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REGIONALLY SPECIFIC EFFECTS OF REPEATED CORTICOSTERONE TREATMENT ON RAT BRAIN SEROTONIN METABOLISM

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Five day corticosterone treatment increased 5-hydroxy indole acetic acid (5-HIAA) concentration in the hypothalamus of rats; 5-hydroxytryptamine (5-HT) increased in the midbrain region. 5-HIAA or 5-HT concentrations in the cortex hippocampus and striatum remained unaffected. Tryptophan levels increased in the hypothalamus and midbrain region. The treatment decreased plasma total tryptophan concentration. The concentration of free tryptophan was however not affected. Food intakes and growth rates of corticosterone injected rats were smaller than in the saline injected animals. The results may help to elucidate neurochemical alterations in depressive or other psychic states induced by prolonged hypercortisolemia.

Key words: Serotonin, Tryptophan, 5-HT., Corticosterone.

Introduction

Glucocorticoids have been reported to influence brain serotonin (5-hydroxytryptamine; 5-HT) metabolism. The effect however, seems to vary depending in part on dose, duration of corticosteroid administration and on the animal species and some times on specific brain region studied [1-4]. The effects of repeated glucocorticoid administration on brain 5-HT metabolism have not been widely studied.

There is some evidence to think that repeated increases of plasma corticosterone may have maladaptive consequences, associated with impaired 5-HT dependent behavioural responses [5-7]. This paper, therefore, concerns the effect of repeated corticosterone administration on brain regional 5-HT metabolism. Corticosterone was administered in doses to raise its plasma concentration typical to stressed rats [5]. After five daily injections the rats were killed on the sixth day to collect brain and plasma samples.

Materials and Methods

Animal chemicals and treatments. Locally bred male albino Wister rats (bred and grown to weight in the animal room of Biochemistry Department) weighing 230-250 gm were housed individually in the experimental animal room with access to cubes of standard rat food and water for atleast five days before the start of the experiment.

All chemicals used in the study were of analytical grade purchased from Sigma or Merck. Corticosterone used as a pharmacological tool was also obtained from Sigma. Deionized water was used through out the analysis.

Animals were randomly assigned to control and test groups. Corticosterone was suspended in 0.9% NaCl containing BRIJ-35 (polyoxy ethylene lauryl ether) and injected s.c. in a volume of 0.1ml/100gm body weight.

To determine plasma levels of corticosterone one hour after corticosterone (50mg/kg, s.c.) administration, the animals injected with saline or corticosterone alternately in a balanced design were killed exactly one hour after the injection. Plasma samples were collected for the estimation of corticosterone.

For repeated treatment study, daily corticosterone treatment was performed by injecting the drug (50mg/kg) for five days between 09:30-10:30 hrs. Control rats were injected with saline at the same time. On the sixth day both control and repeatedly corticosterone treated animals were decapitated between 10:30-11:30 hrs. Plasma and brain samples were collected for respective biochemical and neurochemical estimations.

Cumulative food intakes during the treatment were calculated as gm/100gm body weight and growth rate as the percentage of the weight on the starting day.

Neurochemical analysis. Brains were taken out within 30 sec. of the death and dipped in ice cold saline before dissecting into regions. The dissected material was frozen immediately in plastic Eppendorf tubes and stored at -70° until used.

Frozen brain samples were homogenized in 5-10 volumes of 0.4M perchloric acid, containing 0.1% sodium metabisulphite, 0.01% ethylene diamine tetra acetic acid and 0.1% cysteine. The homogenates were left in a refrigerator for 10 mins to aid precipitation before removal of precipitate at 12000 *gm in a high speed refrigerated centrifuge for 15 mins.

5-HT and 5-hydroxy indoleacetic acid (5-HIAA) in the extract were determined by HPLC with electrochemical detector [8] using a 5μ Schimadzu ODS (4.6mm *15cm) column. Mobile phase was methanol (18%), octyl sodium sulphate (OSS, 0.023%) and EDTA (0.0035%) in 0.1M phosphoric acid buffer (pH=2.9). Methanol recommended for

liquid chromatography was used for the preparation of mobile phase. The electrochemical detection was provided by glassy carbon electrode versus a Ag/AgCl reference electrode (Schimadzu). The potential was set at 1.0 V and detector sensitivity at 2nA scale deflection.

Determination of plasma corticosterone. The levels of plasma corticosterone were determined by the method of Mattingley [9].

Determination of total and free tryptophan in plasma. Plasma total and free (ultrafiltrable) tryptophan was determined by the fluorimetric method of Denckla and Dewey [10] as revised by Bloxam and Warren [11].

Statistical analysis. Data were statistically tested by t-test (two tailed). Differences between groups were considered significant when $P<0.05$.

Results and Discussions

Effect of corticosterone injection 50mg/kg s.c. on plasma levels of corticosterone. Table 1 shows that rats injected with saline and killed 1 hr. after the injection had plasma corticosterone concentration which were similar to values obtained for non-injected controls [5]. Corticosterone given in doses of 50mg/kg s.c. 1 hr. before decapitation led to plasma values typical to stressed females [5].

Effect of five day corticosterone injection on brain regional concentrations of 5-HT, 5-HIAA and tryptophan. Table 2 shows the effect of five; one daily corticosterone (50mg/kg s.c.) injection on brain regional 5-HT, 5-HIAA and tryptophan concentrations. The treatment increased 5-HIAA (35%, $P<0.0001$) concentration in the hypothalamus. 5-HT increased (28%, $P<0.01$) in the midbrain. 5-HT or 5-HIAA concentrations in the cortex (Table 2), hippocampus and striatum (data not shown) were not altered. Mean values of tryptophan were higher in all the brain regions of corticosterone treated rats but the differences were significant for the midbrain ($P<0.05$) and hypothalamus ($P<0.001$) only.

Effect of corticosterone treatment on plasma tryptophan. Table 3 shows plasma total and free tryptophan concentrations in saline and corticosterone treated rats. Repeated corticosterone injection decreased plasma total tryptophan; free tryptophan concentrations were however, not affected.

Effect of 5 day corticosterone injection on food intake and growth rate. Table 4 shows cumulative food intakes and growth rates of rats given 5 day saline or corticosterone (50mg/kg s.c.) injections. Both of these measures were smaller in the latter group.

Other authors have reported that glucocorticoids influence brain serotonin metabolism depending in part on dose, animal species and also on the specific brain region studied [1-4]. We find an increase in the midbrain and

TABLE 1. PLASMA CORTICOSTERONE LEVELS (µg/100 ml) IN RATS 1 HR. AFTER SALINE OR CORTICOSTERONE (50mg/kg) INJECTION.

	Saline	Corticosterone
1 Hr. after single injection	22.5 ± 2.9	82.3 ± 2.1*

Results are means ±S.D. (n=10). Significant differences by t-test, from saline injected rats, *P<0.01.

TABLE 2. BRAIN REGIONAL 5-HT, 5-HIAA AND TRYPTOPHAN CONCENTRATIONS (ng/gm) IN RATS GIVEN FIVE (ONE DAILY) SALINE OR CORTICOSTERONE (50mg/kg s.c.) INJECTION.

	Saline	Corticosterone	% Change
Hypothalamus			
5-HIAA	569± 39	766± 26*	+35
5-HT	148± 20	175± 35	+18
Tryptophan	5982±508	9210±802*	+54
Midbrain			
5-HIAA	480± 70	525± 75	+09
5-HT	392± 55	502± 30*	+28
Tryptophan	5820±709	7505±812*	+29
Cortex			
5-HIAA	245± 20	252± 30	+03
5-HT	149± 21	130± 17	–13
Tryptophan	4268±507	4459±311	+05

Values are means ±S.D. (n=9) 24 hrs after last saline or corticosterone injection. Significant differences by t-test: *P<0.01.

TABLE 3. PLASMA TOTAL AND FREE TRYPTOPHAN CONCENTRATIONS (n mol/ml) IN FIVE DAY SALINE OR CORTICOSTERONE (50mg/kg s.c.) INJECTED RATS 24 HRS AFTER LAST INJECTION.

	Saline	Corticosterone
Total tryptophan	115 ± 15	81 ± 20*
Free tryptophan	91 ± 1.5	10.2 ± 2.5*

Values are means ± S.D. (n=10). Significant differences by t-test: *P<0.01.

TABLE 4. CUMULATIVE FOOD INTAKES AND GROWTH RATES IN RATS GIVEN FIVE DAY SALINE OR CORTICOSTERONE (50mg/kg s.c.) INJECTION.

	Saline	Corticosterone	% Difference
Food intake (gm/100gm body weight)	32.2 ± 1.2	26.9 ± 1.9*	–16.4
Growth rate (change in body weight as percentage of starting day)	102.5 ± 4.2	92.1 ± 4.1*	–10

Values are means ± S.D. (n=10). Food intakes and body weight changes were monitored as described in methods. Significant differences by t-test: *P<0.01.

hypothalamic 5-HT metabolism, 24 hrs after five day corticosterone treatment. Other regions studied showed negligible effect. The enhancement of 5-HIAA concentration was particularly marked in the hypothalamus, while 5-HT increased in the midbrain region, which contains serotonergic cell bodies.

The enhancement of brain 5-HT metabolism may result from the increase in brain tryptophan concentration observed in the hypothalamus and midbrain region of repeatedly corticosterone treated rats (Table 2). Tryptophan hydroxylase, the rate limiting enzyme of 5-HT biosynthesis exists unsaturated with its substrate [12-14]. Brain levels of tryptophan are therefore, known to play an important role in determining the ongoing rate of 5-HT synthesis [14].

Tryptophan to the brain is supplied from the circulation by a stereospecific, saturable, faciliated transport mechanism shared by all large neutral amino acids (LNAAs; 15-17). Therefore, plasma concentrations of LNAAs other than tryptophan also influence tryptophan transport to the brain [18]. Moreover, unlike other LNAAs, 80-90% of tryptophan in plasma circulates largely bound to albumin [19,20]. The levels of tryptophan both total and free in plasma and the efficiency of carrier-mediated transport have been shown to have some role in determining the brain levels of typtophan in different physiological and pharmacological conditions [18,21,22].

In the present study plasma total tryptophan fell considerably but free tryptophan was not affected (Table 3). Although we have no data on plasma competing aminoacids. However, glucocorticoids have been shown to stimulate the uptake of L-$[H]_3$ tryptophan from synaptosomes [23]. A greater uptake of tryptophan by the brain in the present study is perhaps also interpretable by a similar mechanism.

A greater uptake of tryptophan only in specific brain regions of repeatedly corticosterone treated rats is perhaps not explainable in terms of greater corticosterone uptake or binding density in these brain regions [24]. It is, however, possible that corticosterone administration enhances uptake of tryptophan by serrotonergic neurons only in specific brain regions. Indeed, despite a common supply from the blood circulation, the concentration of tryptophan is different in different brain regions.

An important finding of the present study seems to be that 5-HT concentration increased in the mid brain region which contains serotonergic cell bodies [25]. However, it is not clear whether the changes of 5-HIAA in the hypothalamus result because of greater 5-HT synthesis in the midbrain region which provides input to the hypothalamus [25], or 5-HT synthesis is also increased in the hypothalamus. As while 5-HT concentration did not increase in the hypothalamus, the concentration of tryptophan was considerable greater in this brain region. Moreover, some serotonergic cell bodies perhaps also occur in the hypothalamus [25].

Indirect evidences indicate that the decrease in food intake produced by repeated corticosterone treatment may also have serotonergic mediation. It is relevant that corticosterone treatment enhances 5-HT metabolism (Table 2) and drugs that increase serotonergic activity at post-synaptic sites are anorectic agents [26,27]. Also serotonergic drugs increase hypothalamo-pituitary adrenocortical (HPA) activity [28,29], indeed by stimulating CRF release. While CRF which stimulates pituitary adrenal axis decreased feeding [30,31]. It can be speculated that corticosterone-induced decrement in feeding may be mediated, atleast partially by serotonergic mechanisms. However, we have no evidence on whether the changes of 5-HT metabolism following corticosterone treatment lead to increased 5-HT release at the functional post-synaptic sites or whether they merely reflect intraneuronal metabolism.

The decrement in feeding may also partially explain the impairment of body weight following corticosterone treatment, although catabolic effects of corticosterone perhaps also contribute.

Serotonergic mechanisms are implicated in the control of HPA axis [28]; injection of 8-hydroxy-2-n-dipropylamino tetralin (8-OH-DPAT) into the paraventricular nucleus of the hypothalamus activates the axis [29]. The present results may help to understand, how repeated increases of plasma corticosterone would oppose the process of adaptation [5]; associated with enhanced 5-HT-mediated behavioural responses [5-7].

Since 5-HT malfunctions are often described in human depression [32]; while a subgroup of depressed patients exhibits hypercortisolemia [33]. The present findings may help to elucidate neurochemical alterations occuring in this group of depressed patients.

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