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BRIEF REPORT

Role of Endogenous Morphine in the Attenuation of Opiate Withdrawal Syndrome by N-acetylmuramyl-L-alanine-D-isoglutamine (MDP)

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Opiates, long considered the prototypical addictive drug, cause the phenomenon of tolerance and physical dependence following chronic administration. Although many factors promote the addictive state, our studies have focused on the role of endogenous morphine in modifying physical dependence. Mammalian tissues contain morphine and codeine and have the capacity to synthesize these alkaloids. The present report shows that N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), which elevates the endogenous opiate alkaloids in various brain regions and peripheral tissues, can attenuate the withdrawal syndrome of morphine-addicted rats. ***[Neuropsychopharmacology 15:99–103, 1996]***

KEY WORDS: *Endogenous morphine; Opiate withdrawal; N-acetylmuramyl-L-alanine-D-isoglutamate (MDP)*

Muramyl dipeptide, N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is the smallest subunit of the bacterial peptidoglycan that exerts various biological effects and has been shown to have a wide variety of immunomodulatory effects both in vitro and in vivo (Elloruz et al. 1974). MDP stimulates the production of interleukin-1 (Il-1), tumor necrosis factor α (TNF-α), and interleukin-6 (Il-6) (Vermeulen et al. 1987; Sanceau et al. 1990). MDP also induces numerous neuropharmacological alterations. It effects the unit discharge frequency of neurons in hypothalamus and hippocampus (Dougherty and Dafny 1990), it is somnogenic (Masek and Kadlecova 1987), it has analgesic activity (Masek 1986) and effects the electrophysiological responses of brain regions involved in various opioid activities (Dougherty and Dafny 1988). It also has been reported to modify the severity of naloxone-precipitated morphine withdrawal (Dougherty et al. 1987). The present study indicates that MDP also increases the levels of endogenous morphine and codeine that may be related to the attenuation of the withdrawal syndrome of opiate-addicted rats.

MATERIALS AND METHODS

Sprague-Dawley male rats (200–250 g) were used in these experiments. Rats were housed 0600–1800 with lights on, food and water ad libitum, room temperature at 22°C. Morphine dependence was induced by the subcutaneous injection of 5 mg/kg BID for 3 days. Opiate dependence was assessed by injecting the animals with 1 mg/kg naltrexone (IP) on the morning of the fourth day. The animals were placed in a transparent acrylic cage and monitored for 10 minutes after naltrexone ad-

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Table 1. Effect of Muramyl Dipeptide (3 mg/kg IP) on Opiate Withdrawal Syndrome

Treatment	n^a	Wet Dog Shakes	Teeth Chattering	Fecal Boli[b]
Placebo + naltrexone	6	0	0	7 ± 2
Morphine + naltrexone	6	8 ± 1[c]	8 ± 2[c]	Diarrhea
Morphine + MDP + naltrexone	6	3 ± 1[d]	3 ± 1[d]	4 ± 1
Placebo + MDP + naltrexone	6	0	0	6 ± 1

[a] n = number of rats/gp. Numbers represent mean ± SEM of the times the rats exhibited the behavioral signs during the 10-minute observation.
[b] Fecal boli could not be counted in those animals having diarrhea; consequently statistics could not be applied.
[c] Placebo + naltrexone versus morphine + naltrexone. $p < .05$.
[d] Morphine + naltrexone versus morphine + MDP + naltrexone. $p < .05$.

ministration. MDP test animals received MDP (3 mg/kg IP) 15 minutes prior to naltrexone administration. Withdrawal signs of wet dog shakes, teeth chattering, and fecal boli were assessed as signs of the withdrawal syndrome. Table 1 shows the protective effect of MDP on the withdrawal syndrome, similar to that reported by Dougherty et al. 1987.

Morphine and codeine were analyzed following HPLC and radioimmunoassay as described by Donnerer et al. (1987). Tissues were sonicated in 10 mM HCl (minimum volume, 3 ml). Concentrated HCl was added in a ratio of 6 ml of acid to 10 ml of tissue homogenate and then vortexed. Samples were then hydrolyzed in a water bath for 30 minutes at 95°C, followed by cooling and centrifugation (10,000 × g for 30 minutes). Samples were then applied to a C-18 Sep Pak cartridge that had been prewashed with 5 ml methanol and 10 ml distilled water. The cartridges were then washed with 7 ml distilled water and eluted with 7 ml of a solution containing water 95.5%, pyridine 0.8%, glacil acetic acid 0.3%, and n-propyl alcohol 0.3% (pH 5.2). The eluate was evaporated to dryness and resuspended in HPLC mobile phase (water 98.65%, pyridine 0.8%, glacil acetic acid 0.3%, n-propyl alcohol 0.25%, pH 5.2) and placed over a Lichosorb RP-18, 10-μM column (250 mm). One-ml fractions were collected, corresponding to fractions where standards elute. The fractions were evaporated to dryness and resuspended in 250 μl of phosphate buffered saline (pH 7.4). The alkaloids were analyzed by radioimmunoassay.

RESULTS

We have previously shown that MDP elevated morphine and codeine levels in brain and peripheral organs in mice (Horak et al. 1993). Because exogenously ad-

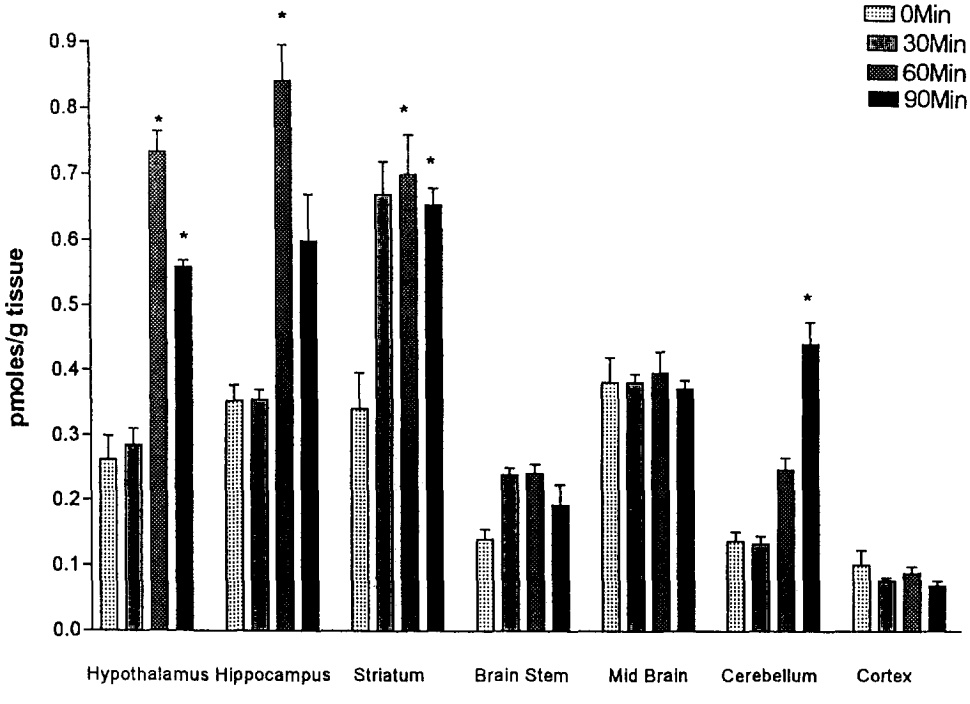

Figure 1. Endogenous morphine levels in different brain regions of rats (n = 6) treated with MDP (3 mg/kg IP). Animals were sacrificed 30, 60 and 90 minutes after MDP administration. Data are presented as mean ± SEM. * $p < .005$ versus control. Bars: 0 minutes, 30 minutes, 60 minutes, and 90 minutes respectively.

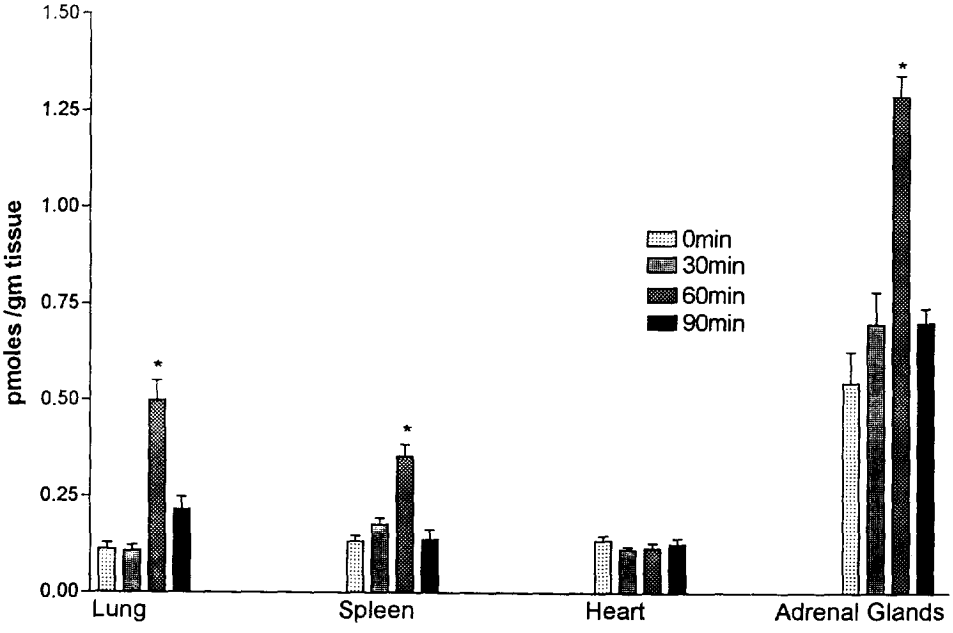

Figure 2. Endogenous morphine levels in lung, spleen, heart, and adrenal glands of rats ($n = 6$) treated with MDP (3 mg/kg IP). Animals were sacrificed 30, 60 and 90 min. after MDP administration. Data are presented as mean ± SEM. * $p < .005$ versus control. Bars: 0 minutes, 30 minutes, 60 minutes, and 90 minutes respectively.

ministered morphine interferes with the determination of endogenous morphine, we were required to determine the effects of MDP on endogenous morphine (Figures 1 and 2) and codeine (Figures 3 and 4) in various brain regions and peripheral organs of morphine-naive rats. The levels of morphine were increased in the hypothalamus, hippocampus, striatum, cortex, and cerebellum. MDP also increased morphine levels in lung and spleen. Codeine, the immediate precursor of morphine, also increased in the same brain regions as morphine, with the exception of the cortex. The same pattern is seen in peripheral tissues, where MDP elevated codeine levels in lung and spleen.

DISCUSSION

The concomitant increase in morphine levels and the attenuation of morphine-dependent withdrawal signs by MDP suggest that the endogenous opiate alkaloids may be involved in this effect. We postulate that the elevated endogenous morphine may substitute for the exogenous morphine in a manner similar to that of methadone. The question arises as to whether the levels of the endogenous morphine are sufficient to compete with naltrexone at relevant receptor sites. Our tissue analysis of morphine is from comparatively large heterogeneous brain regions, and it is possible that the morphine levels

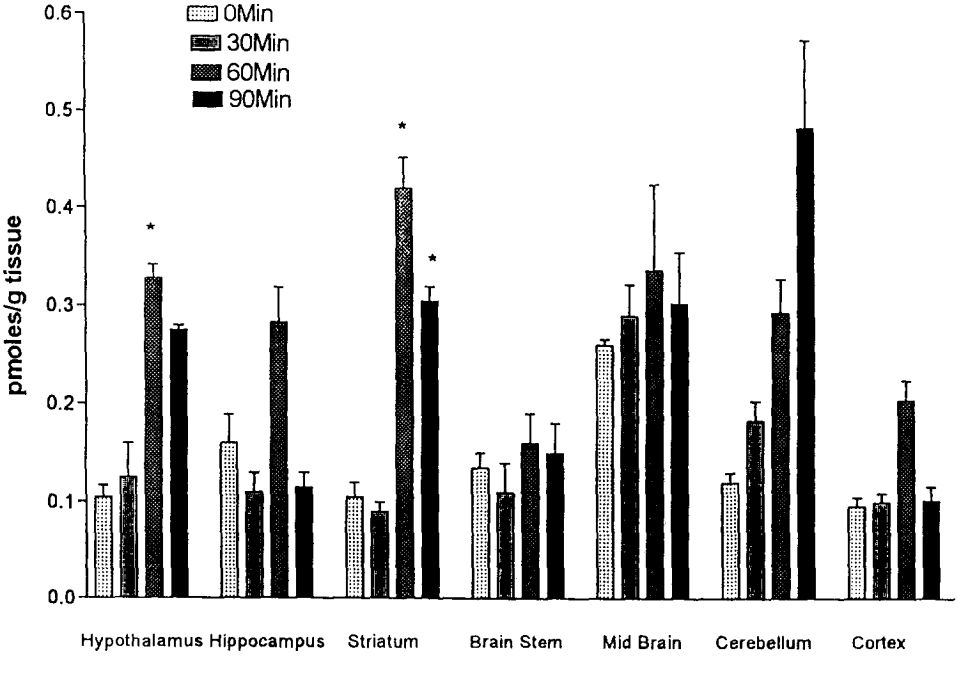

Figure 3. Endogenous codeine levels in different brain regions of rats ($n = 6$) treated with MDP (3 mg/kg IP). Animals were sacrificed 30, 60 and 90 minutes after MDP administration. Data are presented as mean ± SEM. * $p < .005$ versus control. Bars: 0 minutes, 30 minutes, 60 minutes, and 90 minutes respectively.

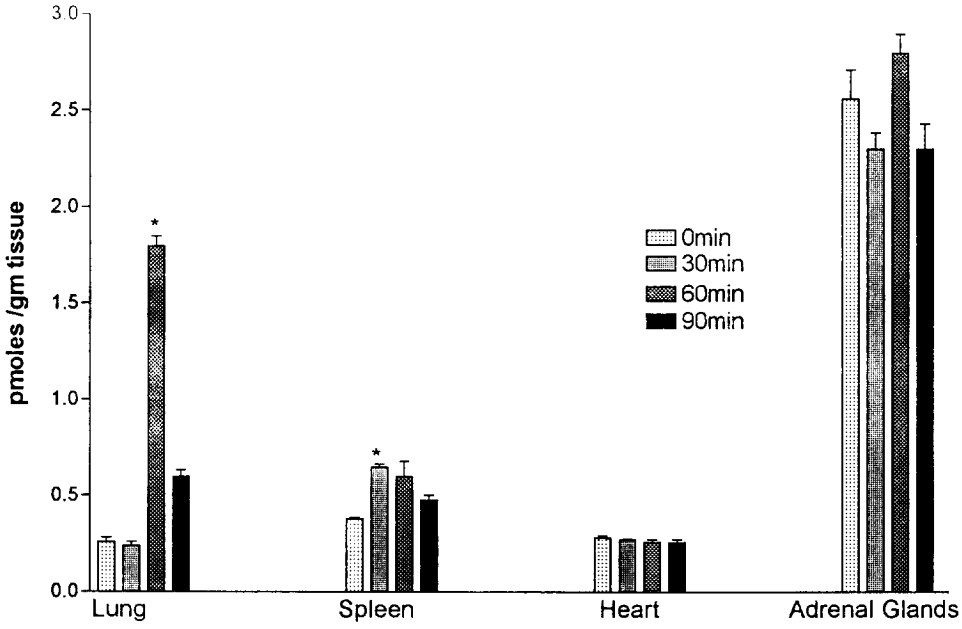

Figure 4. Endogenous codeine levels in lung, spleen, heart, and adrenal glands of rats ($n = 6$) treated with MDP (3 mg/kg IP). Animals were sacrificed 30, 60, and 90 minutes after MDP administration. Data are presented as mean ± SEM. * $p < .005$ versus control. Bars: 0 minutes, 30 minutes, 60 minutes, and 90 minutes respectively.

are much greater at critical sites in the brain microenvironment. It has been shown that chronic morphine treatment upregulates the cAMP system with the consequence of an increased excitability of specific neurons in the central nervous system (CNS) (Nestler et al. 1989). It has also been demonstrated that chronic morphine enhances protein phosphorylation of specific substrates for cAMP-dependent protein kinase, which may play a role in the development and expression of morphine addiction (Guitart and Nestler 1989). The elevated endogenous morphine may maintain the activity of cAMP-dependent protein kinase and thus attenuate the development of the behavioral signs of physical dependence.

Another explanation for the attenuation of naltrexone-precipitated withdrawal is that the brain contains phenolsulfa transferase activity (Foldes and Meek 1974) and the enzyme may produce morphine-3-sulfate and/or morphine-6-sulfate within the brain. In a previous report (Donnerer et al. 1987) we have demonstrated that the sulfated form of morphine exists in the CNS. Both these morphine sulfates have been reported to be more potent analgesics than morphine (Brown et al. 1985), and it is possible that they mediate some of the effects of the endogenous morphine.

Our results suggest a potentially important role for the endogenous morphine or codeine in modifying the effects of physical dependence from exogenous opiates (morphine or heroin) and the clinical potential of drugs that are capable of elevating the endogenous morphine content.

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