Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring

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Muneoka, Katsumasa, Masahiko Mikuni, Tetsuo Ogawa, Katsuki Kitera, Kenji Kamei, Morikuni Takigawa, and Kiyohisa Takahashi. Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1669–R1675, 1997.—In this study, it has been clearly demonstrated that prenatal dexamethasone treatment (Dex; 0.05 mg/kg on gestational days 17, 18, and 19) resulted in the significant reductions of 5-hydroxytryptamine (5-HT) turnover in four brain regions, including the neocortex, hippocampus, hypothalamus, and midbrain plus medulla (M + P - M) but not in the striatum in the offspring at 3 and 14 wk of life, as well as dopamine turnover in the hypothalamus. [3H]paroxetine binding densities were increased in the hypothalamus and M + P - M at 14 wk of life, which corresponded to increased 5-HT contents in both regions. On the other hand, significantly lower norepinephrine contents in the neocortex and hippocampus were observed in the Dex group compared with the control group at 14 wk of life. In addition, the exposure to new environmental condition elevated blood corticosterone levels and enhanced behavioral activities to a greater extent in the Dex group than in controls at 7 wk of life, suggesting that elevated glucocorticoid levels during the pregnancy mimicked prenatal mild stress, producing developmental alterations in brain monoamine metabolism, endocrine response, and behavior in adult offspring.

glucocorticoids; serotonin; hypothalamic-pituitary-adrenal axis; stress; paroxetine

IT HAS BEEN RECOGNIZED that the development of an immature organism is not only determined by genetic factors but also by the postnatal environment during the neonatal period or the maternal environment during gestation (1, 12, 13, 16, 28). Recently, prenatal stress has been reported to affect monoaminergic neuron development and sensitize neuroendocrine systems. Adult offspring from dams stressed during pregnancy are known to show increased 5-hydroxytryptamine (5-HT) contents in several brain regions, e.g., the hypothalamus, as well as alterations of behavioral and hormonal responses to environmental stimuli, including the hypothalamic-pituitary-adrenal (HPA) axis (1, 12, 14, 16). These are not surprising results because serotonin is a major modulator of the HPA axis; corticotropin-releasing hormone-containing cells in the paraventricular nucleus of the hypothalamus are given a projection of the serotonergic neuron from the raphe nuclei of midbrain (4), and neuronal activities in raphe nuclei are regulated by circulating glucocorticoids through the 5-HT1A autoreceptor in adult rats (9). On the other hand, in the developing brain, multiple interactions between the serotonergic neurons and the glucocorticoids have been found; corticosterone, a major glucocorticoid in rat, regulates the activity of serotonergic system, including tryptophan hydroxylase in the raphe nuclei (29), and 5-HT regulates the expression of glucocorticoid and mineralocorticoid receptors in rat brain (13). Moreover, the third trimester of pregnancy is likely to be a most critical period of developing monoaminergic neurons in the rat (16, 21).

The effect of prenatal stress reported in these animal studies is also important to understanding the pathogenesis of affective disorders, because the dysfunctions of serotonergic systems and the HPA axis are often seen in depressed patients (26).

On the other hand, the maternal and fetal response to stressful events is likely to vary depending on the intensity, style, and timing of the stress applied during pregnancy (23, 28). The prenatal stress is likely to influence not only the glucocorticoid secretion but other endocrinological factors such as corticotropin, prolactin, growth hormone (19), and sexual hormone (27) in the mother or fetus. In the absence of any direct neural connections between the mother and fetus, numerous data suggest that some hormones, e.g., glucocorticoids, transported from the maternal blood to the fetal organs through the placenta, are most likely involved (1, 12, 23, 27, 28), but it is little known whether or not the exogenous prenatal glucocorticoid treatment mimics prenatal stress on developmental alterations in brain amine metabolism, endocrine system, and behavior in adult offspring.

Thus, in this study, we challenged dams with dexamethasone (Dex) on gestational days 17-19 to define the direct effect of glucocorticoids in late pregnancy on the development of the central monoaminergic systems as well as behavioral and adrenocortical responses to stressful stimuli in adult offspring. We used a dose of 0.05 mg·kg⁻¹·day⁻¹ Dex phosphate in this experiment to minimize physical impairment of the offspring (22). In this experiment, we did not carry out cross-fostering because previous studies showed that Dex treatment in pregnancy did not affect mother nursing to their pups (22), and cross-fostering may introduce some complex effects on mother-infant interaction and possibly modify the effects of prenatal treatments (12, 28).

METHODS

Animal treatment. Male and female Sprague-Dawley rats were mated in our laboratory. The morning on which sperm-positive smears were obtained was declared gestational day 0.
We used a total of 10 litters in this experiment (5 litters in each group). Pregnant rats were housed individually under standard conditions (12:12-h light-dark cycle) and given food and water ad libitum. On gestational days 17, 18, and 19, dams were given 0.05 mg/kg Dex phosphate or an equivalent volume of saline vehicle (1 ml/kg) by subcutaneous injections (once daily, at 1000). Maternal weight gain was recorded during the period of Dex or saline administration. At birth, all litters were weighed and reduced to 10 pups. All pups were weaned at postnatal day 22 (with birth as postnatal day 0). Only male offspring were used for each experiment. Some male pups were killed at the weaning day (3 wk of life) by decapitation. The killing was performed between 0900 and 1100 (lights on at 0800). The other rats were killed at 14 wk of life. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Tissue preparation. Brain tissues obtained by decapitation were immediately removed and rapidly dissected over ice into the neocortex, hippocampus, striatum, hypothalamus, and midbrain + pons-medulla (M + P-M). The M + P-M is constituted of the caliculi, pons, and medulla oblongata. The tissue was stored at −80°C until it was processed for biological measurements.

Measurement of brain monoamine contents and turnovers. Contents of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindole-3-acetic acid (5-HIAA) in the brain tissues obtained at 3 and 14 wk of life were determined using reverse-phase high-performance liquid chromatography with electrochemical detection (ECD). Tissues were homogenized in 0.1 M perchloric acid containing 1 mM EDTA and 2 mM sodium pyrosulfate, washed with chloroform, and centrifuged (8,800 g) at 4°C for 30 min. The supernatants were separated by a stainless steel reversed-phase column packed with Nucleosil 5C18. As the mobile phase, we used 0.1 M acetate citrate buffer (pH 4.1) containing 15% methanol (vol/vol), 0.7 mM octanesulfonic acid, and 0.01 mM EDTA at a flow rate of 0.6 ml/min. ECD was achieved with a carbon graphite working electrode set at −0.7 V.

As indexes of DA and 5-HT turnover, DOPAC/DA and 5-HIAA/5-HT were calculated, respectively. NE contents in the striatum and DA, DOPAC, and HVA contents in the 5-HIAA were below the detection limit of our procedure.

[3H]paroxetine binding assay. With the use of the hypothalamic and M + P-M obtained at 14 wk of life, [3H]paroxetine (20.5 Ci/mmol, NEN) binding assay was carried out by the method previously described (8). Briefly, 25-µl blood samples were obtained from the tail tip and extracted with 1 ml of ethanol. After centrifugation (2,300 g for 30 min at 4°C), the solvent (20–500 µl) was decanted and dried under nitrogen gas. The dried extract was assayed in duplicate by adding 500 µl phosphate assay buffer, 100 µl diluted antiserum (1:40,000, UCB-Bioproducts), and 100 µl [1,2,6,7-3H (N)]cortisol (−10,000 counts/min) to the tubes. The tubes were incubated at 37°C for 30 min, followed by overnight incubation at 4°C. The standard curve ranged from 4 to 2,000 pg/tube of corticosterone. Separation of bound and free hormone fractions was achieved by adding 500 µl of Dextran (0.025% wt/vol)-coated Chatoil (0.25% wt/vol) suspension. The bound radioactivity was determined in a liquid scintillation counter. The method has a sensitivity of 4 pg/tube and an intra-assay variation between 5.7 and 7.3%. There are cross-reactions with other steroids: 5% with 11-deoxycortisol, 0.18% with 21-deoxycorticosterone, and <0.005% with other related steroids.

Statistics. Data are presented as means ± SE. For data of monoamine contents in the separate age, two-way analysis of variance (ANOVA) (factors of Dex and age) was applied initially, with data log-transformed when heterogeneity of variance was indicated by Bartlett test. Where a significant interaction of Dex and age was found, a subsequent Student’s t-test was conducted for each age individually. Data from [3H]paroxetine binding assay and body weight measurement were analyzed by Student’s t-test. Two-way repeated-measures ANOVA (factors of Dex and times) was used to analyze the results from the repeated open-field test.

RESULTS

General conditions. At birth, a slightly but significantly lower body weight of male pups was found in the Dex group compared with the control group (6.6 ± 0.09 vs. 6.0 ± 0.09 g in controls vs. Dex group, respectively). But the weight loss recovered to control levels until 3 wk of life (53.4 ± 1.02 vs. 56.8 ± 1.62 g in controls vs. Dex group, respectively). Sex ratios at birth (male/total × 100) were 51.1 ± 8.4 and 52.9 ± 6.8 in controls and the Dex group, respectively. After culling to 10 pups per litter, sex ratios were 60.0 ± 4.5 and 62.0 ± 3.7 in controls and the Dex group, respectively. There were no
significant differences in the sex ratios between controls and the Dex group both before and after the culling.

Monoamine contents. Significant effects of age were found in this analysis in the NE, DA, and 5-HT systems but not in DA contents in M + P-M; DOPAC and 5-HIAA contents in the hypothalamus; and DOPAC contents in the striatum. The effects of Dex on each transmitter system are described below.

NE system. Two-way ANOVA (factors of Dex and age) indicated that prenatal Dex administration significantly reduced NE contents in the hippocampus (P < 0.01). In the neocortex, there was a significant interaction between Dex and age. Subsequent Student’s t-test indicated that the Dex group showed significantly lower NE contents compared with control group at 14 wk of life (P < 0.01) (Table 1).

DA system. Two-way ANOVA (factors of Dex and age) indicated that prenatal Dex administration resulted in a significant reduction of DA turnover in the hypothalamus (P < 0.01), which was accompanied by lower DOPAC contents (P < 0.01). A higher DA content in the striatum was found in the Dex group (P < 0.05) (Table 2).

Table 1. Effect of prenatal dexamethasone administration on NE contents in each brain region at 3 and 14 wk of life

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dex</th>
<th>M + P-M</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Neocortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 wk</td>
<td>205 ± 7</td>
<td>232 ± 5</td>
<td>240 ± 10</td>
<td>240 ± 9</td>
<td>897 ± 24</td>
<td>181 ± 9</td>
</tr>
<tr>
<td>14 wk</td>
<td>240 ± 10</td>
<td>240 ± 9</td>
<td>904 ± 28</td>
<td>146 ± 7</td>
<td>92 ± 4</td>
<td>52 ± 3</td>
</tr>
</tbody>
</table>

Table 2. Effect of prenatal dexamethasone administration on contents of DA, DOPAC, and HVA, and DA turnover in each brain region at 3 and 14 wk of age

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dex</th>
<th>M + P-M</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Neocortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 wk</td>
<td>82 ± 3</td>
<td>83 ± 5</td>
<td>82 ± 5</td>
<td>83 ± 5</td>
<td>86 ± 2</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>14 wk</td>
<td>82 ± 5</td>
<td>86 ± 2</td>
<td>86 ± 2</td>
<td>82 ± 5</td>
<td>86 ± 2</td>
<td>86 ± 2</td>
</tr>
</tbody>
</table>

5-HT system. Significant reductions of 5-HT turnover were observed in the Dex group compared with the control group in M + P-M (P < 0.01) and the hypothalamus (P < 0.01), as indicated by two-way ANOVA (factors of Dex and age). In the hypothalamus, hippocampus, and neocortex, there were significant interactions between Dex and age. Subsequent Student’s t-test indicated that the reduced 5-HT turnovers of Dex group in the hypothalamus were significant at 3 (P < 0.01) and 14 wk (P < 0.05), whereas the reductions in the neocortex and hippocampus were significant only at 3 wk of life (P < 0.05 in both regions). In M + P-M and the hypothalamus, significantly higher 5-HT contents were found in the Dex group compared with the control group (P < 0.01 in both regions) (Table 3).

Table 3. Effect of prenatal dexamethasone administration on 5-HT contents and turnover in each brain region at 3 and 14 wk of age

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dex</th>
<th>M + P-M</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Neocortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 wk</td>
<td>160 ± 6</td>
<td>166 ± 6</td>
<td>202 ± 8</td>
<td>216 ± 8</td>
<td>89 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>14 wk</td>
<td>202 ± 8</td>
<td>216 ± 8</td>
<td>89 ± 1</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid. *No significant difference by Student’s t-test.
Table 3. Effect of prenatal dexamethasone administration on contents of 5-HT and 5-HIAA and on 5-HT turnover in each brain region at 3 and 14 wk of life

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>3 wk Control</th>
<th>3 wk Dex</th>
<th>14 wk Control</th>
<th>14 wk Dex</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M + P-M</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5-HT, ng/g</td>
<td>576 ± 20</td>
<td>716 ± 22</td>
<td>808 ± 15</td>
<td>920 ± 21</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA, ng/g</td>
<td>982 ± 47</td>
<td>1055 ± 69</td>
<td>828 ± 25</td>
<td>900 ± 25</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA/S-HT</td>
<td>1.702 ± 0.044</td>
<td>1.480 ± 0.109</td>
<td>1.026 ± 0.027</td>
<td>0.979 ± 0.019</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT, ng/g</td>
<td>623 ± 24</td>
<td>755 ± 8</td>
<td>790 ± 21</td>
<td>944 ± 13</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA, ng/g</td>
<td>901 ± 43</td>
<td>872 ± 25</td>
<td>859 ± 24</td>
<td>912 ± 28</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA/S-HT+</td>
<td>1.444 ± 0.024</td>
<td>1.155 ± 0.035</td>
<td>1.092 ± 0.039</td>
<td>0.965 ± 0.024</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
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<tr>
<td>5-HT, ng/g</td>
<td>322 ± 11</td>
<td>346 ± 11</td>
<td>407 ± 11</td>
<td>428 ± 12</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA, ng/g</td>
<td>782 ± 29</td>
<td>793 ± 9</td>
<td>573 ± 21</td>
<td>570 ± 23</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA/S-HT</td>
<td>2.433 ± 0.081</td>
<td>2.304 ± 0.081</td>
<td>1.410 ± 0.032</td>
<td>1.329 ± 0.030</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT, ng/g</td>
<td>266 ± 11</td>
<td>275 ± 10</td>
<td>348 ± 11</td>
<td>344 ± 14</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA, ng/g</td>
<td>494 ± 20</td>
<td>431 ± 32</td>
<td>357 ± 9</td>
<td>360 ± 18</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA/S-HT†</td>
<td>1.860 ± 0.03</td>
<td>1.572 ± 0.113</td>
<td>1.031 ± 0.042</td>
<td>1.049 ± 0.049</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td><strong>Neocortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT, ng/g</td>
<td>134 ± 9</td>
<td>150 ± 4</td>
<td>331 ± 4</td>
<td>325 ± 10</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA, ng/g</td>
<td>260 ± 11</td>
<td>250 ± 8</td>
<td>294 ± 7</td>
<td>295 ± 5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5-HIAA/S-HT†</td>
<td>1.959 ± 0.086</td>
<td>1.666 ± 0.057</td>
<td>0.888 ± 0.020</td>
<td>0.915 ± 0.032</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindole-3-acetic acid. * †-Test: control > Dex at 3 (P < 0.01) and 14 (P < 0.05) wk. †-Test: control > Dex at 3 wk (P < 0.05). †-Test: control > Dex at 3 wk (P < 0.05).

Discussion

In this experiment, significant differences in turnovers of 5-HT and DA and in NE contents between rats at 3 and 14 wk of life were found in a wide area in rat brain. These age-dependent changes of neuronal activities in central monoaminergic systems suggest that the maturation of central transmitter systems has continued until the adult period. Several investigations have clearly demonstrated that functional changes of the central monoaminergic system continue even after the weaning period (25, 30).

Besides the effects of aging on neuronal activity, the present study demonstrated that prenatal Dex administration affected the developments of 5-HT and DA systems. These changes were region specific. The reduction of DA turnover in the Dex group was found in the hypothalamus alone. The reductions of 5-HT turnover, however, were found in all brain regions, except the striatum, in the Dex group, although these changes were more persistent in M + P-M and the hypothalamus in adulthood than in the hippocampus and neocortex. In our laboratory, similar reductions of 5-HT and DA turnovers in the brain were found in the offspring given prenatal saline injection stress (14). Therefore, the present data support a possibility that the effect of prenatal stress on the development of the monoaminergic system can be due to the activation of adrenocortical function in the dams.

The persistent changes of 5-HT and DA turnovers in the hypothalamus or 5-HT turnover in M + P-M indicate that these regions are more vulnerable to prenatal manipulations than the other brain regions. This finding is consistent with several reports suggesting the vulnerability of the hypothalamus, midbrain, and pons-medulla to adverse manipulations in late pregnancy, e.g., glucocorticoids or serotonergic agents (15, 21, 30). Such regional selectivity may depend on the relationship between the period of drug exposure and tissue maturation in each brain region (21). Late pregnancy is considered to be an important period for the synaptic formation of the serotonergic neuron, because axonal growth from cell bodies existing in the brain stem and dendritic arborization are known to occur during the late pregnancy in rat (29). Slotkin et al. (21), especially, have emphasized that the midbrain and brain stem is a most prominent target, with exogenous Dex on gestational days 17-19. In late pregnancy, the HPA axis is functional in the fetus, unlike in the early postnatal period when the HPA axis is suppressed (13). Interestingly, increases in the expression of glucocorticoid
receptors are found in raphe nuclei and the paraventricular hypothalamic area in this period (3). Our data suggest that regions such as the brain stem and hypothalamus are sensitive to glucocorticoids in the developing brain as well as hippocampal and cortical regions in which glucocorticoid receptors are well documented as targets of corticosteroids or stress in gestation (13).

The pattern of changes in the noradrenergic system found in this experiment was different from the other systems. Lower NE contents in the Dex group were observed in the hippocampus and neocortex. In addition, the finding in the neocortex, in which the reduction of NE content was observed only at the adult period, is in contrast to the effects of Dex on 5-HT and DA contents, which were already increased at 3 wk of life. Such delayed appearance of the change in the NE contents might reflect altered NE turnovers, although it is not possible to estimate the turnover of NE in this experiment, because an elevation of NE turnover in the first month and a later deficit after weaning in the forebrain were observed by Slotkin et al. (21). The mechanism by which this region-specific difference is produced is still unclear; however, the functional changes in the noradrenergic system in the neocortex observed in the adult period may be a compensation for the persistent changes in the dopaminergic or serotonin signaling in the limbic system or brain stem induced by prenatal Dex treatment.

[3H]paroxetine binding capacity was increased in M+P-M and the hypothalamus. These results confirmed the earlier finding of elevated serotonin transporter density, which was labeled by [3H]paroxetine, in brain stem by prenatal Dex exposure (20). In addition, the present data indicated that the increased [3H]paroxetine binding was found in the brain regions in which the persistent elevations of 5-HT contents were ob-
serviced. Thus these results suggested that the higher 5-HT contents in both regions could be attributed to increases in the terminal density of serotonergic neurons. There are numerous studies reporting that 5-HT has a role as a growth factor to their own system (29) and regulates proliferation and differentiation in their own neurons. Accordingly, it is a possibility that prenatal Dex treatment influenced the serotonergic innervations through modifications of the serotonergic transmissions, e.g., synthesis or degradation in immature brain (29). On the other hand, it is another possibility that glucocorticoids directly changed the pattern of innervation of serotonergic neuron, because it was suggested that glucocorticoids, acting through their receptors, led to cessation of cell division and induction of differentiation in the developing brain (7). In any case, the increased terminal densities of serotonergic neuron in M + P-M and the hypothalamus in this experiment suggest a structural change in the brain; glucocorticoids in this period might promote short axonal connections rather than longer projections directed toward terminal fields.

Effects of prenatal Dex administration in the offspring were observed not only in biochemical assays but also in behavioral response, as shown in the open-field test at 7 wk of life. Enhancement of ambulation activity and rearing activity was observed to a greater extent in the Dex group than in the control group. However, at the second trial, there were lesser differences in both groups in behavioral alterations during exposure to the open field. The hyperactivity displayed in Dex group in the first trial can be considered to reflect a higher degree of sensitivity to the novel environment because the enhanced activity was abolished by the repeated exposure to the same environment. In addition, higher corticosterone secretions were induced by the open-field exposure in the Dex group than controls. Thus it may be supported that the rats in Dex group, exposed to novel environmental conditions, were more anxious than the control group, although no differences were found in defecation scores between control and Dex groups. In this laboratory, the hypersecretion of corticosterone during conditioned fear stress and low entry number in elevated maze as well as low 5-HT turnover in the hypothalamus were found in the offspring given prenatal saline injection stress (14). Therefore, it is clearly demonstrated in this study that an exogenous prenatal glucocorticoid treatment is able to mimic prenatal stress on the developmental alterations in brain amine metabolism, adrenocortical response, and behavior in adult offspring. These results are consistent with the recent reports that offspring of a mother stressed during pregnancy showed a prolonged stress-induced corticosterone secretion, whereas prenatally stressed offspring from adrenalectomized mother did not differ from rats of control mothers for any endocrine parameters, suggesting that stress-induced increase in maternal glucocorticoids impairs the development of adult offspring's glucocorticoid response (1).

Peters (16) reported that a similar enhancement of behavioral and HPA responses to open-field exposure was induced by prenatal "mild" stress, in which the dams received daily saline injections. The author has also pointed out the higher corticosterone levels in the dams that received the stressful treatment during pregnancy. These effects were also accompanied by both pre- and postsynaptic functions in the central serotonergic system. Takahashi et al. (23, 24) also indicated that prenatal inescapable stress resulted in the hypersecretions of adrenocorticotropic hormone and corticosterone and increased defensive behavioral responses to stress in the offspring, although the authors have emphasized the changes of the noradrenergic or dopaminergic systems rather than the serotonergic system. It is difficult to find some behavioral alterations attributed to one or several biochemical factors because various neuron systems in many brain regions and their networks were involved in stress responses (5). Data in this study, however, are consistent with the reports by Burnet et al. (2) comparing Lewis rats with Fischer rats, in which 5-HT levels in the hippocampus, midbrain, and hypothalamus parallel the activity of the HPA axis. In addition, the altered serotonin transporter density in M + P-M demonstrated in the [3H]paroxetine binding assay might be associated with "anxiety" demonstrated in open-field test because a recent study reported that a polymorphism of the serotonin transporter gene regulatory region was related to anxiety in human (10). There is evidence that somatodendritic 5-HT1A autoreceptor functions in raphé nuclei are regulated by corticosteroids through glucocorticoid receptors (9). Thus a possible explanation is that some factors involved in serotonergic activities in the brain stem, e.g., the serotonin transporter density, determine responsiveness to a novel environment or anxiety in cooperation with adrenocortical activity.

In other studies, it has been reported that prenatal stress resulted in reduced activities (6, 17) or a feminization of male sexual behavior (5). In both cases, the investigators used severe forms of prenatal stress in which a reduction of body weight of the offspring was often observed. It is considered that the effects of severe prenatal stress may be associated with testosterone levels or opioid activities in the fetus rather than corticosterone levels (5, 28).

In conclusion, the present study clearly demonstrated that the increased glucocorticoid level during the pregnancy was a potent factor that could directly influence the development of the central monoaminergic systems, e.g., noradrenergic, dopaminergic, and serotonergic systems. In addition, the elevated glucocorticoid levels during pregnancy enhanced adrenocortical and behavioral responses to stress, suggesting that prenatal glucocorticoid treatment mimicked prenatal mild stress, producing developmental alterations in brain monoamine metabolism, adrenocortical response, and behavior in adult offspring.

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