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Effects of Acute Administration of L-Arginine on Morphine Antinociception and Morphine Distribution in Central and Peripheral Tissues of Mice

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BHARGAVA, H. N. AND J.-T. BIAN. *Effects of acute administration of L-arginine on morphine antinociception and morphine distribution in central and peripheral tissues of mice.* PHARMACOL BIOCHEM BEHAV **61**(1) 29–33, 1998.—The effect of acute treatment with L-arginine, a substrate for nitric oxide synthase (NOS) that forms NO, an important second messenger, on morphine antinociception and distribution of morphine in central and peripheral tissues of male Swiss–Webster mice was determined. The antinociception activity of morphine (10 mg/kg, SC) was attenuated by 400 and 800 mg/kg doses of L-arginine, but a lower dose (200 mg/kg) had no effect. D-Arginine (200–800 mg/kg) did not modify morphine antinociception. The dose of L-arginine (200 mg/kg) that did not modify morphine antinociception also did not alter the distribution of morphine in brain regions and spinal cord. A dose of 800 mg/kg of L-arginine produced a significant decrease in the concentration of morphine in midbrain and spinal cord. The highest dose of L-arginine (800 mg/kg) also increased the concentration of morphine in spleen. None of the doses of L-arginine modified the concentration of morphine in serum or urine. The results suggests that acute activation of the NO system attenuates morphine antinociception possibly by inhibiting its uptake in central sites (midbrain and spinal cord) involved in antinociceptive actions. © 1998 Elsevier Science Inc.

Morphine Nitric oxide Antinociception Morphine concentration Brain regions Peripheral tissues

MECHANISMS by which opioid drugs like morphine produce their antinociceptive action have not been delineated. Recent studies have implicated nitric oxide (NO) in the nociceptive. NO, a second messenger involved in the regulation of cell function, is formed enzymatically by the action of NO synthase (NOS) on L-arginine (12). Drugs that modify the concentration of NO in the central nervous system appear to modify morphine-induced antinociception. Acute administration of L-arginine orally or intraperitoneally but not intracerebroventricularly attenuates morphine antinociception in mice. The effect of L-arginine was reversed by inhibitors of NOS like N^G-nitro-L-arginine (L-NNA) or N^G-monomethyl-L-arginine (L-NMMA) (10). Studies from this laboratory indicate that chronic administration of L-arginine decreases morphine induced antinociception (1,2). Although the study of Brignola et al. (10) suggested that NO was involved in the action of L-arginine in decreasing morphine antinociception because such an action was reversed by NOS inhibitors, we have demonstrated that decreases in morphine antinociception by chronic treatment with L-arginine was associated with decreases in morphine concentration in several brain regions and spinal cord (2) and increases in peripheral tissues like

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lungs and spleen (1). Additionally, chronic administration of L-arginine did not alter antinociception induced by intracerebroventricular administration of morphine (9).

The present studies were undertaken to determine the effects of acute administration of L-arginine or D-arginine on morphine antinociception. To determine the mechanisms involved in altered morphine antinociception by acute treatment with L-arginine, the distribution of morphine in brain regions, peripheral tissues, serum, and urine of mice was determined under the conditions in which antinociceptive response to morphine was found to be altered.

METHOD

Animals

Male Swiss–Webster mice weighing 25–30 g (Sasco King Animal Co., Oregon, WI) were acclimated to a room with controlled ambient temperature (23 ± 1°C), humidity (50 ± 10%) and a 12 L:12 D cycle (0600–1800 h). The animals were housed under these conditions for at least 4 days prior to being used. The animals were given food and water continuously.

Chemicals

Morphine sulfate was purchased from the Mallinckrodt Chemical Co., St. Louis, MO. L-Arginine and D-arginine were purchased from the Sigma Chemical Co., St. Louis, MO. The drugs were dissolved in physiological saline and injected intraperitoneally (IP) or subcutaneously (SC) in a volume of 10 ml/kg body weight. The RIA kit for morphine was obtained form the Diagnostic Products Corporation, Los Angeles, CA.

Acute Treatment With L-Arginine or D-Arginine

Mice were injected IP with vehicle, L-arginine (200–800 mg/kg) or D-arginine (200–800 mg/kg). Ten minutes later, the animals were injected with morphine (10 mg/kg, SC) and antinociceptive response was measured as described below.

Measurement of the Antinociceptive Response

The antinociceptive response to morphine was measured by the tail-flick test as described earlier (5,8,11). At the beginning of the study, the light intensity in the tail-flick apparatus was adjusted so that the mean basal latencies for the tail-flick response were approximately 2 s. To minimize tail skin tissue damage, the cut-off time was set at 10 s. The tail-flick latencies were determined before and 30-min intervals after the injection of morphine. The basal tail-flick latencies were subtracted from the effect induced by the drug for each mouse. The antinociceptive response for each mouse was converted into area under the curve (AUC). AUC was used to evaluate the effect of antinociception of morphine. The baseline of tail flick latency in each mouse was first determined for around 2 s. And then the tail flick latencies were measured every 30 min after the SC injection of morphine until 210 min when the effect of morphine antinociception almost recovered the baseline. These data points at 0, 30, 60, 90, 120, 150, 180, and 210 min before and after the injection of morphine were taken for calculation of AUC for each mouse. AUC is the area of tail flick latency at each data point times the time from 0 to 210 min. The AUC in every dose in Fig. 1 is the mean value that the total AUC for 0–210 min subtracted the baseline AUC for the determination in each mouse, and such AUC values were for the 10 mice in every dose group (1,2). Data were expressed as AUC (means ± SEM). Ten mice were used for each treatment group. The differences in the antinociceptive response in vehicle and drug-treated mice were determined by ANOVA followed by the Students' *t*-test. A value of $p < 0.05$ was considered to be significant.

Measurement of Morphine Concentration in Central and Peripheral Tissues of L-Arginine or D-Arginine-Treated Mice

Mice were treated with appropriate doses of L-arginine, D-arginine, or their vehicles. Ten minutes later the animals were injected with morphine (10 mg/kg, SC) and killed 60 min later. Brain was isolated and dissected into several regions namely, cortex, striatum, hypothalamus, midbrain, hippocampus, amygdala, and pons and medulla. Spinal cords were also isolated. The peripheral tissues (lungs, liver, kidneys, and spleen), serum, and urine were collected. The tissues samples were stored in a deep freeze at −80°C. The concentration of morphine in the tissues was determined by RIA as described previously (3–7). This detection method uses ^{125}I-labeled morphine, which competes with morphine in the sample for antibody sites. The antibody is immobilized to the wall of a polypropylene tube. The reaction of morphine and antibody was terminated by decanting the supernatant. The antibody-bound radiolabeled morphine was counted in a gamma counter. The tissues were weighed and homogenized in 3–10 times the volume of water using a Polytron homogenizer PT 10 (setting 6 for 15 s). The final homogenate contained approximately 60 mg of tissue/ml. The concentration of morphine in the homogenate was expressed as nanograms per gram of tissue. The recovery of morphine from the tissues was found to be quantitative. The limit of detection was 0.8 ng/ml of homogenate. The specificity and the cross-reactivity of the antibody with morphine metabolites and other analogs has been described previously (5). The antibody had cross-reactivity with the two morphine glucuronides to only 0.2% and for normorphine 10%. Eight mice were used for each treatment group.

The differences in the morphine concentrations in various tissues, serum and urine of vehicle, L-arginine, and D-argi-

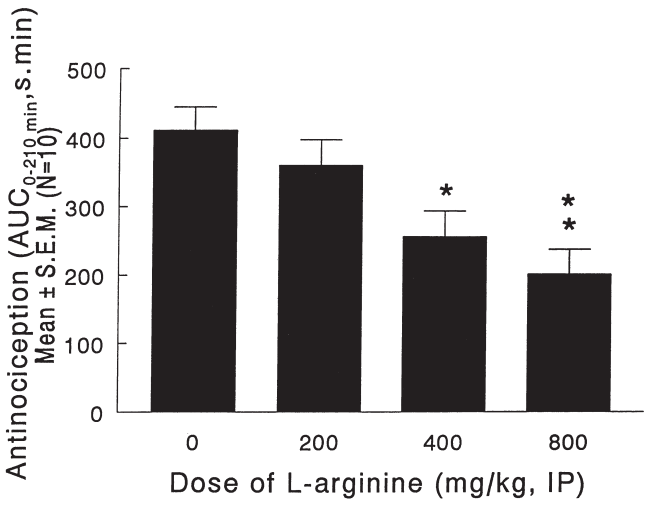

FIG. 1. Effect of different doses of L-arginine given acutely on the morphine (10 mg/kg, SC) induced antinociception in mice. L-arginine was injected 10 min prior to the injection of morphine.

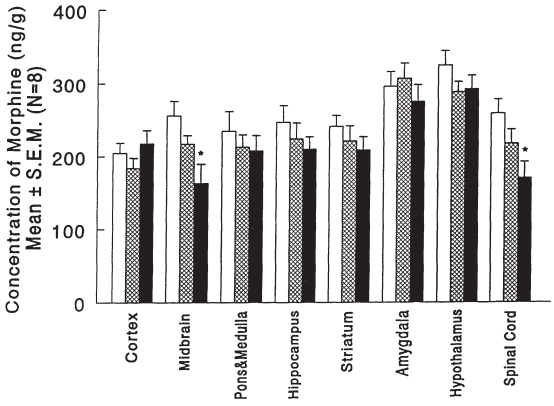

FIG. 2. Effect of different doses of L-arginine on the distribution of morphine in brain regions and spinal cord of mice. Morphine (10 mg/kg, SC) was injected 10 min after the injection of an appropriate dose of L-arginine (open column) vehicle; (crosshatched column) L-arginine (200 mg/kg, IP); (filled column) L-arginine (800 mg/kg, IP).

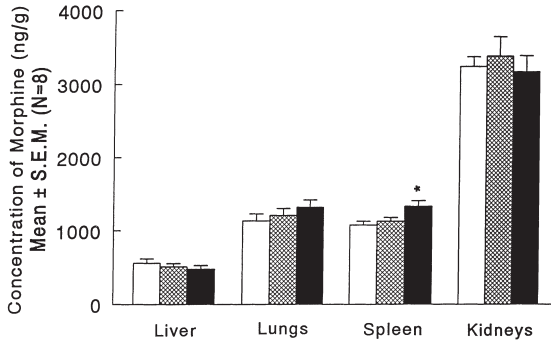

FIG. 4. Effect of different doses of L-arginine on the distribution of morphine in peripheral tissues. Morphine (10 mg/kg, SC) was injected 10 min after the injection of an appropriate dose of L-arginine (open column) vehicle; (crosshatched column) L-arginine (200 mg/kg, IP); (filled column) L-arginine (800 mg/kg, IP).

nine–treated mice were determined by using ANOVA followed by the Newman–Keuls test. A value of $p < 0.05$ was considered to be significant.

RESULTS

Acute administration of L-arginine produced a dose-dependent inhibition in the antinociceptive response to morphine in mice. A dose of 200 mg/kg of L-arginine had no effect on morphine antinociception, but the 400 and 800 mg/kg doses of L-arginine decreased morphine antinociception by 40 and 50%, respectively, with p-values of 0.05 and 0.001, respectively (Fig. 1). D-Arginine (200–800 mg/kg), on the other hand, had no effect on morphine antinociception (data not shown).

Because L-arginine 400 and 800 mg/kg showed similar effects on morphine antinociception, a dose of 400 mg/kg of L-arginine was not used to study the distribution of morphine in brain. The effects of 200 and 800 mg/kg of L-arginine on the distribution of morphine, 60 min after its administration, in brain regions and spinal cord are shown in Fig. 2. L-Arginine (200 mg/kg did not alter the concentration of morphine in any brain region or the spinal cord. However, the higher dose (800 mg/kg) of L-arginine significantly decreased the concentration of morphine in midbrain ($p < 0.05$) and spinal cord ($p < 0.05$), but the concentration of morphine in other brain regions did not change. On the other hand, D-arginine at 200 or 800 mg/kg dose did not affect the distribution of morphine in brain regions and spinal cord (Fig. 3).

The effects of L-arginine (200 and 800 mg/kg) on the distribution of morphine in the liver, lungs, spleen, and kidneys are shown in Fig. 4. The lower dose of L-arginine had no effect on the distribution of morphine in the peripheral tissues. The higher dose of L-arginine (800 mg/kg) produced a significant ($p < 0.05$) increase in the concentration of morphine in the spleen, but it was unchanged in the liver, lungs, and kidneys (Fig. 4). The distribution of morphine was unaffected by D-arginine (200 and 800 mg/kg) (Fig. 5). The concentration of mor-

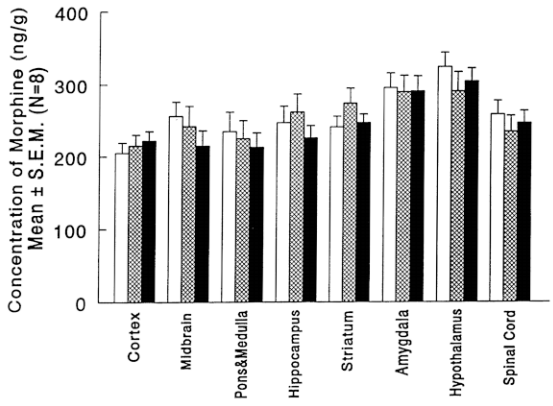

FIG. 3. Effect of different doses of D-arginine on the distribution of morphine in brain regions and spinal cord of mice. Morphine (10 mg/kg, SC) was injected 10 min after the injection of an appropriate dose of D-arginine (open column) vehicle; (crosshatched column) D-arginine (200 mg/kg, IP); (filled column) D-arginine (800 mg/kg, IP).

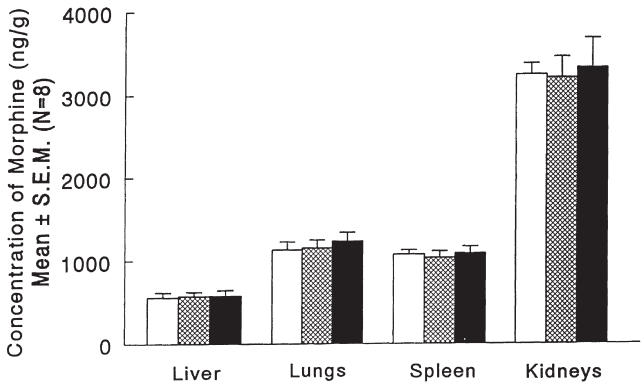

FIG. 5. Effect of different doses of D-arginine on the distribution of morphine in peripheral tissues. Morphine (10 mg/kg, SC) was injected 10 min after the injection of an appropriate dose of D-arginine (open column) vehicle; (crosshatched column) D-arginine (200 mg/kg, IP); (filled column) D-arginine (800 mg/kg, IP).

TABLE 1

EFFECT OF ACUTE ADMINISTRATION OF L-ARGININE OR D-ARGININE ON THE CONCENTRATION OF MORPHINE IN SERUM AND URINE FOLLOWING PERIPHERAL MORPHINE INJECTION

	Serum Concentration of Morphine Mean ± SEM (n = 8)	
Treatment (IP)*	Serum (ng/ml)	Urine (μg/ml)
Vehicle	233.6 ± 21.1	198.8 ± 20.0
L-Arginine (200 mg/kg)	207.4 ± 17.9	188.7 ± 18.1
L-Arginine (800 mg/kg)	242.2 ± 24.3	193.8 ± 28.8
D-Arginine (200 mg/kg)	263.8 ± 19.9	179.0 ± 28.0
D-Arginine (800 mg/kg)	219.0 ± 27.1	191.0 ± 26.6

*Mice were injected with L-arginine, D-arginine, or the vehicle. Ten minutes later mice were injected with morphine (10 mg/kg, SC) and were killed 60 min later.

phine in both the serum and urine was unaffected by acute treatment with either L-arginine or D-arginine (Table 1)

DISCUSSION

The present studies demonstrate that acute administration of L-arginine, a precursor to NO, dose dependently decreases antinociceptive response to morphine in mice. When the same dose of D-arginine were used, morphine antinociception was not modified. Thus, the effect of arginine was stereospecific. For the inhibition of morphine antinociception, high doses of L-arginine 400 and 800 mg/kg were required in the present study. These results are similar to those reported by Brignola et al. (10), who observed the effects with 300 and 1000 mg/kg doses.

Previous study from this laboratory have demonstrated that chronic treatment with L-arginine produces a robust decrease in morphine antinociception and was associated with decreases in morphine concentration in midbrain, pons and medulla, hippocampus, corpus striatum, and spinal cord. On the other hand, chronic treatment with D-arginine, which did not modify morphine antinociception, also did not affect the distribution of morphine in the spinal and supraspinal structures (1,2). Further studies revealed that whereas chronic treatment with L-arginine decreased morphine concentration in the central nervous system, the concentration of morphine was increased in peripheral tissues like lungs and spleen (9).

The distribution of morphine in brain regions, spinal cord, peripheral tissues like lungs, liver, kidneys and spleen, and serum and urine was determined in mice that were treated acutely with L-arginine or D-arginine. The dose of L-arginine that produced a decrease in morphine antinociception decreased the concentration of morphine in midbrain where there is an area that contains periaqueductal gray matter rich in endogenous opioid peptides and is involved in antinociceptive actions of drugs like morphine. At the same time, there was an increase in the concentration of morphine in the spleen. D-Arginine given acutely neither modified morphine antinociception nor changed morphine concentration in any brain region, spinal cord, or peripheral tissues. Acute treatment with L-arginine or D-arginine did not affect the concentration of morphine in serum or urine. These results suggest that high doses of acutely administered L-arginine are required to inhibit the entry of morphine into the deeper structures of the brain like midbrain to cause a decrease in antinociception. On the other hand, as indicated earlier, chronic administration of even lower doses of L-arginine causes a decrease in many brain regions and spinal cord.

The differential effects of acute and chronic administration of L-arginine on tissue distribution of morphine may also explain the differential effects on morphine antinociception.

The role of NO in the action of L-arginine cannot be ignored. Studies have shown that the action of L-arginine given acutely or chronically on morphine antinociception can be reversed by concurrent treatment with NOS inhibitors (2,10). We have also demonstrated that L-arginine–induced changes in morphine distribution in the central sites (1) and peripheral sites (1) can be reversed by concurrent treatment with L-NNA, an inhibitor of NOS. Thus, it appears that NO–NOS systems may be regulating the uptake of morphine across the blood–brain barrier.

It should be noted that L-arginine given orally or intraperitoneally but not intracerebroventricularly attenuated antinociception induced by peripherally (subcutaneously) administered morphine (9). It indicates that intracerebroventricularly administered L-arginine apparently does not interfere with the entry of morphine into the CNS, however large doses of L-arginine given peripherally apparently decreased it.

It is also possible that decreased morphine antinociception by L-arginine may be related to alteration in the characteristics of μ-opioid receptors, for which morphine is an agonists. Chronic administration of L-arginine was shown not to affect the binding characteristics of [^{3}H][D-Ala^2, MePhe^4, Gly-ol^5]enkephalin to mouse brain homogenates (2). Thus, changes in opioid receptor characteristics by acute or chronic treatment with L-arginine may not be a likely explanation for the altered morphine antinociception.

In summary, the present studies show a dose-dependent and stereospecific inhibition of morphine antinociception in mice by acute treatment with arginine. This effect was associated with a selective decrease in morphine concentration in midbrain, an important area for nociceptive and antinociceptive processing and rich in endogenous opioid peptides, and an increase in morphine concentration in the spleen.

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