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Lack of Adaptive Changes in 5-HT Sites in Perinatally Undernourished Rats After Chronic Stress: Opioid Influence

E. A. KELLER,[1] L. M. CANCELA, V. A. MOLINA AND O. A. ORSINGHER

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Sucursal 16, C.C. 61, 5016 Córdoba, Argentina

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KELLER, E. A., L. M. CANCELA, V. A. MOLINA AND O. A. ORSINGHER. *Lack of adaptive changes in 5-HT sites in perinatally undernourished rats after chronic stress: Opioid influence.* PHARMACOL BIOCHEM BEHAV **47**(4) 789–793, 1994.—The reactivity of 5-HT receptors following repeated immobilization sessions or after immobilization plus morphine was measured through 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) or 8-hydroxy-2-(dipropyl-amino)tetralin (8-OH-DPAT)-induced serotonergic syndrome in adult rats undernourished at perinatal age. Repeated stress enhanced the scores of forepaw treading and hindlimb abduction elicited by 5-MeODMT in control animals. In a similar way, forepaw treading induced by 8-OH-DPAT was enhanced in chronically stressed control rats. These results indicate the development of supersensitivity in 5-HT_1 receptors. Conversely, this effect was not observed in undernourished animals. Morphine injections before each stress session instaured the increased reactivity to 5-HT_1 sites in malnourished animals. An injection of naloxone prior to morphine before each stress session fully antagonized the increased behavioral reactivity to 5-MeODMT observed in deprived animals. A possible deficiency in the functional role of the opiate system involved in the process of adaptation to chronic stress in early undernourished rats is suggested.

Protein malnutrition 5-HT receptors Chronic stress Opiate agent

EARLY undernutrition leads to several behavioral disturbances at adult age. Among these changes, malnourished rats present alterations in different behavioral tasks which involve stressful stimuli (2,4,9,15,21–23). Therefore, it seems likely that perinatal undernutrition could permanently affect the ability to display an adequate behavioral coping strategy during highly aversive events.

It is well known that exposure to a single stress event induces different adverse effects such as reduced locomotion, marked anorexia, and decreased growth rate and body weight (1,11,14,18). Upon chronic exposure, most of these changes disappear, indicating that a process of adaptation to stress has developed to protect the organism against recurrent stressors (11,14,18). Parallel to the relief of these effects, chronic stress induces several adaptive changes on monoamine receptors (5–8,14,24–26); therefore it has been suggested that these changes on monoamine sites are functionally linked to the onset of the adaptation process to stress. Among these adaptive changes, chronic stress leads to a reduced reactivity of presynaptic DA sites and of α_2-adrenoceptors as well as to an increased behavioral reactivity of 5-HT sites (6–8,14). Unlike controls, stressed animals undernourished in early life did not present modifications in their behavioral reactivity either on presynaptic DA sites or on α_2-adrenoceptors (13). Consequently, to explore whether this deficiency may be extended to 5-HT sites, we have evaluated the behavioral reactivity of these receptors to 5-MeODMT (an agonist of 5-HT sites) and 8-OH-DPAT (agonist of 5-HT_1 sites) following repeated exposure to restraint.

According to previous observations (5,7,8), activation of an endogenous opiate system seems to be critically involved in the development of the adaptive changes following chronic stress. A reduced release of β-endorphin has been reported following shock exposure in early malnourished rats (19, 20,29). Therefore, an additional goal of the present study was to analyze whether an alteration of an opiate process could be underlying the lack of adaptive responses to chronic stress shown by malnourished rats. Thereby, we studied the influ-

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

ence of the combined treatment with morphine and stress on the behavioral response induced by 5-MeODMT and 8-OH-DPAT. Furthermore, each immobilization session was preceded by naloxone (opioid antagonist) before morphine, and the behavioral response induced by 5-MeODMT was evaluated in one group of deprived animals.

METHODS

Deprivation Schedule

A protein deprivation schedule was used as previously described (17). Briefly, female rats (Wistar strain) were divided into two groups at 14 days of pregnancy and fed with isocaloric diets containing 24% and 8% casein (controls and deprived, respectively). Diets contained 4 g/kg DL-methionine. After weaning (30 days), pups continued consuming the same diet as their dams until 50 days of age. Both groups were given balanced standard chow thereafter for at least 90 days prior to the experiments (i.e., at trials, rats were at least 140 days old). Deprived and control male rats used in these experiments came from different litters (i.e., sibling replication was consistently avoided). Body weights for control and deprived rats were 312 ± 8.7 g and 239 ± 6.1 g, respectively, when experiments were performed. Animals were maintained at 22 ±

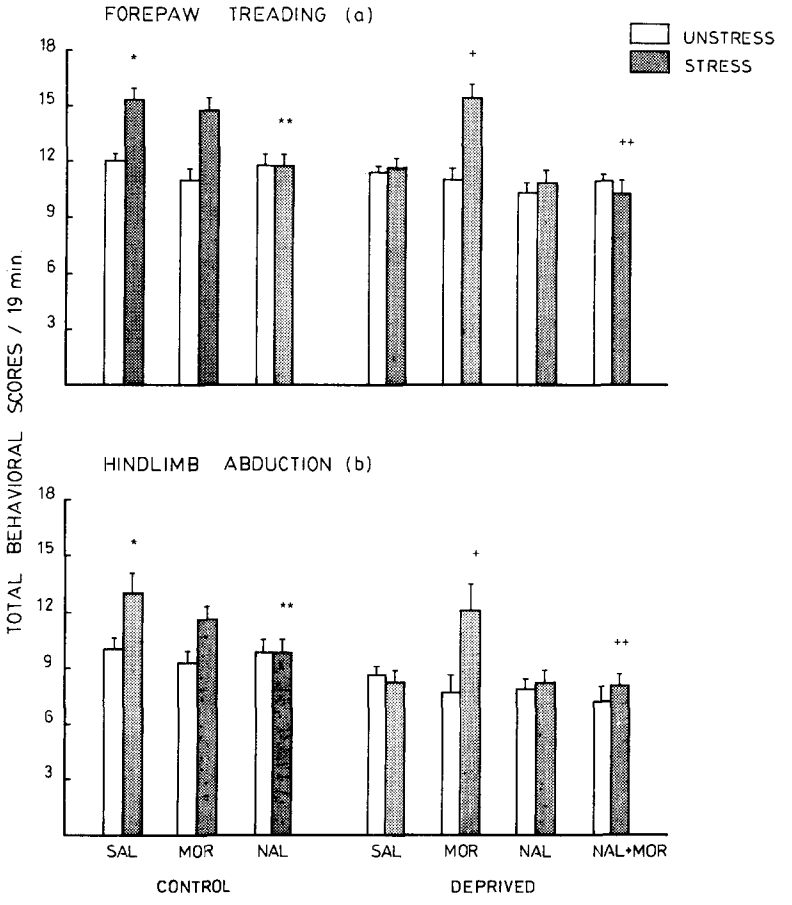

FIG. 1. Effect of chronic immobilization alone, associated with morphine or with naloxone on the behavioral responses to 5-MeODMT in deprived and control rats. Saline (1 ml/kg IP), morphine (1 mg/kg IP), or naloxone (2 mg/kg IP) were injected before each immobilization session. Additional groups of rats were daily injected with saline, morphine, or naloxone for seven days. In one group of deprived animals each immobilization session was preceded by naloxone 5 min before morphine; the respective control group received daily injections of naloxone + morphine for seven days. All rats were injected with 5-MeODMT (5 mg/kg IP) 24 h after the last drug administration and/or immobilization session. Bars represent mean ± SEM of 8–14 animals for each group. (a) *Different from control unstressed rats ($p < 0.001$). **Different from control stressed rats ($p < 0.001$). $^{+}$Different from deprived stressed rats treated with saline ($p < 0.002$). $^{++}$Different from deprived stressed rats treated with morphine ($p < 0.001$) (Mann-Whitney U test). (b) *Different from control unstressed rats ($p < 0.003$). **Different from control stressed rats ($p < 0.002$). $^{+}$Different from deprived stressed rats treated with saline ($p < 0.002$). $^{++}$Different from deprived stressed rats treated with morphine ($p < 0.001$) (Mann-Whitney U test).

2°C in a 12-h light–dark cycle, lights on at 0700 with food and water ad lib.

Procedure

Adult male rats from both groups (control and deprived) were immobilized for 2 h a day, beginning always between 1000 and 1200, by placing them in a Plexiglas restraining device adjusted for the body size of the animals for seven consecutive days. Additional groups of control and deprived rats were immobilized with the same schedule preceded by saline (1 ml/kg IP), morphine (1 mg/kg IP), or naloxone (2 mg/kg IP) 10 min before the onset of restraint. The respective control groups (unstress + saline, unstress + naloxone, or unstress + morphine) received daily injections of saline, naloxone, or morphine for seven days. In one group of deprived animals each immobilization session was preceded by naloxone 5 min before morphine; the respective control group (unstress + naloxone + morphine) received daily injections of naloxone + morphine for seven days. In all experimental and control groups of animals the behavioral response induced by 5-MeODMT was determined 24 h after the last restraint session and/or injection.

In another set of experiments, the behavioral response induced by 8-OH-DPAT was analyzed in groups of control and deprived rats submitted to the same schedule of immobilization with saline (1 ml/kg IP) or morphine (1 mg/kg IP) injection 10 min before the onset of restraint. The behavioral response induced by 8-OH-DPAT was determined 24 h after the last restraint session and/or injection.

Behavioral Studies

Behavioral experiments were performed in a quiet room at 20–24°C between 1200 and 1600. Animal behavior was observed while subjects were in a plastic cage (50 cm diameter) to which they had been accustomed by placing them individually there for at least 30 min before the experiments. Behavior was observed by one of the authors who was blind to the treatments. Following 5-MeODMT administration (5 mg/kg IP) rats were observed for continuous scoring periods of 2 min separated by 1-min nonscoring intervals over a total observation period of 19 min beginning 2 min after 5-MeODMT administration. As previously described by Kennett et al. (14), intermittent behavior (forepaw treading) in each scoring period was scored on a 0–4 scale: 0 = absent, 1 = present once, 2 = present several times, 3 = present frequently, and 4 = present continuously. Continuous behavioral responses (hind limb abduction, Straub tail, and tremor) were scored on a 0–4 range of relative intensity: 0 = absent, 1 = perceptible, 2 = weak, 3 = medium, and 4 = maximal.

In the experiment with 8-OH-DPAT, the behavioral response was evaluated for periods of 2 min separated by 1 min of nonscoring intervals over a total observation period of 25 min beginning 2 min after 8-OH-DPAT (0.5 mg/kg SC) administration. Forepaw treading or head weaving (intermittent behavior) and flat body posture (continuous behavior) were scored as mentioned above.

Drugs

Morphine-HCl (Lab. Verardo, Buenos Aires), naloxone-HCl (Sigma Chemical Co., St. Louis), and 8-hydroxy-2-(dipropyl-amino) tetralin (Research Biochemicals Inc., Natick, MA) were dissolved in saline. 5-Methoxy-*N,N*-dimethyltryptamine (Sigma) was dissolved in saline using a few drops of glacial acetic acid. Injections were made at a volume 0.1 ml/100 g body weight for all drugs.

Statistics

The 5-HT behavioral syndrome induced by 5-MeODMT and 8-OH-DPAT was analyzed using a Kruskal–Wallis test followed by a two-tailed Mann–Whitney *U* test.

RESULTS

As described in previous works from other laboratories and from our own (8,14), a schedule of immobilization for 2 h daily for seven days in control rats induced, 24 h after the last session, an enhanced behavioral response to 5-MeODMT with significant increases in forepaw treading and hindlimb abduction scores as revealed by Kruskal–Wallis test (Fig. 1). Other behavioral effects of 5-MeODMT such as tremor or Straub tail were not significantly increased (data not shown). Pretreatment with naloxone completely abolished the increase in the 5-HT-dependent behavioral response to 5-MeODMT in control rats chronically stressed by seven days (Fig. 1). In deprived rats, chronic immobilization failed to enhance the behavioral responses to 5-MeODMT as observed in control rats (Fig. 1). However, when morphine was administered prior to each restraint session, the scores of forepaw treading and hind limb abduction were enhanced to a level similar to that obtained in chronically stressed control rats (Fig. 1). The pretreatment with morphine in chronically stressed control animals did not modify the degree of enhancement with respect to the observed with immobilization alone. The administration of naloxone prior to morphine abolished the increased behavioral response to 5-MeODMT observed in chronically stressed deprived rats treated with morphine only (Fig. 1). The single administration of seven daily injections of morphine and/or naloxone in deprived and control rats did not affect

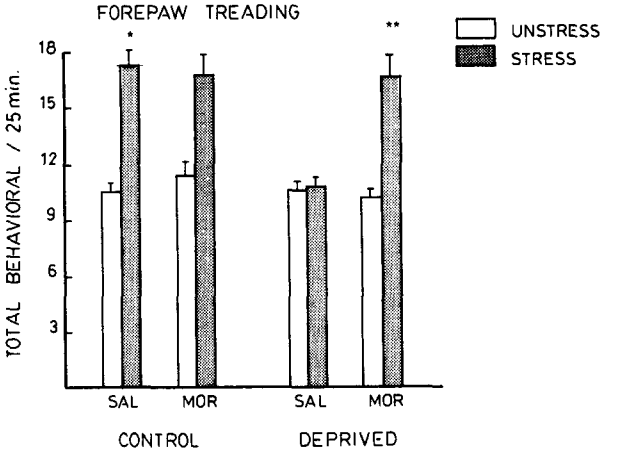

FIG. 2. Effect of chronic immobilization alone or associated with morphine on the behavioral response to 8-OH-DPAT in deprived and control rats. Saline or morphine (1 mg/kg IP) was injected before each immobilization session. Additional groups of rats were daily injected with saline or morphine for seven days. All rats were injected with 8-OH-DPAT (0.5 mg/kg SC) 24 h after the last immobilization session. Bars represent mean ± SEM of 6–10 animals for each group. *Different from control unstressed rats ($p < 0.001$). **Different from deprived stressed rats treated with saline ($p < 0.002$) (Mann-Whitney *U* test).

the different components of the behavioral response to 5-MeODMT (Fig. 1). It should be addressed that the behavioral response to 5-MeODMT was similar when unstressed deprived and control groups were compared. The only exception was the hind limb abduction component, which is attenuated in unstressed deprived rats as compared to that observed in unstressed controls.

In the same line, the chronic immobilization schedule in control rats induced an increase in the forepaw treading response elicited by 8-OH-DPAT as revealed by Kruskal-Wallis test (Fig. 2). Other behavioral effects of 8-OH-DPAT such as head weaving and flat body posture were not modified (data not shown). In a way similar to the previously described with 5-MeODMT, the forepaw treading induced by 8-OH-DPAT was not enhanced in chronically stressed deprived rats as in chronically stressed control rats (Fig. 2). However, when morphine was administered prior to each stress session, the score of forepaw treading was enhanced to a level similar to that obtained in chronically stressed control rats (Fig. 2).

DISCUSSION

As previously observed in our own and in other laboratories (8,14), chronically immobilized control animals displayed higher scores of forepaw treading and hindlimb abduction than unstressed rats following 5-MeODMT administration. Other behavioral components such as tremor and Straub tail remained unmodified. Conversely, the same regime of chronic stress did not alter the behavioral reactivity to 5-MeODMT in malnourished animals, since similar behavioral scores were obtained in stressed and nonstressed deprived rats. When behavioral response after 5-MeODMT was observed in nonstressed deprived and control rats, similar forepaw treading scores were found. However, hindlimb abduction values from nonstressed deprived rats were lower than those of nonstressed control rats. Though the latter results could, at least in part, agree with previous work (10), it should be noted that no difference was found in the hypotermic effect of 5-MeODMT between deprived and control rats (3).

In the same way, similar results were obtained when the behavioral response induced by 8-OH-DPAT was determined. Thus, chronically stressed control rats showed higher scores of forepaw treading than unstressed control rats. Deprived stressed and unstressed animals presented the same scores. It is well known that several components of the 5-HT behavioral syndrome, mainly forepaw treading, are closely linked to 5-HT_1 receptor activation (16,27,28). Thus, the present results, obtained with a specific 5-HT_1 agonist, 8-OH-DPAT, confirm that chronic restraint brings about adaptive changes of 5-HT_1 receptors (8,14). Therefore, in deprived animals the lack of an enhanced behavioral response to 5-HT agonists indicates that early undernutrition may impair the development of adaptive changes on 5-HT_1 sites following chronic stress in adult rats. This assumption extends previous findings, which have reported similar deficiencies in other neurotransmitter systems. In fact, evidence obtained using a similar restraint schedule has shown that the subsensitivity of presynaptic DA and of α_2-adrenoceptor sites observed after chronic stress has not been observed in deprived animals (13). Furthermore, the lack of adaptive changes in monoamine sites does not seem to be restricted to the effects of stress. Thus, behavioral and neurochemical data obtained in deprived rats show that chronic antidepressant treatments failed to induce adaptive changes on catecholaminergic receptors, which are usually observed in control rats (12,13).

Numerous experiments indicated that endogenous opioids play a modulatory role in the development of adaptive changes on monoaminergic receptors after chronic stress (5, 7,8). Thus, it has been described that naloxone pretreatment fully antagonized the appearance of adaptive changes on α_2 and 5-HT_1 receptors after chronic stress (7,8). Furthermore, an associated treatment with morphine or β-endorphin and each restraint accelerated the onset of these adaptive responses (7,8). In the present work, a combined treatment with stress and morphine in undernourished rats instaured the appearance of an increased behavioral reactivity of 5-HT_1 sites to a degree similar to that observed in chronically stressed control rats. Consequently, naloxone pretreatment abolished the morphine-induced effects. These results suggest that perinatal undernutrition impairs the activation of an opiate mechanism implicated in the response to aversive experiences. In accordance with this view, several authors have reported that adult rats undernourished at early life present a reduced release of endorphines following shock or novel training procedures (19,20,29). Besides, alterations in different behavioral paradigms, such as an exaggerated behavioral response to stress, have been consistently noticed in rats submitted to perinatal undernutrition (9,15,21), suggesting that this insult during early life probably affects the ability to cope with environmental demands at adult age. The process of adaptation to stress, which is functionally linked to the development of adaptive changes, serves in part to maintain a functional homeostasis during recurrent aversive experiences. Hence, our evidence concerning the deficit of these rats in the promotion of adaptive changes may account, at least in part, for different behavioral disturbances observed when these rats are confronted with highly stressful situations. Moreover, the fact that the combination of morphine and stress resets these changes may indicate that the deficit of these rats might be related to a functional deficiency in stress-induced activation of an endogenous opiate process.

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