EFFECT OF NEONATAL HYDROCORTISONE TREATMENT ON BRAIN MONOAMINES IN DEVELOPING RATS

Atsushi KUROSAWA, Hiroyasu KAGEYAMA, Thoppil M. JOHN*,
Ryoji HIROTA and Shinji ITOH
Shionogi Research Laboratory, Shionogi and Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

* Present address: Department of Zoology, University of Guelph, Guelph, Ontario, Canada

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Abstract—In our previous study, neonatal treatment with hydrocortisone was shown to produce a marked retardation of pituitary-adrenocortical development in infant rats. The present investigation was an attempt to determine whether or not the retarded activity is caused by functional changes in brain monoamine systems. In rats treated with hydrocortisone (0.5 mg/rat, s.c.) on the 2nd day of life, the development of whole brain was suppressed significantly. However, norepinephrine, dopamine and serotonin contents in the brain were higher in these rats than in controls. These changes of monoamine contents were apparent in the hypothalamus, diencephalon and pons-medulla oblongata. Our data suggest that monoaminergic nervous systems are potentiated with hydrocortisone in these brain regions, although the results do not necessarily explain the retarded hypothalamo-pituitary function.

Neonatal treatment of rats with several corticosteroids caused a marked atrophy of the adrenal gland and the thymus, these producing the wasting syndrome (1). In the treated animals, circadian rhythm of plasma corticosterone appeared at 4 weeks of age, but the plasma levels of corticosterone were significantly lower than those of controls until 6 weeks (2). Since the secretion of ACTH is likely to be affected by brain monoamines (3), the lowered plasma corticosterone levels in neonatally hydrocortisone-treated rats might be related to changes in monoamine contents in the brain. In this regard, Ulrich et al. (4) reported that both norepinephrine and serotonin in the medial hypothalamus were elevated, but dopamine levels did not increase in those rats at 30 days of age. However, these workers determined the content in the hypothalamus at one age only. More recently, Nyakas (5) found an increased norepinephrine content in the hindbrain of 4 month old rats given corticosterone on the 3rd to 5th postnatal days. The present study was undertaken to observe serial changes in monoamine contents from 1 to 7 weeks of age in the brain of rats given hydrocortisone neonatally. Our results are related to the ontogenic pattern of brain monoamines, as affected by hydrocortisone treatment.

MATERIALS AND METHODS

Male and female Wistar rats were housed at a constant temperature of 25±2°C under controlled lighting (fluorescent illumination from 07:00 to 19:00). Rat biscuits (Oriental Yeast Co.) and water were given ad lib.
Newborn rats were given 0.5 mg hydrocortisone acetate (Merck) in 0.05 ml solvent s.c., on the 2nd day of life. The solvent consisted of 0.4% polysorbate-80, 0.5% carboxymethylcellulose and 0.5% benzyalcohol in 0.9% NaCl. Control rats were given the solvent only. About three-fourths of the litters were treated with hydrocortisone and the remaining served as controls. The rats were left with their mothers until weaning at 21 days of life. Almost 1,000 newborn rats were used, but more than 50% of the infant rats given hydrocortisone died within 10 days.

The animals were decapitated between 13:00 and 14:00 hr. The whole brain was quickly removed, weighed and frozen. In some experiments, the brain was placed on ice and dissected into the telencephalon, diencephalon, hypothalamus, pons + medulla oblongata and cerebellum, according to the method of Glowinski and Iversen (6). Tissue samples thus obtained from 2 to 4 infant rats were pooled and frozen at −20°C until analysis. The samples were homogenized in cold 0.4 N perchloric acid, centrifuged for 10 min at 1000 g, and the supernatants analyzed for the norepinephrine, dopamine and serotonin contents by the method of Karasawa et al. (7). The supernatants were first passed through double columns consisting of an aluminum oxide column (0.6 × 1.0 cm) and an Amberlite CG-50 column (0.6 × 1.5 cm). Norepinephrine and dopamine were adsorbed on aluminum oxide in the upper column while serotonin was retained by Amberlite CG-50 in the bottom column. After washing the columns with distilled water, the amines were eluted with diluted hydrochloric acid. Catecholamines in the eluate were then transformed to fluorescent trihydroxyindole compounds by an oxidation method. As the oxidant, iodine and ferricyanide were used for norepinephrine and for dopamine, respectively. Fluorescence development of serotonin was performed by o-phthalaldehyde conjugation.

Fluorescent intensities of these samples were measured on an Aminco-Bowman spectrophotofluorometer. Student’s t-test was used to determine statistical significance of the final data.

RESULTS

Brain weight: The brain weight of hydrocortisone-treated rats was significantly less than that of controls in both sexes, as shown in Fig. 1. Percentage difference was particularly marked at 1 week of age, averaging 33% in males and 30% females. The greatest difference was seen in the telencephalon as indicated in Fig. 2, which shows the mean values of 10 to 14 male rats at ages from 2 to 4 weeks, the percentage differences in telencephalon weight being 14% at 2 weeks, 6% at 3 weeks and 12% at 4 weeks of age.

Norepinephrine content: Norepinephrine content in the whole brain of hydrocortisone-treated rats showed a small elevation in periods during 3–4 weeks of age in males and 4–7 weeks in females (Table 1). In male treated rats, the mean contents at 5 and 7 weeks of age were higher than the levels in respective controls, but the difference was not significant. When regional distribution of norepinephrine was examined in male infant rats (Fig. 3), the level was highest in the hypothalamus. In comparison with the levels in this area of control rats, the treated animals showed appreciably elevated values at 3 and 4 weeks of age,
Fig. 1. Brain weights of normal and neonatally hydrocortisone-treated rats. Data indicate mean values of 5–11 determinations. Solid circles show the hydrocortisone treatment and open circles are their controls. *P<0.05, **P<0.01, ***P<0.001

Fig. 2. Regional brain weights of normal and neonatally hydrocortisone-treated rats at 2–4 weeks of age. The tissues of two animals were pooled and weighed as a sample. Each value is mean of 2–3 measurements.

Table 1. Brain norepinephrine contents in normal and neonatally hydrocortisone-treated rats

<table>
<thead>
<tr>
<th>Age (week)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>0</td>
<td>123±2</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>160±8</td>
<td>200±17</td>
</tr>
<tr>
<td>2</td>
<td>211±7</td>
<td>235±9</td>
</tr>
<tr>
<td>3</td>
<td>263±3</td>
<td>283±6*</td>
</tr>
<tr>
<td>4</td>
<td>303±7</td>
<td>328±9*</td>
</tr>
<tr>
<td>5</td>
<td>331±11</td>
<td>378±20</td>
</tr>
<tr>
<td>7</td>
<td>380±12</td>
<td>417±9</td>
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</table>

Data indicate the mean±S.E. of 5–11 determinations. *P<0.05, **P<0.01
the percent difference being 19% at 3 weeks and 32% at 4 weeks. A similar elevation of norepinephrine content, albeit to a lesser extent was also observed in the pons+medulla oblongata.

**Dopamine content:** In contrast to the gradual increase of norepinephrine content in the brain, dopamine content increased more rapidly during postnatal days and at 3 weeks of age had already reached the levels seen in adult animals. Here again the levels were higher in the hydrocortisone-treated group than in the controls throughout the period tested (Table 2). Although regional differences of dopamine content between the two groups were rather small, the levels in the diencephalon and hypothalamus were slightly higher in the treated rats at 3 and 4 weeks of age (Fig. 4).

**Serotonin content:** In control rats, the whole brain serotonin content showed a tendency to decrease during the first two postnatal weeks, and then increased gradually at least until 7 weeks of age. On the other hand, in the hydrocortisone-treated group, the content began to increase at 2 weeks of life and during the next week, a pronounced increment was observed, in both sexes. In these rats the serotonin contents remained at a similar high level for the following 2 weeks, but further elevation was seen at 7 weeks of age (Fig. 5.).

**Table 2.** Brain dopamine contents in normal and neonatally hydrocortisone-treated rats

<table>
<thead>
<tr>
<th>Age (week)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>0</td>
<td>209±7</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>303±10</td>
<td>365±11***</td>
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<tr>
<td>2</td>
<td>390±13</td>
<td>532±27***</td>
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<tr>
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</tr>
<tr>
<td>4</td>
<td>642±48</td>
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</tr>
<tr>
<td>7</td>
<td>801±12</td>
<td>1042±15***</td>
</tr>
</tbody>
</table>

Data indicate the mean ± S.E. of 5–11 determinations. *P<0.05, **P<0.01, ***P<0.001
Postnatal changes in regional serotonin content are shown in Fig. 6. In treated rats the content was particularly high in the pons + medulla oblongata at the 2nd to 4th week, in the hypothalamus at the 2nd and 3rd weeks, and in the diencephalon at the 3rd week, while there was little difference in the telencephalon and cerebellum.

FIG. 4. Regional brain dopamine contents in normal and hydrocortisone-treated rats at 2-4 weeks of age. Samples for dopamine assay were prepared as described in the legend of Fig. 3. Data indicate mean values of 2-3 determinations.

FIG. 5. Brain serotonin contents in normal and neonatally hydrocortisone-treated rats. Data indicate mean values of 5-11 determinations. (●) hydrocortisone-treated, (○) Control  *P<0.05, **P<0.01, ***P<0.001

Postnatal changes in regional serotonin content are shown in Fig. 6. In treated rats the content was particularly high in the pons + medulla oblongata at the 2nd to 4th week, in the hypothalamus at the 2nd and 3rd weeks, and in the diencephalon at the 3rd week, while there was little difference in the telencephalon and cerebellum.

DISCUSSION

Postnatal development of monoamine-containing neurons in the rat brain was extensively studied by Loizou (8). At birth the dopamine-containing neurons were found to be more developed than the norepinephrine-containing neurons, which in turn were more developed than the serotonin-containing neurons. It was suggested that the increments in the biochemically measured levels of the three amines correspond to morphological
development and production of increasing amounts of synthesizing enzymes and storage particles. In the present study, postnatal increase of these three amines was not in parallel; that is, the increment of dopamine was the fastest, followed by norepinephrine and serotonin, in that order. A sharp increment in brain dopamine from low values at early stages of postnatal life to practically adult values by the 30th day were also reported by Hrdina et al. (9).

At birth, the level of norepinephrine was about 30% of the level at the 7th week. The concentration then increased linearly approaching that seen in adult animals. The serotonin level increased more rapidly during the period from the 2nd to 3rd week of life. According to Benetato (10), the serotonin concentration in the hypothalamus decreased from 30 days of age to the time of vaginal opening. This decrease of serotonin content was assumed to be related to increased gonadotropin secretion before the onset of puberty. In the present study, a marked increase in the serotonin concentration in the hypothalamus was observed at the 4th week of age in control rats and the level showed further increase at the 5th week. Therefore, reduction of serotonin content in the hypothalamus is probably not involved in the onset of puberty.

As to the effect of corticosteroids on brain monoamine content, conflicting data have been reported. Following administration of corticosteroids, brain serotonin content was decreased (11–13), unchanged (14, 15), or increased (11, 16). According to Telegdy and Vermis (17), a single dose of corticosterone increased the serotonin content in the mesencephalon, amygdala and hypothalamus. Curzon and Green (18) reported that following a single injection of hydrocortisone, a significant fall in the serotonin content occurred, while daily injections resulted in a normal or slightly elevated brain serotonin. Kizer et al. (19) found an increased tyrosine hydroxylase activity in rats treated with dexamethasone. There are also reports that tryptophan hydroxylase activity increases with corticosterone injection (20, 21), while others were not able to confirm the data (22). In addition, an increased turnover of serotonin was demonstrated after corticosterone treatment (23–25). The mechanism by which corticosteroids influence the monoamine level is not clearly under-
stood. In investigations of regional changes of brain monoamines, Ulrich et al. (4) reported that hydrocortisone implanted in the hypothalamus of rats elevated serotonin levels in the basal hypothalamus, while the norepinephrine levels remained unchanged. Recently, Fekete et al. (26) have shown that corticosterone increased norepinephrine content of the supraoptic nucleus and norepinephrine and dopamine levels in the median eminence, while it had no effect on the amine concentration in the locus coeruleus.

The conflicting results might be due to different experimental conditions such as age of animals, dose and route of administration, etc. It is likely that the findings in adult rats differ from those observed in developing animals. As already noted, Ulrich (4) and Nyakas (5) found increased levels of norepinephrine and serotonin in the hypothalamus and hindbrain of neonatally corticosteroid-treated rats. In the present study, a significant elevation of brain monoamines was observed not only in the hypothalamus, but also in other regions of the brain throughout all stages of development in the treated rats, as compared with the respective levels in the controls. Despite the fact that the telencephalon is a major area of the whole brain and the absolute amount of monoamines is larger than in other discrete regions, the amine content itself did not change significantly after treatment with hydrocortisone. The elevation of monoamine levels observed in a whole brain are likely to be based on change of amine content in the hypothalamus, midbrain and pons-medulla oblongata. The relative value might be expanded by reduction of brain weight, since decrease of tissue weight was mainly observed in the telencephalon. Regional changes in dopamine contents were not clear in comparison with the contents of norepinephrine or serotonin. The increment of norepinephrine and/or serotonin content suggests that function of the monoaminergic nervous system is potentiated in the hypothalamus, midbrain and pons-medulla oblongata. The potentiated activities of monoaminergic neurons may influence the physiological functions of these brain regions, for example, functions of the hypothalamo-pituitary systems. In a previous study, we observed that neonatal treatment with hydrocortisone induced a marked retardation of pituitary-adrenocortical development in infant rats (1). The relationship between the monoaminergic systems and the hypothalamo-pituitary function remains to be elucidated.

REFERENCES

6) GLOWINSKI, J. AND IVERSEN, L.L.: Regional studies of catecholamines in the rat Brain. I.


