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Permanent Dopaminergic Alterations in the N. Accumbens After Prenatal Stress

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ALONSO, S. J., E. NAVARRO AND M. RODRIGUEZ. *Permanent dopaminergic alterations in the n. accumbens after prenatal stress.* PHARMACOL BIOCHEM BEHAV 49(2) 353–358, 1994. – It has been suggested that stress during the initial stages of human life may serve as a predisposing factor to mental illness. Recently, we reported that in pregnant rats, stress induces an increase of behavioral depression in the female offsprings when adult. This article describes the effect of prenatal stress on central dopaminergic transmission during adulthood. The offspring of stressed mothers showed an increase of behavioral depression in the Porsolt test and a reduction of DOPAC, HVA, and DOPAC/DA index in the n. accumbens. The effect on the right accumbens was more marked than on the left. A great body of information exists to suggest that depression is related to a decrease of dopaminergic neurotransmission, and the present data provide new evidence in support of the hypothesis that maternal stress during gestation increases the risk of depression in the offspring. We are also reporting a hitherto uncommented relationship between behavioral depression in the Porsolt test and the decrease of dopamine transmission in the n. accumbens.

Dopamine Depression Stress Development

MATERNAL stress during pregnancy alters behavioral development in the offspring (18,30,42,51) and may even serve as a predisposing factor to mental illness (26,38). The biological basis of this phenomenon remains unknown. Recently, we found in two different animal models that maternal stress during gestation induces an increase in depression-related behavior in the female offspring during adulthood (4). Among the various behavioral models of human depression, the Porsolt et al. test (45) and the modified version of Hilakivi and Hilakivi (25) were used for these studies because (3,4) they are, nowadays, the animal depression analogs of depression most widely used (64) and bear important similarities to human depression (9). However, the validity of animal models for human mental disorders should be assessed by the criteria proposed by Goodwin and Bunney (21), one of which is that the brain biochemistry in the animal model should be similar to that reported in humans. In this article, accordingly, we study if the prenatal stress that induces behavioral depression in female rat offspring also modifies brain biochemistry in the same way as has been reported for depressive patients.

A great deal of data has been adduced to support the hypothesis that human depression is related to the alteration of monoaminergic neurons. Although initially the monoamine hypothesis of depression was associated mainly with 5-HT and norepinephrine brain neurotransmission, over the last years the idea has been mooted that alteration of central dopamine (DA) also is involved in the pathogenesis of some of the major symptoms of depression (58). Thus, clinical (1,11,20–23,27, 28,33,34,37,39,43,60,63) and experimental data in animal analogs (7,8,48,49,53) strongly suggest that brain dopaminergic neurotransmission is decreased in depression. Although the exact location of dopaminergic alteration in the brain in depressive patients has not yet been ascertained, studies on animal analogs suggest that DA alteration takes place in the mesolimbic system, particularly the DA innervation of n. accumbens (14,31,39,43,52,60).

Dopaminergic central activity was quantified in rats whose mothers were subjected to stress during gestation. Special attention was paid to the study of the nucleus accumbens. Because emotional processes have been consistently linked to the right cerebral hemisphere in human beings (41) and rats (12) and prenatal (15) and postnatal (12,32) stress, which reduces

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the reaction to stressful stimuli in adulthood and causes an increase in dopaminergic brain asymmetry, in the present article the brain lateralization of DA systems was also evaluated.

METHOD

Experiments were carried out on Sprague-Dawley female rats (Letica, Barcelona) weighing 180–250 g. Animals were housed at 22°C, three or four per cage, under normal laboratory conditions on a standard 12 L : 12 D schedule (with 0300–1500 lights on) and with free access to food and water.

Stress Procedure

Pregnant rats were stressed from day 15 of gestation until parturition. Timed pregnancies were obtained by daily checking of vaginal washings for sperm, the day on which sperm was found being defined as day 0.

The stress procedure was similar to that previously reported by Alonso et al. (4). Briefly, rats were suspended by the thorax with a tight belt, and remained suspended for 3 h each day with their back legs 100 mm above the table surface. The control group had its food cups and water removed from the cages for the same period of time that the stress group was denied access to food and water.

Porsolt's Behavioral Despair Test

Rats forced to swim in a confined space assume an immobile posture after an initial frenzied attempt to escape. On subsequent immersion, the onset of immobility is more rapid and marked. Porsolt et al. (45–49) named this phenomenon behavioral despair and offered this phenomenon as an animal model of depression. Among the various animal models of depression this test has the following advantages: a) the antidepressant procedures useful in humans (including atypical antidepressant drugs such as iprindole and mianserin, electroconvulsive shock, deprivation of REM sleep, etc.) delay the onset of immobility; b) there is a significant correlation (not found in any other model) between clinical potency and potency of antidepressants in the behavioral despair test; c) it is widely used and much information is now available about it (9,64). So, in the present article we used the behavioral despair test.

Rats were plunged individually into a vertical crystal cylinder (height 30 cm; diameter 15 cm) containing 15 cm of water maintained at 25°C. After 15 min in the cylinder the animals were removed and allowed to dry for 30 min in a heated enclosure (28°C) before being returned to their individual cages. One day later, the rats were replaced in the cylinder and the seconds immobilized quantified during the following 5 min (45–49).

Preparation of Tissue

Rats were sacrificed (over 4–6 h of the light period) by decapitation and the brains quickly removed and dissected on ice. The regional dissection of nucleus accumbens, medial preoptic area, and striatum was carried out according to Gonzalez et al. (19) and of the s. nigra (A9 cells group), ventral tegmental area (A10 cells group), and hippocampus according to Diaz Palarea et al. (13). The retrorubral nucleus (A8 cells group) was dissected in a slice 1 mm posterior to the slice of the A9 and A10 groups and according to the Paxinos and Watson (44) stereotaxic coordinates. Brain pieces were weighed in conical 1.5 ml test tubes and 300 μl of 0.1 M perchloric acid containing 4×10^{-5} M sodium metabisulphite was pipetted into the tubes. The mixture was sonicated at 100 W for about 12 s while on ice, and the homogenate was centrifuged for 15 min at 15000 $\times$ g. The supernatant was used to quantify monoamines and the pellet to determine the total proteins. The protein in each sample pellet was determined according to the method of Bradford (10).

Biochemical Analysis

Dopamine, DOPAC, and HVA, were simultaneously measured by liquid chromatography with electrochemical detection according to Magnusson et al. (35) and Sperk (57). So, an aliquot of the supernatant was injected into the chromatographic column (300 $\times$ 3.9 mm stainless steel column packed with μBondapack c18, 10 μm, Waters Assoc. Milford, MA). The mobile phase consisted of 0.07 M $NaH_2PO_4H_2O$, 0.1 mM EDTA : methanol (92 : 8 v/v) containing heptyl sulphate 1.7×10^{-3} M and 20 ml of acetic acid. The final solution (pH = 3.1) was filtered (0.45 μm Millipore filter) before using. Standards of tyrosine, dopamine, and DOPAC (Sigma, St. Louis, MO) were dissolved in perchloric acid (PCA) containing 4×10^{-5} M sodium metabisulphite and kept as stock solutions at −20°C. They were diluted with ice-cold PCA/$Na_2O_5S_2$ shortly before chromatographic injection. All separations were performed isocratically at a flow-rate of 1.0 ml/min at room temperature. Quantification was determined from standard curves of peak height.

Statistics

Biochemical and behavioral data were evaluated with the STATISTIC-SX program and comparative analysis of variance (Kruskal-Wallis) followed by Mann–Whitney U Rank Sum Test. Differences were judged significant when associated with a probability of 5% or less.

Experimental Procedure

The behavioral despair of 29 female (15 control group and 14 stress group) rats were quantified in 90–110-day-old animals. Twelve days after this initial study all rats were sacrificed to evaluate the dopaminergic brain neurotransmission using the biochemical procedures previously commented. Because the estrous cycle does not modify behavioral despair (3), the cytological determination of the estrous cycle stage was omitted to avoid experimental manipulations affecting behavioral or biochemical data.

RESULTS

Exposure of pregnant rats to stress had a significant effect on depression in the Porsolt test. Figure 1 shows the time (in seconds) that rats remain immobile (depression). Immobility was higher in the prenatally stressed group than in the control group ($p < 0.05$ stress group vs. control group).

The quantification of the dopamine, DOPAC, HVA, DOPAC/DA index, and HVA/DA index showed no statistical differences between the control and stress groups in the MPOA, hippocampus, striatum, A8, A9, or A10 groups (Table 1).

There is, however, an increase of dopamine (not statistically significant) and a decrease of DOPAC ($p < 0.05$ stress group vs. control group) HVA ($p = 0.02$ stress group vs. control group) (Fig. 2), DOPAC/DA index ($p = 0.007$ stress group vs. control group and HVA/DA index ($p = 0.049$ stress group vs. control group) (Fig. 3) in the nucleus accumbens.

Dopaminergic lateralization was also modified by prenatal stress. The percentage of dopamine in the right n. accumbens was higher for dopamine ($p = 0.05$ stress group vs. control

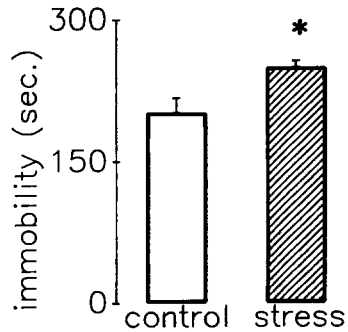

FIG. 1. Immobility in the Porsolt test for female rats: effect of suspension stress during gestation on behavioral despair of adult offspring. Data represent means + SEM of time that each rat remains immobile. *$p < 0.05$ vs. control group.

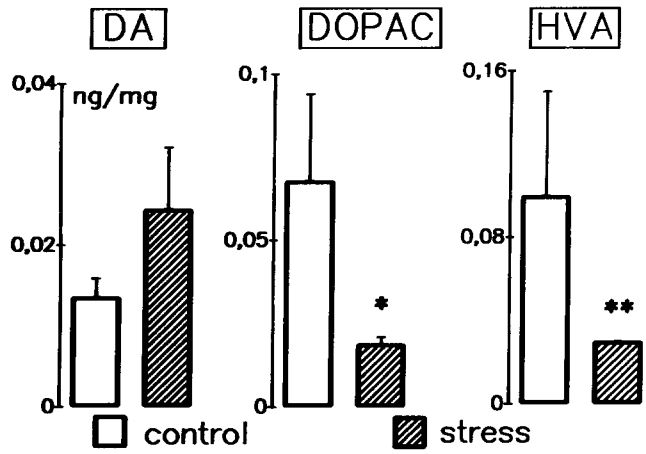

FIG. 2. Effect of prenatal stress on dopaminergic transmision in nucleus accumbens. The data presented is the mean + SEM of dopamine, DOPAC, and HVA concentration (ng/mg protein). *$p < 0.05$ vs. control group; **$p = 0.02$ vs. control group.

group) and lower for DOPAC/DA index ($p = 0.041$ stress group vs. control group) than that found in the control groups (Fig. 4).

These data, thus, provide evidence that: 1) decreases the DA turnover in the nucleus accumbens but not in the other brain regions studied; b) decrease of DA turnover is more marked in the nucleus accumbens of the right brain.

DISCUSSION

The sex differentiation during ontogeny is controlled by the action of the following principal biological factors: a) genetic constitution and b) gonadal steroids. In humans, there are important sex differences for the clinical characteristics of depression (41). However, the biological basis of these sex-related clinical differences is unknown. Many authors have reported that after modifying the steroid level in the fetus (61,62) stress during pregnancy induces morphological (5,6, 24), biochemical (50), and behavioral (2,42,54) alterations of sexual differentiation in rats and may even serve as a predisposing factor to mental illness in man (38). Recently, we reported that: a) there are significant sexual differences in animal models of depression (3); b) prenatal stress modifies depressive behavior in female rats but not in male rats (4); c) sexual differences in these behavioral tests are not seen in adult rats whose mothers were stressed during gestation (4). So, these studies suggest that stress in the initial stages of development: a) decreases the sexual differences in animal models of depression and b) increases the risk for depression in females. Figure 1 shows that immobility in the Porsolt test is higher in prenatally stressed rats than in the control group, and these data are, therefore, in agreement with previously reported findings and allow the neurochemical differences between the depressed and nondepressed groups to be studied.

Over the last few years, evidence from both clinical and preclinical studies has been used to argue that depletion of CNS dopamine (DA) is involved in the pathogenesis of some

TABLE 1

VALUES ARE THE MEAN (±SEM) OF DOPAMINE, DOPAC, AND HVA CONCENTRATION (ng/mg PROTEIN) AND TURNOVER RATE OF DOPAMINE (DOPAC/DA, HVA/DA) IN THE BRAIN AREAS STUDIED

	MPOA	Striatum	Hippocampus	A8	A9	A10
DA						
C	0.22 ± 0.02	0.8 ± 0.09	0.18 ± 0.06	0.17 ± 0.03	0.69 ± 0.01	0.07 ± 0.03
S	0.33 ± 0.10	0.65 ± 0.10	0.22 ± 0.06	0.32 ± 0.18	0.94 ± 0.31	0.05 ± 0.00
DOPAC						
C	0.35 ± 0.03	0.41 ± 0.11	0.03 ± 0.01	0.13 ± 0.03	0.72 ± 0.11	0.11 ± 0.03
S	0.43 ± 0.04	0.43 ± 0.11	0.06 ± 0.09	0.15 ± 0.06	0.98 ± 0.37	0.09 ± 0.01
HVA						
C	–	0.56 ± 0.07	–	–	–	0.25 ± 0.12
S	–	0.64 ± 0.19	–	–	–	0.23 ± 0.04
DOPAC/DA						
C	0.22 ± 0.02	0.52 ± 0.08	0.61 ± 0.24	–	1.23 ± 0.22	1.98 ± 0.12
S	0.33 ± 0.10	0.54 ± 0.06	1.28 ± 0.41	–	1.30 ± 0.52	1.96 ± 0.10
HVA/DA						
C	–	0.77 ± 0.11	–	–	–	3.59 ± 0.10
S	–	0.75 ± 0.12	–	–	–	3.18 ± 0.20

C = control group; S = stress group.

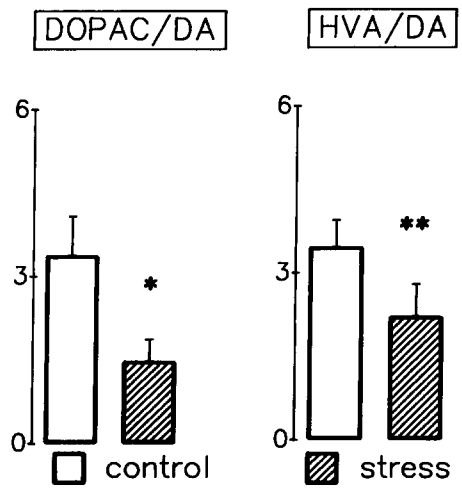

FIG. 3. Effect of prenatal stress on dopaminergic transmision in nucleus accumbens. The data presented is the mean + SEM of turnover rate of dopamine (DOPAC/DA, HVA/DA). *p = 0.007 vs. control group; **p = 0.049 vs. control group.

of the major symptoms of depression (58). Clinical support for this hypothesis comes from several observations: a) decreased tyrosine concentrations were found in CSF of depressed patients (11,22); b) drugs that decrease central DA transmission precipitate depressive episodes (1); c) drugs that increase DA transmission such as L-DOPA (20), different tricyclic antidepressants (23), monoamine-oxidase inhibitors (33), etc., have been shown to have antidepressant properties in depressed patients; d) some depressed patients exhibit greatly diminished motor activity which is believed to result from disruption of brain DA activity (28); e) symptoms of major depression often precede (27) or accompany (28,37) loss of central DA in Parkinson's disease; f) the DA metabolite homovanillic acid (HVA) (its measurement is the most direct method available for studying DA turnover in the human brain) is diminished in CSF of depressed patients (21,39,43,53, 55,56,59,60,63). Moreover, it has been reported in animal models of depression that: a) rats exposed to uncontrollable electric shock in the heplessness paradigm are subsequently deficient in both learning and DA levels in the nucleus accumbens (53); b) behavioral alteration induced by the helplessness paradigm is antagonized by DA agonists (8) and exacerbated by DA blockers (7); c) behavioral despair in rats induced by the Porsolt paradigm is decreased by DA agonists and increased by DA antagonists (48).

Although the brain location of dopaminergic alteration in depressive patients is as yet unknown, studies on animal analogs suggest that DA alteration takes place in the mesolimbic system and especially in the DA inervation of n. accumbens. The animal evidence includes the following: a) antidepressant procedures like chronic treatment with tricyclic antidepressant drugs (39,40,60), REM sleep deprivation (14,43), or electroconvulsive shock (16,17,29) all increase the response to DA agonist drugs in behavioral patterns mediated by DA innervation of nucleus accumbens (36); b) unavoidable aversive events decrease DA turnover in the nucleus accumbens (31); c) chronic treatment with antidepressant drugs like desipramine attenuate alpa$_2$-adrenoceptor--mediated inhibition of ^{3}H-DA release and, thus, facilitate the DA transmission in the nucleus accumbens (52). Figure 2 shows that the group whose mothers were stressed during prenatal life exhibited a lower level of DOPAC and HVA in nucleus accumbens and a higher level of dopamine. The DOPAC/DA index and the HVA/DA index (Fig. 3) were dramatically reduced in prenatally stressed animals. As in depressive patients, there is a marked reduction of central dopaminergic turnover in the group of animals with higher levels of behavioral depression. In addition, these biochemical effects were found in the nucleus accumbens, the brain locus most directly related with the action of dopamine in depression.

Behavioral despair or biochemical differences founded between the stress and the control groups are not necessarily induced by the sole action of prenatal stress. Perhaps any of the physiological stressing conditions during development, or the frustration induced by the first day of the Porsolt et al. test during adulthood, could be activating factors that facilitate the expresion of underlying differences induced direct by prenatal stress. In this case, the prenatal stress could be a predisposing factor rather than an etiological, that reinforcing other can encourages the development of depression during adulthood.

Figure 4 shows accumulation of dopamine and decrease of dopamine turnover in prenatally stressed rats to be higher in the right side of the brain than in the left. Taking into account that emotional processes have been consistently linked to the right cerebral hemisphere in humans (41) and rats (12) and that prenatal (15) and postnatal (12,32) stress, which reduces the reaction to stressful stimuli in adulthood, causes an increase in dopaminergic brain asymmetry, the present data suggest that depression could be related to a biochemical asymmetry of the mesolimbic dopaminergic system. Detailed postmortem studies of the right and left brain mesolimbic systems in depression patients are needed to evaluate this hypothesis.

Taken as a whole, the present data provide further evidence in favor of the hypothesis that maternal stress during gestation increases the risk of depression in the offspring. In addition, we have also identified a hitherto unknown relationship between behavioral depression in the Porsolt test and two neurochemical modifications previously associated with human depression.

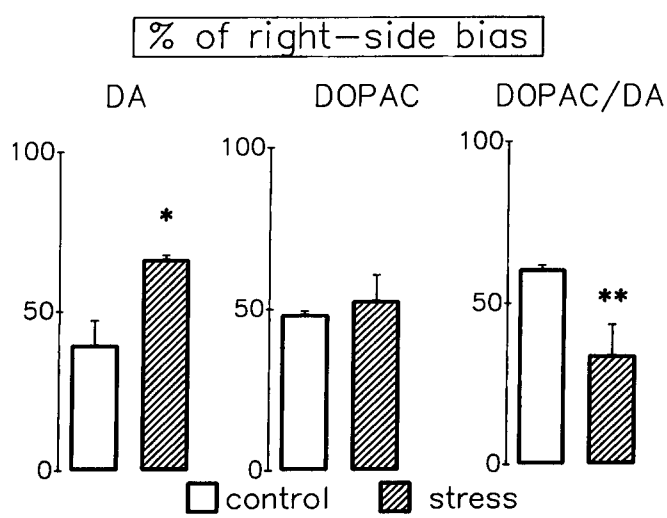

FIG. 4. Effect of prenatal stress on the dopaminergic accumbens asymmetries. The data presented means + SEM of percentage of right-side bias. *p = *0.05 vs. control group;* **p = 0.041 vs. control group.

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