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TECHNICAL REVIEW

N-Methyl-D-Aspartate (NMDA) Receptors, Mu and Kappa Opioid Tolerance, and Perspectives on New Analgesic Drug Development

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This laboratory perspective reviews the pharmagologic approaches that have been used in preclinical animal models to demonstrate the ability of competitive (LY274614) and noncompetitive (MK801 and dextromethorphan) N-methyl-D-aspartate (NMDA) receptor antagonists to attenuate or reverse the development of morphine tolerance. We provide additional data to support previous observations that these NMDA antagonists modulate morphine (mu) opioid tolerance but do not affect U50488H (kappa$_1$) opioid tolerance. A strategy, which utilizes efficacy as an NMDA receptor antagonist and clinical safety, provides the basis for a discussion of the clinical potential of dextromethorphan, ketamine, and felbamate as modulators of opioid tolerance in pain patients or opioid addicts. The potential use of NMDA receptor antagonists and nitric oxide synthase (NOS) inhibitors in neuropathic pain is also discussed. ***[Neuropsychopharmacology 13:347–356, 1995]***

KEY WORDS: *Opioid tolerance; Mu agonist; morphine, kappa agonist; U50488H; NMDA receptor antagonist*

NMDA RECEPTOR ANTAGONISTS AND NITRIC OXIDE SYNTHASE INHIBITORS MODULATE OPIOID TOLERANCE

Tolerance and physical dependence are observed both in patients with pain and in opioid addicts who receive or self-administer opioids chronically. These pharmacologic effects of opioids are undesirable for both the pain patient and the opioid addict. For the pain patient, tolerance to the analgesic effects of opioids necessitates dose escalation, which can result in an increase in the intensity of side-effects (for example, sedation and constipation) or the appearance, at higher doses, of adverse effects (for example, myoclonus) (Inturrisi 1990; Foley 1991). The development of physical dependence exposes the pain patient to the risk of the withdrawal syndrome if opioid administration is abruptly discontinued or an opioid antagonist is inadvertently administered (Inturrisi 1990; Foley 1991). In the opioid addict tolerance to the mood effects of an opioid results in rapid dose escalation and withdrawal is a powerful stimulus, engendering drug seeking behavior. Thus, nonopioid drugs that could attenuate and/or reverse the tolerance and physical dependence would be a very useful adjunct in pain management. These same drugs could be used in the opioid addict to assist in opioid detoxification and during maintenance treatment by reducing or eliminating withdrawal symptoms. Furthermore, nonopioid drugs that modulate tolerance and dependence without altering the analgesic effects of opioids could provide an important new tool with which to investigate the biochemical and molecular mechanisms of opioid analgesia, reward, tolerance, and physical dependence. Thus, a strong argument can be made for

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the pharmacologic evaluation of nonopioid modulators of opioid tolerance in both "analgesic" and "drug abuse" model systems. Studies utilizing the former system will be the focus of this report.

Recent studies (Trujillo and Akil 1991; Marek et al. 1991; Tiseo and Inturrisi 1993; Kolesnikov et al. 1993a; Kolesnikov et al. 1993b; Tiseo et al. 1994; Elliott et al. 1994a; Elliott et al. 1994b; Inturrisi 1994) have demonstrated that the excitatory amino acid (EAA) receptor system and the nitric oxide (NO) system are involved in morphine tolerance and dependence. Since the 1980s, EAAs including glutamate and aspartate, have been identified as neurotransmitters in the vertebrate central nervous system (CNS). An important aspect of the N-methyl-D-aspartate (NMDA) subtype is that it opens a distinctive membrane channel, characterized by voltage dependent Mg^{2+} blockade and high permeability to calcium ions. Physiologic increases in intracellular calcium subsequent to receptor activation can initiate a number of metabolic changes in the cell including a calcium-calmodulin mediated activation of nitric oxide synthase (NOS) leading to the production of NO (Bredt and Snyder 1992). Activation of NMDA receptors can also alter the expression of cellular regulatory genes such as c-fos (Bading et al. 1993; Rasmussen et al. 1994). However, large and prolonged increases in intracellular calcium such as those which can occur from excessive NMDA receptor stimulation are toxic to the cell and stimulation of EAA/NMDA receptors may represent the pathophysiologic basis of neuronal degeneration in acute (cerebral ischemia/hypoxia or traumatic CNS injury) or chronic (for example,, Alzheimer's disease or AIDS dementia) conditions (Meldrum and Garthwaite 1991). Thus, EAA receptor antagonists, especially NMDA receptor antagonists, represent a major area of drug development.

NMDA antagonists including MK801, LY274614, and dextromethorphan, as well as the NOS inhibitor, NorArg (L-N_G-nitroarginine) can attenuate or reverse the development of tolerance to morphine's antinociceptive (analgestic) effects (Trujillo and Akil 1991; Marek et al. 1991; Tiseo and Inturrisi 1993; Kolesnikov et al. 1993a; Kolesnikov et al. 1993b; Tiseo et al. 1994; Elliott et al. 1994a; Elliott et al. 1994b). Figure 1 shows the ability of LY274614 or MK801 to attenuate morphine tolerance in the rat as measured by use of the hot plate test, whereas Figure 2 shows that MK801 can prevent the rightward shift in the morphine dose-response curve indicative of morphine tolerance in the mouse as measured by use of the tail-flick test. In Figure 2 changes

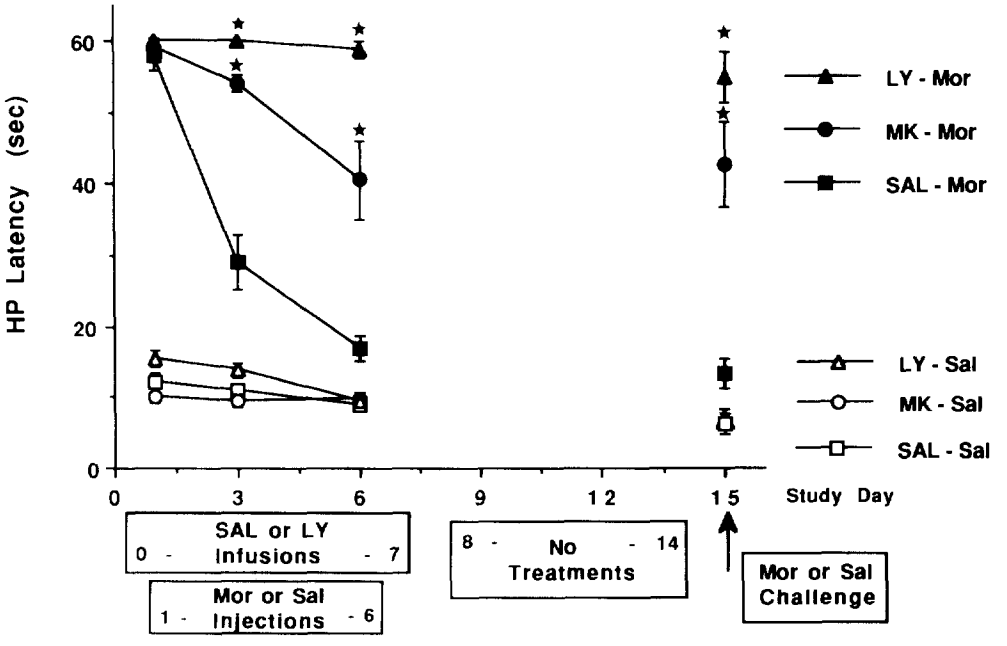

Figure 1. NMDA receptor antagonists attenuate morphine tolerance in the rat. Rats received LY274614 (24 mg/kg/24 hr), MK801 (0.4 mg/kg/24 hr) or saline by continuous SC infusion. Concurrent twice a day injections were saline or morphine (10 mg/kg, SC). Hot plate (HP) latencies were assessed 60 min post morphine or saline injection on days 1, 3, 6. On the evening of day 6 the injections were discontinued and on the morning of day 8 the osmotic pumps were removed. No treatments were administered for 7 days. On day 15 HP latencies were assessed after morphine or saline challenge as above. Abbreviations indicate the infusion treatment and the injection treatment and challenge. LY-Mor refers to LY274614 by SC infusion and morphine by injection. The mean (± SEM) HP latencies of the ★ LY-Mor and ★ MK-Mor groups were significantly longer than the mean values of the SAL-Mor group on days 3, 6 and 15 ($p < .05$). (From Tiseo and Inturrisi, 1993 with permission of the publisher.)

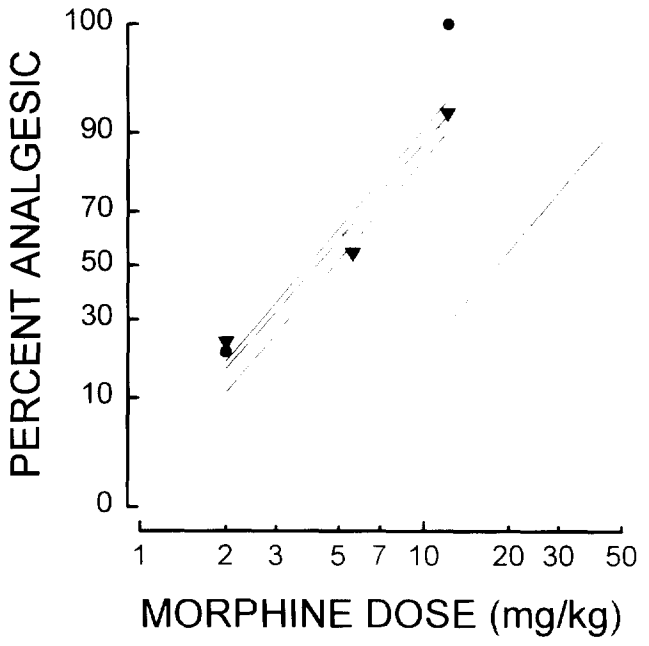

Figure 2. The NMDA receptor antagonist, MK801, prevents morphine tolerance in the mouse. Tolerance, as assessed by a rightward shift in the cumulative dose-response curve in mice was produced by the sc injection of MOR, three times per day at 10 mg/kg, on day 1, 20 mg/kg on day 2 and 40 mg/kg on day 3. The ordinate indicates the percentage of animals that achieved an analgesic response using the tail-flick test. Each morphine dose was preceeded 30 minutes earlier by either saline (SAL) or MK 0.3 mg/kg ip. On day 1 a single pretreatment dose of SAL or MK was followed by a cumulative dose-response curve. On day 4 no pretreatment was administered prior to the cumulative dose-response curve. On day 4 the SAL + MOR curve shifted nearly 5 fold while the curves for day 1 and day 4 MK pretreated groups were not significantly different from the day 1 SAL + MOR group (● day 1 SAL, ▼ day 1 MK, ▽ day 4 MK + MOR, ○ day 4 SAL + MOR). (From Elliott et al., 1994a with permission of the publisher.)

in the relative potency of morphine were determined by constructing cumulative dose-response curves derived from the response of groups of mice administered increasing log doses (approximately 0.25 log increase with each dose escalation) of morphine until each animal became an analgesic responder. Each animal was tested 30 minutes after each dose. This cumulative dose-response procedure reduces the number of animals required to assess a change in the response to morphine (Elliott et al. 1994a).

LY274614, a competitive NMDA receptor antagonist, was chosen for study over the commonly used noncompetitive NMDA antagonist, MK801 (MK), because LY274614 does not produce the PCPlike behavioral effects in animals that are seen with MK801. These behavioral effects in rodents appear to be predictive of PCPlike effects in humans including psychotomimetic effects and abuse liability. Dextromethorphan is a widely used antitussive with demonstrated safety (Elliott et al. 1994b). These NMDA antagonists and the NOS inhibitor, NorArg, do not affect the tail-flick and hot-plate measures of analgesia (Figures 1 and 2) (Trujillo and Akil 1991; Marek et al. 1991; Tiseo and Inturrisi 1993; Kolesnikov et al. 1993a; Kolesnikov et al. 1993b; Tiseo et al. 1994; Elliott et al. 1994a; Elliott et al. 1994b). Therefore, these behavioral measures can be used to assess the adaptations that result from chronic opioid exposure and the effects of NMDA antagonists on opioid tolerance.

The characteristics of the effects of NMDA antagonists and NOS inhibitors on morphine tolerance, as represented by LY274614, are of interest. The attenuation of morphine tolerance by LY274614 is dose-dependent (Tiseo and Inturrisi, 1993). Additionally, animals tested 1 week after the discontinuation of drug treatments (LY274614 plus morphine) were observed to retain their analgesic sensitivity to morphine, whereas control animals (morphine only) remained relatively tolerant (Figure 1) (Tiseo and Inturrisi 1993; Tiseo et al. 1994). To determine whether LY274614 treatment modifies the subsequent development of tolerance, it was administered to nontolerant animals for 1 week. One week after LY274614 treatment was discontinued the animals were challenged with morphine and then implanted with morphine pellets. Neither the expression of morphine analgesia nor the development of morphine tolerance differed when LY274614 and saline-treated animals were compared (Tiseo et al. 1994). Thus, the ability of LY274614 to affect the development of tolerance and the subsequent sensitivity of animals to morphine requires coadministration of LY274614 when morphine is present and therefore occupying opioid receptors. However, LY274614 administration does not alter the affinity or density of opioid receptors in the rat CNS nor does it alter opioid ligand binding in a number of standard equilibrium opioid binding assays (Tiseo et al. 1994).

The reversal of established morphine tolerance by LY274614, dextromethorphan (or NOS inhibitors) shows a pattern of gradual reversal over a period of at least 2 days (Tiseo and Inturrisi 1993; Kolesnikov et al. 1993b; Tiseo et al. 1993; Elliott et al. 1994b). This time-course differs from the immediate reversal of tolerance produced by drugs, such as the CCK antagonist, proglumide (Watkins et al. 1984), that potentiate morphine analgesia (Tiseo and Inturrisi 1993; Elliott et al. 1994b). These results suggest that NMDA receptors and NO are required for both the induction as well as the maintenance of morphine tolerance. Furthermore, they suggest that the induction and maintenance of tolerance involves time lags consistent with cellular biosynthetic processes (see Figure 4).

LY274614 and MK801 attenuate mu opioid tolerance

but not the tolerance produced by U50488H, a kappa$_1$ opioid agonist, or naloxone benzylhydrazone (NalBzoH) a kappa$_3$ opioid agonist (Elliott et al. 1994a). Interestingly, NPC 17742, an NMDA antagonist which differs structurally from LY274614 and MK801 can attenuate both morphine and U50488H tolerance (Kolesnikov 1993a). Like LY274614 and MK801, we and others have found that the nitric oxide synthase inhibitor NorArg can attenuate morphine but not U50488H or NalBzoH opioid tolerance (Kolesnikov et al. 1993b; Elliott et al. 1994a). These results differ from those reported by Bhargava and colleagues (Bhargava and Thorat 1994; Thorat et al. 1993). These investigators found that MK801 and NOS inhibitors can attenuate U50488H tolerance. Different strains of mice, doses of MK801 and pretreatment protocols were used in each study. Pick et al. (1991) compared seven mouse strains using the tail-flick assay and found that the CD-1 strain used by Elliott et al. (1994a) and Kolesnikov (1993b) was the most sensitive to the kappa opioids, U50488H and NalBzoH. In contrast, the Swiss-Webster mouse strain used by Bhargava and Thorat (1994) and Thorat et al. (1993) are more than 3-fold less sensitive to U50488H than CD-1 mice. The large dose of U50488H used by these investigators (Bhargava and Thorat 1994) to induce tolerance may have resulted in some cross-tolerance at mu receptors. Furthermore, Bhargava and Thorat (1994) measured the response to U50488H at 30 minutes after pretreatment with MK801 on each test day. As appropriate MK801-only controls were not included in this study, it is not clear whether the pretreatment dose of MK801 interferred with the development of tolerance, its expression on the test days or both (see Trujillo and Akil 1991; Marek et al. 1991; Tiseo and Inturrisi 1993).

To further clarify the effects of NMDA antagonists on opioid tolerance, we evaluated the ability of selected doses of MK801, LY274614, or dextromethorphan to modulate U50488H tolerance in CD-1 mice. The most rigorous and quantitative test of a putative modulator drug is obtained when the ability of the modulator drug to alter the ED$_{50}$ of an opioid after acute or chronic treatment is determined. The ED$_{50}$ values for U50488H treated mice were obtained from cumulative dose response curves using a tailflick assay described previously. The quantal dose-response data (Figure 3) were analyzed using the BLISS-21 computer program. This program maximized the log-likelihood function to fit a parallel set of gaussian normal sigmoid curves to the dose-response data and provides ED$_{50}$ values, 95% confidence limits (CI), and relative potency estimates (Elliott et al. 1994a). Tolerance was produced by the SC injection of U50488H, three times per day (tid) at 10 mg/kg on day 1, 20 mg/kg on day 2, and 40 mg/kg on day 3. On day 4, each treatment group was subjected to a cumulative dose-response analysis. These results were compared with the ED$_{50}$ values obtained from

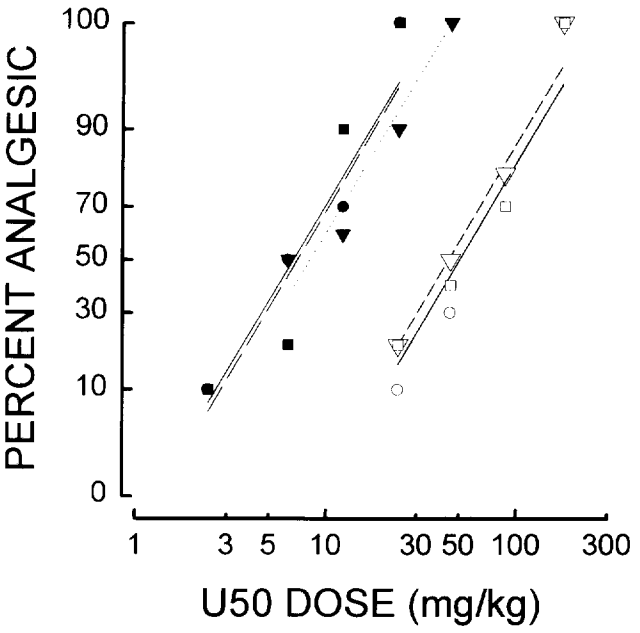

Figure 3. The NMDA receptor antagonist, MK801, does not attenuate U50488H tolerance in the mouse. Tolerance, as assessed by a right-ward shift in the cumulative U50488H (U50) dose-response curve was produced by the sc injection of U50 three times per day at 10 mg/kg on day 1, 20 mg/kg on day 2 and 40 mg/kg on day 3. The ordinate indicates the percentage of animals that achieved an analgesic response using the tail-flick test. Each U50 dose was preceded 30 minutes earlier by a SC injection of either saline (SAL), MK-801 (MK) at 0.1 mg/kg (MK-0.1), or MK at 1 mg/kg (MK-1). On day 1 a single pretreatment dose of SAL or MK was followed by a cumulative U50 dose-response assessment. On day 4 no pretreatment was administered prior to the cumulative U50 dose-response assessment. MK at either dose does not prevent the right-ward shift in the U50 dose-response curve on day 4 (see Table 1 for ED$_{50}$ values) (● day 1 SAL, ▼ day 1 MK 0.1, ■ day 1 MK 1.0, ○ day 4 SAL + U50, ▽ day 4 MK 0.1 + U50, □ day 4 MK 1.0 + U50).

mice that were pretreated with a single dose of the NMDA antagonist or saline followed by a U50488H cumulative dose-response analysis (treatment day 1).

Figure 3 shows an example of the dose-response curves obtained in this type of study. On day 1 pretreatment with MK801 at 0.1 or 1 mg/kg SC did not significantly shift the U50488H dose-response curve or change the U50488H ED$_{50}$ value (Table 1). The escalating U50488H dose schedule resulted in a shift to the right of the U50488H dose-response curve (Figure 3) and a more than 7-fold increase in the U50488H ED$_{50}$ value. Concurrent treatment with either 0.1 or 1.0 mg/kg of MK801 failed to attenuate U50488H tolerance as assessed by the rightward shift in the dose-response curve (Figure 3) and the increased ED$_{50}$ value (compare day 1 and day 4 values). In a previous study in the CD-1 mouse we found that MK801 at 0.3 mg/kg attenuates

morphine tolerance but not U50488H tolerance (Elliott et al. 1994a). We now find that a higher dose of MK801 (1.0 mg/kg) and a lower dose (0.1 mg/kg) are also without effect on U50488H tolerance. The lower dose (0.1 mg/kg) was the same dose of MK801 that Bhargava and Thorat (1994) found to reduce U50488H tolerance in Swiss Webster mice. Similar results were obtained with LY274614 and dextromethorphan.

Previously we had shown that LY274614 given by intraperitoneal injection at 6 mg/kg or at 24 mg/kg/24 hours via a subcutaneous osmotic pump failed to attenuate U50488H tolerance (Elliott et al. 1994a). Higher doses of LY274614 produce sedation in the mouse and, therefore, to avoid nonspecific interactions we tested the ability of lower doses (for instance, 3.0 mg/kg and 0.6 mg/kg) of LY274614 to modulate U50488H tolerance. These doses of LY274614 also failed to attenuate U50488H tolerance (Table 2).

Coadministration of dextromethorphan at 30 mg/kg attenuates and reverses morphine tolerance (Elliott et al. 1994b). However, dextromethorphan at the same dosage, or at 10 mg/kg or 3 mg/kg failed to attenuate the development of U50488H tolerance (Table 3). Surprisingly, the 30 mg/kg dose of dextromethorphan also increased the ED_{50} of U50488H on day 1 (Table 3). This apparent antagonist activity of dextromethorphan on U50488H analgesia was not seen when dextromethorphan at 30 mg/kg was administered three times per day (tid) for three days (Table 3). Kest et al. (1992) found that MK801 antagonized the analgesic effect of U50488H as assessed by the tail-flick test in rats. Because animals coadministered dextromethorphan and U50488H (day 4 DM + U50, Table 3) develop tolerance to U50488H, which is indistinguishable from the saline + U50 group (day 4 SAL + U50, Table 3), dextromethorphan does not appear to act as an opioid antagonist to attenuate tolerance when given repeatedly. These observations indicate that some NMDA antagonists may produce complex interactions with opioids, which require careful evaluation by use of dose-response relationships and generalization to at least more than one rodent species.

When administered to U50488H tolerant mice, dextromethorphan at 30 mg/kg tid for 4 days failed to reverse U50488H tolerance (data not shown). Taken

Table 1. MK-801 (MK) Does Not Affect the Development of Tolerance to the Analgesic Effect of U50488H (U50) in CD-1 Mice

Treatment	Day	U50 ED_{50}	(95% CI)
SAL	1	6.7	(4.6–9.5)
MK 0.1	1	8.2	(5.5–11.7)
MK 1.0	1	7.0	(4.9–10.0)
SAL + U50	4	50.2[a]	(36.1–69.9)
MK 0.1 + U50	4	44.0[a]	(30.9–61.9)
MK 1.0 + U50	4	50.4[a]	(35.7–70.8)

ED_{50} values for U50488H (U50) with the 95% confidence interval (CI) were determined on days 1 and 4 by use of cumulative dose-response analysis. The U50 dosing schedule was 10 mg/kg tid on day 1, 20 mg/kg tid on day 2, and 40 mg/kg tid on day 3. Injections of either saline vehicle, 0.1 mg/kg MK (MK-0.1), or 1 mg/kg MK (MK-1) were given 30 minutes prior to each U50 injection. The route of administration for U50 and MK was SC. On day 4 the SAL + U50 ED_{50} value was increased more than 7-fold. The concurrent MK treatments did not significantly alter the corresponding day 4 ED_{50} values of the MK treated groups compared to the day 4 SAL + U50 group.

[a] Significantly different ($p < .05$) from day 1 SAL group.

Table 2. LY274614 (LY) Does Not Affect the Development of Tolerance to the Analgesic Effect of U50488H (U50) in CD-1 Mice

Treatment	Day	U50 ED_{50}	(95% CI)
SAL	1	4.6	(3.1–6.5)
LY 0.6	1	6.7	(4.0–10.8)
LY 3.0	1	3.8	(2.5–5.6)
SAL + U50	4	38.0[a]	(30.3–47.3)
LY 0.6 + U50	4	62.4[a]	(37.8–100.2)
LY 3.0 + U50	4	36.2[a]	(26.1–49.3)

ED_{50} values for U50488H (U50) with the 95% confidence interval (CI) were determined on days 1 and 4 by use of cumulative dose. The U50 dosing schedule and route of administration was as in Table 1. Injections of either saline vehicle (SAL), 0.6 mg/kg LY (LY-0.6), or 3 mg/kg LY6 (LY-3) were given 30 minutes prior to each U50 injection. On day 4 the SAL + U50 ED_{50} increased more than 8-fold.

[a] Significantly different ($p < .05$) from day 1 SAL group. The concurrent LY treatments did not significantly alter the corresponding day 4 ED_{50} values of the LY treated groups compared to the day 4 SAL + U50 group.

Table 3. Dextromethorphan (DM) Does Not Affect the Development of Tolerance to the Analgesic Effect of U50488H (U50)

Treatment	Day	U50 ED_{50}	(95% CI)
SAL	1	8.4	(6.0–11.5)
DM 3	1	7.7	(4.5–11.9)
DM 10	1	13.6	(9.6–18.8)
DM 30	1	25.8[a]	(20.3–32.7)
SAL + U50	4	56.7[a]	(35.6–90.0)
DM 3 + U50	4	34.7[a]	(22.8–53.0)
DM 10 + U50	4	53.3[a]	(38.3–73.3)
DM 30 + U50	4	57.5[a]	(37.9–88.0)
SALINE	4	8.9	(5.2–13.6)
Dex 30	4	13.3	(8.7–20.3)

ED_{50} values for U50488H (U50) with the 95% confidence interval (CI) were determined on days 1 and 4 by use of cumulative dose-response analysis. The U50 dosing schedule and route of administration was as given in Table 1. Injections of either saline vehicle (SAL), 3 mg/kg DM (DM 3), 10 mg/kg DM (DM 10), or 30 mg/kg DM (DM 30) were given 30 minutes prior to each U50 injection. Additional controls were the SALINE group which received saline tid on days 1, 2, and 3, and the Dex 30 group that received DM at 30 mg/kg tid on days 1, 2, and 3.

[a] Significantly different ($p < .05$) from day 1 SAL Group. The concurrent DM treatments did not alter the corresponding day 4 ED_{50} values of the DM treated group compared to the day 4 SAL + U50 group.

together these results suggest that the sites and mechanisms of tolerance development for kappa$_1$ (U50488H) and/or kappa$_3$ (NalB$_z$OH) agonists may differ from those for mu opioid tolerance.

Mu opioid tolerance appears to be modulated at either NMDA receptors or NOS (or both). Because NMDA antagonists and NOS inhibitors do not alter the analgesic response but rather attenuate or reverse the development of tolerance, it is reasonable to assume that a functional NMDA receptor and the production of NO are required for the development of morphine tolerance but may not be required for analgesia. Whether the NMDA receptors modulating morphine tolerance are co-localized with the mu-opioid receptors activated by morphine remains to be determined. Of particular interest are the studies of Chen and Huang (1991), who found that activation of mu receptors in trigeminal neurons results in an increase in NMDA-gated calcium currents. This mu-opioid effect is modulated by protein kinase C (PKC) which acts by reducing the Mg^{2+}-block of the NMDA channel. One consequence of this mu-opioid-NMDA receptor interaction is increased intracellular calcium ions. Morphine tolerant mice have a higher free intracellular calcium level in brain synaptosomes than nontolerant mice (Welch and Olson 1991). In this context an NMDA antagonist would be expected to reduce the NMDA receptor-mediated calcium influx and decrease the calcium-calmodulin activation of NOS and the production of NO.

The rapid and efficient diffusion of NO would allow postsynaptic NMDA receptors to modulate intracellular events in presynaptic cells expressing mu-opioid receptors. One major implication of these observations is that the homeostatic mechanisms involved in the development of morphine tolerance can be viewed as an antianalgesic system that can be affected by the NMDA-NO systems (see Tiseo and Inturrisi 1993). An understanding of the activation and regulation of this antianalgesic system is key to developing a biochemical basis for morphine tolerance and for implementing new drugs for pain management and opioid drug abuse. A model (Figure 4) can be envisioned in which mu receptor occupancy by morphine results in the mobilization of protein kinase C (PKC), which in turn phosphorylates the NMDA receptor resulting in the removal of the Mg^{2+} block. This sequence results in the opening of the calcium channel portion of the NMDA receptor. This leads to an elevation in intracellular calcium followed by NO production and other calcium-mediated intracellular events including alterations in gene expression. This cascade of intracellular events, as well as the consequences of diffusion of NO and recruitment of other neurons, is manifest as the neuronal plasticity which appears at a systems level to be antianalgesic and is observed behaviorally as morphine tolerance. NMDA receptor antagonists and NOS inhibitors attenuate or reverse morphine tolerance by interfering with the generation of intracellular tolerance events without directly altering the analgesia cascade as measured by the tail-flick or hot-plate tests. For simplicity the model in Figure 4 is presented as an intracellular mechanism. Each of these events can, of course, affect transmitter release and synaptically mediated neurotransmission. The utility of the model is that it allows for a consideration of the biochemical and molecular intracellular events associated with morphine tolerance separately from those associated with analgesia.

PERSPECTIVES ON NEW DRUG DEVELOPMENT

Next, we discuss perspectives that can be used to identify drugs for future development as nonopioid modulators of analgesic tolerance.

Neuronal plasticity most likely underlies many of the nervous system responses associated with chronic pain of various mechanisms and the development of mu opioid tolerance. The future will produce a clearer understanding of the functional changes, the biochemical changes, and the gene changes important for the pathogenesis of these disorders. Much of this experimental work will depend upon the elucidation of newer animal models that more closely mimic the patient with chronic pain and opioid tolerance.

Central sensitization of the spinal cord projection neurons may be modulated by the release of EAAs, especially glutamate, and the subsequent activation of the NMDA receptor. These neuronal changes may be attenuated experimentally with NMDA receptor antagonists (Woolf and Thompson 1991). Specific behavioral correlates of the sciatic nerve ligation model of neuropathic pain are suppressed with experimental NMDA receptor antagonists (Mao et al. 1992; Mao et al. 1993; Yamamoto and Yaksh 1992). A recent case report describes the first published use of an experimental NMDA receptor antagonist, CCP, administered intrathecally to a patient with intractable neuropathic leg pain (Kristensen et al. 1992). Unfortunately, this trial was complicated by the development of drug-induced psychomimetic side-effects.

The NMDA receptor antagonists known as the open-channel blockers occupy the NMDA channel only when it is open. Because these open channel blockers act at a different site, they may have an advantage over the competitive NMDA receptor antagonists when pathological concentrations of glutamate are present (Lipton and Rosenberg 1994). Ketamine and dextromethorphan (Lipton and Rosenberg 1994) are two open-channel blockers with proven clinical utility and safety. Spinal administration of ketamine suppresses experimental neuropathic pain behavior (Mao et al. 1993). Ketamine has demonstrated efficacy in patients with

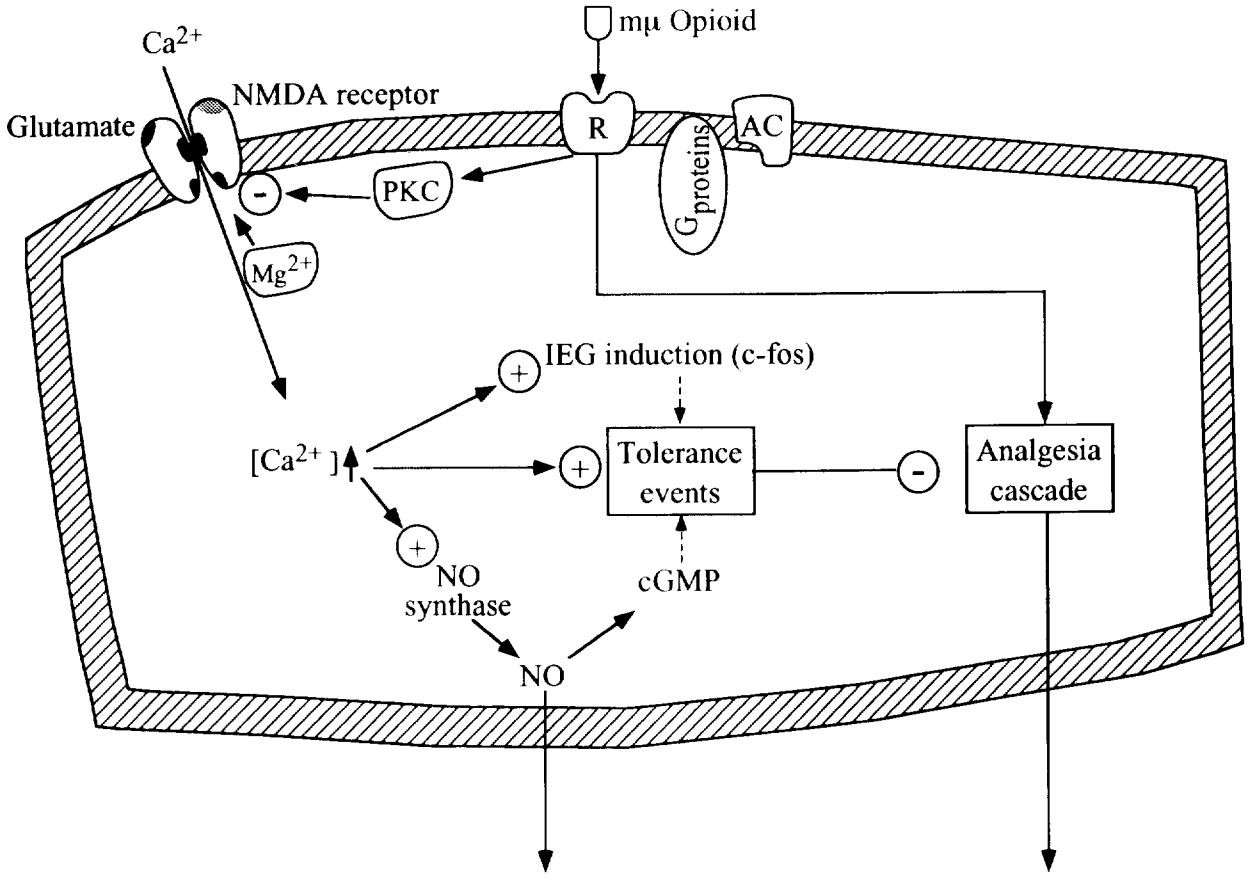

Figure 4. A model which suggests some of the receptor mediated signal transduction events that may be involved in the attenuation of morphine (mu) opioid tolerance by NMDA receptor antagonists and/or NOS inhibitors. Morphine (mu opioid agonist) occupation of the receptor results in an intracellular cascade mediated, in part by, a G protein coupled inhibition of adenyl cyclase (AC). This cascade results in the cellular changes, e.g., inhibition of neurotransmitter release, that are reflected at the behavioral level (i.e., tail-flick or hot plate tests) as analgesia. Concurrently, mu opioid receptor occupancy results in the activation of protein kinase C (PKC) which then phosphorylates the NMDA receptor resulting in the removal of a Mg^{2+} block, and the opening of Ca^{2+} channel of the NMDA receptor. Ca^{2+} influx triggers the activation of a number of signal transduction systems including nitric oxide synthase (NO synthase) leading to NO production, immediate early gene activation resulting in induction of c-fos, a transcription factor for a number of genes, and the activation of Ca^{2+} mediated protein kinases. These signal transduction events result in neuronal plasticity changes (tolerance events) that exert an antianalgesic effect on the analgesia cascade. NMDA receptor antagonists and NOS inhibitors interfere with these tolerance events but have no direct effects on the analgesia cascade.

postoperative nociceptive-somatic pain (Maurset et al. 1989). Long-term subcutaneous infusion appeared to benefit three patients with phantom limb pain (Stannard and Porter 1993). Ketamine, when administered in a blinded fashion by infusion in patients with established postherpetic neuralgia, attenuated pain intensity, increased pain relief, and decreased "wind-up" pain, as compared with saline (Eide et al. 1994).

Dextromethorphan can block, in a dose-dependent manner, the current induced by NMDA in cortical neurons, as determined by whole cell configuration patch clamp studies (Metzer et al. 1993). This drug can suppress NMDA-provoked seizures (Ferkany et al. 1988), NMDA-induced neuronal firing of spinal cord neurons (Church et al. 1985), glutamate-induced neurotoxicity (Choi 1987), and the "wind-up" phenomenon (Dickenson et al. 1991). In other animal studies, dextromethorphan suppressed formalin-induced nociceptive behavior after systemic or intraspinal administration (Elliott et al. 1993; Elliott et al. 1995). LY274614 has antinociceptive activity in the formalin model (Elliott et al. 1992).

Dextrorphan, a major metabolite of dextromethorphan, reduces experimental neuropathic pain when administered intrathecally (Mao et al. 1993). Pilot efficacy and safety studies of dextromethorphan have been undertaken in several neurologic disorders, including patients with Parkinson's disease (Bonuccelli et al. 1992; Saenz and Tanner 1993), patients with intractable seizures (Fisher et al. 1990), and patients at risk for cerebral ischemia (Albers et al. 1991). One case report describes suppression of intractable seizures in a young

patient with nonketotic hyperglycemia (Schmitt et al. 1993). These data suggest that dextromethorphan is safe and potentially efficacious in other neurologic disorders characterized by alterations of the NMDA receptor. Recently, Price et al. (1994) reported that the temporal summation of second pain, a psychophysical correlate of wind-up in humans, is attenuated by dextromethorphan. Whereas McQuay et al. (1994) using a double-blind cross-over design found no significant difference in analgesic effectiveness between dextromethorphan and placebo in patients with neuropathic pain. The relatively modest dosing scheduled (27 mg of dextromethorphan, three times a day) may have limited the efficacy of the drug in these patients.

Felbamate is a new anticonvulsant that has properties of both a competitive NMDA receptor antagonist (at the strychnine insensitive glycine site) (McCabe et al. 1993) and a sodium channel antagonist (White et al. 1992). This drug has demonstrated efficacy in suppressing damage from experimental ischemia (Wasterlain et al. 1992). Unfortunately, felbamate has been linked with cases of fatal aplastic anemia and its future clinical utility is presently unknown.

The utility of ketamine, dextromethorphan (or felbamate) for the management of intractable pain or opioid analgesic tolerance awaits future controlled clinical trials. Studies in laboratory animals suggest that repetitive dosing will be required to reverse established opioid analgesic tolerance (Elliott et al., unpublished observations). It is not known whether a concomitant reversal in tolerance to the respiratory depressant effects of the opioids may occur in patients and will require evaluation in Phase I safety studies.

The development of nitric oxide synthase (NOS) inhibitors is another potential area of future therapy for patients with chronic pain syndromes and opioid tolerance. Nitric oxide is generated from the enzyme nitric oxide synthase (NOS), which is activated after EAA-NMDA receptor interaction (Bredt and Snyder 1991). NOS inhibitors suppress experimental nociception resulting from intraplantar formalin injection (Moore et al. 1991; Elliott et al. 1992), NMDA-induced hyperalgesia (Kitto et al. 1992), and sciatic nerve ligation-induced hyperalgesia (Meller et al. 1992). NOS inhibition also decreases the neurophysiologic response to intraplantar formalin (Haley et al. 1992) in the rat and the neurophysiologic responses to NMDA and SP in the cat (Radhakrischaar and Henry 1993). The clinical potential of NOS inhibitors is discussed elsewhere in this volume. In models of nociception characterized by central sensitization, NOS inhibitors have antinociceptive properties. However, we and others have selected doses of these NOS inhibitors that are not antinociceptive, as assessed by baseline measurements, in the models (tail-flick and hot-plate) that we have used to measure opioid tolerance (Elliott 1994; Kolesnikov 1993b). This approach provides the opportunity to separate the modulation of tolerance from the confounding effects of drug-induced antinociception.

Neuropathologic evaluation after large doses of both competitive and noncompetitive NMDA receptor antagonists in the adult rat reveal neuronal vacuolation in the cingulate cortex and retrosplenial cortex and other neuronal regions suggestive of a neurotoxic effect from excessive NMDA receptor blockade (Olney et al. 1989; Olney et al. 1991). After a careful evaluation of the nature and time course of the neuronal vacuolation induced by MK801, Auer and Coulter (1994) suggested that this vacuolation may actually represent a fixation artifact with the clinical significance as yet unclear. Importantly, these studies need to be extended to include the range of doses that have recently been established in rodents using nociceptive and tolerance models. These studies should also include primates. Neither neurotoxicity nor permanent psychosis has been reported to result from the clinical use of either dextromethorphan or ketamine. This controversial topic is reviewed elsewhere in this volume.

In summary, the utility of NMDA receptor antagonists for use in patients with pain or in opioid addicts requires appropriate evaluation of their safety in a therapeutic dose range in preclinical neurotoxicity models (rodent and primate) and demonstration of the safety and efficacy in opioid tolerant patients. The information developed in this workshop provides the basis for focused preclinical and clinical studies of this important class of drugs.

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