Br. J. Pharmacol. (1994), **113**, 1372–1376

Modulation of morphine antinociception in the mouse by endogenous nitric oxide

Giovanni Brignola, [1]Antonio Calignano & Massimo Di Rosa

Department of Experimental Pharmacology, University of Naples 'Federico II', Via Domenico Montesano 49, 80131 Naples, Italy

1 L-Arginine (100–1000 mg kg^{-1}) administered orally (p.o.) or intraperitoneally (i.p.), but not intracerebroventricularly (i.c.v., 0.08 mg per mouse), reduced the antinociceptive effect of morphine (0.5–10 mg kg^{-1} s.c.) assessed in mice using three different tests: hot plate, tail-flick and acetic acid-induced writhing. D-Arginine (up to 1000 mg kg^{-1} p.o. or i.p.) was ineffective.

2 N^G-Monomethyl-L-arginine (L-NMMA, 5–50 mg kg^{-1} i.p.) and N^G-nitro-L-arginine methyl ester (L-NAME, 5– 30 mg kg^{-1} i.p.), but not N^G-nitro-D-arginine methyl ester (D-NAME, 30 mg kg^{-1} i.p.), reversed in all assays the effect of L-arginine on morphine-induced antinociception.

3 Morphine (10 mg kg^{-1} s.c.), L-arginine (1000 mg kg^{-1} p.o.) or L-NAME (30 mg kg^{-1} i.p.), either alone or in combination, did not produce changes in locomotor activity or sensorimotor performance of animals.

4 These results suggest that the L-arginine–nitric oxide pathway plays a modulating role in the morphine-sensitive nociceptive processes.

Keywords: Antinociception; L-arginine; morphine; nitric oxide

Introduction

The biosynthesis of nitric oxide (NO) from L-arginine is a pathway for the regulation of cell function and communication (Moncada *et al.*, 1991). In all cell types so far studied NO is generated following oxidation and cleavage of the terminal nitrogen atom(s) of L-arginine by an enzyme, NO synthase. This enzyme is competitively inhibited by some L-arginine analogues including N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) (Rees *et al.*, 1990).

Histochemical studies using antibodies to the constitutive NO synthase have shown that this enzyme occurs widely in brain and in peripheral nerves (Bredt *et al.*, 1990). There is now considerable evidence that NO modulates synaptic transmission in both the central and peripheral nervous system (Meller & Gebhart, 1993), and it has been suggested that NO is involved in nociceptive processes either in the periphery or within the spinal cord (Haley *et al.*, 1992). It has been shown in mouse and rat that NO mediates the *N*-methyl-D-aspartate (NMDA)-produced facilitation of the nociceptive tail-flick reflexes which depends on the activity of spinal cord neurones (Kitto *et al.*, 1992; Meller *et al.*, 1992a). Furthermore, it has been reported that L-NAME enhances morphine antinociception in rats (Przewlocki *et al.*, 1993).

In this study we have investigated in mice the interaction between the L-arginine–NO pathway and the antinociceptive effect of morphine using three experimental models: hot plate, tail-flick and acetic acid-induced writhing.

Methods

Animals

Male Swiss mice (25–30 g, Nossan, Italy) were used. Animals were housed in a room maintained at 22 ± 1°C with an alternating 12 h light–dark cycle. Food and water were available *ad libitum*. All experiments were carried out between 09.00 and 12.00 h.

[1] Author for correspondence.

Drugs

Morphine hydrochloride was obtained from SALARS (Italy), *N*-methylmorphine iodide was synthetized in our laboratory, N^G-nitro-D-arginine methyl ester (hydrochloride-D-NAME) was obtained from Bachem (Switzerland), N^G-monomethyl-L-arginine acetate (L-NMMA) was kindly donated by Dr S. Moncada (Wellcome Research Laboratories, U.K.), and L-arginine hydrochloride, D-arginine hydrochloride and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma (U.S.A.). Drugs were dissolved in saline and administered by various routes: morphine and *N*-methylmorphine were given subcutaneously (s.c., 10 ml kg^{-1}); L-arginine orally (p.o., 0.1 ml per mouse), intraperitoneally (i.p., 10 ml kg^{-1}) or intracerebroventricularly (i.c.v., 0.005 ml per mouse): D-arginine p.o. (0.1 ml per mouse); and L-NMMA, L-NAME and D-NAME i.p. (10 ml kg^{-1}).

Hot plate

Mice were placed on a hot plate (55.5 ± 0.5°C) according to the procedure described by Eddy and Leimback (1953). The reaction time(s) measured was either jumping off the plate or hind paw licking. The cut-off imposed was 60 s to avoid tissue damage (Porreca *et al.*, 1984). Each mouse was tested twice before administration of drugs and the reaction times were averaged to obtain a baseline. The reaction time was subsequently assessed 30 min after morphine administration or 60 min after other drugs which were given either alone or in combination. Control mice received saline instead of drugs. The antinociceptive response of each mouse was expressed as the maximum percentage effect (MPE), which was calculated as:

$$\frac{\text{Post-drug reaction time} - \text{baseline reaction time}}{\text{Cut-off value} - \text{baseline reaction time}} \times 100$$

(Hong *et al.*, 1993).

Tail flick

The tail-flick was evoked by a source of radiant heat, which was focused on the dorsal surface of the tail (D'Amour &

Smith, 1941, modified by Dewey *et al.*, 1970). Each mouse was tested twice before drug administration and the reaction times were averaged to obtain a baseline. The intensity of heat stimulus was adjusted so that the mouse flicked its tail after 2–4 s. Each mouse was then tested 30 min after morphine administration or 60 min after other drugs, which were given either alone or in combination. Control mice received saline instead of drugs. The cut-off imposed was 10 s to prevent tissue damage. The antinociceptive response of each mouse was calculated as MPE.

Acetic acid-induced writhing

Writhing was induced by the i.p. injection of 0.5 ml per mouse of acetic acid [0.6% (v/v) in saline] and the abdominal constrictions were counted for 20 min thereafter. Results were expressed as number of abdominal constrictions per mouse. Morphine and all the other drugs used were administered, respectively, 30 min and 60 min before acetic acid. Control animals received appropriate volumes of saline by different routes (i.p., p.o., s.c.). Saline was given by single or multiple administrations according to the drug treatment schedule. Since the number of abdominal constrictions in these groups did not differ significantly, the corresponding data were cumulated and shown as controls in the Results section (see Figures 4 and 5).

Locomotor activity

Locomotor activity was recorded for 20 min in an activity cage (Basile, Italy). Mice were acclimatized to the activity cage at least once prior to starting the experimental session. Morphine and all other drugs used were administered, respectively, 30 and 60 min before the session.

Sensorimotor performance

To evaluate the muscle-relaxing and sedative effects of drugs, the mice were tested on a Rotarod (Rosland *et al.*, 1990). Briefly, the Rotarod apparatus (Basile, Italy) consists of a rod 30 cm long and 3.0 cm in diameter, subdivided into five compartments by discs 24 cm in diameter. The rod rotated at a constant speed of 16 r.p.m. Sensorimotor performance was assessed 30 min after morphine administration or 60 min after L-NAME or L-arginine, which were given either alone or in combination. Results are expressed as percentage of animals that succeeded in remaining on the rod for 45 s, which was the cut-off time.

Statistical analysis

Results are expressed as mean ± s.e.mean of 6–18 animals. Statistical comparisons were made using the Student's *t*-test. *P*-values less than 0.05 were considered statistically significant.

Results

Hot plate

L-Arginine or D-arginine, up to 1000 mg kg^{-1} p.o., did not modify mouse nociception. Thus the MPEs in these groups were respectively 0.5 ± 2.6 and 3.8 ± 2.1 ($n = 6$ for each group) and were similar to those observed in control mice (1.7 ± 2.0, $n = 6$).

Morphine caused a dose-dependent increase in the reaction time (Figure 1). The MPEs after 5 and 10 mg kg^{-1} morphine were 47.0 ± 3.5 ($n = 18$, $P<0.01$) and 82.8 ± 6.4 ($n = 18$, $P<0.01$) respectively.

L-Arginine (100–1000 mg kg^{-1} p.o.), but not D-arginine (up to 1000 mg kg^{-1}) reduced, in a dose-related manner, morphine-induced antinociception. Similar results were obtained when L-arginine was administered i.p. (data not shown). In contrast, L-arginine when given i.c.v. (0.08 mg per mouse) was ineffective in reducing the effect of 5 or 10 mg kg^{-1} morphine (MPEs 50.3 ± 6.8 and 85.7 ± 7.0 respectively, $n = 6$ for each group).

L-NAME (10, 30 mg kg^{-1}), but not D-NAME (30 mg kg^{-1}), produced a slight antinociceptive effect which was significant at the highest dose (MPE 19.5 ± 5.0, $n = 12$, $P<0.01$). This effect was reversed by 1000 mg kg^{-1} p.o. L-arginine (MPE 2.4 ± 1.3, $n = 6$, $P<0.01$).

A combined treatment of L-arginine (p.o.) and L-NAME, depending on the dose ratio of these two drugs, reduced (dose ratio 1000:10), did not modify (dose ratio 300:10) or increased (dose ratio 300:30) the antinociceptive effect induced by 5 mg kg^{-1} morphine (Figure 2).

The ability of L-arginine (300 mg kg^{-1} p.o.) to reduce the antinociception induced by 5 mg kg^{-1} morphine was significantly antagonized by the administration of 50 mg kg^{-1} L-NMMA (MPE 45.8 ± 9.1, $n = 6$, $P<0.01$).

Tail-flick

No changes in tail-flick response were observed in mice treated with L-arginine or D-arginine (each group up to

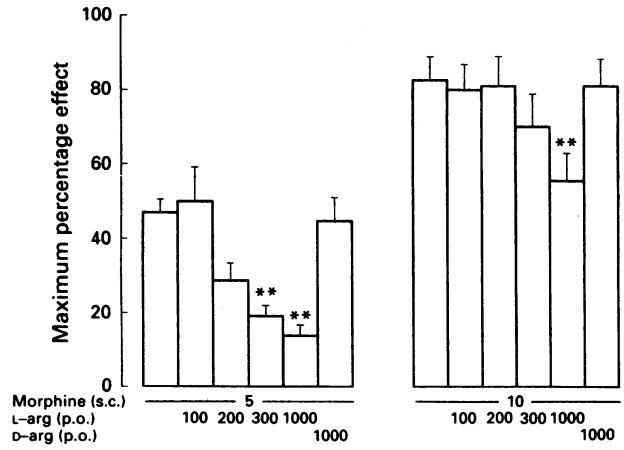

Figure 1 Effect of L-arginine (L-arg) and D-arginine (D-arg) on morphine-induced antinociception in mice assessed by the hot plate test. Doses are expressed in mg kg^{-1}. Each column represents the mean ± s.e.mean (vertical bar) of 6–18 animals. $^{**}P<0.01$ vs. morphine alone.

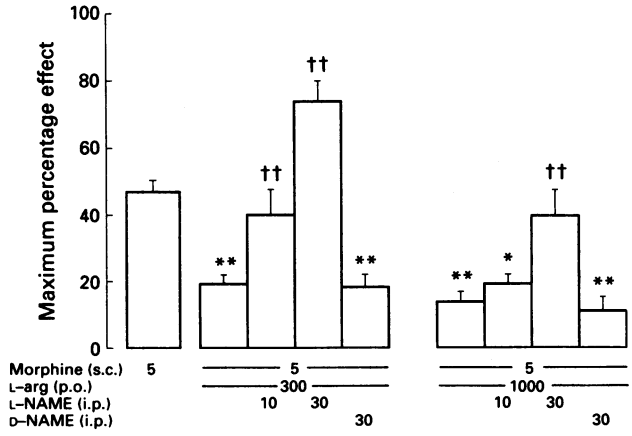

Figure 2 N^G-nitro-L-arginine methyl ester (L-NAME), but not N^G-nitro-D-arginine methyl ester (D-NAME), modulates the effect of L-arginine (L-arg) on morphine-induced antinociception in mice assessed by the hot plate test. Doses are expressed in mg kg^{-1}. Each column represents the mean ± s.e.mean (vertical bar) of 6–18 animals. $^{*}P<0.05$, $^{**}P<0.01$, vs. morphine alone; $\dagger\dagger P<0.01$, vs. morphine + L-arg.

1000 mg kg^{-1} p.o., $n = 6$). Thus the MPEs in these groups were respectively 2.4 ± 5.6 and 4.3 ± 3.1 and were not significantly different from those observed in control mice (3.5 ± 3.1, $n = 6$).

Morphine caused a dose-dependent increase in the reaction time (Figure 3). The MPEs after 2.5 or 5 mg kg^{-1} morphine were respectively 68.3 ± 12.4 ($n = 12$, $P<0.01$) and 91.2 ± 8.8 ($n = 6$, $P<0.01$).

L-Arginine (100–1000 mg kg^{-1} p.o.), but not D-arginine (1000 mg kg^{-1}), dose-dependently reduced the morphine effect on the tail-flick reflex.

The ability of L-arginine (300 mg kg^{-1} p.o.) to reduce the antinociception induced by 2.5 mg kg^{-1} morphine was significantly antagonized by the administration of 10 mg kg^{-1} L-NAME (MPE 74.5 ± 9.8, $n = 6$, $P<0.01$) or 30 mg kg^{-1} L-NMMA (MPE 65.2 ± 10.7, $n = 6$, $P<0.05$).

Acetic acid-induced writhing

Administration of 0.6% acetic acid (0.5 ml per mouse i.p.) in mice ($n = 18$) induced 51.0 ± 1.3 abdominal constrictions per mouse in the 20 min test period. L-Arginine or D-arginine treatment (up to 1000 mg kg^{-1} p.o.) did not modify the incidence of abdominal constrictions (53.2 ± 2.6 and 52.4 ± 4.1 respectively, $n = 6$ for each group). Morphine (0.5 mg kg^{-1}) significantly reduced by 55.9 ± 6.5% the number of abdominal constrictions ($P<0.01$, Figure 4). L-Arginine (100–1000 mg kg^{-1} p.o.), but not D-arginine (1000 mg kg^{-1}), dose-dependently reduced the effect of morphine. L-NAME (5 mg kg^{-1}) on its own did not modify the number of abdominal constrictions, whereas it was able to antagonize the L-arginine (300 mg kg^{-1} p.o.) effect on morphine-induced antinociception (Figure 5). Similar results were observed with L-NMMA (data not shown). *N*-Methylmorphine (5 mg kg^{-1}) significantly reduced the number of abdominal constrictions (25.4 ± 4.7, $n = 6$, $P<0.01$). L-Arginine (300 mg kg^{-1} p.o.) reversed the antinociceptive effect of this quaternarized morphine analogue (48.5 ± 4.8, $n = 6$, $P<0.01$).

Locomotor activity and sensorimotor performance

Locomotor activity of control mice was 730 ± 20 counts per session ($n = 6$). The administration of morphine (10 mg kg^{-1}), L-arginine (1000 mg kg^{-1} p.o.) or L-NAME (30 mg kg^{-1}), either alone or in combination, did not modify spontaneous activity. In fact, in these groups the locomotor activity ranged between 720 ± 23 counts and 746 ± 25 ($n = 6$ for each group).

The sensorimotor performance of control mice was 95.2 ± 4.8% ($n = 6$). The administration of morphine (10 mg kg^{-1}), L-arginine (1000 mg kg^{-1} p.o.) or L-NAME (30 mg kg^{-1}), either alone or in combination, did not modify sensorimotor performance. In fact, in these groups the percentage of animals with success in the Rotarod test ranged between 90.3 ± 6.7% and 97.4 ± 2.6% ($n = 6$ for each group).

Discussion

In this study we have shown that oral or intraperitoneal administration of L-arginine dose-dependently and stereospecifically reduces the antinociceptive effect of morphine assessed in mice using three different tests: hot plate, tail-flick and acetic acid-induced writhing.

L-Arginine is a substrate for NO synthase, the enzyme that releases NO from the terminal nitrogen atom(s) of the guanidino group of this amino acid (Moncada *et al.*, 1991). Since the L-arginine effect was reversed by concomitant

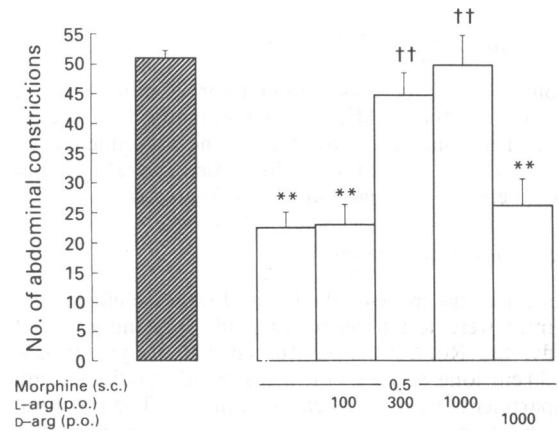

Figure 4 Effect of L-arginine (L-arg) and D-arginine (D-arg) on morphine-induced antinociception in mice assessed by the acetic acid-induced abdominal constrictions test. Doses are expressed in mg kg^{-1}. Each column represents the mean ± s.e.mean (vertical bar) of 6–18 animals. **$P<0.01$ vs. control (hatched column); ††$P<0.01$ vs. morphine alone.

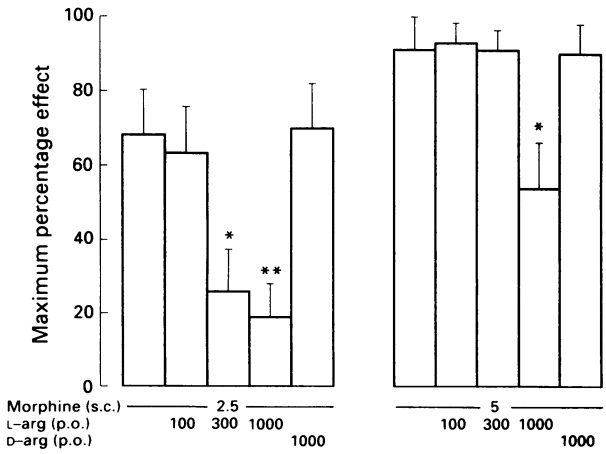

Figure 3 Effect of L-arginine (L-arg) and D-arginine (D-arg) on morphine-induced antinociception in mice assessed by the tail-flick test. Doses are expressed in mg kg^{-1}. Each column represents the mean ± s.e.mean (vertical bar) of 6–12 animals. *$P<0.05$, **$P<0.01$ vs. morphine alone.

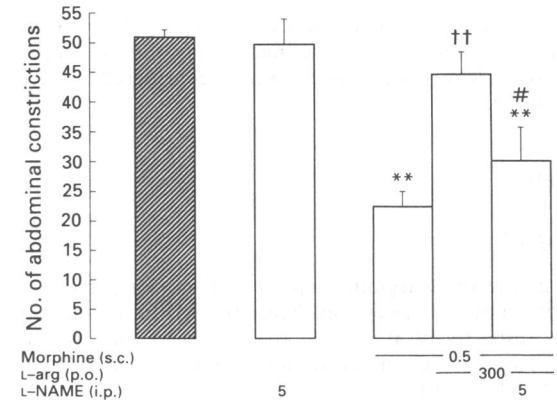

Figure 5 N^G-nitro-L-arginine methyl ester (L-NAME) modulates the effect of L-arginine (L-arg) on morphine-induced antinociception in mice assessed by the acetic acid-induced abdominal constrictions test. Doses are expressed in mg kg^{-1}. Each column represents the mean ± s.e.mean (vertical bar) of 6–18 animals. **$P<0.01$ vs. control (hatched column); ††$P<0.01$ vs. morphine alone; #$P<0.05$ vs. morphine + L-arg.

administration of competitive inhibitors of NO synthase such as L-NAME or L-NMMA (Rees *et al.*, 1990), our results strongly suggest that the reduction in morphine-induced antinociception by L-arginine is dependent on NO formation. This hypothesis is strengthened by the observation that neither D-arginine, which is not a substrate for NO synthase, nor D-NAME, which does not inhibit the NO synthase, was able to modify the antinociceptive effect of morphine.

Interestingly we have shown that following combined treatment with L-arginine and L-NAME, depending on the dose ratio of the two drugs, the antinociceptive effect of morphine was either unchanged, reduced or enhanced. Most likely, since both drugs compete for the active site of NO synthase, when a balanced dose combination of L-arginine and L-NAME was used the morphine effect was not modified, whereas when the dose ratio was shifted towards L-arginine the effect of the amino acid prevailed and the morphine effect was reduced. On the other hand, when the dose ratio was shifted towards L-NAME, this drug, even in the presence of L-arginine, was able to exhibit its own antinociceptive action and the morphine effect was increased. In this light it is interesting that low doses (1–2 mg kg^{-1} i.p.) of the NO synthase inhibitor N^G-nitro-L-arginine did not modify the antinociceptive effect of morphine (Kolesnikov *et al.*, 1992; Elliott *et al.*, 1994).

The involvement of NO in the nociceptive process is also supported by our results showing that L-NAME not only reversed the L-arginine effect, but produced a slight antinociceptive effect on its own, which is in agreement with previous reports (Moore *et al.*, 1991; Mustafa, 1992). We have also shown that the antinociceptive effect of L-NAME may be reversed by L-arginine.

L-NAME is a selective inhibitor of NO biosynthesis in a variety of mammalian tissue and cells, including both peripheral (Gibson *et al.*, 1990) and central neurones (Murad *et al.*, 1990). Although it has been suggested that L-NAME produces antinociception by a direct effect within the central nervous system (Moore *et al.*, 1991), L-NAME may also act on peripheral nerves where, according to our results, the interaction with L-arginine may take place.

It is important to point out that L-arginine also antagonizes the antinociceptive effect of *N*-methylmorphine in the acetic acid writhing test. This morphine derivative does not cross the blood–brain barrier and is therefore devoid of central effects.

The existence of a peripheral antinociceptive action of opiates is supported by several studies on the effects of quaternary analogues of opiates. It has been reported that *N*-methylmorphine induces an antinociceptive effect in the writhing test and in the hyperalgesia induced by prostaglandin E_2 in the rat paw (Smith *et al.*, 1982; Lorenzetti & Ferreira, 1982). Furthermore, Bentley *et al.*, (1981) have shown that opiate receptors are present in the mouse peritoneum. Our results clearly suggest that the L-arginine–NO pathway modulates both the central and peripheral antinociceptive effects of morphine and that this modulation operates at peripheral sites. In fact, the interaction between L-arginine and morphine does not occur at supraspinal sites because in the hot plate test, which is widely believed to be sensitive solely to drugs acting supraspinally (Yaksh & Rudy, 1977), the amino acid was ineffective when given i.c.v. Furthermore we have shown that morphine-induced constipation in the mouse, which mainly depends on action(s) within the central nervous system (Schulz *et al.*, 1979), is modulated by L-arginine acting at peripheral sites (Calignano *et al.*, 1991). However, a spinal interaction cannot be ruled out. Indeed, an involvement of NO in spinally mediated hyperalgesia has been described (Kitto *et al.*, 1992; Meller *et al.*, 1992a,b). Our results showing that L-arginine reverses the antinociceptive effect of *N*-methylmorphine in acetic acid-induced writhing suggest that endogenous NO may well play a role in the modulation of peripheral pain mechanism(s).

It has been reported that NO mediates both the peripheral and central antinociceptive effects of morphine evaluated using a modification of the Randall–Sellitto rat paw pressure test (Ferreira *et al.*, 1991; Duarte & Ferreira, 1992). The reason for the discrepancy may be related to differences between species or nociceptive stimuli.

Since animal models of nociception may be influenced by factors such as muscle relaxation, sedation or behaviour of the animals, we also investigated the possible sedative and behavioural effects of the drugs used. At the maximum doses used in the nociceptive assays, morphine, L-NAME and L-arginine, either alone or in combination, did not modify the spontaneous locomotor activity of animals or their performances in the Rotarod test.

We have previously shown that L-arginine modulates other morphine effects in mice. Thus, morphine-induced constipation is reduced by L-arginine, whereas this amino acid increases the effects of morphine on locomotor activity and food intake (Calignano *et al.*, 1991; 1993). Therefore it appears that L-arginine reduces morphine-induced antinociception by mechanisms other than interference with the absorption/distribution of the opioid. In conclusion, our results further support the hypothesis that NO is involved in the modulation of morphine effects and suggest that the L-arginine–NO pathway modulates morphine-sensitive nociceptive processes. Further studies will clarify the possible mechanism(s) of the modulatory role played by L-arginine in morphine-induced effects.

References

BENTLEY, G.A., NEWTON, S.H. & STARR, J. (1981). Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum. *Br. J. Pharmacol.*, **73**, 325–332.

BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, **347**, 768–770.

CALIGNANO, A., MONCADA, S. & DI ROSA, M. (1991). Endogenous nitric oxide modulates morphine-induced constipation. *Biochem. Biophys. Res. Comm.*, **181**, 889–893.

CALIGNANO, A., PERSICO, P., MANCUSO, F. & SORRENTINO, L. (1993). Endogenous nitric oxide modulates morphine-induced changes in locomotion and food intake in mice. *Eur. J. Pharmacol.*, **231**, 415–419.

D'AMOUR, F.E. & SMITH, D.L. (1941). A method for determination of pain sensation. *J. Pharmacol. Exp. Ther.*, **72**, 74–79.

DEWEY, W.L., HARRIS, L.S., HOWES, J.F. & NUITE, J.A. (1970). The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J. Pharmacol. Exp. Ther.*, **175**, 435–442.

DUARTE, I.D.G. & FERREIRA, S.H. (1992). The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine-nitric oxide-cGMP pathway. *Eur. J. Pharmacol.*, **221**, 171–174.

EDDY, N.B. & LEIMBACK, D. (1953). Synthetic analgesics. II. Dithienylbutenyl and dithienylbutylamines. *J. Pharmacol. Exp. Ther.*, **107**, 385–393.

ELLIOTT, K., MINAMI, N., KOLESNIKOV, Y.A., PASTERNAK, G.W. & INTURRISI, C.E. (1994). The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, N^G-nitro-L-arginine, attenuate analgesic tolerance to the mu-opioid morphine but not to kappa opioids. *Pain*, **56**, 69–75.

FERREIRA, S.H., DUARTE, I.D.G. & LORENZETTI, B.B. (1991). The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release. *Eur. J. Pharmacol.*, **201**, 121–122.

GIBSON, A., MIRZAZADEH, S., HOBBS, A.J. & MOORE P.K. (1990). L-N^G-Monomethyl arginine and L-N^G-nitroarginine inhibit non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus. *Br. J. Pharmacol.*, **99**, 602–606.

HALEY, J.E., DICKENSON, A.H. & SCHACTER, M. (1992). Electrophysiological evidence for a role of nitic oxide in prolonged chemical nociception in the rat. *Neuropharmacology*, **31**, 251–258.

HONG, M., SUTAK, M. & JHAMANDAS, K. (1993). Inhibition of spinal opioid antinociception by intrathecal β-endorphin$_{1-27}$ in the rat. *Br. J. Pharmacol.*, **108**, 1137–1142.

KITTO, K.F., HALEY, J.E. & WILCOX, G.L. (1992). Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. *Neurosci. Lett.*, **148**, 1–5.

KOLESNIKOV, Y.A., PICK, C.G., & PASTERNAK, G.W. (1992). N^G-Nitro-L-arginine prevents morphine tolerance. *Eur. J. Pharmacol.* **221**, 399–400.

LORENZETTI, B.B. & FERREIRA, S.H. (1982). The analgesic effect of quaternary analogues of morphine and nalorphine. *Brazil. J. Med. Biol. Res.*, **15**, 285–290.

MELLER, S.T. & GEBHART, G.F. (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain*, **52**, 127–136.

MELLER, S.T., DYKSTRA, C. & GEBHART, G.F. (1992a). Production of endogenous nitric oxide and activation of soluble guanylate cyclase are required for *N*-methyl-D-aspartate-produced facilitation of the nociceptive tail-flick reflex. *Eur. J. Pharmacol.*, **214**, 93–96.

MELLER, S.T., PECHMAN, P.S., GEBHART, G.F. & MAVES, T.J. (1992b). Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. *Neuroscience*, **50**, 7–10.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, patophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

MOORE, P.K., OLUYOMI, A.O., BABBEDGE, R.C., WALLACE, P. & HART, S.L. (1991). L-N^G-Nitro arginine methyl ester exhibits antinociceptive activity in the mouse. *Br. J. Pharmacol.*, **102**, 198–202.

MURAD, F., POLLOCK, J.S., SCHMIDT, H.H.H.W., FORSTERMANN, U., ISHII, K., HEILER, M. & GORSKY, I.D. (1990). Characterization and purification of a rat brain enzyme which catalyses the formation of an EDRF-like factor from L-arginine. *Eur. J. Pharmacol.*, **183**, 647–648.

MUSTAFA, A.A. (1992). Mechanisms of L-N^G-nitro arginine methyl ester-induced antinociception in mice: a role for serotonergic and adrenergic neurons. *Gen. Pharmacol.*, **23**, 1117–1182.

PORRECA, F., MOSBERG, H.I., HURST, R., HRUBY, V.J. & BURKS, T.F. (1994). Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. *J. Pharmacol. Exp. Ther.*, **230**, 341–348.

PRZEWLOCKI, R., MACHELSKA, H. & PRZEWLOCKA, B. (1993). Inhibition of nitric oxide synthase enhances morphine antinociception in the rat spinal cord. *Life Sci.*, **53**, PL 1–5.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.* **101**, 746–752.

ROSLAND, J.H., HUNSKAAR, S. & HOLE, K. (1990). Diazepam attenuates morphine antinociception test-dependently in mice. *Pharmacol. Toxicol.*, **66**, 382–386.

SCHULZ, R., WUSTER, M. & HERZ, A. (1979). Centrally and peripherally mediated inhibition of intestinal motility by opioids. *Naunyn Schmied. Arch. Pharmacol.*, **308**, 255–260.

SMITH, T.W., BUCHAN, P., PARSONS, D.N. & WILKINSON, S. (1982). Peripheral antinociceptive effects of N-methyl morphine. *Life Sci.*, **31**, 1205–1208.

YAKSH, T.L. & RUDY, T.A. (1977). Studies on the direct spinal action of narcotics on the production of analgesia in the rat. *J. Pharmacol. Exp. Ther.*, **202**, 411–428.

(Received May 24, 1994
Revised August 3, 1994
Accepted August 9, 1994)