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Morphine Physical Dependence Intensification by Hypoglycemia: NMDA Receptor Involvement

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KOYUNCUOĞLU, H. I. HATİPOĞLU AND Ö. SARICA. *Morphine physical dependence intensification by hypoglycemia: NMDA Receptor involvement.* PHARMACOL BIOCHEM BEHAV **48**(3) 571–574, 1994.—The destruction of *N*-methyl-D-aspartate (NMDA) receptor-bearing neurons by insulin-induced hypoglycemia has long been known to be due to excessively released aspartate and glutamate. In this study, the effects of NMDA-bearing neuron destruction by insulin-induced hypoglycemia on the development of morphine (M) physical dependence, which was found releated to functional states of NMDA receptors, were investigated. NMDA receptor antagonists CGP 39551 and MK-801 were used to see whether they could change intensity of precipitated abstinence syndrome by preventing destruction. Therefore, two groups of fasting rats injected IP with physiological saline, and another two groups given IP 10 mg/kg CGP 39551 and 0.5 mg/kg MK-801 received 15 IU/kg crystalline zinc insulin IP. After 2 h, the rats were orally given 2 × 4 ml of 5% glucose solution. On the third day, two pellets containing 75 mg base M were SC implanted to all rats. On the sixth day, they were IP given 2 mg/kg naloxone (NL). Then jumps, wet-dog shakes, and defecation were counted while diarrhea and ptosis were rated for 15 min. The rats given insulin manifested significantly more intense NL-precipitated abstinence syndrome than controls. The rats administered CGP 39551 showed a less intense physical dependence than those injected with only insulin. But, the intensity was still significantly higher than controls. In the rats that received MK-801, the abstinence syndrome was more or less equal to that in controls. The results were considered as evidence for the blockade by M of NMDA receptors and the prevention by NMDA receptor antagonists of NMDA-bearing neurons from destruction of insulin-induced hypoglycemia.

Morphine physical dependence Intensification by hypoglycemia of morphine dependence CGP 39551 MK-801

A UNIFYING excitotoxic mechanism has long been proposed to underlie many types of brain injury and evokes the release of excitatory amino acids (i.e., glutamate, aspartate), their activation of postsynaptic receptors, and the accumulation of intracellular calcium (1,2,22,25). Glutamate neurotoxicity has been considered an inherent feedforward, self-propagating event. That is, damage to some neurons leads to leakage of their endogenous excitatory amino acids that produce further damage, even though the original challenge may have been removed or neutralized (5,11,27). On the other hand, among the non-*N*-methyl-D-aspartate (NMDA) of glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) has also especially been claimed to be involved in the excitotoxicity of excitatory amino acids, and its antagonists have been shown to diminish ischemia-related damage (9,13,24).

On the basis of our previous experimental results, it has been postulated that the mechanism of physical dependence upon and tolerance to opiates would be related to the inhibition of aspartate and glutamate-producing enzymes L-asparaginase and glutaminase, the blockade by opiates of especially NMDA subtypes of the aspartatergic/glutamatergic receptors, and the upregulation and supersensitivity of NMDA receptor associated with opiate blockade (14,18). As a result, the production and release of aspartate and glutamate, and the number and functional state of NMDA receptors appeared to be three key points in the development of opiate physical dependence and the determination of the intensity of the abstinence syndrome. In regard to the number and functional states of NMDA receptors, a noncompetitive NMDA receptor antagonist dextromethorphan has been used in the treatment of heroin addicts (14,19).

The purpose of the present study is to investigate the effects of hypoglycemia-induced brain damage on the development of morphine physical dependence, thinking that the reduction

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of the NMDA receptors can be considered a chronic blockade of the receptors that results in the intensification of opiate physical dependence (15). Additionally, it has been thought that it would be interesting to search whether a competitive NMDA receptor blocker CGP 39551 [(DL-4-methyl-APPA or DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid)-carboxyethylester] (27,28) and noncompetitive blocker MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo-a,d-cyclohepten-5,10-imine maleate] (29,30) (administered before insulin-induced hypoglycemia) could prevent the changes by hypoglycemia-elicited brain damage that would be reflected in the development of morphine physical dependence.

METHOD

Rats fasting for about 16 h were divided into four groups. The third group was intraperitoneally (IP) injected with 10 mg/kg CGP 39551 (CGP + hypoglycemia group), while the others received same volume of physiological saline IP. Forty-five minutes later the rats of the fourth group were given 0.5 mg/kg MK-801 IP (MK-801 + hypoglycemia group), whereas the other rats were IP administered the same volume of physiological saline. One hour following administration of CGP 39551 or 15 min after MK-801 injection, all rats with the exception of those belonging to the first group (control) were IP injected with 15 IU/kg crystalline zinc insulin (26). The control rats were given physiological saline IP. Ninety minutes after insulin administration, the blood glucose level of the insulin-injected rats was determined by the glucose oxidase and peroxidase methods (39.3 ± 9.8 mg %). The rats whose blood glucose levels were found more than 50 mg/100 ml were discarded. Two hours following insulin injection the remaining rats were given 4 ml of 5% glucose solution by means of gavage twice, with an interval of 10 min.

On the third day of insulin-induced hypoglycemia, two pellets containing 75 mg base morphine (total 150 mg) were subcutaneously implanted in the back of all rats, including controls, under light ether anesthesia (28). Three days following pellet implantation, all rats were IP injected with 2 mg/kg naloxone. Immediately after injection, rats were placed in a metal cage (base area 20 × 22 cm, height 20 cm) and strictly observed by the experimenters blind to group identification. The number of jumps, wet-dog shakes, and defecation were counted for 15 min. During this observation period, diarrhea and ptosis were rated 1, 2, or 3, whereas teeth chattering was rated 1–10, according to their intensity. Additionally, on the basis of Himmelsbach's Degree Method (12), 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 signs according to the morphine content of the implanted pellet(s), and the amount of naloxone injected to precipitate withdrawal syndrome (3), each sign was rated as follows. In every jump, wet-dog shake, maximum degree of teeth chattering, diarrhea, and defecation, the ptosis was separately scored 10, 4, 5, 10, 1, and 3, respectively. For over all information of six signs for each group, the total score of each group was given as a total evaluation of the abstinence syndrome intensity. The precipitated abstinence syndrome was induced only once in each rat. All the results were first analyzed by analysis of variance (ANOVA). When F-values were less than 0.05, the statistical evaluation of the group differences was carried out by t-test. $p < 0.05$ was considered statistically significant.

Materials

Male Wistar inbred rats (weighing 140–160 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 and CGP 39551 were generous gifts from Merck Sharp and Dohme (Rahway, NJ) and Ciba-Geigy (New Jersey), respectively. Crystalline zinc insulin (Insulin Organon), morphine, and naloxone were purchased from N. V. Organon (Holland), Verenigde Pharmaceutische Fabrieken B.V. (Holland), and Sigma Chemical Co. (St. Louis, MO), respectively.

TABLE 1

MEAN VALUE OF THE ABSTINENCE SYNDROME SIGNS AND THEIR STATISTICAL EVALUATIONS

Signs	Control (22)	Hypoglycemia (10)	CGP + Hypoglycemia (10)	MK-801 + Hypoglycemia (10)
Jumping $F = 112$	6.09 ± 1.38	18.1 ± 3.14*	8.00 ± 1.33*§	6.00 ± 1.25§
Wet dog shake $F = 13.17$	3.95 ± 0.84	1.60 ± 0.70*	2.40 ± 1.26†	2.90 ± 1.45‡#
Teeth chattering $F = 12.46$	6.09 ± 2.92	8.60 ± 1.35†	3.30 ± 0.67*§	3.80 ± 1.69†§
Diarrhea $F = 2.52$	0.77 ± 0.53	1.30 ± 0.95	0.70 ± 1.06	0.50 ± 0.53¶
Defecation $F = 0.99$	7.82 ± 3.69	8.70 ± 0.67	6.30 ± 3.30	8.00 ± 3.50
Ptosis $F = 1.30$	1.00 ± 1.02	1.50 ± 0.53	0.80 ± 0.42	1.30 ± 1.16
Total evaluation of signs	96.3	242.06	105.35	87.80

Mean (± SD).
The figures in parentheses indicate the numbers of rats in each group.
*$p < 0.001$, †$p < 0.01$, ‡$p < 0.05$ referring to control values.
§$p < 0.001$, #$p < 0.01$, ¶$p < 0.05$ referring to the values of hypoglycemia group.

RESULTS

The mean values (± SD) and their statistical evaluations of counted or rated abstinence syndrome signs observed in rats first receiving CGP 39551 or MK-801 and then being subject to a severe insulin-induced hypoglycemia before morphine physical dependence are shown in Table 1. The mean values of jumping, teeth chattering, and wet-dog shakes were found significantly more and less intense, respectively, in the hypoglycemia group than in controls. Even though jumping showed a significantly higher increase in the CGP + hypoglycemia group than controls, both wet-dog shakes and teeth chattering were significantly lower in the CGP + hypoglycemia group than in controls. Only wet-dog shakes had a significant decrease in the MK-801 + hypoglycemia group when observed abstinence syndrome signs were compared to controls.

If the values of the CGP + hypoglycemia and MK-801 + hypoglycemia groups are compared with those of the hypoglycemia group, jumping and teeth chattering values in the CGP + hypoglycemia group appear significantly lower than those in the hypoglycemia group. Instead, jumping, wet-dog shakes, teeth chattering, and diarrhea in the MK-801 + hypoglycemia group were found significantly less intense than those in the hypoglycemia group.

DISCUSSION

Before starting to discuss the results of the present study, the point with respect especially to wet-dog shakes, which seems to be inconsistent with an increase or decrease of the other abstinence syndrome signs, should be clarified. Among the signs, those such as flying, jumping, and diarrhea are considered dominant, while some others, such as wet-dog shakes and teeth chattering, are classified recessive. When the intensity of dominant ones increases, the intensity of some, if not all, recessive ones may decrease, and vice versa (3). So, this was taken into consideration when increases or decreases in wet-dog shakes are considered in determining intensification or attenuation of precipitated abstinence syndrome, as well as in scoring abstinence syndrome signs for total evaluation of signs (Table 1). Additionally, vomitus and diarrhea occur as two late signs during a severe abstinence syndrome in humans, and they are symptoms that appear the most life threatening and resistant to any kind of drug management. For these reasons, diarrhea was regarded as indicative of flying and jumping (3,11) in total evaluation of signs in the present study and elsewhere (16,17).

The experimental results displayed in Table 1 clearly show that previously insulin-induced hypoglycemia intensifies morphine physical dependence developing 3 days after hypoglycemia. Hypoglycemia has long been known to augment the release of excitatory amino acids, to impair their uptake and detoxification through the reduction of intracellular energy levels (4,20,21,25,29). As a result of these, hypoglycemia causes the destruction of neurons in the CNS, most probably via NMDA subtype receptors of the aspartatergic/glutamatergic system (23,29,30). More or less the same biological processes such as upregulation, supersensitivity, etc. may occur when some of the receptors belonging to a well-established receptor category are blocked by their antagonists. In this context, the striking results of our previous studies can be shown distinct examples (15,16). For these reasons, the results of those experiments have been considered to be another body of evidence for the fact that opiate also block NMDA receptors (14,19). As the upregulation and supersensitivity of NMDA receptors appear to be the most important mechanism underlying the development of opiate physical dependence (14,19), the reduction in the number of functioning NMDA receptors due to the destruction of NMDA receptor-bearing neurons can be assumed as the blockade by antagonists of the receptors.

As seen in Table 1, the administration of the competitive NMDA receptor antagonist CGP before insulin-induced hypoglycemia reduces the intensity of morphine physical dependence observed in the hypoglycemia group even though the severity of dependence is still significantly higher in the CGP + hypoglycemia group than controls. The statistically lower number of wet-dog shakes in the CGP + hypoglycemia group may be interpreted as evidence for the higher intensity of morphine physical dependence in the CGP + hypoglycemia group, as explained before. A less intense development of morphine physical dependence in the CGP + hypoglycemia group than in the hypoglycemia group should be related to the prevention by the competitive NMDA receptor antagonist CGP (6,7) of NMDA receptor-bearing neurons from the destruction evoked by excessively released excitatory amino acids under the effects of insulin-induced hypoglycemia. However, this does not necessarily indicate that CGP showed a complete prevention of NMDA receptor-bearing neurons, and there were no upregulation and supersensitivity. The upregulation and supersensitivity related to the destruction of neurons by insulin-induced hypoglycemia in the CGP + hypoglycemia group may not be as high as in the hypoglycemia group. It is likely that, for this reason, the intensity of morphine physical dependence is significantly lower in the CGP + hypoglycemia group than in the hypoglycemia group, while it is still significantly higher in the CGP + hypoglycemia group than in controls.

With the exception of wet-dog shakes, all abstinence syndrome signs did not show any statistically significant change in the MK-801 + hypoglycemia group when compared to controls (Table 1). A significant decrease in wet-dog shakes without any significant change in a dominant sign (3) could normally be attributed to a less severe physical dependence in the MK-801 + hypoglycemia group than in controls.

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