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Effects of MK 801 on Morphine Physical Dependence: Attenuation and Intensification

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KOYUNCUOĞLU, H., Y. DIZDAR, F. ARICIOĞLU AND Ü. SAYIN. *Effects of MK 801 on morphine physical dependence: Attenuation and intensification.* PHARMACOL BIOCHEM BEHAV 43(2) 487–490, 1992.—It has previously been reported that the noncompetitive NMDA receptor antagonists ketamine and dextromethorphan suppressed the naloxone-induced morphine abstinence syndrome. In addition, the previous blockade by ketamine and dextromethorphan of NMDA receptors has been shown to intensify the naloxone-elicited morphine abstinence syndrome. On the basis of this information, another noncompetitive NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo-*a*,*d*-cyclohepten-5,10-imine maleate (MK 801), was administered to rats in which two morphine-containing (75 × 2 morphine base) pellets had been implanted. The naloxone-precipitated abstinence syndrome in rats injected with 0.3 mg/kg MK 801 36 h after pellet implantation was found significantly more intense than controls whereas the abstinence syndrome in rats that received 0.1 mg/kg MK 801 before naloxone injection was less intense. The intensification by MK 801 given 36 h following pellet implantation was attributed to the further increase in upregulation and supersensitivity of NMDA receptors caused by morphine. The attenuation was explained by the blockade by MK 801 of NMDA receptors as occurred in the case of ketamine and dextromethorphan.

MK 801 Intensification of morphine dependence Attenuation of precipitated abstinence syndrome

IN our previous studies, we were able to show that the endogenous NMDA receptor agonists aspartic or glutamic acids or glycine can antagonize some effects of morphine (13,16). Consequently, we found that morphine inhibits the enzymes producing aspartic and glutamic acids (12,17) from asparagine and glutamine (2,22). Then it has been reported that opioid receptor agonists antagonize the effects of intratechally administered excitatory amino acid receptor agonists that preferably stimulate NMDA-type receptors of aspartatergic/glutamatergic system (1). Moreover, it has been shown that an endogenous opioid peptide, dynorphin (1–13), that interacts with NMDA receptors (4) attenuates the intensity of the opiate abstinence syndrome (5). These results suggested that the probable mechanism of the physical dependence upon and tolerance to opiates would be related to: a) the inhibition of aspartic and glutamic acids, producing enzymes that result in less production of the endogenous excitatory amino acids, namely, aspartic/glutamic acids; b) the blockade by opiates of especially NMDA subtypes of the aspartatergic/glutamatergic receptors, which would lead to the upregulation and supersensitivity of the receptors. As a result, the production and release of aspartic/glutamic acids and the number and functional state of NMDA receptors appear to be three key points in the development of opiate physical dependence and the determination of the intensity of the abstinence syndrome. A decrease in the production and release during the development of opiate physical dependence would provide suitable conditions for a more intense opiate physical dependence due to the weaker quantitative competition of endogenous excitatory amino acid neurotransmitters aspartic/glutamic acids for NMDA receptors that are going to be blocked by opiates and subsequently upregulated and become supersensitive. In fact, when the production and release of aspartic/glutamic acids, respectively, are inhibited by *D*-aspartic acid and prolyl-leucyl-glycine amide (14) and tizanidine during the development of opiate physical dependence a more severe opiate physical dependence is observed (11,14). In contrast, when the production and/or release is inhibited just before abrupt withdrawal the severity of the abstinence syndrome appears to be attenuated (11,14) because of the lesser stimulation of probable upregulated and supersensitive NMDA receptors. In regard to the third key point, which implies the number and functional state of NMDA receptors, the following experiments were carried out. Young rats that had neonatally been injected with monosodium glutamate developed a less intense opiate physical dependence than their control littermates, most probably due to the destruction by monosodium glutamate of some neurons bearing NMDA receptors (10). The blockade by the noncompetitive NMDA receptor blockers ketamine and dextromethorphan of NMDA receptors has been reported to attenuate or completely suppress the naloxone-induced morphine abstinence syndrome in rats (15). Encouraged by these promising

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results obtained from animal experiments, dextromethorphan has been successfully used in the treatment of heroin addicts for about 4 years (8,18,24). After attenuation of the naloxone-precipitated abstinence syndrome by tizanidine via decreasing the release of aspartic/glutamic acids had been observed in rats (11), dextromethorphan has been combined with tizanidine in the treatment of heroin addicts (11).

(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo-*a*,*d*-cyclohepten-5,10-imine maleate (MK 801) is a selective noncompetitive NMDA receptor antagonist. It blocks NMDA-induced excitation by interacting with open ion channels associated with NMDA receptors (7,23,29). It has recently been reported that MK 801 inhibits morphine tolerance and dependence (19, 26,27) and prevents the neurochemicals and behavioral signs of the morphine abstinence syndrome (26).

Because the noncompetitive NMDA receptor blockers ketamine and dextromethorphan and the inhibitor of aspartic/glutamic acid release tizanidine injected just before naloxone-precipitated abstinence syndrome attenuate the intensity of the syndrome (11,15), and in addition administration of ketamine and dextromethorphan prior to morphine dependence development and administration of tizanidine during development intensify the naloxone-precipitated abstinence syndrome (9,11), it would be of interest to administer also MK 801 during development of morphine physical dependence and before the abstinence syndrome. The intensification or attenuation of the naloxone-precipitated abstinence syndrome would confirm the previous experimental findings.

METHOD

Two pellets containing 75 mg morphine base (total 150 mg) (26) were SC implanted on the back of rats lightly anesthetized with ether (28). Then, rats were divided into three groups at random. Thirty-six hours after pellet implantation, rats of the third group were IP injected with 0.3 mg/kg MK 801 (chronic MK 801 group) whereas the others IP received the same volume of physiological saline. Seventy-two hours following pellet implantation, 0.1 mg/kg MK 801 was IP administered to rats of the second group (acute MK 801 group). The first group (control) and the chronic MK 801 group were IP given the same volume of physiological saline. Thirty minutes later, 2 mg/kg naloxone was IP injected into rats and they were immediately placed in a metal cage (base area: 20 × 22 cm, height 20 cm) and observed for 15 min by an experimenter blind to group identification. During this observation period, the number of jumps, wet-dog shakes, and defecations were counted. According to their intensity, teeth-chattering and ptosis were rated 1, 2 or 3 and 1–10, respectively. In addition, on the basis of Himmelsbach's Degree Method (6), which characterizes the abstinence syndrome into four grades to reflect the clinical severity, and the correlations among the occurrence, onset, and fading of each abstinence syndrome sign in accordance with the morphine content of the implanted pellet(s), exposure time of animals to different morphine content pellet(s), and amount of naloxone used for precipitated abstinence syndrome (3), each sign was rated as follows. Every jump, wet-dog shake, maximum degree of teeth-chattering, defecation, and ptosis was separately scored 8, 4, 1, and 3, respectively. The total score of each category was shown as a total evaluation of the abstinence syndrome intensity in the last column of Table 1 to give overall information of the signs of each group. All results were first analyzed by a one-way analysis of variance (ANOVA); subsequently, the statistical evaluation was carried out by Student's *t*-test. $p < 0.05$ was considered statistically significant.

Material

Male Wistar inbred rats (150–170 g) kept in a room 22–23°C on a 12 L : 12 D cycle and fed with a standard regimen ad lib were used. MK 801 was a generous gift from Merck Sharp & Dohme (Rahway, NJ). Naloxone and morphine were purchased from Sigma Chemical Co. (St. Louis, MO) and Verenigde Pharmaceutische Fabrieken B.V. (Holland), respectively.

RESULTS

The mean values (± SE) and their statistical evaluation of the abstinence syndrome signs during the first 15 min immediately after 2 mg/kg naloxone administration are shown in Table 1. Jumping and teeth-chattering were found to be significantly lower in the acute MK 801 than the control group. In the total evaluation, the signs of the control and acute MK 801 groups are 64.80 and 28.52, respectively. The mean value of jumping in the chronic MK 801 group appeared significantly higher than that in the control group. The total evaluation of the signs of the chronic MK 801 group is 176.71. When the values of the acute and chronic MK 801 groups were statis-

TABLE 1

MEAN VALUES (± SE) AND STATISTICAL EVALUATION OF ABSTINENCE SYNDROME SIGNS DURING THE FIRST 15 MIN IMMEDIATELY AFTER 2 mg/kg IP NALOXONE

	Groups			
Signs	Control (n = 10)	Acute MK 801 (n = 12)	Chronic MK 801 (n = 9)	ANOVA
Jumping	6.60 ± 0.60	2.42 ± 0.38*	20.22 ± 2.41*†	$F = 51.09, p < 0.05$
Wet-dog shakes	0.70 ± 0.21	0.75 ± 0.18	1.11 ± 0.26	$F = 1.01, p > 0.05$
Teeth-chattering	4.40 ± 0.37	2.08 ± 0.26*	3.56 ± 0.34	$F = 14.41, p < 0.05$
Defecation	4.90 ± 0.43	4.17 ± 0.30	5.11 ± 0.45	$F = 1.71, p > 0.05$
Ptosis	0.70 ± 0.21	0.33 ± 0.14	1.22 ± 0.15†	$F = 6.84, p < 0.05$
Total evaluation of signs	64.80	28.52	176.71	

The figures in parentheses ineicate the number of rats in the groups.
*Statistical significance referring to control values.
†Statistical significance betwen acute MK 801 and chronic MK 801 groups.

tically compared, jumping and ptosis were significantly higher in the chronic MK 801 than in the acute MK 801 group.

DISCUSSION

The noncompetitive NMDA receptor antagonists ketamine and dextromethorphan have recently been reported to significantly attenuate signs of the naloxone-induced abstinence syndrome in rats (15). For this reason, dextromethorphan has successfully been used in the treatment of heroin addicts for about 4 years (8,18,24). The well-known noncompetitive NMDA antagonist MK 801 (7,23,29), which has been claimed to inhibit morphine tolerance and physical dependence (19,26,27) and prevent neurochemical and behavioral manifestations of the morphine withdrawal syndrome (26), significantly attenuated the intensity of naloxone-elicited abstinence syndrome (Table 1) when administered to morphine-dependent rats 30 min prior to naloxone injection. This is quite consistent with the previous results obtained with the use of ketamine and dextromethorphan administered before the naloxone-induced abstinence syndrome (15), supporting the before-mentioned hypothesis regarding the development of opiate physical dependence (8,18,24) and the previous results (15). On the other hand, it has been shown that previous blockade of NMDA receptors by administration of multiple doses of ketamine, dextromethorphan, morphine, and naloxone for 5 days before development of morphine physical dependence intensified the severity of the naloxone-induced abstinence syndrome (19). The intensification was attributed to the probable upregulation and supersensitivity of NMDA receptors by the previous blockade (19). As a matter of fact, the following high-dose morphine application by means of pellet implantation led rats to have further upregulation and supersensitivity of NMDA receptors due to the further binding of morphine to the already upregulated and supersensitive NMDA receptors as if the exposure time to and amount of morphine had increased (3). Consistent with this, MK 801 pretreatment has been reported to enhance the neurotoxicity of NMDA due to the persistent increase of brain NMDA recognition sites (upregulation) by 30–50% (20,21). In addition, it has been reported that a single dose of 100 mg/kg morphine sulphate 22 h before administration of the single priming dose of 10 mg/kg morphine sulphate could cause the development of opiate physical dependence proved by naloxone administration performed 2 h later (25,30). This method, which provides acute opiate dependence, should be based upon the upregulation of NMDA receptors by 100 mg/kg morphine sulphate administration 22 h before the single priming dose of 10 mg/kg morphine sulphate. The upregulated NMDA receptors are primed with the priming dose of morphine sulphate, which would be moved by naloxone injected 2 h later (25,30). Information concerning the upregulation by MK 801 (20,21) and the method for acute morphine dependence development (25,30) suggested that the single dose of an NMDA receptor antagonist administered during development of morphine physical dependence could be enough to upregulate NMDA receptors and intensify morphine physical dependence as occurred in the present study. The implanted morphine-containing pellets could continuously provide morphine, which would block NMDA receptors or consequently upregulate the receptors. As the additionally upregulated receptors would be blocked by the present morphine in the bodies of rats, the number of NMDA receptors that would not be covered by morphine following naloxone administration would be much more than in those rats not administered MK 801 or any NMDA receptors antagonist.

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