Review Article

Opioid Tolerance Development: A Pharmacokinetic/Pharmacodynamic Perspective

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Abstract. The opioids are commonly used to treat acute and severe pain. Long-term opioid administration eventually reaches a dose ceiling that is attributable to the rapid onset of analgesic tolerance coupled with the slow development of tolerance to the untoward side effects of respiratory depression, nausea and decreased gastrointestinal motility. The need for effective-long term analgesia remains. In order to develop new therapeutics and novel strategies for use of current analgesics, the processes that mediate tolerance must be understood. This review highlights potential pharmacokinetic (changes in metabolite production, metabolizing enzyme expression, and transporter function) and pharmacodynamic (receptor type, location and functionality; alterations in signaling pathways and cross-tolerance) aspects of opioid tolerance development, and presents several pharmacodynamic modeling strategies that have been used to characterize time-dependent attenuation of opioid analgesia.

KEY WORDS: opioid; pharmacodynamics; pharmacokinetics; tolerance.

INTRODUCTION

Opioid analgesics are commonly used to treat acute and chronic pain associated with surgical interventions or disease states such as cancer. Opioids produce a diverse spectrum of centrally- and peripherally-mediated responses, including respiratory depression, nausea, sedation, euphoria or dysphoria, decreased gastrointestinal motility, and itching (1). Long-term use of opioids can be problematic due to the rapid development of profound tolerance to the analgesic effects coupled with slow development of tolerance to many of the untoward effects of these agents. It is the inability to tolerate these undesirable side effects that eventually limits dose escalations and analgesic efficacy.

The World Health Organization (WHO) has recommended adoption of a three-step "analgesic ladder" to meet the therapeutic challenges presented by opioid tolerance. In this approach, analgesic therapy is initiated with a non-opioid analgesic co-administered, if necessary, with an adjuvant. As the underlying condition progresses and pain becomes more intractable, a weak opioid is substituted for the non-opioid. Eventually, a strong opioid is introduced as a final step (2). While these recommendations are designed to improve quality of life for chronic pain sufferers, the underlying problem of opioid tolerance persists, and the clear and compelling need for effective long-term analgesics, or for effective strategies for applying existing analgesics to control chronic pain, remains. This goal may be achieved through the specific design and development of new agents that are not subject to tolerance development, or through the use of adjunct treatments that can minimize or reverse tolerance. In either case, a comprehensive understanding of the mechanisms underlying tolerance, as well as the kinetics of tolerance development and regeneration of responsivity, is required.

The purpose of this paper is to review the pharmacokinetic and pharmacodynamic mechanisms involved in the development of opioid tolerance. Pharmacokinetic considerations such as the production of metabolites, alterations in metabolic enzyme activity, and modulation of drug transporter expression or function are addressed. Pharmacodynamic processes such as receptor binding type and location, alterations in signal transduction, and cross-tolerance also are considered. Finally, approaches to comprehensive pharmacokinetic and pharmacodynamic (PK-PD) modeling of opioid antinociception and tolerance development are discussed.

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ABBREVIATIONS: AC, adenylyl cyclase; BBB, blood brain barrier; CREB, cAMP-responsive element binding protein; CYP, cytochrome P450; DOR, δ-opioid receptor; GABA, γ-amino-butyric acid; Glu, glutamate; GPCR, G-protein coupled receptor; GRK, G-protein receptor kinase; hPXR, human pregnane X receptor; KOR, κ-opioid receptor; $K_{p,brain}$, brain-to-serum ratio; L-Arg, L-arginine; L-Cit, L-citrulline; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; NMDA, N-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NR, NMDA receptor; OR, opioid receptors; PD, pharmacodynamic; P-gp, P-glycoprotein; PK, pharmacokinetic; PKA, protein kinase A; PSD-95, post-synaptic density complex; sGC, soluble guanylyl cyclase; SNP, single nucleotide polymorphism; UGT, uridine-5′-diphosphate-glucuronosyltranserase.

ETIOLOGY OF TOLERANCE

Tolerance is defined as a decrease in pharmacologic response following repeated or prolonged drug administration. Tolerance can be separated into two main classifications: innate or acquired. Innate tolerance is a predisposition to exhibit drug sensitivity or insensitivity due to pharmacogenetic makeup. In most situations, innate tolerance is determined upon administration of the initial dose. In contrast, acquired tolerance is a consequence of repeated drug exposure, and can be subdivided into three general types based on the prevailing mechanism: pharmacokinetic, pharmacodynamic, or learned. Pharmacokinetic tolerance occurs when drug disposition or metabolism is altered as a function of time, often a consequence of the drug being an inducer or inhibitor of a specific metabolic enzyme or transporter system, resulting in a time-dependent decrease in presentation of the active moiety to the receptor biophase. Pharmacodynamic tolerance occurs when the intrinsic responsivity of the receptor system diminishes over time.

Acute tolerance is mediated predominantly by pharmacodynamic mechanisms, manifested as a decreased response following a single administration of the agent or during repeat-dosing but over a short time frame. This phenomenon is exemplified by nasally-administered cocaine. Initially, the relationship between cocaine-associated euphoria and blood cocaine concentrations is proportionate. However, at later points in time, the euphoric response decreases despite continued, or even increased, circulating concentration (3). In contrast to acute tolerance, chronic tolerance can be mediated through either pharmacokinetic or pharmacodynamic mechanisms, with an end result of a long-term decrease in drug response in the face of constant systemic exposure. In cases in which chronic tolerance develops, cross-tolerance within the pharmacologic class also may occur. Replacing the initial drug with a comparable agent will result in a lower pharmacologic response compared to that experienced by a drug-naive individual. Cross-tolerance is the principle underlying methadone substitution in the treatment of heroin addicts. Although methadone produces opioid effects in heroin-addicted subjects, euphoria and side effects are minimized by the tolerance produced by long-term heroin exposure. The significance of pharmacokinetic and pharmacodynamic mechanisms in relation to tolerance development will be examined in depth in subsequent sections of this review.

The final class of acquired tolerance is attributed to learning, either behavioral or conditioned. Behavioral tolerance occurs when an individual learns to function despite repeat exposure to a drug. Chronic alcohol abusers, for example, may not exhibit an outward appearance of motor impairment as a consequence of intoxication because of learned motor function adaptations and awareness of their impairment. Conditioned tolerance follows Pavlovian principles in which situational cues are associated with drug administration. Removal of these environmental cues will result in an enhancement in pharmacologic effect. For example, when morphine-tolerant rats are placed in a novel environment and challenged with morphine, antinociceptive tolerance is reduced (4). Analysis of behavioral tolerance has been reviewed in detail elsewhere (5).

MORPHINE AND RELATED AGONISTS

The medicinal value of opium has been acknowledged for centuries. Despite possessing an extensive side-effect profile, morphine, isolated from opium more than 200 years ago, remains the gold standard for treating pain. Structurally similar, semisynthetic morphine-like derivatives as well as structurally distinct opioids have been synthesized in a search for compounds that improve analgesia and minimize side effects. The common, clinically-used opioid analgesics are semisynthetic morphine derivatives, as well as the synthetic phenylpiperidine, anilidopiperidine, and diphenylpropylamine derivatives (Table I). Due to the structural similarities between these drugs and morphine, they primarily bind to the same receptor subtypes (μ-opioid receptors), produce analgesia, and exhibit similar side effect profiles. However, divergence in congener pharmacokinetic–pharmacodynamic parameter values allows the clinician to select a drug based on specific patient needs (1). For example, an ideal drug for a short surgical procedure would have rapid onset and offset of effect, such as the piperidine derivative alfentanil. Overall, opioid selection depends on a number of factors, including the nature of pain, predisposition to opioid responsivity, and previous opioid exposure.

PHARMACOKINETIC MEDIATORS OF OPIOID TOLERANCE

Receptor occupancy theory holds that pharmacologic response will be proportional to the fraction of the target receptor population that is occupied at a particular drug concentration (6). As the drug concentration in the vicinity of the receptor increases, the likelihood of drug binding to the

Table I. Categorization of the Common Opioids Based on Structural Similarities

Common opioids
Semisynthetic derivatives
Morphine-related agonists
Hydromorphone
Hydrocodone
Oxycodone
Oxymorphone
Codeine
Morphine-related partial agonists and antagonists
Buprenorphine
Naloxone
Naltrexone
Synthetic derivatives
Phenylpiperidines
Meperidine
Loperamide
Diphenylpropylamines
Methadone
Propoxyphene
Piperidines
Fentanyl
Alfentanil
Sufentanil
Remifentanil

receptor and producing an effect also increases. Gibaldi and Levy (7) applied this principle in describing the sigmoidal relationship between tubocurarine-mediated paralysis and dose or blood concentration. For this simplest of pharmacodynamic systems, drug concentrations in the receptor biophase must be in immediate equilibrium with concentrations in blood, and response must be instantaneous, reversible and time-independent. However, the relationship between the magnitude or time course of drug response and the kinetics of drug disposition generally is more complex, evidenced by time-dependent dissociations between pharmacologic response and systemic pharmacokinetics. A variety of factors, including slow equilibration of drug between the target receptor and blood or slowly-elaborated pharmacologic responses post-receptor binding, can cause such kinetic–dynamic dissociation. However, a particularly complicated underlying factor is the development of tolerance.

Metabolic and Distributional Mediators of Tolerance

One source of the extensive variability in drug concentrations, and consequently, response, within a population can be attributed to differences in drug absorption, distribution and metabolism. The opioids undergo significant phase I and II biotransformation, and polymorphisms in the cytochrome P450s (CYPs) and uridine-5′-diphosphate-glucuronosyltranserases (UGTs) will influence individual opioid disposition and response. For example, depending on the allelic combinations of the highly polymorphic CYP2D6, patients are characterized as one of four phenotypes: poor, intermediate, extensive and ultrarapid metabolizers (8). Subjects who exhibit the poor-metabolizer phenotype will not convert codeine to morphine efficiently, and therefore will exhibit a reduced effect compared to patients who can form morphine (9). In addition to the important role these enzymes play in mediating opioid concentrations and responses, it has been suggested that biotransformation processes contribute to morphine tolerance secondary to induced enzymatic expression (10,11). However, there is a lack of evidence to support this observation, as long-term administration of methadone or morphine does not alter the parent drug to metabolite concentration ratios, indicating that autoinduction is unlikely (12,13). Ultimately, metabolite formation could influence tolerance development through pharmacodynamic mechanisms, but there is a lack of evidence supporting time-dependent changes in the formation clearance of opioid metabolites.

In addition to metabolism, drug transport can play a significant role in mediating drug exposure at the target site, and therefore, the time course and magnitude of pharmacologic response. Many of the common opioids are substrates of the efflux transporter P-glycoprotein (P-gp), an ATP-dependant, 170-kDa transmembrane protein encoded by the *MDR1* (human) and *mdr1a/mdr1b* (rodent) genes(s) (14). P-gp is expressed in a number of tissues in which it plays a protective role as a barrier transporter, limiting absorption from the intestines or penetration into organs such as testes or brain, as well as an excretory transport system in the kidney and liver (15). P-gp also functions to modulate the secretion of centrally synthesized opioid peptides and neurotransmitters from the brain to the systemic circulation (16).

Studies with P-gp-deficient mice have revealed that P-gp-mediated efflux attenuates the brain-to-serum ratio ($K_{p,brain}$) and antinociceptive effect of fentanyl, methadone and loperamide by ~2-, 7-, and 45-fold, respectively (17). Considering the extent to which P-gp mediates transport of some opioids, upregulation of this barrier transporter could further limit CNS penetration and antinociceptive effects of some opioids. Indeed, exposing transgenic mice expressing human pregnane X receptor (hPXR) to rifampin, an acute P-gp inhibitor and long-term P-gp inducer, upregulated expression of P-gp in brain capillary endothelium and attenuated the antinociceptive effect of the P-gp substrate methadone by 70% (18). Based on the results of this study, if an opioid is both a substrate and inducer of P-gp, chronic treatment could result in higher blood brain barrier (BBB) P-gp expression, with a consequent decrease in CNS penetration and antinociception (i.e., pharmacokinetic tolerance). Although autoinduction of BBB P-gp remains a possible mechanism underlying opioid tolerance, increases in P-gp expression following chronic treatment with morphine and oxycodone appear to be modest (2- and 1.3-fold, respectively) (19, 20). This minor increase in expression (≤2-fold) coupled with a negligible alteration in function (a 1.4-fold reduction in paclitaxel brain distribution) suggests that opioid-mediated P-gp upregulation, at least in experimental animals, does not influence opioid antinociceptive tolerance to an extent that can be distinguished from population PK-PD variability (19).

Metabolite Contributions to Opioid Response and Tolerance

Tolerance also may result from the production of metabolites that accumulate in the systemic circulation over time, penetrate the site of action, and interfere with pharmacologic response whether by competing with the parent compound for receptor binding (e.g., antagonists or partial agonists) or by down-regulating the responsivity of the receptor system or downstream events. The contribution of derived metabolites to morphine pharmacodynamics has received significant attention. In humans, morphine is metabolized in the liver by UGT2B7 to form morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Although morphine is metabolized preferentially to M3G (~5:1 M3G to M6G formation ratio), this metabolite does not contribute to antinociceptive response. In contrast, M6G is considered to be an unusual Phase II metabolite in that it is biologically active, and in fact appears to be somewhat more potent (2- to 4-fold) than morphine (21).

Some studies have suggested that, rather than contributing to antinociception, M3G attenuates the response to morphine. In this scenario, time-dependent M3G accumulation could be responsible for the appearance of morphine tolerance. For example, Smith and Smith (22) observed an inverse relationship between morphine-associated antinociception and M3G concentrations. However, careful examination of the influence of M3G pretreatment on morphine antinociception in rats revealed that acute M3G exposure, at concentrations similar to those produced by pharmacologically-relevant doses of morphine, does not attenuate antinociception to a significant extent (23,24). In contrast, M3G concentrations that are far in excess of those produced by pharmacologic doses of the parent drug can evoke neuro-

excitatory behavioral responses that oppose the analgesic effects mediated by morphine binding to opioid receptors. Such neuroexcitation would account for the observed M3G-associated decrease in morphine-related antinociception (25). These excitatory responses are independent of the opioid receptor system, as evidenced by the absence of naloxone reversibility, and may be mediated through interactions at the N-methyl-D-aspartate (NMDA) and the γ-amino-butryic acid (GABA$_A$) receptors (25,26). Overall, it appears that M3G may influence antinociception and tolerance, although through a mechanism other than μ-opioid receptor (MOR)-mediated interactions, and it is unlikely that accumulation of this metabolite, as it is formed from morphine, contributes substantially to the development of functional morphine tolerance.

PHARMACODYNAMIC MEDIATORS OF OPIOID TOLERANCE

While inherent inter-individual pharmacokinetic variability can influence analgesia within a population, time-dependent changes in systemic disposition appear to play a minimal role in the development of opioid tolerance. In contrast, a number of pharmacodynamic processes have been characterized as potential mediators of opioid tolerance. These adaptations include, but are not exclusively limited to, genetic predisposition, receptor subtypes, cross-tolerance, receptor affinity, alterations in secondary mediators such as the nitric oxide synthase pathway and transcription alterations, and receptor binding in the central *versus* the peripheral nervous system.

Mu-Opioid Receptor-Mediated Changes

The biologic effects that are characteristic of opioids are a consequence of agonist binding to opioid receptors with subsequent alterations in signal transduction and ion conductance that reduce neuronal pain transmission. The three major mammalian types of opioid receptors (OR) are designated μ, δ, and κ (27–29). Pharmacologic studies with opioid receptor ligands and receptor knockout mice identified the MOR as the primary mediator of both the therapeutic and side effects associated with opioids such as morphine (30–32). The role of the δ- and κ-opioid receptors in mediating analgesia and tolerance will be discussed more thoroughly in subsequent sections of this review.

The MOR is a seven-transmembrane protein belonging to the G-protein coupled receptor (GPCR) superfamily. As shown in Fig. 1, the binding of an agonist to the MOR results in the replacement of the α-subunit GDP with GTP and the subsequent dissociation of the α- and $\beta\gamma$-subunits from the G$\alpha\beta\gamma$ heterotrimer (33,34). These mechanisms result in acute inhibition of neuronal transmission due to alterations in the conductance of ion channels, the stimulation of protein kinase A (PKA), and the transient inhibition of the adenylyl cyclase (AC) pathway (35). Chronic opioid use enhances the cAMP pathway via the superactivation of AC, alterations in ion channel conductance, an increase in neurotransmitter release, and altered gene expression through changes in cAMP-responsive element binding protein (CREB) phosphorylation and expression (36,37).
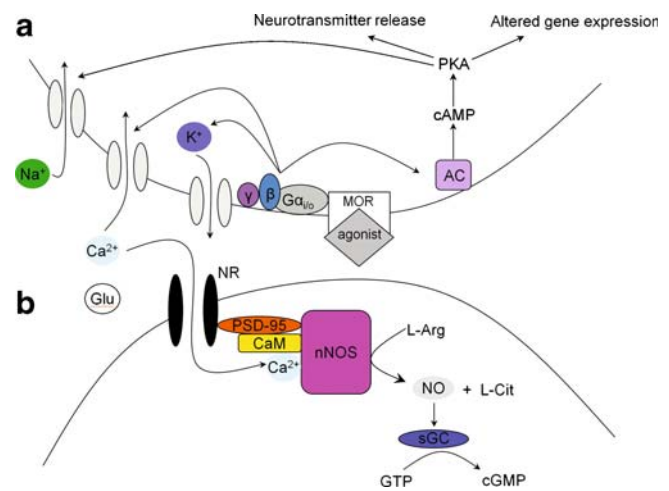

Fig. 1. Downstream mediators of antinociception following opioid agonist binding to the MOR. **a** Binding of the agonist changes ion channel conductance and alters the cAMP pathway. **b** Activation of the NMDA receptor/NO pathway. Chronic opioid exposure alters synaptic cleft ion conductance and glutamate expression, leading to the displacement of the Mg^{2+} block of the NMDA receptor, an influx of Ca^{2+} and conversion of L-Arg to NO, a mediator of tolerance. Administering an NMDA receptor antagonist attenuates and delays the onset of tolerance by blocking Ca^{2+} influx and associated alterations

Overall, antinociception is mediated through a complex sequence of downstream effectors, which can differ following acute *versus* chronic opioid exposure.

In addition to alterations in signaling, opioid tolerance is associated with decreases in opioid receptor sensitivity and expression in the plasma membrane. Following receptor binding of some, but not all (e.g., morphine), opioids, the MOR is phosphorylated by G-protein receptor kinase (GRKs), with opioid efficacy related to the extent of phosphorylation (38,39). Receptor phosphorylation is the first step in the desensitization and internalization of the MOR, and precedes association of β-arrestin to the receptor complex, which uncouples the receptor from G proteins. This uncoupling event desensitizes the receptor and attenuates the second messenger signal cascade, reducing agonist efficacy (40). The role of β-arrestin in receptor desensitization was characterized in β-arrestin KO mice that exhibited enhanced and prolonged analgesia with a lack of antinociceptive tolerance (41,42). β-arrestin also can modulate tolerance by recruiting adaptor proteins that link the receptor complex to clathrin, resulting in endocytosis in clathrin-coated pits. Following internalization, the receptor is retained and recycled to the plasma membrane as a resensitized receptor or, upon recognition of an interaction with the GPCR-associated sorting protein (GASP), the receptor will be sent to the lysosome for degradation (33,43). Agonist binding therefore results in diverse cellular adaptations that mediate antinociception and onset of tolerance.

The mechanisms underlying morphine tolerance appear to be different from those of other opioid agonists. Despite significant and prolonged efforts directed towards understanding morphine tolerance, the specific pathway has remained obscure. In contrast to most opioids, morphine-

receptor binding does not initiate GRK-mediated MOR phosphorylation that results in receptor internalization (40). This observation is further supported by the absence of increased membrane internalization protein expression (e.g. β-arrestin and GRK) that accompanies most opioids (44). Even in the absence of receptor internalization, the interaction between the morphine-bound receptor and β-arrestin persists, evidenced by a reduction in tolerance in β-arrestin KO mice, suggesting that morphine induces tolerance by sustaining signaling pathways and cellular adaptations such as AC superactivation (41,45). An alternative explanation for the reduction in morphine tolerance observed in β-arrestin KO mice is that, although it is not an internalizing opioid, morphine binds to MORs that exist as heterodimers, with the second OR subtype interacting with β-arrestin and the subsequent internalization of the morphine-heterodimer complex contributes to tolerance (46,47). However, the validity of this hypothesis appears unlikely, as the results were not reproducible in later experiments and depended on the co-administration of morphine with an internalizing opioid for dimer endocytosis, and chronic morphine exposure does not change MOR expression (46,48,49).

Another contributor to morphine tolerance is the slow recovery of resensitized MORs because morphine desensitized receptors do not participate in the traditional pathway of internalization, recycling and reinsertion into the plasma membrane (50). Ultimately, the specific molecular mechanisms governing morphine tolerance have not been fully elucidated, and the possibility that mechanisms of morphine tolerance vary depending on the specific brain and spinal cord region requires further investigation (51).

MOR Receptor Polymorphisms

Analgesic variability within a population can, in part, be attributed to opioid pharmacogenetics. The human MOR has over 100 identified single nucleotide polymorphisms (SNPs), some of which are thought to influence opioid responsiveness, abuse potential, and tolerance (52,53). Of these SNPs, the most well-characterized is the A118G nucleotide substitution, which changes the amino-acid sequence at position 40 from asparginine to aspartic acid, eliminating an extracellular N-linked glycosylation site (54,55). Clinically, this variant reduces the analgesic effects of alfentanil and M6G (56,57). These alterations are not likely a consequence of altered binding affinity, as *in vitro* studies demonstrated that the N40D substitution did not affect binding of the exogenous opioids morphine, fentanyl, or methadone, nor that of the endogenous opioids Met- and Leu-enkephalin, endomorphin-1 and-2 and D-Ala^2, N-methyl-Phe^4, Glycol^5]enkephalin (DAMGO) (54). Although this study demonstrated a 3-fold increase in binding affinity of the endogenous opioid peptide β-endorphine, subsequent studies did not detect any difference in β-endorphine binding, or receptor endocytosis, desensitization or resensitization in the N40D substitution (54,58,59). Additional work on human MOR polymorphisms characterized amino acid substitutions of N15D, R265H, and S268P. The latter substitution, located in the intracellular domain, exhibited a significant loss of receptor signaling (58). The human MOR intracellular loop polymorphisms reduced the extent of MOR-calcium calmodulin CaM interactions

(R265H and S268P) and the R260H and R265H variants exhibited a reduction in basal MOR signaling (60).

Despite these detailed studies, the relationship between MOR polymorphisms and binding affinity, signaling pathways and ion channel conductance is not entirely clear. These alterations may, in part, mediate differential development of opioid tolerance within a population. For example, individuals expressing the N40D variant exhibit decreased analgesia, and may require larger opioid doses to achieve equipotent pain relief. It is conceivable that this dose differential could influence the development of tolerance in these subjects compared to normal MOR expressers. Research focused on MOR polymorphisms is continuing, and should shed light on the specific physiologic implications of these variants, including opioid responsivity and tolerance development.

Multiple Opioid Receptors

While less is known about the specific roles of the two remaining ORs, δ and κ, these proteins are expressed throughout the body and mediate antinociception, although to a lesser extent than the MOR (61–64). The κ-opioid receptor (KOR) exhibits tolerance following prolonged opioid exposure, a consequence of GRK-mediated phosphorylation of the KOR that initiates receptor internalization (65,66). While dephosphorylation is the typical mechanism of recovering receptor sensitivity, the KOR appears to require regeneration and reassembly of the receptor complex for restoration of antinociception (67). The net effect of these processes is a reduction in KOR expression that results in a decreased pharmacologic response following long-term opioid exposure. Similar to the KOR, the δ-opioid receptor (DOR) manifests tolerance to specific DOR-agonists via receptor phosphorylation and internalization (68,69). Despite receiving less attention than the MOR, opioid binding to the DOR and KOR also can result in antinociception and tolerance.

Cross-tolerance

Long-term exposure to one drug often results in the development of tolerance to the effects of other structurally similar drugs in the same pharmacologic class. This phenomenon is termed cross-tolerance. Although opioids frequently exhibit cross-tolerance, it is rarely complete, as evidenced by the utility of opioid rotation from morphine to hydromorphone or oxycodone, particularly in treatment of cancer pain (70,71). However, complete cross-tolerance can develop, as evidenced by the lack of analgesia elicited by high-dose morphine in patients receiving methadone maintenance therapy (72). The more common scenario of incomplete cross- tolerance has been attributed to multiple pharmacologic mechanisms. This preservation of effect may be a function of the inverse relationship between intrinsic opioid efficacy and tolerance, as well as that in situations of tolerance, replacing a low efficacy opioid such as morphine with fentanyl, a high-efficacy opioid with a high receptor reserve, will enhance analgesia (73,74). Furthermore, multiplicity in MOR subtypes may play an important role in determining the magnitude of cross-tolerance. For example, when one opioid is substituted for another, the secondary

opioid may bind to a different receptor subtype than the initial compound, thereby limiting the apparent extent of cross-tolerance (75). Alternatively, incomplete cross-tolerance may be attributed to the ability of the opioid receptors to homo- and heterodimerize. For example, substituting an initial opioid with an agonist that binds to a different dimer combination can result in the recovery of analgesia because of incomplete tolerance to the new opioid-receptor combination. (47,76). Further investigation of the interplay between opioid receptors likely will reveal novel mechanisms that mediate antinociception and contribute to opioid tolerance. Clinically, opioid cross-tolerance presents a challenge when a patient switches to a new opioid regimen. Despite this possibility, the lack of complete tolerance generally results in a reduction in pain and fewer side effects compared to the initial, tolerant regimen.

NMDA Receptor Contributions to Opioid Tolerance

In addition to the direct actions of opioids, neurotransmitters such as dopamine, serotonin and glutamine have been implicated as mediators of opioid tolerance and antinociception (77–79). Of particular interest is the N-methyl-D-aspartate (NMDA)-sensitive glutamate receptor (NR). The intertwined relationship between the MOR and NMDA receptor systems is in part attributed to co-localization in central tissues (80,81). Mg^{2+} is bound to the receptor channel of the NMDA receptor under normal physiologic conditions, and serves to block the entrance of ions such as Ca^{2+} (Fig. 1). Small changes in synaptic cleft concentrations of glutamate (Glu) and inorganic ions can temporarily displace Mg^{2+} and allow an influx of Ca^{2+}, which will bind to and activate the neuronal nitric oxide synthase (nNOS), CaM and PSD-95 complex, which converts L-arginine (L-arg) to L-citrulline (L-Cit) and nitric oxide (82,83). Finally, NO mediates the conversion of GTP to cGMP, a mediator of tolerance, via soluble guanylyl cyclase (sGC). Chronic opioid exposure enhances NMDA receptor function by increasing intracellular Ca^{2+}, NO and cGMP levels, which contribute to the development of opioid tolerance. The use of NMDA receptor antagonists, or inhibitors of NOS production from L-arginine to enhance antinociception and delay the onset of tolerance has proven effective (82). Of these approaches, the use of NMDA receptor antagonists has shown preclinical and clinical potential in minimizing opioid tolerance. The potential role of NOS in determining the time course and magnitude of opioid tolerance is considered in detail in the final section of this review.

Initially viewed as a "miracle" drug class, the clinical use of NMDA receptor antagonists has yet to be fully realized. NMDA receptor antagonists are particularly promising drug targets due to a number of modulatory sites with different structural subunit combinations, most commonly the NR1 subunit combined with NR2A-D or NR3A-B subunits (84,85). These modulatory sites result in four general classes of NMDA receptor antagonists: the uncompetitive receptor channel blockers (MK-801, ketamine, PCP, memantine), the competitive glutamate antagonists (LY235959, D-CPPene), the glycine site antagonists [(+)(R)-HA-966], and the polyamine site antagonists (86). Preclinical and clinical models have been used to demonstrate that NMDA receptor antagonists can attenuate tolerance development and enhance antinociception, the latter effect allowing decreases in dose which may delay tolerance onset (87).

The majority of studies characterized the NMDA receptor antagonists as enhancing or having no effect on analgesia, whereas a minority demonstrated attenuation of acute morphine analgesia (88–93). One challenge in this area is the absence of consistent results identifying which opioids exhibit NMDA receptor antagonist-enhanced antinociception. For example, Redwine and Trujillo (94) found that morphine analgesia was enhanced only when co-administered with LY235959 (3 mg/kg); fentanyl analgesia was enhanced in the presence of LY235959 (3 mg/kg), dextromethorphan (30 mg/kg) and (+)(R)-HA-966 (30 mg/kg); and no difference was observed for morphine or fentanyl when co-administered with MK-801 (0.1 and 0.3 mg/kg), memantine (3 and 10 mg/kg), or ifendopril (1 and 3 mg/kg). Additional work clearly is needed to identify the specific interactions between each opioid and NMDA receptor antagonist. For example, NMDA receptor antagonist pharmacokinetics are poorly characterized. However, it is clear that they are capable of penetrating central tissues, evidenced by the appearance of centrally-mediated side effects such as hallucinations, memory impairment, blood pressure elevation, catatonia and anesthesia (95). NMDA receptor antagonists of greater selectivity and lower affinity were developed in response to these undesirable central effects, and promising alternatives may include the use of low affinity channel blockers (e.g., memantine), NR2B subunit selective drugs (ifendopril), or the partial glycine site antagonists (96). Based on these varied observations, further characterization of the underlying pharmacologic mechanisms and pharmacokinetic/pharmacodynamic relationships between the opioids and NMDA receptors remains to be elucidated.

The Role of Peripheral Opioid and NMDA Receptors In Mediating Antinociception

While NMDA receptor antagonists may represent a feasible approach to delay the onset and attenuate the extent of tolerance, these compounds manifest intolerable central side effects that limit use. One alternative strategy that may prove effective in treating pain is targeting non-centrally located NMDA receptors and/or ORs to achieve antinociception while minimizing centrally-mediated side effects and, potentially, the development of tolerance. The feasibility of this approach is, in part, supported by the broad peripheral distribution of these receptors. For example, MORs are present in a number of peripheral tissues, including the small and large intestines, kidney, lung, spleen, testis, ovaries and uterus (97). While these sites are important in mediating a number of physiologic processes, molecular and functional studies suggest that MORs and NMDA receptors localized on unmyelinated, cutaneous sensory axons contribute to antinociception (98). Moreover, the density of ORs and NMDA receptors on peripheral sensory axons increases in conditions of inflammation, suggesting a role in mediating pain perception (99,100). Overall, the physiologic distribution of the ORs and NMDA receptors suggests a role in mediating peripheral antinociception.

The functionality of peripheral ORs was demonstrated when a systemically inactive, subcutaneous dose of morphine

into the rat tail exhibited naloxone-reversible antinociception (101,102). Additional studies identified the ability of locally-administered morphine and loperamide to mediate antinociception in a radiant heat tail-flick assay and in an inflammatory model, respectively (103). Furthermore, repeated immersion of the tail in opioid-containing DMSO solutions resulted in local antinociception and tolerance development, the latter being reversible following topical or systemic administration of the NMDA receptor antagonist MK-801 (104–106). These results suggest that peripherally-restricted opioids contribute to antinociception and tolerance, which may be minimized by co-administration with non-centrally penetrating NMDA receptor antagonists.

PHARMACOKINETIC–PHARMACODYNAMIC MODELS OF OPIOID TOLERANCE

"Black-Box" Approach

Although it is clear that complex cellular, mechanistic and homeostatic alterations contribute to tolerance development, PK-PD models, including those that incorporate tolerance as a time-dependent loss of intrinsic activity, often utilize a black-box approach that accounts for changes in the response-concentration relationship over time rather than incorporating specific biologic mechanisms that serve to drive the development of tolerance. Such empirical models tend to exhibit a broad applicability, are useful in characterizing and summarizing the temporal aspects of loss of drug response, and can be used to describe numerous tolerance scenarios (107). One such model developed by Porchet *et al.* (108) characterized acute tolerance to the cardio-accelerating effects of nicotine by integrating a hypothetical noncompetitive antagonist formed by a first-order process, with nicotine concentrations serving as the driving force for formation. Furthermore, optimal recovery from acute nicotine corresponded with longer intervals between nicotine exposure (108).

The generic approach to modeling tolerance development, which included consideration of the relationship between the development of opioid tolerance and administration strategy, was utilized by Ouellet and Pollack (109) for two morphine treatment regimens: multiple increasing i.v. bolus doses over a 12-h period (24 mg/kg total exposure in seven doses) or one i.v. bolus dose per day (beginning with 1.85 mg/kg on day 1, and increasing to 6 mg/kg maximum) for 13 days. The hypothesis underlying this experimental design was that tolerance development would be attenuated in the once-daily administration group if the kinetics of tolerance offset were rapid (i.e., if significant return to basal responsivity occurred within 24 h after morphine exposure). Morphine disposition did not differ significantly between the 12-h and 13-day treatment groups, indicating that changes in systemic morphine disposition could not account for tolerance development. Concentration-normalized peak analgesic effects remained relatively constant over 12 h, while the 13-day treatment group exhibited a decrease in normalized peak effect from day 1 to 8. In addition, the extent of analgesic tolerance development was similar whether morphine was administered as a multiple i.v. bolus or constant infusion. Taken together, these observations suggested that morphine

tolerance development was dependent on systemic drug concentrations (i.e., the extent of exposure), but was independent of the kinetics of morphine administration (i.e., the rate of morphine exposure or the presence of relatively brief "drug holidays" in the administration regimen).

The apparent independence of the rate or extent of tolerance development and the kinetics of drug administration was an unexpected observation, as conventional wisdom suggested that continuous presentation of opioids such as morphine would result in the production of profound tolerance. An explanation for this observation was formulated through comprehensive PK-PD modeling of morphine concentration-time and antinociceptive effect-time data obtained from rats during and following continuous morphine infusion (109). In this experiment, morphine was infused for 12 h at several different rates to achieve one of five preselected steady-state concentrations. Blood morphine concentrations and antinociceptive response (tail flick) were determined at timed intervals during infusion. Separate groups of rats were studied for 18 days after termination of a 12-h morphine infusion, with pharmacologic response compared to that produced 15 min after a single intravenous bolus dose (presumably representing a "no-tolerance" state). The data were fit with a tolerance model similar to that displayed schematically in Fig. 2. This model assumed accumulation of a hypothetical "inhibitor" that was formed from morphine by a first-order process; several different "inhibitor" scenarios (reverse agonist, competitive antagonist, non-competitive antagonist, or partial agonist) were evaluated,
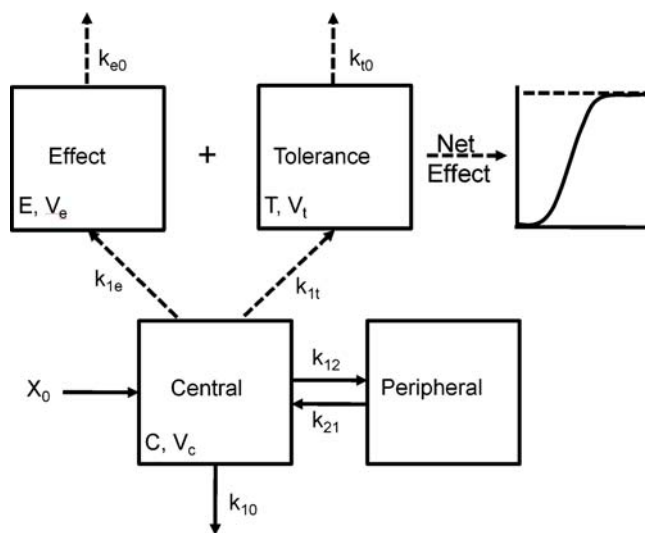

Fig. 2. Scheme depicting the PK-PD model of tolerance following multiple morphine i.v. bolus doses. The time course of morphine concentrations following multiple i.v. bolus doses was described by a two-compartment model with a central volume of distribution V_c, distribution between the central and peripheral compartment according to the rate constants k_{12} and k_{21}, and elimination from the central compartment by the first-order rate constant k_{10}. The time course of antinociception was described using an approach derived by Porchet *et al.* (108) where the first-order rate constants of effect onset, k_{1e} and k_{1t}, link the central compartment to the effect (E) and tolerance (T) compartments of volumes V_e and V_t and, effect offset is governed by the first-order rate constants k_{e0} and k_{t0} [Adapted from Ouellet and Pollack (118)]

with the net effect (E) produced under each scenario shown in Eqs. 1, 2, 3, and 4, respectively:

$$E = \frac{E_{\max} \cdot C_e^{\gamma}}{EC_{50}^{\gamma i} + C_e^{\gamma}} - \frac{I_{\max} \cdot C_i^{\gamma i}}{IC_{50}^{\gamma i} + C_i^{\gamma i}} \qquad (1)$$

$$E = \frac{E_{\max} \cdot C_e^{\gamma}}{C_e^{\gamma} + (EC_{50}/IC_{50}) \cdot C_i^{\gamma} + EC_{50}^{\gamma}} \qquad (2)$$

$$E = \frac{E_{\max} \cdot IC_{50}^{\gamma i} \cdot C_e^{\gamma}}{EC_{50}^{\gamma} \cdot IC_{50}^{\gamma i} + C_e^{\gamma} \cdot IC_{50}^{\gamma i} + C_i^{\gamma i} \cdot EC_{50}^{\gamma} + C_e^{\gamma} \cdot C_i^{\gamma i}} \qquad (3)$$

$$E = \frac{E_{\max} \cdot IC_{50}^{\gamma} \cdot C_e^{\gamma} + I_{\max} \cdot C_i^{\gamma} \cdot EC_{50}^{\gamma}}{EC_{50}^{\gamma} \cdot IC_{50}^{\gamma} + C_e^{\gamma} \cdot IC_{50}^{\gamma} + C_i^{\gamma} \cdot EC_{50}^{\gamma}} \qquad (4)$$

The results of this study indicated that morphine tolerance development was dependent on both morphine concentration and the duration of morphine exposure. As morphine concentrations increased, the net change in antinociceptive response (peak effect minus effect at 12 h) increased; regardless of the steady-state concentration produced, antinociceptive responses at 12 h were similar, resulting in a truncated effect *versus* concentration relationship at 12 h (Fig. 3). The PK-PD model was capable of describing the time course of loss (Fig. 4) and return (Fig. 5) of pharmacologic effect. When the loss-of-response data were fit alone, it was not possible to distinguish between the four alternative "inhibitor" scenarios. However, the return-of-response data suggested that the kinetics of morphine tolerance behaved as if driven by a partial agonist with a very low intrinsic activity (<10% of the maximum response to
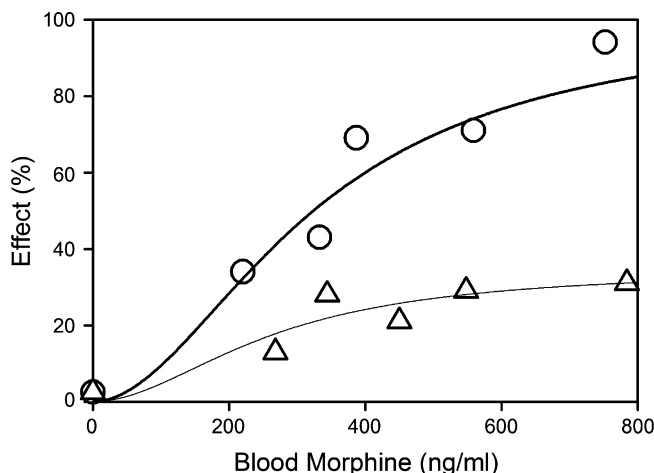

Fig. 4. Time course of antinociceptive response during morphine infusion in rats. Infusion rates were selected to produce steady-state concentrations of 200–299 ng/ml (*squares*), 300–399 ng/ml (*inverted triangles*), 400–499 ng/ml (*diamonds*), 500–599 ng/ml (*circles*), or >600 ng/ml (*triangles*). *Closed circles* indicate animals that did not receive morphine. Data are represented as mean for clarity; *lines* indicate the fit of an integrated PK-PD model (Fig. 2) with the assumption that tolerance is driven by accumulation of a hypothetical partial agonist (Eq. 4) [Adapted from Ouellet and Pollack (118)]

morphine) and a high affinity ($IC_{50} \sim 14$ ng/ml). In addition, the combined data set allowed estimation of the rate constant associated with tolerance offset (~ 0.12 day^{-1}), which corresponded to a half-life of 5.7 days.

This modeling effort revealed the explanation, if not the underlying mechanism, for the apparent independence between the kinetics of tolerance development and the kinetics of morphine administration, a situation starkly different from that for nicotine. In fully-tolerant animals, a "drug holiday" of nearly 6 days would be required in order to regenerate 50% of the intrinsic responsivity to morphine lost during the

Fig. 3. Relationship between antinociceptive response and morphine concentrations in blood during continuous infusion at the time of peak response (*circles*) or at 12 h into the infusion (*triangles*). Data are presented as mean for clarity; lines indicate the fit of the Hill equation to the data with the following parameter values: peak effect *versus* concentration data ($E_{\max}=100\%$, $EC_{50}=324$ ng/ml, $\gamma=1.92$); effect at 12 h *versus* concentration ($E_{\max}=34.8\%$, $EC_{50}=262$ ng/ml, $\gamma=1.92$) [data were obtained from Ouellet and Pollack (118)]
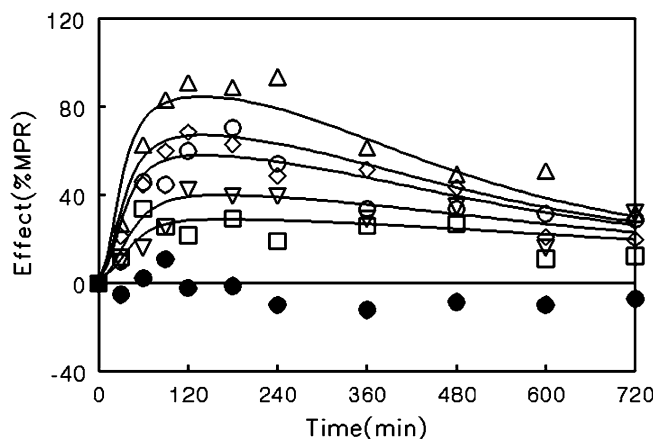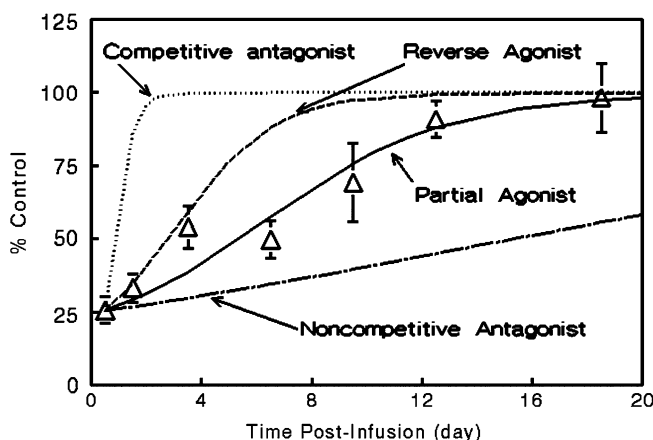

Fig. 5. Recovery of antinociceptive response to a 2-mg/kg bolus dose of morphine following termination of a 12-h morphine infusion (2 mg kg^{-1} h^{-1}). Data are presented as mean±SE; lines indicate model simulations based on an assumption that tolerance is driven by accumulation of a hypothetical reverse agonist (*dashed line*), competitive antagonist (*dotted line*), non-competitive antiagonist (*dot-dashed line*), or partial agonist (*solid line*) [Adapted from Ouellet and Pollack (118)]
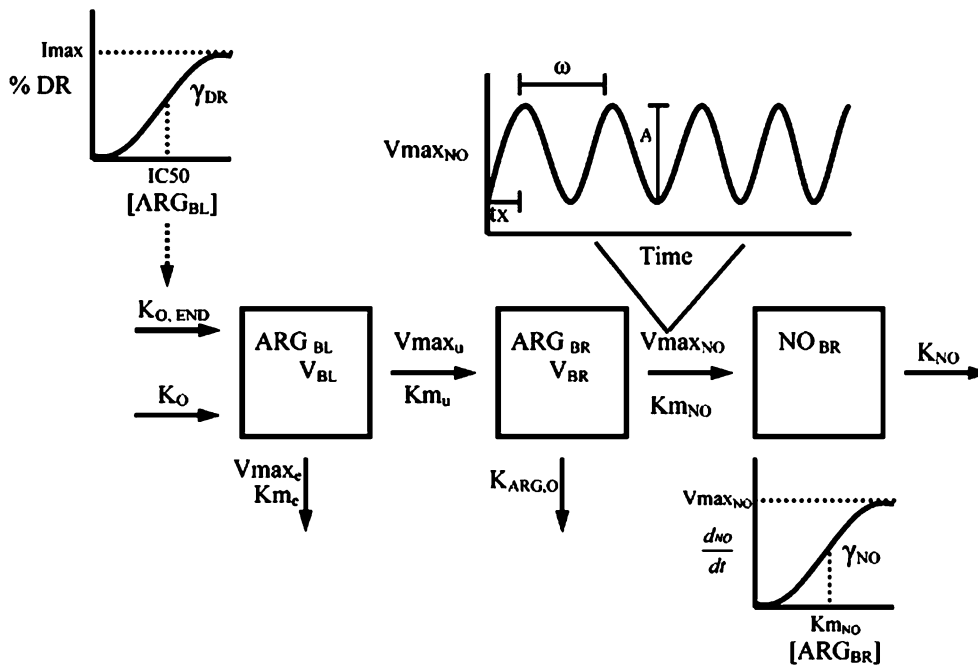
Fig. 6. PK-PD model for L-arginine-associated stimulation of nitric oxide production in rats [From Heinzen and Pollack (115)]

development of tolerance. In contrast, the half-life associated with return of responsivity to nicotine (35 min) is consistent with nearly complete recovery and resensitization when doses are separated by three or more hours (108,109).

As these examples demonstrate, "black-box" modeling of tolerance can provide useful information regarding system dynamics, are capable of describing the loss or return of

pharmacologic effect over time, and can predict responsivity under differing administration scenarios. By their very nature these models have a limited ability to reflect the specific mechanisms underlying tolerance development in a given system. However, when used appropriately, such modeling efforts can direct further mechanistic experimentation. Subsequent elaboration of mechanistic detail then can be used to
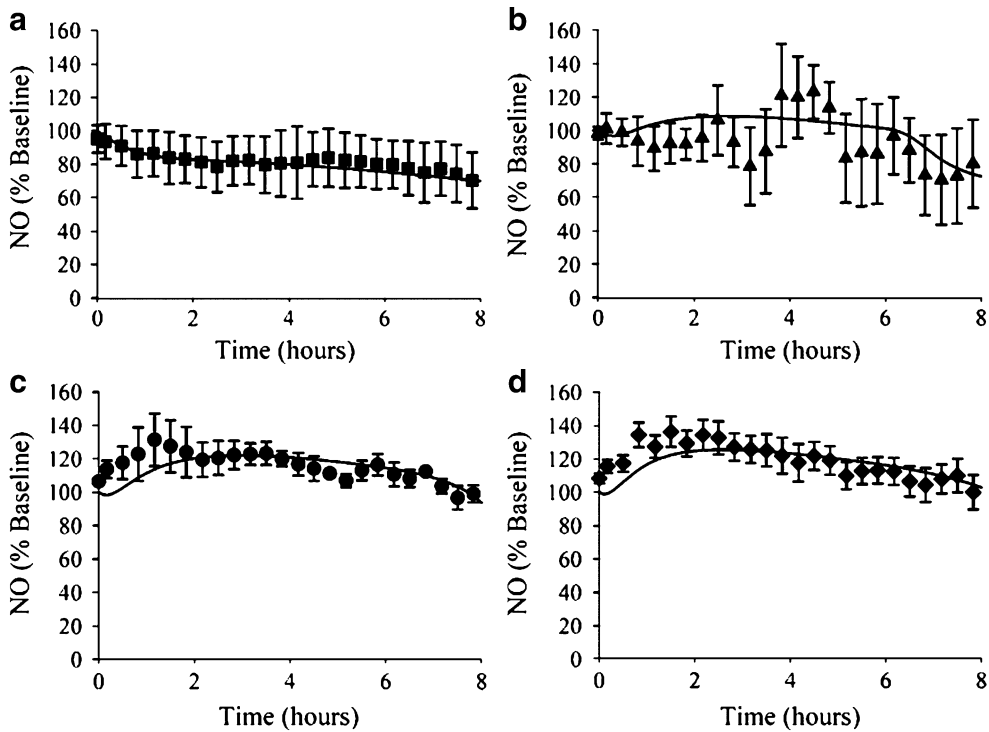

Fig. 7. Concentration-time profiles for brain NO during (**a**) saline or administration of L-arginine (**b** 250 mg kg^{-1} h^{-1}, **c** 500 mg kg^{-1} h^{-1}, or **d** 1,000 mg kg^{-1} h^{-1}). Data are mean±SE. *Lines* indicate the fit of the PK-PD model (Fig. 6) to the data [From Heinzen and Pollack (115)]

inform the PK-PD model of tolerance, which in turn provides additional understanding of system dynamics and an increased capacity to predict system response under differing conditions. An example of this iterative approach to modeling opioid tolerance is provided in the next section of this review.

Mechanistic Approach

When the specific physiologic alterations that lead to time-dependent loss of pharmacologic effect are known, they can be incorporated into mechanistic PK-PD models. As discussed earlier in this review, chronic opioid exposure enhances NMDA receptor function thereby permitting greater Ca^{2+} influx; Ca^{2+} will attach to and activate the NOS complex that mediates the conversion of L-Arg to NO. One method of limiting NO production is to coadminister an opioid with an NMDA receptor antagonist or a NOS inhibitor (85,87,110). Furthermore, coadministering exogenous L-Arg with an opioid increased NO levels and attenuated antinociception (84,111,112). These observations suggested an important, and perhaps causative, role of NO in the induction of opioid tolerance.

The significance of NO in mediating the time dependent loss of morphine-associated antinociception was elaborated in a series of experiments by Heinzen and coworkers. Following long-term morphine administration, the extent of antinociceptive tolerance that developed in mice deficient in neuronal

nitric oxide synthase (nNOS) was significantly less than that in wild-type mice (30% *versus* 80% loss of effect, respectively) (113). In rats, infusion of the NO precursor L-arg increased the concentration of NO in brain tissue and decreased the intrinsic antinociceptive activity of morphine (114–116). An integrated PK-PD model was developed (Fig. 6) that was capable of describing the time course of changes in brain NO in response to L-arg infusion (Fig. 7). This model required inclusion of circadian control of NO production to accurately describe the observed data. Furthermore, morphine administration increased the brain concentration of NO, and the temporal pattern of apparent nNOS stimulation by morphine was consistent with the time course of loss of morphine-associated antinociception due to tolerance development (116). Subsequent experiments at the receptor level confirmed that NO alters MOR function (117). Taken together, these observations demonstrate that elevated brain content of NO is both necessary and sufficient to induce functional antinociceptive tolerance to morphine in rats and mice.

The relationship between morphine concentrations in blood and brain tissue (measured by microdialysis sampling), NO production in brain (measured with an indwelling amperometric probe), and the development of antinociceptive tolerance (measured by electrical stimulation vocalization) was characterized with a comprehensive PK-PD model (116). In this model (Fig. 8), a two-compartment PK model was fit to morphine blood and brain concentrations during
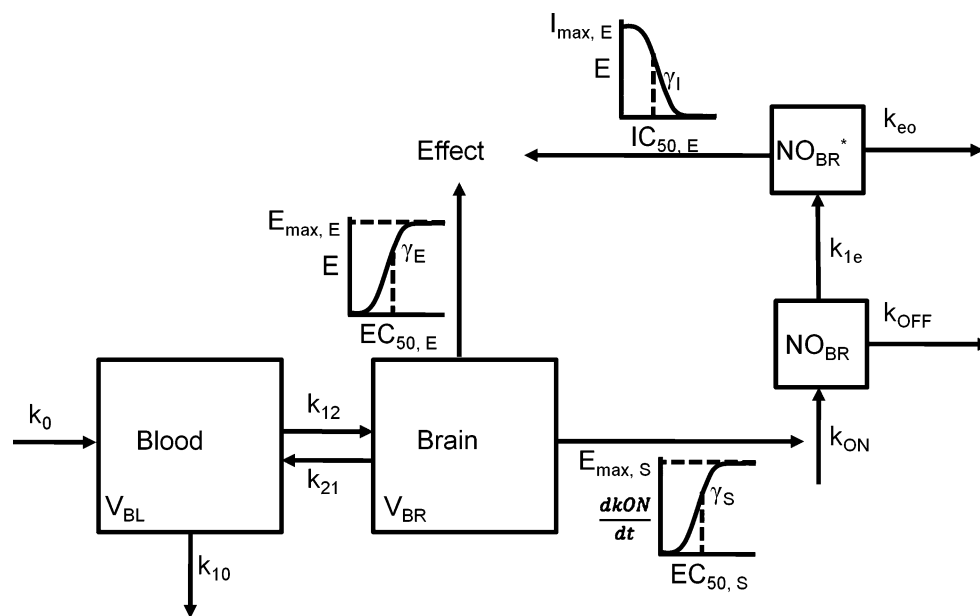

Fig. 8. Scheme depicting the PK-PD model of morphine disposition, NO production and antinociceptive effect. The disposition of morphine administered as a zero-order infusion (k_0) into the blood of volume V_{BL} was described by the first-order rate constants of transfer between the blood and brain, k_{12} and k_{21}, and by the first-order rate constant of elimination k_{10} from the blood. Antinociception was mediated by morphine in the brain of volume V_{BR} by acting as an agonist at the MOR and by indirectly stimulating the production of NO, which indirectly inhibited antinociception. The actions of morphine on NO production and antinociception was described using sigmoidal E_{max} models, where $E_{max, E \text{ or } S}$ is maximum effect or stimulation; $EC_{50, E \text{ or } S}$ is the concentration that elicits 50% effect or stimulation; $\gamma_{E \text{ or } S}$ is the shape factor dictating the relationship between concentration and effect or stimulation of NO production (k_{ON}); k_{OFF} is the first-order rate constant of NO degradation; $NO_{BR}*$ is the concentration of NO in the hypothetical compartment that indirectly inhibits effect; $I_{max,E}$ is the maximum inhibitory effect of NO; $IC_{50,E}$ is the concentration of NO that inhibits 50% of the effect; and γ_I is the shape factor of the inhibitory effect of NO on antinociception [Adapted from Heinzen and Pollack (116)]
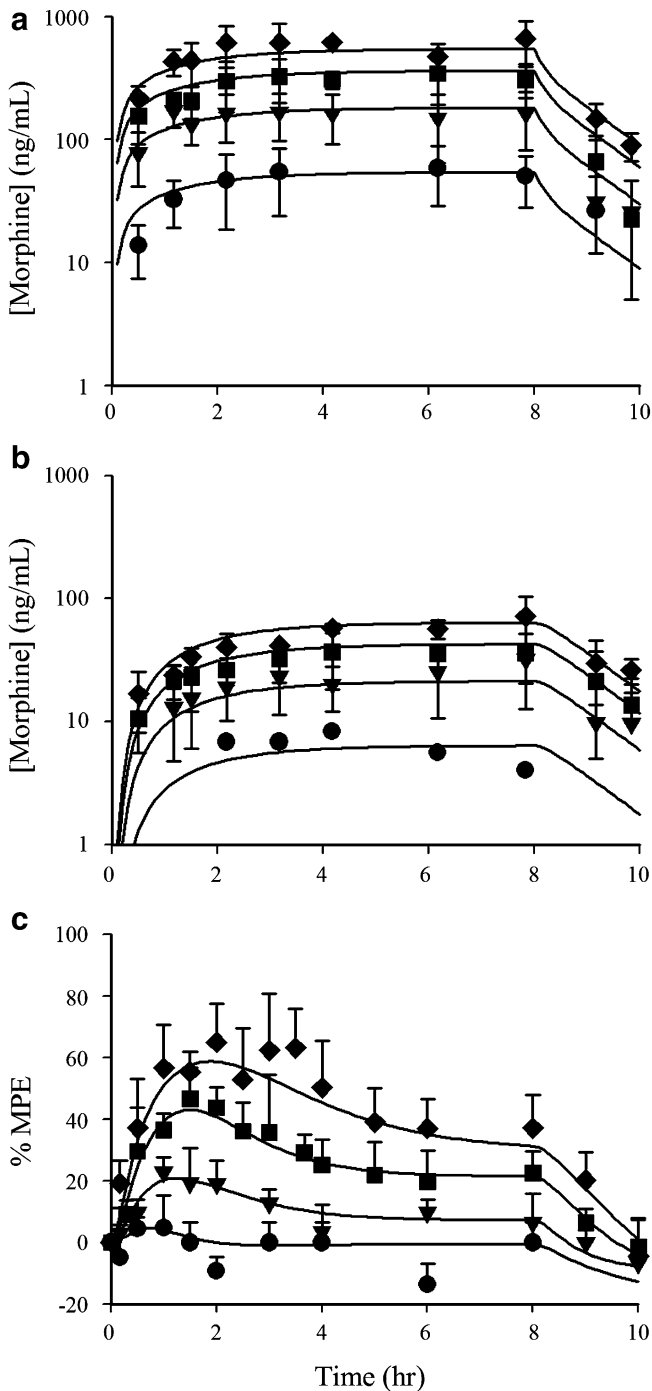
Fig. 9. Blood morphine concentrations (*top*), brain morphine concentrations (*middle*), and antinociceptive effect (*bottom*) during and following an 8-h morphine infusion at 0.3- (*circles*), 1- (*triangles*), 2- (*squares*), or 3- (*diamonds*) mg kg^{-1} h^{-1}. Lines indicate the fit of the PK/PD model to data [Adapted from Heinzen and Pollack (116)]

and after an 8 h i.v. morphine infusion in rats. A multi-component PD submodel was required to incorporate morphine-associated changes in the production of neuronal NO, morphine associated agonism of the mu-opioid receptor (expressed as an increase in the threshold for electrical stimulation vocalization), and NO-associated decreases in the threshold for electrical stimulation vocalization. This relatively complex PK-PD model was similar in structure to

the preceding "black-box" approaches, but utilized an experimentally-determined variable (brain concentration of NO), rather than a hypothetical construct, as the driving force for tolerance development. The model was able to provide a good description of the time course of morphine-associated antinociception during and following infusion in relation to changes in the blood and brain tissue concentrations of morphine (Fig. 9). Despite the complexity of the model, the data obtained were capable of supporting parameter estimates with a reasonable degree of precision (Table II).

Two aspects of the comparison between the "black box" and "mechanistic" modeling approaches for morphine tolerance are noteworthy. First, although both models are capable of describing the time course of changes in pharmacologic response, there are fundamental differences in the interpretation of those models. With the "black box" approach, the hypothetical "inhibitor" of response appeared to be a partial agonist of low intrinsic activity and high affinity; in the "mechanistic" approach, NO (presumably the analog of the hypothetical "inhibitor") clearly functioned as a reverse agonist. This difference underscores the difficulty in extrapolating specific mechanisms from compartmental modeling when mechanistic information is absent. Secondly, although the "black box" approach did not provide mechanistic detail, it did inform further experimentation that was target towards obtaining the missing mechanistic information. Thus, such modeling efforts can be extraordinarily useful in the design and interpretation of studies aimed as elucidating fundamental mechanisms of kinetic or dynamic behavior. As additional steps in the NO pathway for opioid tolerance development are elaborated, or as alternative pathways for opioid tolerance are elucidated, for the development of opioid tolerance, more detailed mechanistic models can be developed, assuming that the relevant steps in those pathways can be studied *in vivo*.

Table II. Pharmacokinetic/Pharmacodynamic Parameter Estimates and Corresponding Coefficients of Variation Obtained from Simultaneous Modeling of Morphine Disposition, NO Production, and Antinociceptive Effects

Parameter	Estimate	CV %
V_{BL} (l/kg)	2.5	25.9
V_{BR} (l/kg)	26	22.6
k_{10} (h^{-1})	2.17	26.3
k_{12} (h^{-1})	2.15	45.2
k_{21} (h^{-1})	1.77	10.8
$E_{max,S}$ (% stim)	0.19	36.8
$EC_{50,S}$ (ng/ml)	15.2	36.7
γ_S	3.1	115
k_{ON}	258	126
k_{OFF}	25.8	127
$E_{max,E}$ (% MPE)	100	21.9
$EC_{50,E}$ (ng/ml)	26.8	29.8
γ_E	1.04	14.8
$I_{max,E}$ (% MPE)	2726	133
$IC_{50,E}$ (% SAL)	264	157
γ_I	5.2	52.4
$k_{eo,low\ dose\ morphine}$ (h^{-1})	1.56	19.3
$k_{eo,intermediate\ dose\ morphine}$ (h^{-1})	1.01	15.8
$k_{eo,high\ dose\ morphine}$ (h^{-1})	0.61	21.4

See Fig. 6 for model structure [from Heinzen and Pollack (116)]

CONCLUSIONS

Opioid tolerance is a complex phenomenon mediated by diverse behavioral and cellular adaptations. Many of the specific alterations in intracellular signaling, trafficking and neurotransmitter activity have been thoroughly characterized using *in vitro* approaches and knockout mice. Despite these advances, there is in general a lack of integrative system approaches characterizing how these cellular adaptations interact within the whole organism. Such an approach will be critical in developing comprehensive understanding of opioid tolerance. As mechanistic information continues to be generated, increasingly sophisticated PK-PD models, with increasing biologic relevance, can be developed. The availability of such models, in turn, will support diverse activities ranging from drug discovery to adjunct therapy in order to minimize or reverse opioid tolerance. The ability to control the tolerance process is a requisite step in devising appropriate pharmacologic strategies for the treatment of chronic pain.

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