

# The Nitric Oxide/Cyclic GMP System at the Supraspinal Site Is Involved in the Development of Acute Morphine Antinociceptive Tolerance<sup>1,2</sup>

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## ABSTRACT

The role of the supraspinal nitric oxide (NO)/cyclic GMP system in the development of acute morphine antinociceptive tolerance was investigated by use of the mouse 55°C warm-water tail-flick test. A single intracerebroventricular (i.c.v.) pretreatment of mice with morphine (3 nmol, 140 min before testing) produced an acute antinociceptive tolerance to subsequent i.c.v. doses of morphine, as demonstrated by a 120-fold rightward shift of the morphine dose-response curve. When co-administered with morphine (140 min before testing), the NO synthase inhibitors: N-nitro-L-arginine methyl ester (L-NAME), 3-bromo-7-nitroindazole, 7-nitroindazole and N<sup>G</sup>-monomethyl-L-arginine, attenuated the development of morphine tolerance. All four NO synthase inhibitors completely blocked the rightward shift of the morphine dose-response curve caused by i.c.v. morphine pretreatment (3 nmol, 140 min before testing). This effect was partially antagonized by L-arginine, but not

D-arginine, in a dose-dependent manner. Also, D-NAME did not block the development of tolerance. Like the NO synthase inhibitors, LY-83,583, a guanylyl cyclase inhibitor, blocked the development of tolerance, which suggests that NO acting through the cyclic GMP pathway is involved in the development of acute antinociceptive tolerance. The effects of increased NO production on acute morphine antinociceptive tolerance were also studied. When co-administered with morphine (140 min before testing), neither L-arginine (100 nmol) nor the NO donors, sodium nitroprusside (5 nmol) and isosorbide dinitrate (10 nmol), had any effect on the magnitude of morphine antinociceptive tolerance. These results suggest that NO, acting through the cyclic GMP pathway, mediates the development of acute antinociceptive tolerance, but that NO production does not alter the magnitude of antinociceptive tolerance.

NO has been implicated as a biological messenger molecule in the central nervous system (Moncada *et al.*, 1989; Garthwaite, 1991; Brecht and Snyder, 1992). NO is derived from one of two equivalent guanidino nitrogens of the amino acid L-arginine by the enzyme NOS, yielding NO and L-citrulline, as a coproduct. NOS is among the largest and most complicated of enzymes, and as many as eight isoforms of NOS have been identified from neurons, macrophages and endothelial cells (Nathan and Xie, 1994; Murad, 1994). These isoforms have been classified as either constitutive or inducible. The neuronal NOS is constitutive and calmodulin-dependent (Brecht

and Snyder, 1992). Activation of NOS and release of NO stimulates the soluble form of guanylyl cyclase, which results in an increase in cyclic GMP levels within the target cells (Deguchi, 1977; Brecht and Snyder, 1992).

The phenomenon of opioid tolerance and dependence has been investigated for many years, but its mechanism is still not completely understood. This phenomenon involves changes of a variety of nonopioid systems, such as adrenergic and cholinergic neurotransmission (Satoh *et al.*, 1976; Schulz and Herz, 1977; Llorens *et al.*, 1978; Hamburg and Tallman, 1981), in addition to the changes in opioid systems, such as the changes in affinity and number of opioid receptors (Puttfarcken *et al.*, 1988), and desensitization of opioid-mediated inhibition of adenylyl cyclase activity (Sharma *et al.*, 1975). The NMDA receptor has been implicated in the development of opioid-induced tolerance and dependence because several NMDA antagonists, such as MK-801, inhibited morphine tolerance and dependence (Trujillo and Akil, 1991; Marek *et*

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**ABBREVIATIONS:** NO, nitric oxide; NOS, nitric oxide synthase; L-NAME, N-nitro-L-arginine methyl ester; L-NOARG, N-nitro-L-arginine; D-NAME, N-nitro-D-arginine methyl ester; L-NMMA, N<sup>G</sup>-monomethyl-L-arginine; LY-83,583, 6-(phenylamine)-5,6-quinolinedione, 6-anilino-5,8-quinolinedione; NMDA, N-methyl-D-aspartate; SNP, sodium nitroprusside; ISDN, isosorbide dinitrate; DAMGO, [D-Ala<sup>2</sup>,(Me)Phe<sup>4</sup>,Gly(ol)<sup>5</sup>]enkephalin; i.c.v., intracerebroventricular(ly); i.t., intrathecal(ly); i.p., intraperitoneal(ly); s.c., subcutaneous.

*al.*, 1991; Tiseo and Inturrisi, 1993). NO produced by the constitutive neuronal NOS has been linked to the NMDA complex. The activation of NMDA receptors has enhanced the entry of extracellular  $Ca^{++}$ , thus stimulating enzymatic production of NO, which in turn increases the formation of cyclic GMP by activating guanylyl cyclase (Deguchi, 1977; Bredt and Snyder, 1992). Therefore, the NO/cyclic GMP system has been postulated to be involved in the development of morphine tolerance and dependence. Indeed, NOS inhibitors, such as L-NAME and L-NOARG, prevented morphine tolerance and dependence after chronic morphine administration in rodents (Adams *et al.*, 1993; Kolesnikov *et al.*, 1992; Kimes *et al.*, 1993; Cappenkijk *et al.*, 1993). Systemic administration of L-NAME and L-NOARG have been reported to attenuate the development of tolerance to the systemic morphine administration (Elliott *et al.*, 1994; Kolesnikov *et al.*, 1992; 1993; Majeed *et al.*, 1994). Other studies suggest that L-NAME has little effect on morphine tolerance and withdrawal at the spinal site (Dunbar and Yaksh, 1996). Therefore, we speculated that the NO/cyclic GMP system at supraspinal site might play an important role in morphine tolerance. In most studies reported so far, tolerance was induced by chronic and systemic administration of morphine. However, acute morphine antinociceptive tolerance has been observed after a single i.c.v. injection of morphine (Jiang *et al.*, 1995). An advantage of studying acute antinociceptive tolerance, induced by a single i.c.v. injection of morphine, is that the subsequent morphine dose-response line is shifted by more than 30-fold, after a single pretreatment of mice with 3 nmol morphine, at 120 min before the second injection of morphine (Jiang *et al.*, 1995). The study reported here focused on the role of supraspinal NO/cyclic GMP system in the development of acute morphine antinociceptive tolerance. The results demonstrate that inhibition of NO production and inhibition of guanylyl cyclase activity blocked the development of acute morphine antinociceptive tolerance.

## Materials and Methods

**Animals.** Male, ICR mice (25–30 g, Harlan Sprague Dawley, Inc., Indianapolis, IN) were used for all experiments. Mice were kept in groups of nine in a temperature-controlled room with a 12-hr light-dark cycle (lights on 7:00 A.M. to 7:00 P.M.). Food and water were available *ad libitum* until the time of the experiment.

**Injection techniques.** Intracerebroventricular injections were made directly into the lateral ventricle according to the modified method of Haley and McCormick (1957). The mouse was lightly anesthetized with ether, an incision was made in the scalp and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm with a 10- $\mu$ l Hamilton microliter syringe. The volume of all i.c.v. injections was 5  $\mu$ l.

**Tail-flick assay.** The thermal nociceptive stimulus was 55°C water with the latency to tail flick or withdrawal taken as the endpoint (Vaught and Takemori, 1979). After determining control latencies, the mice received graded i.c.v. doses of opioid agonists or antagonists at various times. Morphine was given as a single i.c.v. injection with testing taking place 20 min after the injection, at which time the maximal response had been established in the preliminary study. A cut-off time of 15 sec was used; if the mouse failed to display a tail flick, the tail was removed from the water and that animal was assigned a maximal antinociceptive score of 100%. Mice, showing no response within 5 sec in the initial control test, were eliminated from the experiment. Antinociception at each time point

was calculated according to the following formula: % antinociception =  $100 \times (\text{test latency} - \text{control latency}) / (15 - \text{control latency})$ .

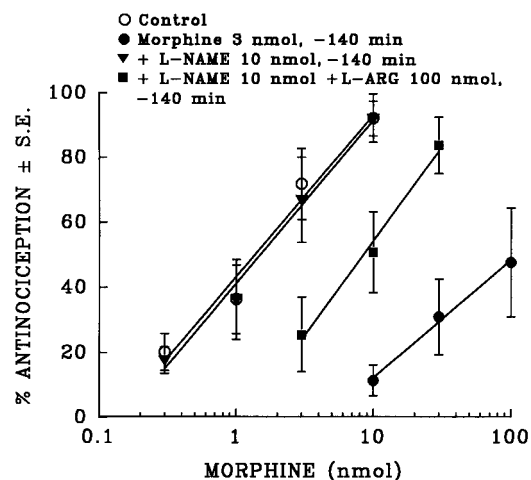
All NOS inhibitors, NO donors and guanylyl cyclase inhibitors were given by i.c.v. injection in a volume of 5  $\mu$ l, except where noted. L-NAME was used at doses ranging from 0.1 to 10 nmol; previously, this dose range was shown to inhibit NO production (Salter *et al.*, 1995). L-NMMA, 7-nitroindazole and 3-bromo-7-nitroindazole were administered by i.c.v. injection at a dose of 100 nmol. This dose of the NOS inhibitors has been an effective dose in blocking NO production (Salter *et al.*, 1996). The NO donors, SNP, L-arginine and ISDN, were administered by i.c.v. injection at doses of 5 nmol, 100 nmol and 10 nmol, respectively, doses that have been shown to be effective in generating NO (Gonzalez *et al.*, 1996). Methylene blue and LY-83,583, guanylyl cyclase inhibitors, were administered at a dose of 10 nmol. This dose of methylene blue and LY-83,583 has been shown to inhibit guanylyl cyclase activity (Melis and Argiolas, 1995).

**Chemicals.** Morphine sulfate was purchased from Mallinckrodt Chemical Company (St. Louis, MO). L-NAME, D-NAME, L-arginine, D-arginine, methylene blue, SNP and ISDN were purchased from Sigma Chemical Company (St. Louis, MO). LY-83,583, 3-bromo-7-nitroindazole, 7-nitroindazole and L-NMMA were purchased from Alexis Corp. (San Diego, CA). DAMGO was purchased from Bachem, Inc. (Torrance, CA). All compounds were dissolved in distilled water just before use.

**Statistics.** All dose-response lines were analyzed by regression methods as described by Tallarida and Murray (1986). Regression lines,  $D_{50}$  (dose producing 50% analgesia) values were determined with each individual data point with use of the computer program by Tallarida and Murray (1986). Statistical significance was determined by analysis of variance, followed by Neuman-Keul's test for multiple group comparison. All data points shown are the mean of 8 to 12 mice and error bars represent the S.E.

## Results

**The effect of NOS inhibitors on the development of acute morphine antinociceptive tolerance.** When mice were given a single i.c.v. injection of 3 nmol morphine, a dose that produced  $70 \pm 10\%$  antinociception, acute antinociceptive tolerance was produced and was measured as a 120-fold rightward shift in the morphine dose-response curve at 140 min after the initial morphine injection (fig. 1). Studies were directed at determining whether NO was involved in the



**Fig. 1.** Dose-response lines for i.c.v. morphine (20 min before testing) in mice treated either alone or with morphine (3 nmol), morphine (3 nmol) plus L-NAME (10 nmol) or morphine (3 nmol) plus L-NAME (10 nmol) and L-ARG (100 nmol) for 140 min before testing in the mouse 55°C warm-water tail-flick assay.

development of acute morphine antinociceptive tolerance. When co-injected with the initial 3 nmol morphine, L-NAME, at a dose of 10 nmol, completely blocked the development of acute antinociceptive tolerance (fig. 1). L-Arginine, the endogenous substrate for NOS, partially inhibited the blockade of tolerance caused by L-NAME, which suggests that L-NAME was producing its effect by acting as a NOS inhibitor (fig. 1). Table 1 summarizes the  $D_{50}$  values obtained for morphine-induced antinociception in the absence and presence of pretreatment with morphine and in the presence of NOS inhibitors. The suppression of acute antinociceptive tolerance by L-NAME was concentration (fig. 2A) and time (fig. 2B) dependent. L-NAME reached its peak effect when co-administered along with the 3-nmol morphine pretreatment, then its effect subsided gradually with time when it was administered before the 3-nmol morphine pretreatment, and lasted less than 8 hr (fig. 2B). An i.c.v. injection of 10-nmol L-NAME did not produce any antinociception, and the same dose of L-NAME, when given i.c.v. either 140 min before morphine administration, or co-administered with morphine, did not alter antinociception, induced by i.c.v. morphine (data not shown). L-Arginine, in a dose-dependent manner, partially blocked effect of L-NAME (fig. 3; table 1). However, D-arginine produced no effect, which demonstrates the stereoselectivity of the L-arginine effect (fig. 3). Similarly, D-NAME, at a dose of 10 nmol, had no effect on the development of acute morphine antinociceptive tolerance (fig. 4).

To further demonstrate that L-NAME was producing its effect by acting on NOS, three other NOS inhibitors were tested to determine whether they blocked the development of acute morphine antinociceptive tolerance. As shown in table 1, the NOS inhibitors, 3-bromo-7-nitroindazole, 7-nitroindazole and L-NMMA, in addition to L-NAME, completely blocked the development of acute tolerance.

**Determining whether cyclic GMP was involved in the development of acute morphine antinociceptive tolerance.** The target action of NO is to activate soluble guanylyl cyclase and increase the production of cyclic GMP. To determine whether inhibition of guanylyl cyclase activity would also affect the development of morphine tolerance, the

TABLE 1

Effect of inhibition of NO/cyclic GMP by 7-nitroindazole, 3-bromo-7-nitroindazole, L-NMMA, L-NAME, LY-83,583 and methylene blue on antinociceptive  $D_{50}$  values for i.c.v. morphine in mice pretreated with i.c.v. morphine for 140 min before testing

Pretreatment consisted of administering morphine and the compounds to mice by i.c.v. injection at 120 min before the subsequent morphine injection. Varying doses of morphine were then injected i.c.v. and tail-flick response was measured 20 min thereafter, as described under "Materials and Methods." The morphine control consisted of varying doses of morphine administered 20 min before testing.

Pretreatment	$D_{50}$ Values nmol (95% C.L.)
Morphine control	0.9 (0.7–1.2)
Morphine 3 nmol	110 (54–210)
Morphine 3 nmol + 7-nitroindazole 100 nmol	1.2 (0.8–1.6)
Morphine 3 nmol + 3-bromo-7-nitroindazole 100 nmol	1.8 (1.3–2.5)
Morphine 3 nmol + L-NMMA 100 nmol	1.4 (1.1–1.8)
Morphine 3 nmol + L-NAME 10 nmol	1.5 (0.9–2.2)
Morphine 3 nmol + L-NAME 10 nmol + L-arginine 100 nmol	8.5 (5.0–14)
Morphine 3 nmol + LY-83,583 10 nmol	1.1 (0.8–1.5)
Morphine 3 nmol + methylene blue 10 nmol	7.7 (4.8–12)

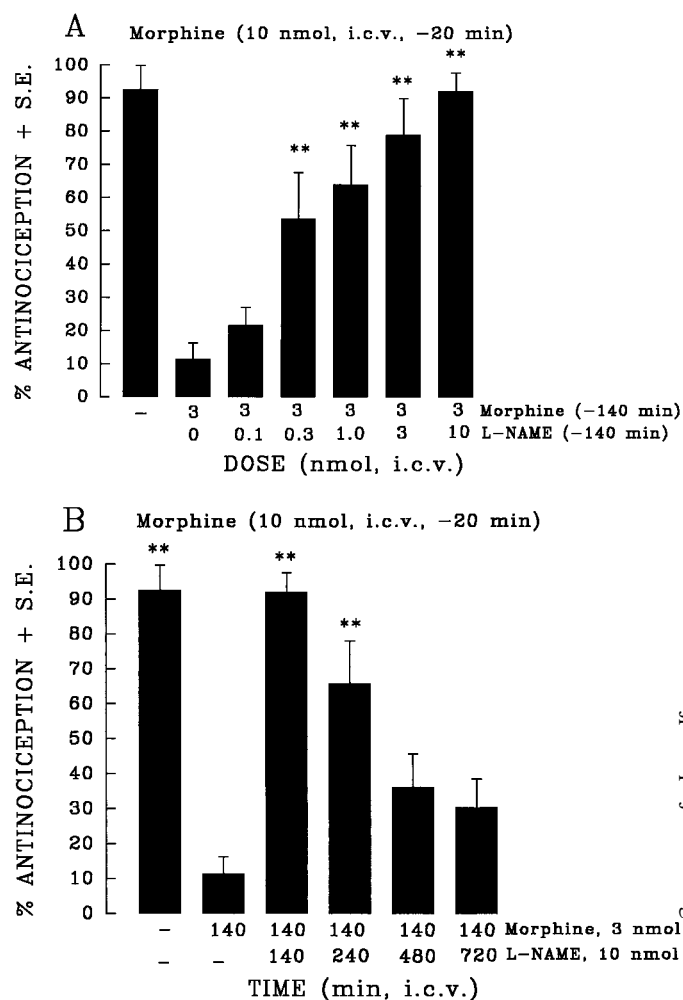
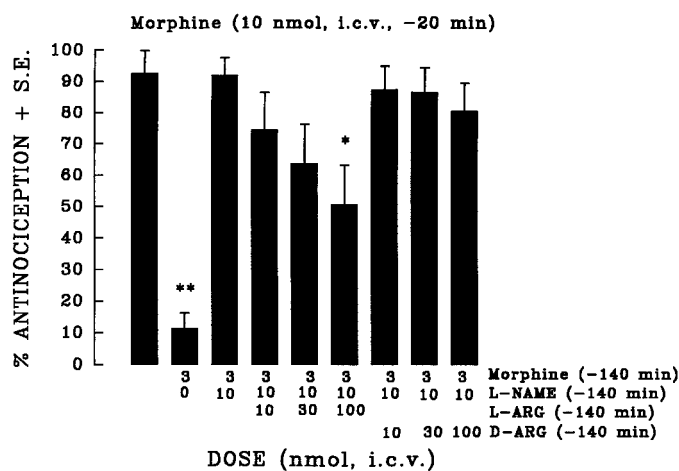


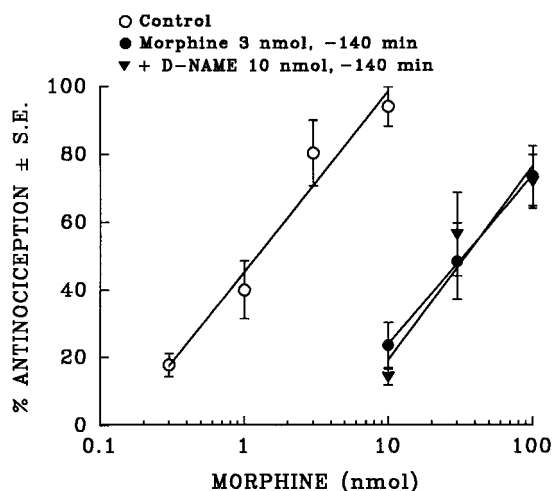
Fig. 2. Dose (A) and time (B) response of the effect of L-NAME on antinociception induced by i.c.v. morphine (10 nmol, 20 min before testing) in mice pretreated with a single i.c.v. pretreatment of morphine (3 nmol, 140 min before testing) in the mouse 55°C warm-water tail-flick assay. \*\* $P < .01$  in comparison with the group of mice pretreated with i.c.v. morphine (3 nmol, 140 min before testing) only.

effects of LY-83,583 and methylene blue, two guanylyl cyclase inhibitors, were studied by use of the same protocol. LY-83,583, at a dose of 10 nmol, completely blocked the development of acute morphine antinociceptive tolerance when LY-83,583 was co-administered with the initial injection of 3 nmol morphine (fig. 5 and table 1). Methylene blue, at a dose of 10 nmol, when co-administered with the 3-nmol morphine pretreatment, partially blocked the rightward shift of the morphine dose-response line caused by the morphine pretreatment (table 1).

**Determining whether NO production would enhance the development of acute morphine antinociceptive tolerance.** Babey *et al.* (1994) suggested that L-arginine, a NO precursor, when co-administered chronically with morphine, accelerates the development of morphine tolerance. Therefore, we were interested in determining whether L-arginine, or the NO donors, such as SNP and ISDN, which are able to release NO without the presence of NOS, would affect the acute morphine antinociceptive tolerance. To reveal the possible potentiating effect of these compounds on morphine tolerance, mice were pretreated i.c.v. with a lower

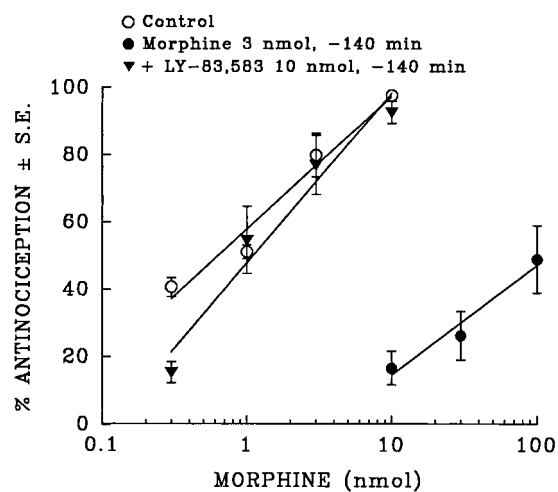


**Fig. 3.** Effect of L-arginine (L-ARG) and D-arginine (D-ARG) on the inhibition of morphine antinociceptive tolerance by L-NAME. Mice were pretreated with either morphine (3 nmol, 140 min before testing) alone, or morphine (3 nmol, 140 min before testing) and L-NAME (10 nmol, 140 min before testing) or morphine (1 nmol, 140 min before testing) and varying doses of L-ARG and D-ARG. A second i.c.v. injection of morphine (10 nmol) was given 20 min before testing in the mouse 55°C warm-water tail-flick assay. \* $P < .05$ , \*\* $P < .01$  in comparison with the group of mice pretreated with i.c.v. morphine (3 nmol) plus L-NAME (10 nmol) for 140 min before testing.

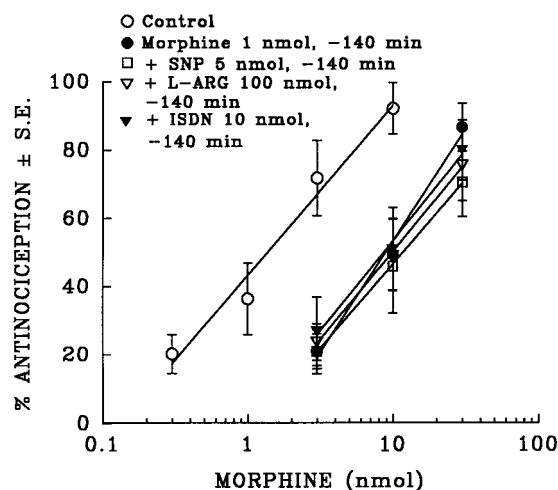


**Fig. 4.** Dose-response lines for i.c.v. morphine (20 min before testing) in mice treated alone or pretreated with either i.c.v. morphine (3 nmol) or i.c.v. morphine (3 nmol) and i.c.v. D-NAME (10 nmol) for 140 min before testing in the mouse 55°C warm-water tail-flick assay.

dose of morphine (1 nmol), so that only 6-fold of the rightward shift of morphine dose-response line was observed, which indicates a lower magnitude of the morphine tolerance (fig. 6). Neither L-arginine (100 nmol), SNP (5 nmol) nor ISDN (10 nmol), when co-administered with the 1-nmol morphine pretreatment for 140 min, affected the magnitude of the morphine antinociceptive tolerance (fig. 6, table 2). The effect of chronic and systemic pretreatment of L-arginine on the morphine tolerance was also investigated. As shown in figure 7, L-arginine, when given i.p. 200 mg/kg daily for 3 days, did not alter the magnitude of the morphine antinociceptive tolerance. These results suggest that increased production of NO did not affect the magnitude of acute morphine antinociceptive tolerance, but that NO was required for the development of acute antinociceptive tolerance.



**Fig. 5.** Dose-response lines for i.c.v. morphine (20 min before testing) in the absence and presence of i.c.v. morphine (3 nmol) and i.c.v. morphine (3 nmol) plus i.c.v. LY-83,583 (10 nmol) given 140 min before testing in the mouse 55°C warm-water tail-flick assay.



**Fig. 6.** Dose-response lines for i.c.v. morphine (20 min before testing) in the absence and presence of i.c.v. morphine (1 nmol) and in the presence of i.c.v. morphine (1 nmol) plus L-arginine (L-ARG) (100 nmol) or SNP (5 nmol) or ISDN (10 nmol) given by i.c.v. injection 140 min before testing in the mouse 55°C warm-water tail-flick assay.

## Discussion

The mechanisms underlying the tolerance to the antinociceptive action of opioids are not clear and need to be further explored. The phenomenon of opioid tolerance and dependence may involve changes in several opioid and non-opioid systems. Animal studies have revealed that there are two kinds of opioid tolerance, namely acute opioid tolerance and chronic opioid tolerance. The characteristics of acute opioid tolerance may differ from those of chronic opioid tolerance. The present study used an acute morphine antinociceptive tolerance model, which has been well documented in this laboratory (Jiang *et al.*, 1995). Acute antinociceptive tolerance to morphine develops within 2 hr after a single i.c.v. injection of morphine in mice.

The magnitude of opioid tolerance appears to be affected by the route of administration. The i.c.v. or i.t. administration of opioid agonists produces a larger magnitude of tolerance to subsequent i.c.v. or i.t. administration of the same opioid

TABLE 2

Effect of increased production of NO by L-arginine, SNP and ISDN on antinociceptive  $D_{50}$  values for i.c.v. morphine in mice pretreated with i.c.v. morphine for 140 min before testing

Pretreatment consisted of administering morphine and the compounds to mice by i.c.v. injection at 120 min before the subsequent morphine injection. Varying doses of morphine were then injected i.c.v. and tail-flick response was measured 20 min thereafter, as described under "Materials and Methods." The morphine control consisted of varying doses of morphine administered 20 min before testing.

Pretreatment	$D_{50}$ Values nmol (95% C.L.)
Morphine control	1.4 (0.8–2.0)
Morphine 1 nmol	8.8 (6.3–12)
Morphine 1 nmol + L-arginine 100 nmol	9.9 (6.1–15)
Morphine 1 nmol + SNP 5 nmol	11 (6.2–18)
Morphine 1 nmol + ISDN 10 nmol	8.5 (4.9–14)

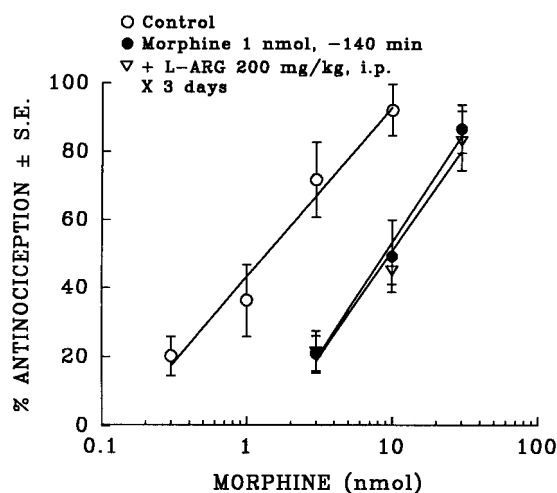


Fig. 7. Dose-response lines for i.c.v. morphine (20 min before testing) in the absence and presence of i.c.v. morphine (1 nmol, 140 min before testing) in mice treated with and without i.p. L-arginine (L-ARG) (200 mg/kg daily for 3 days) in the mouse 55°C warm-water tail-flick assay.

agonists, respectively, in comparison with the systemic administration of opioids. For example, with the  $\mu$  selective peptide DAMGO, Mattia *et al.* (1991) reported a 47-fold rightward shift of the i.c.v. DAMGO dose-response curve in mice pretreated with i.c.v. injections of the  $D_{90}$  dose of DAMGO twice daily for 3 days. The same protocol with the  $\delta$  selective peptide, [D-Ala<sup>2</sup>]deltorphin II, produced a greater than 37-fold shift in the i.c.v. [D-Ala<sup>2</sup>]deltorphin II dose-response curve (Mattia *et al.*, 1991). Other investigators demonstrated a robust 117-fold rightward shift in the i.t. morphine dose-response curve by a chronic i.t. infusion of 20 nmol/hr of morphine into rats for 7 days as measured by the hot-plate test (Stevens and Yaksh, 1989). On the other hand, a s.c. injection of 100 mg/kg of morphine produced only a 3- to 6-fold rightward shift in the s.c. morphine dose-response curve when measured by the radiant heat tail-flick test at 3 hr after the initial morphine injection (Vaught and Take-mori, 1979). With use of the acute antinociceptive tolerance model presented here and used previously (Jiang *et al.*, 1995), we have consistently observed a 30- to 70-fold rightward shift of the i.c.v. morphine dose-response curve in mice pretreated with a single i.c.v. injection of 3 nmol morphine for 140 min as measured by a 55°C warm-water tail-flick test. The reason why i.c.v. or i.t. administration of opioid agonists is more effective than the systemic route of administration in

producing tolerance is not understood. It could be the result of the relatively higher potency produced by systemic administration of opioid agonists, because both supraspinal and spinal opioid receptors are activated and a multiplicative interaction between the two sites may occur (Roerig and Fujimoto, 1989). This phenomenon fits the concept proposed by Stevens and Yaksh (1989) that the potency of antinociceptive agents is inversely related to magnitude of tolerance after continuous infusion.

The present study demonstrated that supraspinal administration of four different NOS inhibitors attenuated the development of acute morphine antinociceptive tolerance in a dose- and time-dependent manner. A dose of 10 nmol L-NAME completely blocked the acute morphine antinociceptive tolerance induced by a pretreatment of mice with 3 nmol morphine for 140 min. This action of L-NAME was partially antagonized by L-arginine but not D-arginine, which indicates the stereoselectivity of the L-arginine effect. This stereoselectivity of L-NAME effect was further supported by the ineffectiveness of the D-NAME in attenuating morphine tolerance. These results strongly suggest that inhibition of supraspinal NO production resulted in an inhibition of acute morphine antinociceptive tolerance. This finding is in line with studies reported by others that systemic administration of NOS inhibitors attenuates the development of tolerance to systemic morphine administration (Kolesnikov *et al.*, 1992, 1993; Babey *et al.*, 1994; Majeed *et al.*, 1994). Recently, i.t. administration of L-NAME has also been reported to have little effect in attenuating tolerance to i.t. morphine (Dunbar and Yaksh, 1996). Taken together, these studies suggest that NO at supraspinal but not the spinal site may play an important role in the mediation of morphine antinociceptive tolerance.

Studies by others suggest that L-NAME exhibits antinociceptive activity in the mouse (Moore *et al.*, 1991; Malmberg and Yaksh, 1993). In those studies, the antinociceptive activity of L-NAME was demonstrated in the formalin-induced paw licking test, as well as the acetic acid-induced writhing test and hot-plate test after L-NAME was administered by i.p. injection. Also, L-NAME produced antinociception in the formalin-induced paw licking test after i.c.v. or oral administration (Moore *et al.*, 1991). Przewlocki *et al.* (1993) reported that i.t. L-NAME potentiated i.t. morphine-induced antinociception. Based on these findings, it is logical to suspect that the reversal effect of L-NAME on morphine-induced antinociception after the development of tolerance might be caused by the additive or synergistic actions between the possible L-NAME-induced antinociceptive effect and morphine-induced antinociceptive effect. However, we were able to exclude this possibility for the following reasons. First, in the mouse 55°C warm-water tail-flick test, L-NAME at doses up to 10 nmol, when given i.c.v., did not produce a significant antinociceptive effect. Second, L-NAME at 10 nmol, when given along with, or 140 min before i.c.v. morphine injection, did not affect morphine-induced antinociception (data not shown). These results are consistent with the study reported by Xu and Tseng (1995), which demonstrated that L-NAME is not effective in modulating morphine-induced antinociception in the tail-flick test at the supraspinal site.

One of the actions of NO is to activate soluble guanylyl cyclase, thus increasing the level of cyclic GMP (Deguchi, 1977; Bredt and Snyder, 1992). This action of NO can be inhibited by LY-83,583 and methylene blue, which are inhib-

itors of guanylyl cyclase. In the present study, we found that LY-83,583, when given along with the i.c.v. morphine pretreatment, completely attenuated the development of morphine antinociceptive tolerance. Methylene blue, which is not as selective for guanylyl cyclase as LY-83,583, partially blocked the development of tolerance. These results suggest that the cyclic GMP system may also participate in the mediation of the morphine tolerance. There may be both cyclic GMP-dependent and -independent mechanisms involved in the effect of NO on the development of tolerance. Indeed, NO has been demonstrated to modulate certain neuronal proteins through a cyclic GMP-independent process. For example, Hess *et al.* (1994) reported that exogenous and endogenously generated NO resulted in the modification of cysteine residues on neuronal proteins. In particular, exposure of synaptosomes to NO inhibited subsequent thiol-linked ADP-ribosylation of the heterotrimeric G-protein by pertussis toxin.

Other studies with L-arginine further support the involvement of NO in morphine tolerance. L-Arginine, but not D-arginine, when given with morphine, appears to accelerate tolerance to systemic morphine (Babey *et al.*, 1994). L-Arginine is the natural substrate for NOS. Administration of L-arginine may increase the formation of NO and, thereby, possibly enhance the rate of development of morphine antinociceptive tolerance. In the present study, we focused on the effect of L-arginine on the magnitude of morphine tolerance. We clearly demonstrated that neither L-arginine, nor NO donors such as SNP and ISDN, which do not require NOS to produce NO, altered the magnitude of the acute morphine antinociceptive tolerance.

In summary, we found that inhibition of supraspinal NO/cyclic GMP system prevented the development of acute morphine antinociceptive tolerance. However, increased production of NO did not affect the magnitude of the morphine tolerance.

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