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Effects of Neonatal Monosodium Glutamate and Aging on Morphine Dependence Development

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KOYUNCUOĞLU, H., F. ARICIOĞLU AND Y. DIZDAR *Effects of neonatal monosodium glutamate and aging on morphine dependence development.* PHARMACOL BIOCHEM BEHAV 43(2) 341-345, 1992.—Administration of monosodium glutamate (MSG) to neonatal rats has been reported to destroy aspartatergic (ASPergic) and glutamatergic (GLUergic) neurons. Ageing has been shown to induce cell loss, a rather general CNS atrophy, and slowness in the CNS functions. On the other hand, it has been hypothesized that two of the main reasons for opiate dependence development are the blockade by opiates of the NMDA receptors and their associated upregulation and supersensitivity. Accordingly, the abstinence syndrome precipitating effect of naloxone (NL) has been assumed to be the consequences of the removal by NL of opiate from NMDA receptors without being able to prevent upregulated and supersensitive NMDA receptors from being stimulated stronger than normal. To investigate the role of the decrease in the number of NMDA receptors in the development of morphine (M) physical dependence, 4 g/kg MSG was SC injected into neonatal rats on days 2,4,6,8 and 10 after birth. Their littermate controls SC received equimolar NaCl solution. Three or 14 months later, three pellets containing 75 mg base M were SC implanted into male rats treated neonatally with MSG or equimolar NaCl solution. Seventy-two hours after pellet implantation, all rats were injected with 2 mg/kg NL intraperitoneally. Some abstinence syndrome signs were counted or rated for 15 min immediately after NL injection and then statistically evaluated. The NL-precipitated abstinence syndrome was less intense in 3-month-old MSG-treated rats than in controls, most probably due to the decrease in the number of NMDA receptors in MSG-treated rats. The intensity of the NL-induced abstinence syndrome was found stronger in 14-month-old MSG-treated rats than in controls. The more intense development of physical dependence in 14-month-old MSG-treated rats was attributed to the upregulation and supersensitivity developed before pellet implantation to compensate for the destruction of NMDA receptors by MSG administered in the neonatal period. The attenuation of the M physical dependence intensity in 14-month-old control rats was explained by cell loss, CNS atrophy, and slowness in the CNS function.

Monosodium glutamate Ageing Intensification of morphine physical dependence
Attenuation of morphine physical dependence

ADMINISTRATION of monosodium glutamate (MSG) results in the destruction of several parts of the CNS (21-23). With associated alterations of some hormones and neurotransmitters, neonatal MSG treatment causes a syndrome of obesity, sexual dysfunction, growth stunting, and behavioral and learning deficits (3,20,21,24). The obesity of MSG-treated rodents appears to be metabolic in nature because food intake is not increased; opiate modulation of food intake is disrupted (5,8). In addition, MSG treatment has been reported to decrease morphine (M) analgesia (1). Glutamate (GLU) neurotoxicity occurs via the aspartatergic (ASPergic) glutamatergic (GLUergic) receptors, especially NMDA subtypes. NMDA receptor antagonists can block some NMDA-mediated neurotoxic effects such as neuronal death (6,12,25).

Aging is associated with a reduction in many distinct physiological and pathologic forms of neuronal plasticity. Neuronal plasticity is required for learning spatial tasks. NMDA receptor antagonists reduce the rates of learning spatial tasks (7,19) and aged rats are slower than young rats in learning spatial tasks (2,9). Kindling development, which depends upon excitatory amino acid neurotransmission mediated by NMDA receptors, is slower in aged rats than young mature animals (10,11). All these experimental results suggest that NMDA receptor-related functions are reduced with ageing. The reduction of M analgesia in elderly mice and rats (26,28) and the altered thermoregulatory responses to M in senescent rats (17,18) also can be considered evidence for the altered functions of NMDA receptors with age. Because opioids have been assumed to act on NMDA receptors (14,15), this has been regarded as one of the main reasons underlying opiate

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physical dependence (13–16). In fact, the noncompetitive NMDA receptor blockers ketamine and dextromethorphan have been found to suppress the precipitated abstinence syndrome in rats (15) and dextromethorphan has successfully been used in the treatment of heroin addicts (13,16).

In light of the experimental findings mentioned above, it was thought of interest to investigate whether destruction of some of the ASPergic/GLUergic receptors by means of neonatal MSG treatment and ageing could influence development of M physical dependence. If the intensity of the abstinence syndrome is less in young rats neonatally treated with MSG than in controls, this will enable us to say that the lower number of NMDA receptors does not allow rats to develop a strong M physical dependence. Similarly, if M dependence is more intense in young rats it will be possible to claim, to some extent, that aged animals fail to upregulate NMDA receptors.

METHOD

Newborn rats were SC administered 4 g/kg MSG (23.6 mM/kg) from a 50% aqueous solution of the compound on days 2,4,6,8, and 10 after birth. Total volume injected ranged between 0.1–0.3 ml. Littermate controls SC received a NaCl solution equimolar to that of MSG (23.6 mM/kg) in proportional volume to those employed for MSG-treated rats.

Pups of every four dam rats were equally divided into two groups. The first group (14–16 pups) was treated with MSG and housed with two dams in a cage. The remaining pups were given NaCl solution and housed with other dams in another cage. Animals were weaned on the 21st day. They were fed pelleted rat chow and free access to tapwater. All rats and pups were kept in a room 22–23°C on a 12 L:12 D cycle throughout the experiment.

Three or 14 months after birth, neonatally MSG-treated male rats and their male littermate controls were SC implanted with three pellets containing 75 mg M base (total 225 mg) (27) under light ether anaesthesia. Seventy-two hours following pellet implantation, rats were IP given 2 mg/kg naloxone (NL). Then, they were immediately placed in a metal cage (base area: 20 × 20 cm, height 20 cm) and strictly observed (2). The number of flyings, jumpings, wet-dog shakes, writhings, and defecations were counted for 15 min. For rating the severity of diarrhoea, ptosis, and teeth-chattering, the ratio between the counted diarrhoea or measured ptosis and teeth-chattering and the highest number of diarrhoea or the longest duration of ptosis and teeth-chattering were used, respectively.

The group consisting of neonatally MSG-treated rats that were implanted with M-containing pellets and then injected with 2 mg/kg NL at the end of 3 months after birth was called the neonatally MSG-treated young rat group (MSG-Y). The group consisting of their littermate controls was called the young littermate control group (Control-Y). When similar experiments were performed with the neonatally MSG-treated rats 14 months after birth, the groups were called the neonatally MSG-treated aged rat group (MSG-A) and the aged littermate control group (Control-A), respectively.

The statistical evaluation of each abstinence syndrome sign between groups was carried out by the Mann-Whitney *U*-test.

Materials

Pups of inbred Wistar rats were used. MSG and NL were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

The mean values (± SE) and their statistical evaluation by the Mann-Whitney *U*-test of the abstinence syndrome signs manifested by rats belonging to Control-Y and MSG-Y groups during the first 15 min immediately after 2 mg/kg IP injection are shown in Table 1. Jumping, teeth-chattering, defecation, and ptosis were significantly lower in the MSG-Y than in the Control-Y group. Wet-dog shakes were significantly higher in the MSG-Y than in the Control-Y group. In Table 2, the mean values (± SE) and their statistical evaluation by the Mann-Whitney *U*-test of the NL-precipitated withdrawal signs observed during the first 15 min in rats of Control-A and MSG-A groups are displayed. Jumping, teeth-chattering, defecation, diarrhoea, and ptosis showed a significant increase in the MSG-A group in comparison to the Control-A group.

When the values of abstinence syndrome signs of the Control-Y group were statistically compared to those of the Control-A group, jumping and defecation were found significantly lower in the Control-A than in the Control-Y group.

TABLE 1

MEAN VALUE (± SE) AND STATISTICAL EVALUATION BY THE MANN-WHITNEY *U*-TEST OF ABSTINENCE SYNDROME SIGNS DURING THE FIRST 15 min IMMEDIATELY AFTER 2 mg/kg IP NALOXONE INJECTION: YOUNG RATS

	Groups		
Signs	Control-Y (11)	MSG-Y (9)	U_{max}
Jumping	4.09 ± 0.27	2.78 ± 0.26*	83
Teeth-chattering	5.27 ± 0.34	3.89 ± 0.19*	136
Wet-dog shakes	1.91 ± 0.20	3.67 ± 0.45*	83.5
Writhing	0.91 ± 0.24	0.55 ± 0.29	61.5
Defecation	6.63 ± 0.27	4.66 ± 0.22*	94
Diarrhoea	1.00 ± 0.22	0.77 ± 0.26	57.5
Ptosis	2.09 ± 0.20	1.44 ± 0.16*	74

Figures in parentheses indicate the number of the rats in each group. Control-Y, control group of young littermate rats; MSG-Y, group of young rats treated neonatally with MSG .

*$p < 0.05$ (statistically significant).

TABLE 2

MEAN VALUES (± SE) AND THEIR STATISTICAL EVALUATION BY THE MANN-WHITNEY *U*-TEST OF THE ABSTINENCE SYNDROME SIGNS DURING THE FIRST 15 min IMMEDIATELY AFTER 2 mg/kg IP NALOXONE ADMINISTRATION: AGED RATS

	Groups		
Signs	Control-A (12)	MSG-A (11)	U_{max}
Jumping	2.58 ± 0.25	5.00 ± 0.34*	124
Teeth-chattering	4.41 ± 0.27	5.91 ± 0.42*	106
Wet-dog shakes	3.42 ± 0.34	2.36 ± 0.32*	98
Writhing	0.75 ± 0.21	1.18 ± 0.22*	86.5
Defecation	4.92 ± 0.27	6.45 ± 0.24*	115
Diarrhoea	0.58 ± 0.18	1.27 ± 0.23*	97.5
Ptosis	1.41 ± 0.29	2.27 ± 0.19*	94

Figures in parentheses indicate the number of the rats in each group. Control-A, control group of aged littermate rats; MSG-A, group of aged rats treated neonatally with MSG.

*$p < 0.05$ (statistically significant).

Instead, wet-dog shakes were significantly higher in the Control-A than in the Control-Y group (Table 3). The mean values (± SE) and their statistical evaluation of the NL-precipitated abstinence syndrome signs of the MSG-Y and MSG-A groups can be seen in Table 4. Jumping, teeth-chattering, writhing, defecation, diarrhoea, and ptosis were significantly higher in the MSG-A than in the MSG-Y group.

DISCUSSION

The statistically significant decrease in the "dominant" abstinence syndrome signs—jumping, teeth-chattering, defecation, and ptosis—and the significant increase in the "recessive" sign—wet-dog shakes—indicate a less severe M physical dependence development in the MSG-Y group than in their littermate controls (4). Instead, the significant increase in the dominant abstinence syndrome signs and the significant decrease in the recessive sign reflect a more intense development of M physical dependence (4). This less intense development by neonatally MSG-treated rats is most probably related to the destruction by neonatally MSG treatment of some ASPergic/GLUergic receptor-bearing neurons in the CNS (6,12). The destruction of these neurons decreases the number of sites on which M can act (1,5,8,13–16). If the development of opiate physical dependence is mainly associated with the consequences of the blockade of ASPergic/GLUergic receptors, especially the NMDA subtype (13–16), it is quite normal that the severity of M physical dependence development would be less in neonatally MSG-treated rats than in their littermate controls (Table 1).

The intensity of M physical dependence development in neonatally MSG-treated rats at 14 months of age is significantly higher than that in their littermate controls (Table 2). The significantly higher body weight (208 g ± 6.2) of neonatally MSG-treated rats than of their littermate controls (186 g ± 7.4) might unfavoarably have affected expression of abstinence syndrome signs, especially jumping. This obesity, the consequence of the destruction by MSG of the some parts within the CNS and rather metabolic in nature (3,20,21,24), cannot be avoided when the probable changes with age in the development of M physical dependence are investigated in neonatally MSG-treated rats, as well as in normal control rats. The exposure of the CNS cells to excessive levels of M may make a negligible difference in responding and/or adapting to

TABLE 3

STATISTICAL DIFFERENCES BY THE MANN-WHITNEY *U*-TEST BETWEEN THE VALUES (±SE) OF THE ABSTINENCE SYNDROME SIGNS OF THE CONTROL-Y GROUP (GIVEN IN TABLE 1) AND THOSE OF THE CONTROL-A GROUP (GIVEN IN TABLE 2)

	Groups		
Signs	Control-Y (11)	Control-A (9)	U_{max}
Jumping	4.09 ± 0.27	2.58 ± 0.25*	102
Teeth-chattering	5.27 ± 0.34	4.41 ± 0.27	82
Wet-dog shakes	1.91 ± 0.20	3.42 ± 0.34*	111.5
Writhing	0.91 ± 0.24	0.75 ± 0.21	71
Defecation	6.63 ± 0.27	4.92 ± 0.27*	105
Diarrhoea	1.00 ± 0.22	0.58 ± 0.18	78
Ptosis	2.09 ± 0.20	1.41 ± 0.29	81.5

*$p < 0.05$ (statistically significant)

TABLE 4

STATISTICAL DIFFERENCES BY THE MANN-WHITNEY *U*-TEST BETWEEN THE VALUES (±SE) OF THE ABSTINENCE SIGNS OF THE MSG-Y GROUP (GIVEN IN TABLE 1) AND THOSE OF THE MSG-A GROUP (GIVEN IN TABLE 2)

	Groups		
Signs	MSG-Y	MSG-A	U_{max}
Jumping	2.78 ± 0.26	5.00 ± 0.34*	113
Teeth-chattering	3.89 ± 0.19	5.91 ± 0.42*	111
Wet-dog shakes	3.67 ± 0.45	2.36 ± 0.32	54.5
Writhing	0.55 ± 0.29	1.18 ± 0.22*	92.5
Defecation	4.66 ± 0.22	6.45 ± 0.24*	114.5
Diarrhoea	0.77 ± 0.26	1.27 ± 0.23*	87
Ptosis	1.44 ± 0.16	2.27 ± 0.19*	101.5

*$p < 0.05$ (statistically significant).

those excessive levels of M, once the CNS completes its natural development and has the higher number of its mature neurons. For this reason, the highest number of M-containing pellets usually used for this purpose were implanted into all rats used in the experiments. In addition, exposure to the highest blood M levels minimizes the individual differences depending upon absorbing, distributing, metabolizing, excreting, etc. capacities and abilities that can often be seen in the dose regimens based upon body weight. On the other hand, the statistical evaluation between the results obtained from Control-Y and Control-A groups shows a less intense development of M physical dependence in control rats (not treated neonatally with MSG) at 14 months of age than in control rats at 3 months of age (Table 3). By contrast, the intensity of M physical dependence in neonatally MSG-treated rats at 14 months of age is higher than in neonatally MSG-treated rats at 3 months of age (Table 4). As a result of the statistical evaluations seen in Tables 3 and 4, it can be said that the intensity of M physical dependence decreases with ageing under normal circumstances. The destruction by neonatal MSG treatment of some parts of the ASPergic/GLUergic system has an intensifying effect on the development of M physical dependence in aged rats. The attenuation of the severity of M physical dependence with ageing cannot be explained only with the well-known age-dependent cell loss in the aged CNS. Otherwise, neonatally MSG-treated rats, which had been subjected to cell loss in the CNS, would not have developed a significantly stronger M physical dependence at 14 months of age than their littermate controls did (Table 2). This might be explained by the necessity of neonatally MSG-treated rats to upregulate NMDA receptors and/or make them supersensitive to compensate for the MSG-destroyed ASPergic/GLUergic receptor-bearing neurons, especially after adolescence, for which additional hormonal, metabolic, etc. activities are needed. Because the upregulation and supersensitivity of NMDA receptors may occur to keep some important central and peripheral functions normal just in case of blockade by NMDA antagonists (14) or possibly destruction by MSG of NMDA receptor-bearing neurons, the upregulation and supersensitivity of NMDA receptors at normal advanced ages would not necessarily be seen because many central peripheral functions concomitant with gradual loss of the related biochemical cellular processes are getting obliterated. The reduction by NMDA antagonists of the rates of spatial learning tasks (7,19), the slower learning of spatial tasks (2), and the slower kindling rates (9,11) in aged rats than in young ones can be considered supporting experimental findings.

On the basis of the present experimental findings, the following conclusions can be drawn:

1. The hypothesis implying that one of the main causes for development of opiate physical dependence is the blockade by opiates of the ASPergic/GLUergic receptors has been supported by other experimental findings.
2. The decrease by neonatal MSG treatment of the ASPergic/GLUergic receptors may play a determining role in the intensity of opiate dependence development in young rats.
3. Ageing and associated slowness in the functions of ASPergic/GLUergic systems, which result in alterations in upregulating of and becoming supersensitive to their receptors, can be the main reason for the relatively less severe opiate physical dependence development.

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