JP. Peptide kappa opioid receptor ligands: potential for drug development. *AAPS* 2009; 11: 312-22.
22. Ballet S, Pietsch M, Abell AD. Multiple ligands in opioid research. *Protein Peptide Lett* 2008; 15: 668-82.
23. Freye E, Schenk G. Prolonged mu- and delta-receptor occupancy may result in naloxone-reversible respiratory depression and naloxone-irreversible antinociception. Prog Clin Biol Res 1990; 328: 379-84.
24. Ling GSF, Spiegal K, Lockhart SH, Paternal GW. Separation of opioid analgesia from respiratory depression: evidence for different receptor mechanisms. *J Pharm Exp Ther* 1985; 232:144-55
25. Kilpatrick GH, Smith TW. Morphine-6-glucuronide: actions and mechanisms. *Med Res Rev* 2005; 5: 521-44.
26. Hanna MH, Elliott KM, Fung M. Randomized, double-blind study of the analgesic efficacy of morphine-6-glucuronide versus morphine sulfate for postoperative pain in major surgery. *Anesthesiology* 2005; 102: 815-21.
27. Thompson PI, Joel SP, John L, Wedzicha JA, Maclean M, Slevin ML. Respiratory depression following morphine and morphine-6-glucuronide in normal subjects. *Br J Clin Pharmacol* 1995; 40: 145-52.
28. Romberg R, Olofsen E, Sarton E, Teppema L, Dahan A. Pharmacodynamic effect of morphine-6-glucuronide versus morphine on hypoxic and hypercapnic breathing in healthy volunteers. *Anesthesiology* 2003; 99: 788-98.
29. Hucks D, Thompson PI, McLoughlin L, et al, Explanation at the opioid receptor level for differing toxicity of morphine and morphine-6-glucuride. *Br J Cancer* 1992; 65: 122-126.
30. Alexander SPH, Mathie A, Peters JA. Guide to receptors and channels (GRAC). 3rd edn. *Br J Pharmacol* 2008; 153 Suppl 2, S1-209.
31. Lötsch J. Pharmacokinetic-pharmacodynamic modeling of opioids. *J Pain Symptom Manage* 2005; 29 Suppl 5: S90-103.
32. Olofsen E, van Dorp E, Teppema L, et al. Naloxone reversal of morphine- and morphine-6-glucuronide-induced respiratory depression in healthy volunteers: A mechanism-based pharmacokinetic-pharmacodynamic modeling study. *Anesthesiology* 2010; 112: 1417-27.
33. Boas RA, Villiger JW. Clinical actions of fentanyl and buprenorphine. The significance of receptor

binding. *Br J Anaesth* 1985; 57: 192-6.

34. Yassen A, Olofsen E, Romberg R, et al. Mechanism-based PK/PD modeling of the respiratory depressant effects of buprenorphine and fentanyl in healthy volunteers. *Clin Pharmacol Ther* 2007; 81: 50-8.
35. Dahan A, Yassen A, Bijl H, et al. Comparison of the respiratory effects of intravenous buprenorphine and fentanyl in humans and rats. *Br J Anaesth* 2005; 94: 825-34.
36. Lötsch J, Skarke C, Schmidt H, Grösch S, Geisslinger G. The transfer half-life of morphine-6-glucuronide from plasma to effect site assesssed by pupil size measurement in healthy volunteers. *Anesthesiology* 2001; 95: 1329-38.
37. Carrupt PA, Testa B, Bechalany A, et al. Morphine-6-glucuronide and morphine-3-glucuronide as molecular chameleons with unexpected lipophilicity. *J Med Chem* 1991; 34: 1272-5.
38. Cowan A, Doxey JC, Harry EJ. The animal pharmacology of buprenorphine, an oripavine analgesic agent. *Br J Pharmacol* 1977; 60: 547-54.
39. Walsh SL, Preston KL, Stitzer ML, Cone EJ, Bigelow GE. Clinical pharmacology of buprenorphine: ceiling effects at high doses. *Clin Pharmacol Ther* 1994; 55: 569-80.
40. Chevillard L, Mégarbane B, Risède P, Baud FJ. Characteristics and comparative severity of respiratory response to toxic doses of fentanyl, methadone, morphine and buprenorphine in rats. *Toxicol Lett* 2009; 191: 327-40.
41. Budd K. High dose buprenorphine for post-operative analgesia. *Anaesthesia* 1981; 36: 900-3.
42. Christoph T, Kögel B, Schiene K, Méen M, De Vry J, Friderichs E. Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. *Eur J Pharmacol* 2005; 507: 87-98.
43. Budd K, Collett BJ. Old dog – new (ma)trix (editorial). *Br J Anaesth* 2003; 90: 722-4.
44. Tantucci C, Paoletti F, Bruni B, et al. Acute respiratory effects of sublingual buprenorphine: comparison with intramuscular morphine. *Int J Clin Pharmacol Ther Toxicol* 1992; 30: 202-7.
45. Pergolizzi J, Aloisi AM, Dahan A. et al. Current knowledge of buprenorphine and its unique pharmacological profile. *Pain Pract*. 2008; 8: 287-313.
46. Villiger JW, Taylor KM. Buprenorphine: characteristics of binding sites in the rat central nervous system. *Life Sci* 1981; 29: 2699-708.
47. Toll L, Berzetei-Gurske IP, Polgar WE, et al. Standard binding and functional assays relating to medications development division testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res Monogr* 1998;178: 440-66.
48. McCormack K, Prather P, Chapleo C. Some new insights into the effects of opioids in phasic and tonic nociceptive tests. *Pain* 1998; 78: 79-98.
49. Koppert W, Ihmsen H, Körber N, et al. Different profiles of buprenorphine-induced analgesia and antihyperalgesia in a human pain model. *Pain* 2005;118: 15-22.
50. le Roux PJ, Coetzee JF. Tramadol today. *Curr Opin Anesthesiol* 2000 ; 13 : 457-61.
51. Budd K. The role of tramadol in acute pain management. *Acute Pain* 1999; 4: 189-96.
52. Budd K, Langford R. Tramadol revisited. *Br J Anaesth* 1999; 82: 493-5.
53. Raffa RB, Friderichs E, Reimann W, Shank RP, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an "atypical" opioid analgesic. *J Pharmacol Exp Ther* 1992; 260: 275-85.
54. Dayer P, Collart L, Desmeules J. The pharmacology of tramadol. *Drugs* 1994; 47: 3-7.
55. Raffa RB. A novel approach to the pharmacology of analgesics. *Am J Med* 1996; 101: 40S-6S.
56. Raffa RB, Friderichs E, Reimann W, et al. Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. J *Pharmacol Exp Ther* 1993; 267: 331-40.
57. Ide S, Minami M, Ishihara K, Uhl GR, Sora I, Ikeda K. Mu opioid receptor-dependent and independent components in effects of tramadol. *Neuropharmacology* 2006; 51: 651-8.
58. Stamer UM, Stüber F, Muders T, Musshoff F. Respiratory depression with tramadol in a

patient with renal impairment and CYP2D6 gene duplication. *Anesth Analg* 2008; 107: 926-9.

59. Nossamen VE, Ramadhyani R, Kadowitz PJ, Nossamen BD. Advances in perioperative pain management: use of medications with dual analgesic mechanisms, tramadol and tapentadol. *Anesthesiol Clin* 2010; 28: 647-66.
60. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nature Neurosci* 2007; 10: 1361-8
61. Watkins LR, Hutchinson MR, Rice KC, Maier SV. The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. *Trends Pharmacol Sci* 2009; 30: 581-591.
62. Chao CC, Hu S, Shark KB, Sheng WS, Gekker G, Petersen PK. Activation of mu opioid receptors inhibits microglial cell chemotaxis. J *Pharmacol Exp Ther* 1997; 281: 998 – 1004.
63. Cao H, Zhang YQ. Spinal glial activation contributes to pathological pain states. *Neurosci Biobehav Rev* 2008; 32: 972-983.
64. Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC. Fractaline (CX3CL1) and fractaline receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. *Eur J Neurosci* 2004; 20: 1150-60.
65. McGaraughty S, Chu KL, Namovic MT, et al. P2X7-related modulation of pathological nociception in rats. *Neuroscience* 2007; 146: 1817-28.
66. Watkins LR, Hutchinson MR, Milligan ED, Maier SF. "Listening" and "talking" to neurons: implications of immune activation for pain control and increasing the efficacy of opioids. *Brain Res Rev* 2007; 56: 148-69.
67. Watkins LR, Hutchinson MR, Rice, Maier SF. The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. *Trends Pharmacol Sci* 2009; 30: 581-91.
68. Hutchinson MR, Bland ST, Johnson KW, Rice KC, Maier SF, Watkins LR. Opioid-induced glial activation: Mechanisms of activation and implications for opioid analgesia, dependence and reward. *ScientificWorldJournal* 2007; 7: 98-111.
69. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface toll-like receptors. *Semin Immunol* 2007; 19: 3-10.
70. Hutchinson MR, Zhang Y, Shridhar M, et al. Evidence that opioids may have toll like receptor 4 and MD-2 effects. *Brain Behav Immun* 2010; 24: 83-95.
71. Kim D, Kim MA, Cho IH, et al. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. *J Biol Chem* 2007; 282: 14975-83.
72. Obata K, Katsura H, Miyoshi K, et al. Toll-like receptor 3 contributes to spinal glial activation and tactile allodynia after nerve injury. *J Neurochem* 2008; 105: 2249-59.
73. Angst MS, Clark JD. Opioid-induced hyperalgesia. A qualitative systemic review. *Anesthesiology* 2006; 104: 570-89
74. Chu LF, Angst MS, Clark D. Opioid-induced hyperalgesia in humans. Molecular mechanisms and clinical considerations. *Clin J Pain* 2008; 24: 479-96.
75. Fishbain DA, Cole B, Lewis JE, Gao J, Rosomoff RS. Do opioids induce hyperalgesia in humans? An evidence-based structures review. *Pain Med* 2009; 10: 829-39
76. Wu HE, Thompson J, Sun HS, Terashvill M, Tseng LF. Antianalgesia; stereoselective action of dextro-morphine over levo-morphine on glia in the mouse spinal cord. *J Pharmacol Exp Ther* 2005; 314: 1101-8.
77. Lewis SS, Hutchinson MR, Rezvani N, et al. Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like recptor 4/MD-2 and interleukin 1beta. *Neuroscience* 2010; 165: 569-83.
78. Hülsmann S, Oku Y, Zhang W, Richter DW. Metabolic coupling between glia and neurons is necessary for maintaining respiratory activity in transverse medullary slices of neonatal mouse. *Eur J Neurosci* 2000; 12: 856-62.
79. Huxtable AG, Zwicker JD, Alvares TS, et al. Glia contribute to the purinergic modulation of inspiratory rhythm-generating networks. *J Neurosci*

2010; 30: 3947-58.

80. Erlichman JS, Leiter JC. Glia modulation of the extracellular milieu as a factor in central CO2 chemosensitivity and respiratory control. *J Appl Physiol* 2010; 108: 1803-11.
81. Higashimoto Y, Yamagata Y, Taya S, et al. Systemic inflammation in chronic pulmonary disease and asthma : similarities and differences. *Respirology* 2008; 13: 128-33.
82. Rubini A. IL-6 increases airway resistance in the rat. *Cytokine* 2010; 51: 266-73.
83. Longnecker DE, Grazis PA, Eggars GWN Jr. Naloxone for antagonism of morphine-induced respiratory depression. *Anesth Analg* 1973; 52: 447 – 53.
84. Johnstone RE, Jobes DR, Kennell EM, Behar MG, Smith TC. Reversal of morphine anesthesia with naloxone. *Anesthesiology* 1974; 41: 361-7.
85. van Dorp E, Yassen A, Sarton E, et al. Naloxone-reversal of buprenorphine-induced respiratory depression. *Anesthesiology* 2006; 105: 51-7.
86. Cassel JA, Daubert JD, DeHaven RN. Alvimopan binding to the micro opioid receptor: comparative binding kinetics of opioid antagonists. *Eur J Pharmacol*. 2005; 520: 29-36.
87. Yassen A, Olofsen E, van Dorp E, et al. Mechanism-based pharmacokinetic-pharmacodynamic modeling of the reversal of buprenorphine-induced respiratory depression by naloxone. *Clin Pharmacokinet* 2007; 46: 966-80.
88. Amin HM, Sopchak AM, Esposito BF, et al. Naloxone-induced and spontaneous reversal of depressed ventilatory responses to hypoxia during and after continuous infusion of remifentanil or alfentanil. *J Pharm Exp Ther* 1995; 274: 34-9.
89. Takahashi M, Sugiyama K, Hori M, Chiba S, Kusaka K. Naloxone reversal of opioid anesthesia revisited: clinical evaluation and plasma concentration analysis of continuous naloxone infusion after anesthesia with high-dose fentanyl. *J Anesth* 2004; 18: 1–8.
90. Gal TJ. Naloxone reversal of buprenorphine-induced respiratory depression. *Clin Pharmacol Ther.* 1989; 45: 66–71.
91. McGilliard KL, Takemori AE. Antagonism by naloxone of narcotic induced respiratory depression and analgesia. *J Pharmacol Exp Ther* 1978; 207: 494-503.
92. Vaidehi N, Kenakin T. The role of conformational ensembles of seven transmembrane receptors in functional selectivity. *Curr Opin Pharmaol* 2010; 10: 775-81.
93. Sanchez-Blazquez P, Gomez-Serranillos P, Garzon J. Agonists determine the pattern of G-protein activation in μ-opioid receptor-mediated supraspinal analgesia. *Brain Res Bull* 2001; 54: 229-35.
94. Piñeyro G, Archer-Lahlou E. Ligand-specific receptor states: implications for opiate receptor signaling and regulation. *Cell Signal* 2007; 19: 8-19.
95. Hutchinson MR, Zhang Y, Brown K, et al. Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). *Eur J Neurosci* 2008; 28: 20-9.
96. Kim H-S, Suh Y-H. Minocycline and neurodegenerative diseases. *Behav Brain Res* 2009; 196: 168-79
97. Smith KS, Leyden JJ. Safety of doxycycline and minocycline: a systematic review. *Clin Ther* 2005; 27: 1329-42.
98. Converse AK, Larsen EC, Engle JW, Barnhart TE, Nickles RJ, Duncan ID. 11C-(R)-PK11195 PET imaging of microglial activation and response to minocycline in zymosan-treated rats. *J Nucl Med* 2011; epub ahead of print.
99. Hutchinson MR, Northcutt AL, Chao LW, et al. minocycline suppresses morphine-induced respiratory depression, suppresses morphine-induced reward, and enhances systemic morphine-induced analgesia. *Brain Behav Immun* 2008; 22: 1248-56.
100. Morgado C, Pereira-Terra P, Cruz CD, Tavares I. Minocycline completely reverses mechanical hyperalgesia in diabetic rats through microglia-induced changes in the expression of potassium chloride co-transporter 2 (KCC2) at the spinal cord.

Diabetes Obes Metab 2011; 13: 150-9.

101. Ledeboer A, Hutchinson MR, Watkins LR, Johnson KW. Ibudilast (AV-411): a new class therapeutic candidate for neuropathic pain and opioid withdrawal systems. *Expert Opin Investig Drugs* 2007; 16: 935-50.
102. Rolan P, Hutchinson M, Johnson K. Ibudilast: a review of its pharmacology, efficacy and safety in respiratory and neurological disease. *Expert Opin Pharmacother* 2009; 10: 2897-904.
103. Hutchinson MR, Lewis SS, Coats BD, et al. reduction of opioid-withdrawal and potentiation of acute ioioid analgesia by systemic AV411 (ibudilast). *Brain Behav Immun* 2009; 23: 240-50.
104. Richter DW, Manzke T, Wilken B, Ponimaskin E. Serotonin receptors: guardians of stable breathing. *Trends Molec Med* 2003; 12: 542-8.
105. Sahibzada N, Ferreira M, Wasserman AM, Taveira-Dasilva AM, Gillis RA. Reversal of morphine-induced apnea in the anaesthetised rat by drugs that activate 5-hydroxytryptamine$_{1A}$ receptors. J *Pharmacol Exp Ther* 2000; 292: 704-13.
106. Guenther U, Manzke T, Wrigge H, et al. The counteraction of opioid-induced ventilatory depression by the serotonin$_{1A}$ antagonist 8-OH-DPAT does not antagonize antinociception in rats in situ and in vivo. *Anesth Analg* 2009; 108: 1169-76.
107. Dutschmann M, Waki H, Manzke T, et al. The potency of different serotergic agonists in counteracting opioid evoked cardiorespiratory disturbances. *Phil Trans R Soc B* 2009; 364: 2611-23.
108. Wilken B, Lalley P, Bischoff AM, et al. Treatment of apneustic respiratory disturbance with a serotonin-receptor agonist. *J Pediatr 1997;* 130: 89-94.
109. El-Khatib MF, Kiwan RA, Jameleddine GW. Buspirone treatment for apneustic breathing in brain stem infarct. *Respir Care* 2003; 48: 956-8.
110. Oertel BG, Scheider A, Rohrbacher M, et al. The partial 5-hydroxytryptamine$_{1A}$ receptor agonist buspirone does not antagonise morphine-induced respiratory depression in humans. *Clin Pharmacol Ther* 2007; 81: 59-68.
111. Guenther U, Bischoff A, Kettler D, Richter DW. 5-HT$_{1A}$-agonists protect against opioidergic depression of respiration. In: Urban BW, Barann
112. M, Eds. M*olecular and basic mechanisms of anesthesia*. D-49525 Lengerich: Papst Science Publishers 2001; pp. 400-3.
113. Guenther U, Wrigge H, Theuerkauf N, et al. Repinotan, a selective 5-HT$_{1A}$-R-agonist, antagonizes morphine-induced ventilatory depression in anesthetized rats. *Anesth Analg* 2010; 111: 901-7
114. Calabrese EJ. Horesis: basic, generalizable, central to toxicology and a method to improve risk-assessment process. *Int J Occup Environ Health* 2004; 10: 466-7.
115. Manzke T, Dutschmann M, Schlaf G, et al. serotonin targets inhibitory synapses to induce modulation of network functions. *Phil Trans R Sob B* 2009; 364: 2589-602.
116. Manzke T, Niebert M, Koch UR, et al. Serotonin receptor 1A-modulated phosphorylation of glycine receptor α3 controls the breathing in mice. *J Clin Invest* 2010; 11: 4118-28.
117. Manzke T, Guenther U, Ponimaskin EG, et al. 5-HT$_{4(a)}$ receptors avert opioid-induced breathing depression without loss of analgesia. *Science* 2003; 301: 226-9.
118. Manzke T, Preusse S, Richter DW. Developmental changes of serotonin$_{4(a)}$ recepror expression in the rat pre-Bötzinger complex. *J Comp Neurol* 2008; 506: 775-90.
119. Meyer LCR, Fuller A, Mitchell D. Zacopride and 8-OH-DPAT reverse oioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. *Am J Physiol Regul Integr Comp Physiol* 2006; 290: R405-13.
120. Lötsch J, Skarke C, Schneider A, Hummel T, Geisslinger G. The 5-hydroxy-tryptamine 4 recptor agaonist mosapride does not antagonize morphine-induced respiratory depression. *Clin Pharmacol ther* 2005; 78: 278-87.
121. Irving HR, Tochon-Danguy N, Chinkwo KA, et al. Investigations into the binding affinities of different human 5-HT4 receptor splice variants. *Pharmacology*

2010; 85: 224-33.

122. Agosti RM. 5HT1F- and 5HT7-receptor agonists for the treatment of migraines. *CNS Neurol Disord Drug Targets* 2007; 6: 235-7.
123. Adham N, Zgombick JM, Bard J, Branchek TA. Functional characterization of the recombinant human 5-hydroxytryptamine$_{7(a)}$ receptor isoform coupled to adenylate cyclase stimulation. *J Pharmacol Exp Ther* 1998; 508-14.
124. Lynch G. Glutamate-based therapeutic approaches: AMPAKINES. Curr Opin Pharmacol 2006; 6: 82-88
125. Greer JJ, Smith JC, Feldman JL. The role of excitatory amino acids in the generation and transmission of respiratory drive in the neonatal rat. *J Physiol* 1991; 437: 727-49.
126. Arai AC, Xia YF, Suzuki E. Modulation of AMPA receptor kinetics differentially influences synaptic plasticity in the hippocampus. *Neurosci* 2004; 123: 1011-24.
127. Ren J, Ding X, Funk GD, Greer JJ. Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthiology* 2009; 110: 1364-70.
128. Greer JJ, Ren J. Ampakine therapy to counter fentanyl-induced respiratory depression. *Resp Physiol Neurobiol* 2009; 168: 153-7.
129. Oertel BG, Felden L, Tran PV, et al. Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. *Clin Pharmacol Ther* 2010; 87: 204-11.
130. Bhatia J Current options in the management of apnea of prematurity. *Clin Pediatr* 2000; 39: 327-36.
131. Brockhaus J, Ballanyi K. Anticonvulsant adenosine A1 receptor-mediated adenosine action on neuronal networks in the brainstem-spinal cord of newborn rats. *Neuroscience* 2000; 96: 359-71.
132. Fredholm BB, Bättig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999; 51: 83-133.
133. Ruangkittisakul A, Ballanyi K. Methylxanthine reversal of opioid-evoked inspiratory depression via phosphodiesterase-4 blockade. *Resp Physiol Neurobiol* 2010; 94-105.