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Suppression by Ketamine and Dextromethorphan of Precipitated Abstinence Syndrome in Rats

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KOYUNCUOĞLU, H., M. GÜNGÖR, H. SAĞDUYU AND F. ARICIOĞLU. *Suppression by ketamine and dextromethorphan of precipitated abstinence syndrome in rats.* PHARMACOL BIOCHEM BEHAV **35**(4) 829–832, 1990.—The development of physical dependence on opiates appears to involve an inhibition by opiates of L-asparaginase and glutaminase, and the blockade by opiates of aspartatergic (ASPergic)/glutamatergic (GLUergic) receptors. Ketamine (K) (0.5 or 1 mg/kg) or dextromethorphan (DM) (1 or 2 mg/kg), both of which are known to decrease the responsiveness of ASPergic/GLUergic receptors, were administered to the three morphine (M)-containing pellets implanted rats prior to 2 mg/kg naloxone (NL) injection. Whereas 0.5 mg/kg K showed no significant effect on abstinence syndrome signs, 1 mg/kg K and 1 mg/kg DM significantly attenuated some of the signs. The attenuation or prevention of all the signs were observed after 2 mg/kg DM administration. Almost complete prevention was seen from the second minute on during the ten-minute observation period. As ASP and GLU antagonists K and DM have this antagonizing effect on the precipitated abstinence syndrome signs, the manifestation of abstinence syndrome may mainly result from the normalization of ASP and GLU production because of the disinhibition by NL of the enzymes and the stronger stimulation of ASPergic/GLUergic receptors which have no opiate blockade after NL injection.

Ketamine Dextromethorphan Precipitated abstinence Suppression of abstinence syndrome

L-ASPARTIC acid (ASP) has previously been reported to antagonize effects of morphine (M) such as the inhibition by acute intravenous (IV) M injection and M pellet implantation of the brain L-asparaginase activity (19,21), the development of physical dependence and the manifestation of abstinence syndrome signs (18, 20, 22). On the basis of these results it has been hypothesized that the development of physical dependence on and the abstinence syndrome upon withdrawal from opiates might be related to a disequilibrium between L-asparaginase and asparagine synthetase due to the inhibitory effects of opiates on L-asparaginase (12, 14, 32). In addition, the similarity between the effects of M and another L-asparaginase inhibitor (24), D-aspartic acid (13, 16, 17, 23), and the successful use of ASP in the treatment of opiate-addicted persons (12,32), were considered as supporting evidence for the hypothesis.

On the other hand, it has recently been reported that ketamine (K) and dextromethorphan (DM), lacking addiction liability (10, 11, 26), have an attenuating effect on the responses to the stimulation of the aspartatergic (ASPergic)/glutamatergic (GLUergic) receptor subtypes, especially N-methyl-D-aspartate (NMDA) (2, 3, 6, 8, 9). The attenuating effect of K and DM on the actions of NMDA can be related to a noncompetitive blockade of the ion channel associated with the NMDA receptor (2, 7, 9, 25, 27, 29, 31). Additionally, the opioid mu, delta and to lesser extent, sigma receptor agonists have been shown to antagonize some effects of intrathecally administered excitatory amino acid agonists namely NMDA, quisqualate or kainate (1).

The experimental results given above suggested that the probable mechanisms underlying the physical dependence on opiates might be the following ones: 1) The inhibition by opiates of the enzymes which produce neurotransmitter ASP and GLU in the nerve ends of their systems from asparagine and glutamine (4,28), 2) the blockade by opiates of the ASPergic/GLUergic receptors, and 3) the adaptation of the organism to the consequent state created by the lesser production of the neurotransmitter ASP and GLU, and the blockade of the receptors of ASP and GLU. In case of withdrawal from opiates, the normalization of ASP and GLU production and release will occur and these excitatory amino acids will, to greater extent, stimulate ASPergic and GLUergic receptors which are not under the blocking effect of opiates anymore. These will lead the organism to manifest the abstinence syndrome. Thus, we thought it would be of interest to minimize the responsiveness of the ASPergic/GLUergic receptors by means of the K or DM administration in order to see whether the blockade of the ASPergic/GLUergic receptors responsiveness can attenuate and/or prevent the manifestation of abstinence syndrome signs.

METHOD

Three pellets containing 75 mg morphine base (total 225 mg) (33) were subcutaneously implanted on the back of the rats under light ether anesthesia. The rats were divided into 5 groups 72 hours after pellets implantation. The first group (12 rats) were intraperitoneally (IP) given 2 mg/kg naloxone (NL) and they were called Precipitated Abstinence Group (PAG). Immediately after the NL

TABLE 1

THE MEAN VALUES (±SE) AND STATISTICAL EVALUATION OF THE ABSTINENCE SYNDROME SIGNS DURING THE FIRST 10 MIN IMMEDIATELY AFTER 2 mg/kg IP ADMINISTERED NL

	PAG (12)	0.5 mg/kg K + PAG (10)	1 mg/kg K + PAG (10)	1 mg/kg DM + PAG (15)	2 mg/kg DM + PAG (16)
Flying	3.61 ±0.71	3.40 ±0.87	2.29 ±0.49	1.34* ±0.48	0.10* ±0.14
Jumping	13.05 ±1.44	12.46 ±1.88	7.86* ±1.57	10.31 ±1.39	5.77* ±1.29
Teeth Chattering	9.22 ±1.65	7.34 ±0.98	3.78* ±0.53	4.44* ±1.09	2.03* ±1.14
Wet Dog Shake	2.88 ±0.61	3.05 ±0.55	9.97* ±2.83	5.72 ±1.98	1.12* ±0.53
Writhing	0.46 ±0.27	0.38 ±0.42	0.23 ±0.41	0.33 ±0.29	0
Defecation	6.31 ±0.46	7.07 ±0.82	0.85* ±1.12	5.17 ±0.95	2.85* ±1.17
Diarrhoea	1.37 ±0.44	1.20 ±0.63	1.45 ±0.81	1.25 ±0.63	0*
Ptosis	1.54 ±0.37	1.45 ±0.42	1.00 ±0.44	1.40 ±0.41	0*

K was IV administered just before NL. DM was given IP 10 min prior to IP NL administration. Uncountable signs such as diarrhoea, ptosis and chattering were evaluated by given points according to their severity (see the Method section).

The figures in parentheses indicate the numbers of the rats in each group.

PAG: Precipitated abstinence group; NL; naloxone; DM: dextromethorphan; K: ketamine.

*Statistically significant referring to the values of PAG ($p<0.05$).

administration they were placed in a metal cage (base area: 20 × 22 cm, height: 20 cm) and they were strictly observed. The number of flyings, jumpings, wet dog shakes, writhings and defecations were counted for 10 min. Diarrhoea and ptosis were rated 1, 2 or 3, whereas teeth chattering was rated 1, 2, 3, . . . 10 according to their severity.

The second and third groups were IV given 0.5 or 1 mg/kg K just before the IP 2 mg/kg NL administration. The fourth and fifth groups were, 10 min prior to the NL injection, receiving IP 1 or 2 mg/kg DM prepared in saline, respectively. Following the IP injection of 2 mg/kg NL the same abstinence syndrome signs were counted or rated in the manner used for the first group. The precipitated abstinence syndrome was induced only once in each rat. For statistical evaluation the Student *t*-test was used.

Materials

Male Wistar inbred rats (150–160 g) kept in a room 22–23°C on a 12-hour light/dark cycle and fed with a standard regimen ad lib were used. DM and NL were gifts from Roche (Basel, Switzerland) and Endo Laboratories (New York), respectively. K (Ketalar®) was purchased from Padeko (Istanbul, Turkey).

RESULTS

The results can be seen in Table 1. The IP injection of 2 mg/kg NL to the rats made physically dependent on M (PAG) by means of three M-containing pellets implantation caused the precipitated abstinence syndrome characterized by the signs given in Table 1. The IV administration of 0.5 mg/kg K just before NL-induced precipitated abstinence syndrome appeared not to significantly cause any change in the observed abstinence syndrome signs. Instead, 1 mg/kg K significantly decreased the number of jumpings, teeth chatterings and defecations, whereas it significantly increased the number of wet dog shakes. The rats given 1 mg/kg DM prior to NL administration showed significantly less flying and teeth chattering than the rats not receiving DM. The administration of 2 mg/kg DM ten min before NL injection brought about a significant decrease in all the abstinence syndrome signs with the exception of writhing. Even though no writhing was observed in the rats that received 2 mg/kg DM and 2 mg/kg NL, the statistical evaluation did not show any statistically significant difference. The most important observation was almost complete suppression of all the abstinence syndrome signs in 2 mg/kg DM + PAG group from the second min until the end of the observation period.

DISCUSSION

As the doses of K higher than 1 mg/kg cause ataxia, muscular weakness and/or some other signs of light general anesthesia, K was not given more than 1 mg/kg. Since DM is the O-methyl derivative of the uncompetitive NMDA antagonist dextrorphan, and it is rapidly converted into dextrorphan by means of demethylation (6,8), the administration of DM was carried out ten min prior to the NL injection. As the aim of the present study was to investigate whether the uncompetitive ASP/GLU antagonists K

and DM could attenuate opiate abstinence syndrome signs, another control group which had implanted pellets without M was considered unnecessary.

The IV administration of 0.5 mg/kg K just before the IP injection of NL appeared to be insufficient to significantly attenuate any of the abstinence syndrome signs. On the other hand, 1 mg/kg K decreased the number of jumpings, teeth chatterings and defecations, and increased the number of wet dog shakes. Even though these results seem to be discordant, they indicate the suppression by K of the abstinence syndrome because, when "dominant" abstinence syndrome signs such as flying, jumping, etc., decrease, the intensity of "recessive" signs like wet dog shakes may increase and vice versa (5). So the decrease in the number of "dominant" signs, which is accompanied by the increase in the number of "recessive" signs, is considered as the attenuation or decreased intensity of physical dependence (5).

DM as well as dextrorphan is virtually devoid of opioid activity. In other words, they have no antinociceptive action through any of the opioid receptors and no addiction liability (10, 11, 26). Therefore, the attenuating or preventing effects of K and DM on abstinence syndrome signs cannot be explained by the substitution by K and DM of M at the levels of opioid receptors. But the mechanism of the attenuation or prevention can be related to the actions of K or DM at excitatory amino acid-associated ion channels which minimize the responsiveness of the ASPergic/GLUergic receptors no longer having the effects of M after the administration of opioid antagonist NL. In this case, the ASPergic/GLUergic receptors being under the effects of K or DM cannot prepare the normal responses to ASP/GLU whose production normalized following the antagonism by NL of the M-exerted inhibition on L-asparaginase and glutaminase. On the other hand, the eventual up-regulation and supersensitivity developed in the ASPergic/GLUergic receptors due to their blockade and the lower production of ASP/GLU associated with the inhibition of the enzymes may play an important role in the manifestation and intensity of the abstinence syndrome. This certainly remains to be elucidated.

In conclusion, it can be said that the results of the present study can not only be considered as supporting evidence for the hypothesis regarding the development of physical dependence on opiates, but they can provide the possibility of a new treatment model for opiate addiction as well. In fact, the successfully clinical use of DM in the treatment of heroine addicts has already been observed (30).

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