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Prenatal Exposure to Morphine or Naloxone Intensifies Morphine Dependence at Maturity

H. KOYUNCUOĞLU[1] AND F. ARICIOĞLU

Department of Pharmacology and Clinical Pharmacology, Istanbul Medical Faculty, 34390 Capa, Istanbul, Turkey

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KOYUNCUOĞLU, H. AND F. ARICIOĞLU. *Prenatal exposure to morphine or naloxone intensifies morphine dependence at maturity.* PHARMACOL BIOCHEM BEHAV 44(4) 939-941, 1993.—Pregnant rats were SC injected with physiological saline (control) or 10 mg/kg morphine (morphine group) or 2 mg/kg naloxone (naloxone group) three times daily during the last 5 days of gestation. Three weeks after birth, male young rats of each group were taken and placed in separate cages. When their body weight reached 130–150 g, 10 rats from control, morphine, and naloxone groups were SC implanted with two pellets containing 75 mg morphine base (total 150 mg). Three days following implantation, rats were IP given 2 mg/kg naloxone for precipitated abstinence syndrome. Immediately after naloxone injection, rats were strictly observed for 15 min and jumping, wet-dog shakes, teeth-chattering, diarrhoea, defecation, and ptosis counted or rated. All abstinence syndrome signs were significantly higher in the morphine or naloxone group than in control. On the basis of the previous experimental findings supporting the idea that opiate physical dependence is related to the binding of opiate, possibly other than their own, to NMDA receptors and the upregulation and/or supersensitivity associated with the binding, the intensification of morphine dependence has been attributed to the long-lasting NMDA receptor upregulation and/or supersensitivity.

Prenatal exposure to morphine Prenatal exposure to naloxone Intensification of morphine physical dependence
Long-lasting upregulation by morphine of NMDA receptors Long-lasting upregulation by naloxone of NMDA receptors

PERINATAL opiate exposure can alter the antinociceptive action of opioids in mature subjects (2,3,10,11,17). In addition, differences in the reaction of similarly treated subjects to noxious stimuli prior to or without administration of an opioid have been reported when subjects were tested at maturity (1,16–20). Whereas a greater sensitivity to noxious stimuli has been found in some experimental works (1,16–20), the opposite has been reported (2,10,11,18,19). Further, it has been shown that offspring of methadone-treated females self-administered 75–80% of their total fluid intake as morphine solution at a time when control offspring, on an identical self-administration schedule, self-administered 20–25% of their fluid intake as morphine solution (13). As these kinds of behaviourial changes observed in animal models chronically treated with opioids have been accompanied by significant decreases in brain weight and by biochemical and structural alterations, they have been attributed to opiate withdrawal that occurred in utero and/or within 24 h of birth (8).

It is understood from the information given above that perinatal exposure to opioids can cause changes in the CNS areas functioning in pain transmission and pain perception. Further, perinatal exposure to opioids can also be involved in the high preference of morphine-containing solution in normal fluid intake that is greatly mediated by endogenous opioids. As known, some of the changes disappear within days following delivery while others persist for years or into young adulthood. As a result, in this study it was aimed to investigate as to whether perinatal exposure to morphine and naloxone may influence the intensity of physical dependence upon morphine as well as to attract attention to the growing disaster regarding especially the future of children born to women taking opiates or methadone under medical control.

METHOD

Twenty-four inbred pregnant rats were divided equally into three groups and every rat was kept in a cage separately. Approximately 5 days before they had pups, the first group was SC injected with 10 mg/kg morphine (10 mg morphine in 1 ml physiological saline) three times a day with the intervals of 4 h during daytime for 5 days (morphine group). The second (naloxone) group was SC given 2 mg/kg naloxone (2 mg nal-

[1] To whom requests for reprints should be addressed.

oxone in 1 ml physiological saline) in the same administration schedule. The last group (control) SC received the same volume of physiological saline instead. After having given birth to their pups on the fifth day of injections, three dams (having the highest number of pups) that had the same treatment were put in a large cage together with their pups to avoid being differently fed for about 3 weeks. Then, male young rats were taken from each group and placed in cages. Five young rats in a cage were kept until their body weight reached 130–150 g. Subsequently, two pellets containing 75 mg morphine base (total 150 mg) were SC implanted into 10 male rats from each group under light ether anaesthesia (15). Three days after pellet implantation, all rats were IP given 2 mg/kg naloxone and placed in a metal cage (base area 20 × 22 cm, height 20 cm) immediately. During the strict observation period of 15 min by experimenters blind to group identification, jumping, wet-dog shakes, defecation, and diarrhoea were counted. The severity of teeth-chattering and ptosis were evaluated, the experienced experimenter rating them according to their intensity: 1–10 and 1, 2, and 3, respectively.

The statistical evaluation of abstinence syndrome signs referring to controls was carried out by the Mann–Whitney *U*-test after being analyzed by the Kruskal–Wallis method.

Materials

Wistar inbred rats kept in a room 22–23°C on a 12 L : 12 D cycle and fed with a standard regimen ad lib were used. Morphine and naloxone were purchased from Verenigde Farmaceutische Fabrieken B. V. (Holland) and (Sigma Chemical Co., St. Louis, MO), respectively.

RESULTS

To prevent or at least minimize the retardation of in vivo physical and/or mental development, in utero opiate withdrawal, stillbirths, etc., in the present study, morphine or naloxone, both of which have rather short half-lives, was administered three times a day only during the last 5 days of gestation. As a result, no significant difference was observed in birth weights, postpartum weight gain, and abnormal behaviour within 24 h of delivery, as well as until later in the experiments.

The mean values (± SD) and their statistical evaluation by the Mann–Whitney *U*-test of the counted or rated 2 mg/kg naloxone-precipitated abstinence syndrome signs manifested in the 72 h before two morphine-containing pellets were implanted into the rats are shown in Table 1.

The values of jumping, wet-dog shakes, teeth-chattering, diarrhoea, defecation, and ptosis in the morphine group are significantly higher than those in the control group. The intensities of jumping, wet-dog shakes, teeth-chattering, diarrhoea, defecation, and ptosis of the naloxone group were found to be significantly greater than those of the control group.

DISCUSSION

It has recently been shown that previous blockade of NMDA subtype receptors by the noncompetitive NMDA receptor blockers ketamine or dextromethorphan and the opioids morphine alone or naloxone alone or combined with ketamine, dextromethorphan, or morphine intensified development of physical dependence upon morphine (5). The similar effect of noncompetitive NDMA blockers and opioids supports the idea that opioids, including those called opioid antagonists, can block NMDA receptors and cause upregulation and/or supersensitivity of the receptors, as receptor antagonists generally do (4,6,7). Therefore, the intensification of morphine physical dependence observed 5 days after the blockade by ketamine or dextromethorphan or morphine or naloxone alone or combined with ketamine, dextromethorphan, or morphine has been attributed to the upregulation and/or supersensitivity of NMDA receptors (5). As the agonists generally cause downregulation of their receptors, the increase by both opioid agonists and antagonists, called substance of the binding site, suggests that opioid agonists and antagonists can really be antagonists of a certain group of receptors that have recently been assumed to be NMDA subtype of the aspartatergic/glutamatergic receptors (4,7). Naloxone displaces morphine from receptors but it cannot prevent the withdrawal syndrome as seen in the naloxone-precipitated abstinence syndrome, and morphine has additional effects on those of the previously given naloxone in the intensification of morphine physical dependence (5). Naloxone and other

TABLE 1

MEAN VALUES (±SD) AND THEIR STATISTICAL EVALUATION BY THE MANN-WHITNEY *U*-TEST OF THE COUNTED OR RATED ABSTINENCE SYNDROME SIGNS MANIFESTED IN THE FIRST 15 MIN IMMEDIATELY AFTER 2 mg/kg NALOXONE INJECTION IN RATS

	Groups				
Signs	Control (10)	Morphine (10)	U_{mn}	Naloxone (10)	U_{mn}
Jumping (H: 20.68)	4.10 ± 1.22	14.20 ± 3.08*	0	13.1 ± 5.82*	0
Wet-dog shakes (H: 12.01)	0.90 ± 0.73	3.50 ± 2.27*	12	3.30 ± 1.88*	11
Teeth-chattering (H: 8.81)	4.10 ± 1.59	5.60 ± 1.17*	22	6.30 ± 1.41*	16
Diarrhoea (H: 8.33)	0.40 ± 0.51	1.20 ± 0.78*	22	1.30 ± 0.67*	17
Defecation (H: 13.57)	7.10 ± 1.10	11.80 ± 4.36*	5	10.60 ± 3.34*	19
Ptosis (H: 13.91)	0.70 ± 0.67	1.60 ± 0.69*	20	2.10 ± 0.56*	7

Rats whose mothers had sc been given 10 mg/kg morphine (morphine group) or 2 mg/kg naloxone (naloxone group) three times daily during the last 5 days of their pregnancy were carrying two 75-mg base morphine-containing pellets sc implanted for 72 h. The figures in parentheses indicate the number of rats in the groups.

*$p < 0.05$ compared to control value.

opioid antagonists could be opioids with high affinity for and weak blocking effects at NMDA receptors (4–7). The results of the present study not only support the idea that opioid agonists and antagonists are blockers of *N*-methyl-D-aspartate receptors but they also show that the effects of perinatal exposure to morphine or naloxone on the intensification of morphine physical dependence development are long lasting as well.

In conclusion, it can be said that the alterations in the antinociceptive action of opioids in mature subjects perinatally exposed to opiates (2,3,10,11,17) can be considered consequences of the upregulation and/or supersensitivity by prenatal exposure to opiates of NMDA receptors. In addition, differences in the reaction of similarly treated subjects to noxious stimuli prior to or without administration of an opioid at maturity (1,16–20) and the preference by offspring of methadone-treated females for the self-administration of 75–80% of their total fluid intake as morphine solution can be related to the upregulation and/or supersensitivity by prenatal exposure to opiates. Even for treatment under medical control and supervision, offspring of females continuing any kind of opiate intake during gestation would be regarded as prone to and most probable candidates for opiate addiction that should be more intense than normal.

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