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Lack of Neuronal Adaptive Changes Following Chronic Treatments in Perinatally Undernourished Rats

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KELLER, E. A., V. A. MOLINA AND O. A. ORSINGHER. *Lack of neuronal adaptive changes following chronic treatments in perinatally undernourished rats.* PHARMACOL BIOCHEM BEHAV **37**(4) 675–678, 1990.—Reactivity of presynaptic dopaminergic and alpha$_2$-adrenoceptors following repeated stress or desipramine treatment was investigated by means of apomorphine (APO)- or clonidine (CLO)-induced hypoactivity, respectively, in adult rats undernourished at perinatal age. Under basal conditions, a comparable hypoactive response was observed in control and experimental animals following either APO or CLO administration. Chronic DMI or repeated immobilization sessions attenuated the hypoactivity elicited by APO or CLO in control animals; however, this effect was not observed in experimental rats. These findings demonstrate that deprived animals show impairment to produce neuronal adaptive changes in response to appropriate stimuli, which may account for the behavioral alterations attributed to early undernutrition.

Perinatal undernutrition DA receptors (presynaptic) Alpha$_2$-receptors DMI Chronic stress

NUMEROUS studies have pointed out permanent alterations in different anatomical, neurological and neurochemical parameters in adult animals submitted to undernutrition at perinatal age (19,32). However, the relationship between these alterations and the behavioral abnormalities observed in these animals is still not clearly understood. Behavioral studies have shown that early undernutrition induces, among other changes, alterations in emotionality and responsiveness to stress (2, 3, 5, 10, 17, 22–24). These abnormal patterns could be defined as maladaptive responses to stressful situations.

On the other hand, there is increasing evidence that adaptation and resistance to many of the adverse effects of stress are related to adaptive changes in catecholaminergic receptors (27). Accordingly, down-regulation of brain beta-adrenoceptors, as well as a diminished response to cyclic AMP accumulation stimulated by noradrenaline (NA), is evoked after different schemes of repeated stressors, in a temporal correlation with the occurrence of adaptation and resistance to many of the adverse effects of stress (27,28). Moreover, behavioral observations have pointed out a reduced reactivity of presynaptic dopaminergic and alpha$_2$-adrenoceptor sites following chronic but not acute stress, in the same temporal correlation with beta-adrenoceptor down-regulation (6,7). Accordingly, we have recently reported that adult rats, submitted to a protein deprivation schedule at perinatal age, failed to produce down-regulation of beta-adrenoceptors in frontal cortex and hippocampus after prolonged DMI treatment (16). This has led us to suggest that the lack of adaptive changes in response to appropriate stimuli may be responsible for the abnormal behaviors attributed to early undernutrition.

The present work deals with the modulatory capacity of presynaptic dopaminergic and alpha$_2$-adrenoceptors in response to repeated stress situations or DMI treatment. With this purpose we evaluated in perinatally undernourished rats the reactivity of these sites by means of apomorphine (APO)- and clonidine (CLO)-induced hypoactivity in animals previously submitted to repeated sessions of immobilization or chronic DMI treatment.

METHOD

Deprivation Schedule

A protein deprivation schedule as previously described (18) was used. Briefly, female rats (Wistar strain) were divided into two groups at 14 days of pregnancy and fed 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. Animals were maintained at 22 ± 2°C in a 12-hr light-dark cycle, lights on at 07:00 hr. Food and water were ad lib.

Experimental Design

Four experiments were performed. In Experiment I, motor activity in control and deprived rats induced by apomorphine was analyzed in groups with prior DMI treatment. In Experiment II, a similar behavioral parameter induced by clonidine was assessed

after DMI administration. Experiments III and IV examined similar dependent variables when stress induced by restraint was followed by either APO (Experiment III) or CLO (Experiment IV) administration. In each experiment control and experimental conditions were defined by an initial $2 \times 2 \times 2$ factorial design. The factors under consideration were: nutritional treatment (deprived and control); postnatal chronic treatment (DMI or saline for Experiments I and II; stress or nonstress for Experiments III and VI) and drug administration prior to testing (APO or saline in Experiments I and III; CLO or saline in Experiments II and IV).

Postnatal Treatment

Adult male rats from both groups (control and deprived) were treated with DMI HCl (20 mg/kg/day IP) or saline (1 ml/kg/day IP) for 7 days.

Other groups of control and deprived rats were immobilized for 2 hr daily in a Plexiglas restraining device for 7 consecutive days. Animals were restrained between 10:00 and 14:00 hr. The respective control groups (unrestrained) were placed in their home cages in the same room as restraint animals.

Measurement of Hypoactivity

In the groups of animals treated with DMI or saline the locomotor activity was determined 48 hr after the last injection; in the groups of animals restrained chronically and nonrestraint, this activity was determined 24 hr after treatment. Locomotor activity was measured in a square open field ($60 \times 60 \times 60$ cm) with 15×15 cm squares delineated in the floor. Animals (treated with DMI or saline; restrained or unrestrained) were tested 5 min after saline (1 ml/kg, SC) or APO (25 μg/kg, SC), respectively. Other groups of animals (with about the same described treatment) were tested 20 min after saline (1 ml/kg, IP) or CLO (75 μg/kg, IP). Testing was performed between 10:00–17:00 hr under tenuous white light in a quiet room. The number of squares entered with four paws over a period of 10 min was recorded.

Drugs

Clonidine HCl (Catapresán, Boehringer), apomorphine HCl (Sigma Chemical Co.), and desipramine HCl (Laboratories Montpellier, Buenos Aires) were used in these experiments.

Statistical Analysis

Data were analysed by means of ANOVA followed by a least significant difference Fisher test set at 0.05.

RESULTS

Effect of Chronic DMI Administration on APO-Induced Hypoactivity in Deprived and Control Rats

No difference was observed in basal locomotion nor in the hypoactivity response induced by APO, between control and deprived rats without antidepressant treatment. A 7-day treatment with DMI reduced the hypoactivity induced by APO in control animals. In contrast, perinatally deprived rats did not show the attenuating effect of chronic DMI treatment on APO-induced hypoactivity (Fig. 1).

Effects of Chronic DMI Administration on CLO-Induced Hypoactivity in Deprived and Control Rats

As depicted in Fig. 1, similar values of motor activity were obtained after saline or CLO in control and deprived rats without antidepressant treatment. Figure 2 shows the attenuating effect of chronic DMI treatment on CLO-induced hypoactivity in control rats. In contrast, chronic DMI administration failed to reduce CLO sedative effects in deprived rats.

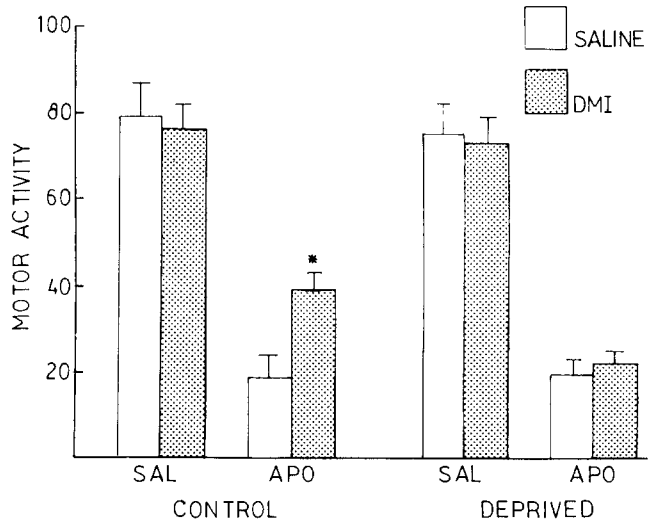

FIG. 1. Effects of chronic administration of DMI on APO-induced hypoactivity in deprived and control rats. Control and experimental rats were administered with DMI (20 mg/kg/day) or saline during 7 days and 48 hr after SAL or APO (25 μg/kg) was given SC. Five minutes after the SAL or APO injection the animals were placed in a open-field and motor activity (number of squares entered) was recorded for 10 minutes. Each column represents the mean ± S.E.M. of 8–13 animals. *Significantly different from comparable control groups, $p<0.05$ (Fisher test).

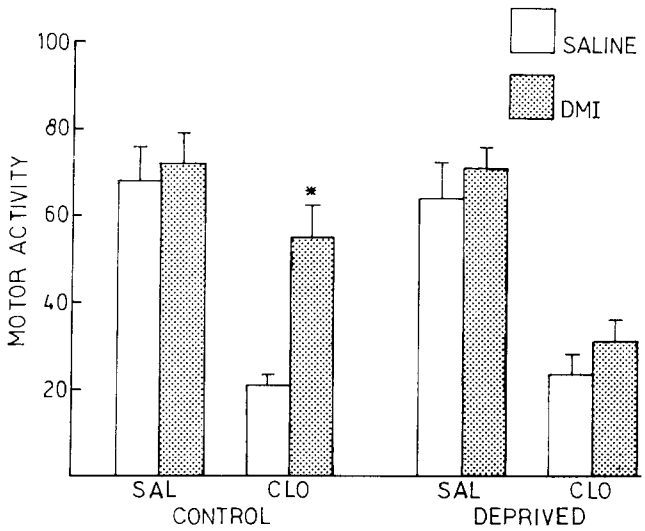

FIG. 2. Effect of 7-day DMI treatment (20 mg/kg/day) on CLO-induced suppression of motor activity in deprived and control rats. Animals received CLO or saline 48 hr after the last DMI or saline injection; motor activity was assayed 20 min after in the open-field. Each column represents the mean ± S.E.M. of 8–13 rats. *Significantly different from comparable control groups, $p<0.05$ (Fisher test).

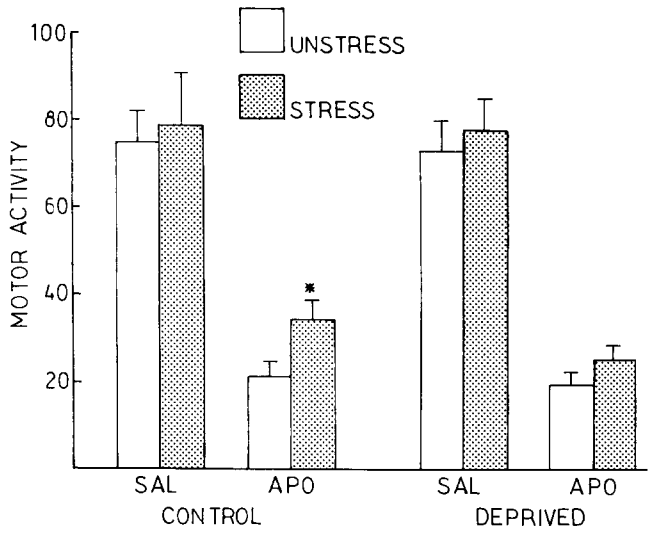

FIG. 3. Effect of chronic stress on APO-induced hypoactivity in deprived and control rats. Locomotor activity was measured 24 hr after the last immobilization session in a square open-field. Animals were tested 5 min after APO or saline. Each column represents the mean ± S.E.M. of 8–13 animals. *Significantly different from comparable control groups, $p<0.05$ (Fisher test).

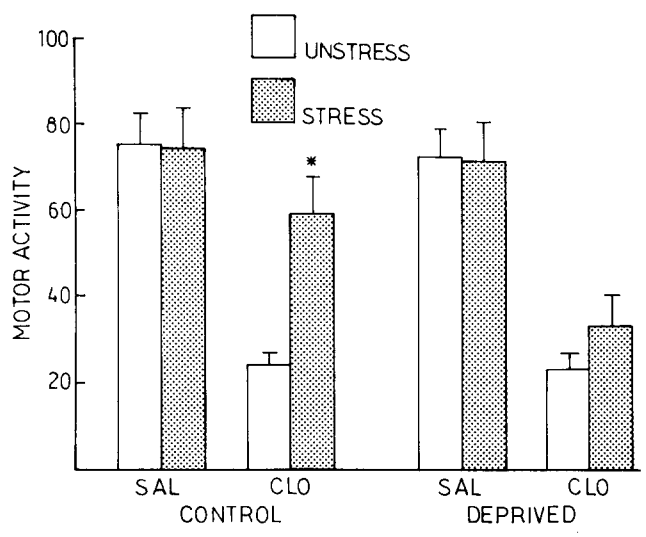

FIG. 4. Effect of chronic stress on CLO-induced hypoactivity in deprived and control rats. Locomotor activity was measured 24 hr after the last immobilization session in a square open-field. Animals were tested 20 min after CLO or saline. Each column represents the mean ± S.E.M. of 8–13 animals. *Significantly different from comparable control groups, $p<0.05$ (Fisher test).

Effects of Chronic Stress on APO- and CLO-Induced Hypoactivity in Control and Deprived Rats

Control rats submitted to repeated immobilization sessions showed a decrease in the sedative response elicited by APO or CLO. In contrast, scores obtained from experimental rats did not show any significant differences between stressed and unstressed animals following either APO or CLO administration (Figs. 3 and 4).

DISCUSSION

As previously described, chronic DMI treatment or immobilization sessions reduced hypoactivity provoked by low doses of APO or CLO in control animals (6, 7, 11, 13, 21, 25). This behavioral response is attributed to stimulation of presynaptic dopaminergic and alpha$_2$-adrenoceptors, respectively (20,26).

Our results demonstrated that experimental rats submitted to chronic stress or DMI treatment failed to induce subsensitivity of presynaptic dopaminergic and alpha$_2$-receptors. This lack of adaptive response was evidenced by the similar hypoactivity response obtained after APO or CLO administration in untreated and treated animals.

We have recently described that rats submitted to a similar deprivation schedule failed to reduce beta-adrenergic receptor density in frontal cortex and hippocampus after prolonged DMI treatment. Since deprived rats have lower density of beta-adrenergic sites in basal conditions (14,16), the ineffectiveness of chronic DMI to elicit down-regulation could be related to an impairment to cause any additional decrease. In the present report, deprived rats showed a similar response to controls after APO or CLO administration in basal conditions. Furthermore, we have reported no basal differences between deprived and control rats in the density of dopaminergic receptors from striatum and accumbens areas (4). These facts suggest that the absence of modulatory capacity observed in undernourished rats following DMI administration or stress sessions may be independent of the basal density of these sites.

In recent years, it has been proposed that habituation and resistance to many adverse behavioral and physiological consequences of stress are related to several adaptive responses in the central monoaminergic system (6, 7, 27, 28). Thus, different schemes of repeated stressors evoke down-regulation of brain beta-adrenoceptors as well as a diminished response to cyclic AMP accumulation stimulated by noradrenaline (27,28). In a similar way, behavioral observations have pointed out a reduced reactivity of presynaptic dopaminergic and alpha$_2$-adrenoceptor sites following chronic but not acute immobilization (6,7). Similar adaptive changes are also evoked in chronically antidepressant-treated rats (1, 8, 21, 25, 30, 31). Therefore, it has been proposed that antidepressant therapy response represents a form of adaptation to stress (27). There is a growing awareness that adaptive changes in catecholaminergic receptors may play a role in the maintenance of normal behaviors after exposure to stressful or aversive events. The fact that our experimental animals showed inability to produce subsensitivity in presynaptic dopaminergic autoreceptors and alpha$_2$-receptors after chronic DMI treatment or repeated sessions of stress suggests that the lack of adaptive process following chronic stimulation that may be responsible for the abnormal behaviors attributed to early undernutrition. This interpretation is supported by previous reports that demonstrated a diminished reactivity to different pharmacological treatments in adult rats deprived at early life, at both peripheral and central levels. In this regard, subsensitivity to adrenergic agonists on the vascular bed (9) and other sympathetic innerved organs (15), in addition to a lower reactivity to the central effects of 5-HT agonist (12), was observed. These data also suggest the possibility that human subjects inadequately fed during childhood may display altered responsiveness to therapeutic drugs later on.

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