TECHNICAL REVIEW

Perspectives on the N-Methyl-D-Aspartate/Nitric Oxide Cascade and Opioid Tolerance

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Opioid tolerance can be modulated by the N-methyl-D-aspartate/nitric oxixde (NMDA/NO) cascade. Evidence exploring a daily injection paradigm indicates that agents antagonizing NMDA receptors can prevent tolerance to morphine and delta drugs, but not kappa agents. Drugs work regardless of whether they act as competitive or noncompetitive antagonists. Even an agent acting as an antagonist on the glycine site of the NMDA receptor is effective. Blockade of nitric oxide synthase has similar effects on opioid tolerance, preventing morphine and delta tolerance but not that of kappa drugs. Even methylene blue, which can inhibit guanylyl cyclase activity, is effective, presumably by blocking cGMP formation resulting from NO release. These results demonstrate the importance of an intact NMDA/NO cascade in the production of opioid tolerance and open new possibilities in the design of agents acting on opioid tolerance. [Neuropsychopharmacology 13:309–313, 1995]

Opioids modulate the perception of pain through a variety of receptor systems (for review, see Pasternak 1993). The identification and classification of the various opiate receptor subtypes initially rested upon their pharmacologic functions in vivo and in bioassays and then moved to basic studies, including receptor binding and now molecular biology (Uhl et al. 1994). There are four major families of opioid receptors that have been defined in binding and pharmacologic assays: mu, delta, $kappa_1$, and $kappa_3$. All the families have been cloned and show a large degree of homology among

themselves. Selective antagonists have been developed that block the actions of the mu, delta, or $kappa_1$ classes (for review, see Pasternak 1993). Although no selective $kappa_3$ antagonists yet have been developed, $kappa_3$ actions are readily reversed by general opioid antagonists such as Win44,441. Antisense studies also readily distinguish among the four major families (Standifer et al. 1994; Chien et al. 1994; Rossi et al. 1994; Bilsky et al. 1994). Short oligodeoxynucleotides complimentary to regions of the mRNA encoding the various different receptors selectively down-regulate the functions of a single receptor class without interfering with the others. Indeed, the ability of oligodeoxynucleotide antisense studies to down-regulate $kappa_3$ analgesia without interfering with mu, delta, or $kappa_1$ provides some of the strongest evidence for a selective $kappa_3$ receptor system (Pan et al., 1994; Pan et al., 1995).

Tolerance to opioids results from a number of mechanisms that lead to a progressive decrease in the opioid activity at the receptor, cellular, and systems levels (Smith et al. 1988). At the receptor level, there is evi-

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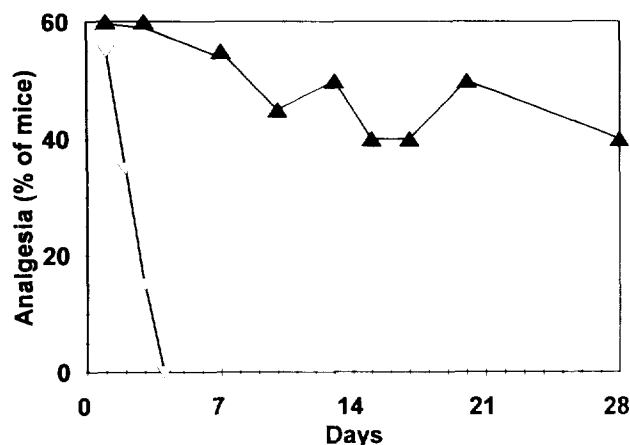
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Figure 1. Blockade of morphine tolerance by NOArg. Groups of mice received morphine (5 mg/kg, SC) alone (−▽−) or with NOArg (2 mg/kg/day) (−▲−). Results are from Kolesnikov et al. (1993a).

Table 1. Effect of NOArg and Arginine on Morphine Analgesia

	Morphine ED_{50} (mg/kg)	
Treatment	Without NOArg	With NOArg
Morphine		
Acute	4.4 (3.6, 5.3)	3.9 (2.8, 5.4)
5 days	8.8 (6.2, 11.3)	4.6 (3.1, 6.7)
10 days	17.2 (11.8, 23.8)	5.8 (4.0, 8.7)
28 days	37.8 (26.6, 51.1)	—
L-arginine		
Acute	6.6 (4.8, 8.5)	—
3 days	9.9 (7.5, 12.5)	—
5 days	10 (7.0, 13.6)	4.0 (3.6, 5.7)
10 ays	8.7 (6.1, 12.2)	—
D-arginine		
Acute	4.6 (2.7, 5.9)	—
5 days	4.4 (2.4, 6.1)	—

Morphine ED_{50} values with 95% confidence limits were determined in mice receiving only the designated treatment alone or coupled with daily injections of NOArg (2 mg/kg). Acute studies examined the actions of the agents 30 min after injection. Chronic dosing was done with once daily injections of morphine (5 mg/kg) alone or in combination with NOArg (2 mg/kg), L-arginine (600 mg/kg, i.p.) alone or in combination with NOArg (2 mg/kg) or only D-arginine (600 mg/kg) for the stated number of days. The ED_{50} values are from the literature (Kolesnikov et al. 1993; Babey et al. 1994).

dence for desensitization and down-regulation, possibly due to phosphorylation of the receptors. At the same time, cellular changes have been associated with tolerance. Early studies demonstrated that chronic exposure to opioids up-regulates adenylate cyclase levels, partially overcoming the inhibitory effects of the opiates on this system. Finally, tolerance may be manifested through the up-regulation of antagonistic systems and/or the concurrent down-regulation of facilitative ones. The mechanisms of tolerance are almost certainly complex and comprise a variety of all three components. Equally important, tolerance develops to each of the opioid receptor families independently, enabling the use of cross-tolerance to define the receptor mechanisms of various drugs. In the current studies, we have focused upon the role of antagonistic nonopioid systems in the production of opioid tolerance.

NMDA RECEPTORS

After the initial observations that MK-801, a noncompetitive N-methyl-D-aspartate (NMDA) antagonist, prevents morphine tolerance, similar effects have been observed with competitive NMDA antagonists (Truijillo and Akil 1991; Ben-Eliyahuy et al. 1992; Marek et al. 1991; Tiseo and Inturrisi 1993; Elliot et al. 1994). These studies have now been extended to the competitive NMDA antagonist LY274614, which also protects against morphine tolerance (Tiseo and Inturrisi 1993; Tiseo et al. 1994; Elliot et al. 1994). However, this action is selective. Like the nitric oxide synthase (NOS) inhibitors (Kolesnikov et al. 1992, 1993a), MK-801 and LY274614 are inactive against kappa₁ (U50,488H) or kappa₃ (naloxone benzoylhydrazone or NalBzoH) tolerance (Elliot et al. 1994). This ability to dissociate the mechanisms

of tolerance produced by mu and kappa systems raises important issues in the understanding of the two different types of systems.

Another competitive NMDA antagonist, NPC17742, also prevents tolerance when co-administered with morphine (Kolesnikov et al. 1993b). However, its profile against the other opioid receptor subtypes is unique. Unlike MK-801, LY274614, or the NOS inhibitors, NPC17742 also prevents tolerance to the kappa₁ analgesic U50,488H. Its inability to prevent tolerance associated with NalBzoH provides further evidence for distinct mechanisms of kappa₁ and kappa₃ tolerances.

1-Aminocyclopropyl carboxylic acid (ACPC) acts as antagonist at the glycine site on the NMDA receptor. Like the other agents acting at the NMDA receptor, ACPC prevents tolerance to morphine but is inactive against kappa₁ and kappa₃ tolerance (Kolesnikov et al., 1994). Like the others, ACPC also reverses preestablished tolerance.

NITRIC OXIDE SYNTHASE INHIBITORS

Morphine

In view of the strong correlation between activation of NMDA receptors and the subsequent production of nitric oxide (NO), we studied the actions of the nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine
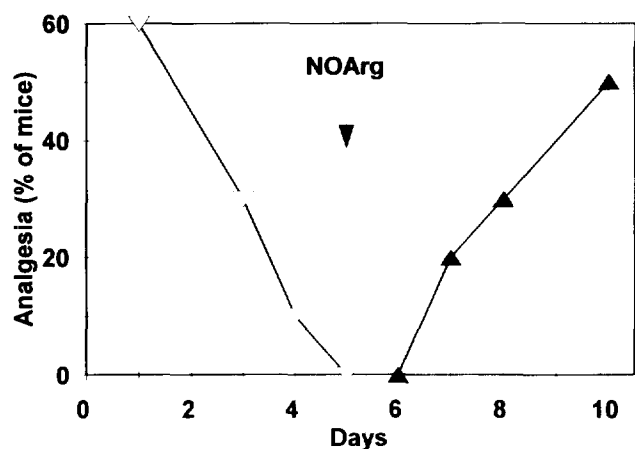
Figure 2. Reversal of morphine tolerance by NOArg. Mice received morphine alone (5 mg/kg, SC) for 5 days (−▽−). Starting on day 6, the mice received daily doses of morphine in conjunction with NOArg through day 10 (−▲−). Results are from Kolesnikov et al. (1993a).

(NOArg; Kolesnikov et al., 1992, 1993a; Babey et al., 1994). NOArg potently blocks morphine tolerance in the traditional morphine pellet paradigm as well as with a daily opioid injection paradigm. Indeed, coadministration of morphine with NOArg led to analgesia for at least 28 days, the longest time tested, whereas complete tolerance developed to morphine alone within 5 days (Figure 1). Like the NMDA drugs, the actions of NOArg are limited to tolerance. Given acutely with morphine, NOArg has no effect upon morphine analgesia (Table 1).

NOArg also reverses preestablished morphine tolerance (Figure 2; Klesnikov et al. 1993a). Animals tolerant to morphine regained their analgesic sensitivity over 5 days when NOArg was given along with the daily morphine injections. The return of morphine sensitivity in the animals receiving both morphine and NOArg is more rapid than another group made abstinent over the same time period (Kolesnikov et al. 1993a).

The ability to block morphine tolerance is not limited to NOArg. Similar results are seen with another NO synthase inhibitor, NAME, although it is not quite as effective and its actions not as long-lasting (Babey et al. 1994).

NO is a readily diffusible gas, making its storage impractical (Bredt and Snyder 1992). Thus, it is generated from arginine by NOS as needed. We next examined possible mechanisms associated with NO and morphine tolerance. Morphine administration does not change the levels of NOS in the brain nor does it change NOS mRNA levels, measured with a solution hybridization assay (Babey et al. 1994). Thus, the NO involved with morphine tolerance is generated from preexisting enzyme without the need to induce more.

L-arginine is the substrate for NOS, leading to the

production of NO. Driving the enzyme to generate NO by administering L-arginine along with morphine accelerates tolerance. D-arginine, the inactive isomer, has no effect. Interestingly, L-arginine given alone lowers morphine's analgesic response after a single dose (Table 1). Repeated dosing with L-arginine alone shifts morphine's analgesic dose-response curves to the right with a timecourse similar to that associated with morphine tolerance. However, unlike morphine, whose continued administration over 28 days leads to a progressive increase in tolerance, the shift due to L-arginine plateaus between 2- and 3-fold after only 4 to 5 days. In a sense, L-arginine makes naive animals "tolerant."

The actions of NOArg are limited to tolerance. Administered acutely with morphine, NOArg has no effect on morphine's analgesic potency. Equally important, NOArg is inactive as an analgesic when given alone. Even continued administration of NOArg for 10 days alone does not influence morphine's analgesic efficacy. Thus, the actions of NOArg appear to be related directly on the mechanisms of tolerance and are not simply a potentiation of morphine's analgesic effectiveness.

NO activates guanylyl cyclase, as well as a number of other actions. Methylene blue inhibits guanylyl cyclase, although the selectivity of its actions remains a matter of controversy. Methylene blue, however, effectively blocks morphine tolerance in our model, consistent with a cascade going from the activation of NMDA receptors, production of NO, and possibly activation of guanylyl cyclase.

Classic studies with morphine have established a close relationship between tolerance and dependence. Current work with both the NMDA antagonists and NOS inhibitors confirm this association. Paradigms which interfere with morphine tolerance have similar effects on morphine dependence (Kimes et al. 1993; Kolesnikov et al. 1993a; Adams et al. 1993). Coadministration of the NMDA antagonists or NOArg along with morphine prevents the development of tolerance. Equally important, they can reverse preestablished tolerance even with the continued administration of morphine.

Delta and Kappa Tolerance

As noted before, NMDA antagonists and NOArg effectively reverse the tolerance induced by morphine. Additional studies have indicated a similar effect on tolerance induced by repeated administration of DPDPE intrathecally. However, kappa tolerance is different. The traditional NMDA antagonists such as MK-801 and LY274614, as well as NOArg, have no effect on tolerance to the kappa$_1$ agent U50,488H or the kappa$_3$ drug NalBzoH in mice. There is one exception: NPC17742, a putative competitive NMDA antagonist. Like the others, NPC17742 blocks morphine and DPDPE toler-

ance. However, it also is effective against U50, 488H, but not NalBzoH tolerance. The reasons behind the difference in selectivity for NPC17742 and the other NMDA antagonists is not clear. However, NPC17742 does provide evidence for distinct kappa₁ and kappa₃ mechanisms of tolerance.

SUMMARY

The ability to selectively modulate opioid tolerance and dependence without interfering with analgesia is an important observation. Clinically, it opens many new potential treatment approaches. It also provides many insights into the mechanisms mediating these actions. First, it provides proof for the presence of separate and distinct mechanisms for analgesia compared to tolerance/dependence. It also provides additional evidence supporting the close association between tolerance and dependence. A second major point is the separation of the mu and delta tolerance from the two kappa systems. Virtually all the drugs acting on this cascade system dissociate kappa tolerance from mu and delta tolerance. The one exception, NPC17746, further differentiates kappa₁ from kappa₃ systems.

In conclusion, studies from our group and others confirm the role of NMDA and NO synthase in opiate tolerance. However, tolerance involves many mechanisms (Smith et al. 1988). Desensitization, downregulation, and cellular changes in signal transduction systems all have been implicated. The mechanisms responsible for tolerance may depend upon the drugs used, their dose, route of administration and frequency, as well as the duration of treatment. Thus, tolerance and dependence remain complex effects, which only have been partially elucidated. Furthermore, it is clear that the final step responsible for tolerance and dependence is neither the activation of the NMDA receptor nor the activation of NO synthase. Blockade of either will reverse preestablished tolerance and dependence, but slowly over several days. If they were the last step, an immediate reversal would have been anticipated. Early studies have suggested that tolerance is dependent upon protein synthesis. The slow reversal of tolerance and dependence might correspond to the turnover of a protein induced by the administration of the opiate. Identification of this putative protein would be important in the further understanding of the tolerance cascade.

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