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REGULATION OF 5-HYDROXYTRYPTAMINE METABOLISM IN MOUSE BRAIN BY ADRENAL GLUCOCORTICOIDS*

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SUMMARY

The effects of glucocorticoid hormone on the metabolism of brain 5-hydroxytryptamine (5-HT) were studied in mice. A single injection of hydrocortisone acetate (HCA; 20 mg/kg, i.p.) accelerated the accumulation of 5-HT in whole brain after inhibition of monoamine oxidase activity by pargyline. The hormone did not appear to change brain tryptophan hydroxylase or 5-hydroxytryptophan decarboxylase activity. However, tryptophan levels in brain were elevated by 50% within 1 h after treatment with HCA. The effect of HCA on brain tryptophan levels was localized mainly in the nerve endings. In *in vitro* synaptosomal preparations, HCA at 10^{-5}–$10^{-7}M$ or corticosterone at 10^{-5} *M* was found to stimulate the uptake of L-[^{3}H]-tryptophan by the synaptosomes while androgenic and progesterone-like steroids were ineffective. These results demonstrate that glucocorticoids may directly act on nerve terminals in the regulation of 5-HT synthesis through an action on the uptake of tryptophan.

INTRODUCTION

Hormonal regulation of neurotransmitters in mammalian brain has not been extensively studied. Some attempts have been made to relate glucocorticoids to brain 5-hydroxytryptamine (5-HT) metabolism. Bilateral adrenalectomy was reported to depress the rate of formation of [5-^{3}H]HT from intracisternally injected [^{3}H]tryptophan[1]. Apparent tryptophan hydroxylase activity in brain was also reported to be reduced by this treatment[2]. Injection of corticosterone tended to restore the reduced enzyme activity toward normal levels and this hormonal effect seemed to be cyclo-

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heximide-sensitive[2]. However, other investigators found no stimulatory effect on 5-HT metabolism when cultured midbrain raphe nuclei of newborn rats were incubated with cortisone or ACTH[10]. Curzon and Green reported a decrease in absolute levels of brain 5-HT in rats following hydrocortisone injection[4]. Several laboratories failed to confirm the effects of corticosterone and adrenalectomy on brain tryptophan hydroxylase activity[16,22] while results from our laboratory also failed to demonstrate any significant increase in tryptophan hydroxylase activity in intact mice following repeated injections of hydrocortisone.

It appears that tryptophan hydroxylase in brain is unsaturated with its substrate since injections of L-tryptophan lead to an increased amount of brain 5-HT within 60 min[8]. Various drugs known to increase brain 5-HT turnover rate were recently reported also to increase brain tryptophan concentrations[26]. For example, the acute inhibitory action of *p*-chlorophenylalanine (PCPA) on 5-HT synthesis was found to be due to its interference with the uptake of tryptophan by brain nerve endings[12]. Thus, 5-HT synthesis may be regulated by the availability of tryptophan, its precursor.

In this work, we present evidence demonstrating the action of glucocorticoids on the uptake of tryptophan in the brain, and propose that this action may be an important mechanism by which the hormone may regulate 5-HT metabolism.

METHODS

Animals

The animals used in this study were male Swiss albino mice, approximately 2 months old, bred in our own colony. The mice were maintained on a 12h: 12h light–dark cycle (with lights on at 06:00 a.m. and off at 18:00 p.m.) at 71 ± 2 °F with 52 ± 2% relative humidity in a pathogen-free environment. All drugs were injected as either solutions or suspensions in 0.9% NaCl at 37 °C. All injections were intraperitoneal and, unless otherwise stated, occurred at 08:30 a.m.

Biochemical assays

5-HT was measured by a modification of the method of Bogdanski[3]. The whole brain was homogenized in 0.1 *N* HCl containing 0.05% ascorbate. The amine was extracted to acidified *n*-butanol and transferred to 0.1 *N* HCl. After the addition of concentrated HCl, the fluorescence was measured at excitation 295 nm and emission 540 nm. Tryptophan was measured by the method of Denkla and Dewey[5]. The tissue was deproteinized with 75% trichloroacetic acid and tryptophan was oxidized to the fluorescent substance norharman by boiling with 1.8% formalin for 1 h. The fluorescence was measured at excitation 373 nm and emission 452 nm. Tyrosine was measured fluorometrically by the method of Waalkes and Udenfriend[29]. Fluorescence was read at activation 460 nm and 570 nm emission. Protein was measured by the method of Lowry *et al.*[17].

Tryptophan hydroxylase activity was determined by measurement of $^{14}CO_2$ formed from L-[1-^{14}C]tryptophan, as described by Ichiyama *et al.*[11]. The final reaction mixture was 1.0 ml containing 100 μmoles Tris–acetate, pH 8.1, 0.033 μCi L-[1-^{14}C]-

tryptophan (spec. act. 8.98 mCi/mmole), 10 μmoles iproniazid phosphate, and 0.5 ml of 20% whole brain homogenate in 0.32 *M* sucrose. 5-Hydroxytryptophan (5-HTP) decarboxylase activity was determined by the procedure of Sims, Davis and Bloom[23]. The final reaction mixture was 1.0 ml containing 100 μmoles Tris–HCl, pH 8.5, 0.25 μCi of L-[1-^{14}C]-5-HTP (spec. act. 55 mCi/mmole) in the presence of 0.05 μmoles of unlabeled L-5-HTP, 7 μmoles pyridoxal phosphate, and 0.5 ml 20% whole brain homogenate in 0.32 *M* sucrose. In both procedures, the $^{14}CO_2$ produced was trapped in hyamine hydroxide after incubation for 1h at 37 °C. The radioactivity was measured by liquid scintillation spectrometry.

Subcellular fractionation

Subcellular fractions of whole brain tissue were prepared by the method of Gray and Whittaker[9]. A crude nuclei fraction was obtained by centrifugation of 15% brain homogenate in 0.32 *M* sucrose at 1,000 × *g* for 10 min. The supernatant was further sedimented at 12,000 × *g* for 20 min to obtain the P_2 pellet (crude mitochondria fraction). Synaptosomes and mitochondria were fractionated from resuspended P_2 by the discontinuous sucrose gradient procedure previously described. The P_2B layer was aspirated and sucrose concentration was adjusted to 0.32 *M*. It was then resedimented at 12,000 × *g* for 20 min to obtain the synaptosomal fraction. The P_2C pellet was washed with 0.32 *M* sucrose and resedimented at 12,000 × *g* for 20 min to obtain the mitochondrial fraction.

Measurement of synaptosomal tryptophan uptake

The uptake of tryptophan by isolated synaptosomal particles was measured by a modification of a procedure previously described[13,14]. One millilitre of the incubation mixture contained 50 μmoles Tris–HCl, pH 7.3, 200 μmoles NaCl, 5 μmoles α-propyldopacetamide, 0.20 μCi L-[U-^{3}H]tryptophan (spec. act. 3.04 Ci/mmole) in the presence of 0.4 nmole of unlabeled L-tryptophan, and resuspended synaptosomal preparation equivalent to 2 mg of particulate protein. The incubation was allowed to proceed for 5 min at 30 °C. The rate of tryptophan uptake was found to be linear within 6 min. After incubation, the synaptosomal suspension was immediately cooled in ice and centrifuged at 48,000 × *g* for 20 min. Radioactivity in both the supernatant and pellet was measured by liquid scintillation spectrometry. In the presence of 5×10^{-3} *M* α-propyldopacetamide, an inhibitor of tryptophan hydroxylase activity, the radioactivity in the pellet after incubation was found to be exclusively from [^{3}H]tryptophan as examined by radiochromatography. After correction for extraparticulate space in the pellet due to entrenched supernatant using [^{14}C]inulin, the rate of uptake was calculated as pmoles [^{3}H]tryptophan/5 min/mg synaptosomal protein.

Statistical analyses

Significance of differences between groups was determined by the use of Student's two-tailed *t*-test. A *P* value of 0.05 or less served as the criterion for significance.

TABLE I

ACCUMULATION OF 5-HT IN BRAIN AFTER HYDROCORTISONE TREATMENT

(a) Hydrocortisone acetate (20 mg/kg) was injected intraperitoneally at 90 min before sacrifice. Controls were injected with saline. (b) All animals were injected with pargyline (80 mg/kg) at the indicated time prior to sacrifice. Injections of pargyline to the 0 min group immediately before sacrifice served to standardize treatment conditions. All values are means ± S.E.M., with the number of animals indicated in the parentheses.

Treatment (a)	*5-HT (ng/g) after pargyline administration (b)*		*Δ 5-HT (ng/g)/90 min*
	0 min	*90 min*	
Control	461 ± 5.4 (9)	829 ± 11.0 (9)	368
Hydrocortisone acetate	378 ± 1.8* (9)	1042 ± 8.0* (9)	664

* $P < 0.001$.

RESULTS

Effect of hydrocortisone on brain 5-HT levels

Table I shows the effect of hydrocortisone acetate (HCA) on brain 5-HT levels in mice with and without treatment with pargyline, an inhibitor of monoamine oxidase (MAO). At 90 min after the administration of HCA (20 mg/kg), the level of 5-HT in the whole brain was reduced by 22% when compared to the controls. However, when both the control and the HCA-administered animals were treated with pargyline (80 mg/kg) at the same time they received saline or HCA (90 min before sacrifice), the level of 5-HT in the whole brain of the hormone-treated animals was 26% higher when compared to the controls. In the presence of the MAO inhibitor, the amount of 5-HT accumulated in 90 min (Δ5-HT/90 min) was 80% greater in the animals receiving HCA than that in the controls.

Activities of tryptophan hydroxylase and 5-HTP decarboxylase in brain after hydrocortisone administration

The activities of the two enzymes involved in the synthesis of 5-HT in brain were measured at 60 min after the injection of HCA (20 mg/kg). The results are shown in Table II. Neither tryptophan hydroxylase activity nor 5-HTP decarboxylase activity of whole brain was changed by the administration of the hormone.

Effect of hydrocortisone on brain tryptophan levels

At 60 min after one injection of HCA (20 mg/kg), the brain level of tryptophan, the amino acid precursor in the synthesis of 5-HT, was determined. As shown in Table III, brain tryptophan level was elevated from 25 to 37 ng/mg protein, an increase of 50%, as a result of the hormone treatment. Since glucocorticoids are known to have proteolytic action[7], the increase of tryptophan could be the result of this general effect on protein degradation. In order to investigate this possibility, tryptophan levels after HCA administration were also measured in liver and kidney, the liver being a

TABLE II

BRAIN TRYPTOPHAN HYDROXYLASE AND 5-HTP DECARBOXYLASE ACTIVITIES AFTER HYDROCORTISONE TREATMENT

(a) Hydrocortisone acetate (20 mg/kg) was injected intraperitoneally at 60 min before sacrifice. Controls were injected with saline. (b) Enzyme activity is expressed as disint./min/100 mg tissue after 60 min incubation. All values are mean ± S.E.M. None of the values are significantly different from the control. The number of animals in each group is indicated in the parentheses.

Treatment (a)	*Enzyme activity (disint./min/100 mg/h) (b)*	
	TRY-H	*5-HTP-D*
Control	2008 ± 22 (24)	25,800 ± 294 (24)
Hydrocortisone acetate	1922 ± 27 (24)	24,000 ± 673 (24)

TABLE III

TRYPTOPHAN CONCENTRATIONS IN BRAIN, LIVER AND KIDNEY AFTER HYDROCORTISONE TREATMENT

(a) Hydrocortisone acetate (20 mg/kg) was injected intraperitoneally at 60 min before sacrifice. Controls were injected with saline. (b) Tryptophan concentration is expressed as ng/mg protein. The number of animals in each group is indicated in the parentheses. All values are mean ± S.E.M.

Treatment (a)	*Tryptophan concentration (ng/mg protein) (b)*		
	Brain	*Liver*	*Kidney*
Control	25 ± 2 (9)	235 ± 25 (10)	360 ± 15 (10)
Hydrocortisone acetate	37 ± 3* (10)	275 ± 35 (10)	410 ± 25 (10)

* $P < 0.01$.

major target organ of glucocorticoids[15] while the kidney is relatively little affected by this class of steroid hormone. However, at 60 min after the injection of HCA, the increase of tryptophan in these two tissues was insignificant. It appeared from these results that the effect of the hormone in increasing tryptophan levels in tissue was rather specific to the brain.

Effect of hydrocortisone on tryptophan concentrations in brain subcellular structures

In order to localize the effect of the hormone on brain tryptophan levels in subcellular structures, subcellular fractions were prepared from whole brain after HCA administration and tryptophan was measured in the various fractions. The results are summarized in Table IV. Essentially there was no significant change in the tryptophan concentration in the crude nuclear fraction (P_1). In the P_2 fraction ('crude mitochondrial fraction'), however, tryptophan was increased by 57% at 60 min after the injection of HCA (20 mg/kg). When the P_2 fraction was further sub-fractionated by ultracentrifugation on a discontinuous sucrose gradient, an increase by 214% of tryptophan was found in the synaptosomal (P_2B) fraction from HCA-treated animals while no change was found in the mitochondrial (P_2C) fraction. Of the three particulate

TABLE IV

TRYPTOPHAN AND TYROSINE CONCENTRATIONS IN SUBCELLULAR BRAIN FRACTIONS AFTER TREATMENT WITH HYDROCORTISONE ACETATE

(a) Hydrocortisone acetate was injected intraperitoneally at 60 min before sacrifice. Controls were injected with saline. (b) Values are expressed as ng amino acid/mg protein. Values are expressed as the mean ± S.E.M. Each mean is the average from 6 preparations. P_1, crude nuclear fraction; P_2, crude mitochondrial fraction; P_2B, synaptosomal fraction; P_2C, mitochondrial fraction.

Treatment (a)	*Tryptophan (ng/mg) (b)*				*Tyrosine (ng/mg) (b)*
	P_1	*P_2*	*P_2B*	*P_2C*	*P_2B*
Control	0.9 ± 0.3	11.8 ± 0.9	9.8 ± 1.9	1.4 ± 0.3	5.4 ± 0.3
Hydrocortisone acetate	0.5 ± 0.1	18.5 ± 2.6*	30.8 ± 0.4**	1.2 ± 0.2	5.7 ± 0.2

* $P < 0.05$.
** $P < 0.001$.

fractions (nuclear, synaptosomal, and mitochondrial) examined, the synaptosomal fraction showed the only and remarkable increase of tryptophan after HCA treatment. It is interesting to note that the hormone showed no effect on synaptosomal tyrosine, another aromatic amino acid and precursor in the synthesis of catecholamines.

Effect of hydrocortisone on tryptophan uptake in vitro by synaptosomes

Since the effect of HCA *in vivo* in increasing brain tryptophan appears to be

TABLE V

TRYPTOPHAN UPTAKE BY SYNAPTOSOMES INCUBATED *in vitro* WITH VARIOUS STEROIDS

(a) Incubation medium is as described in text. Hydrocortisone acetate, corticosterone, 17α-hydroxyprogesterone or 5α-dihydrotestosterone was present in the indicated concentration in the incubation medium. Incubation was carried out in air at 30 °C for 5 min with no pre-incubation. (b) Tryptophan uptake is expressed as pmole [^{3}H]tryptophan/5 min/mg synaptosomal protein after correction for extra-particulate space (see text). All values are mean ± S.E.M. Numbers in parentheses refer to the number of experiments.

Treatment (a)	*Tryptophan taken up by synaptosomes (b) (pmoles/5 min/mg protein)*
Control	214.4 ± 13.4 (10)
Hydrocortisone acetate (10^{-5} *M*)	361.8 ± 13.8** (6)
Hydrocortisone acetate (10^{-6} *M*)	375.2 ± 12.9 ** (6)
Hydrocortisone acetate (10^{-7} *M*)	291.2 ± 5.6 ** (6)
Hydrocortisone acetate (10^{-8} *M*)	223.6 ± 1.1 (6)
Corticosterone (10^{-5} *M*)	348.4 ± 14.2 ** (4)
17α-Hydroxyprogesterone (10^{-4} *M*)	201.0 ± 26.8 (6)
5α-Dihydrotestosterone (10^{-4} *M*)	201.0 ± 15.3 (6)

* $P < 0.01$.
** $P < 0.001$.

mainly localized in the nerve endings, the possible direct action of the hormone on the transport of tryptophan into nerve endings was examined in an *in vitro* system. Isolated brain synaptosomes were suspended in a medium containing [^{3}H]tryptophan, and the rate of [^{3}H]tryptophan taken up by the particulate structures from the medium was measured at 30 °C (Table V). In the absence of added hormone, an uptake rate of 214 pmoles tryptophan/5 min was obtained per mg of synaptosomal protein. In the presence of 10^{-5} or $10^{-6}M$ HCA in the medium, the rate was accelerated to 362 pmoles/5 min/mg protein, with an increase of 70% above the control value. HCA concentration at $10^{-7}M$ produced an increase of 36%, whereas HCA at 10^{-8} M was ineffective. Corticosterone, the major glucocorticoid in the mouse, showed a similar stimulatory effect on the rate of tryptophan uptake by the synaptosomes. Neither 17α-hydroxyprogesterone nor 5α-dihydrotestosterone, even at the concentration of 10^{-4} M, could substitute HCA or corticosterone for the stimulatory effect on the synaptosomal uptake of tryptophan.

DISCUSSION

Our finding that a single injection of hydrocortisone acetate (HCA) lowered the steady-state level of brain 5-HT is in agreement with the results of Curzon and Green[4]. However, when the oxidative degradation of 5-HT was prevented by treatment with pargyline, the accumulated level of brain 5-HT was found to be significantly higher in mice administered with HCA than in the controls. If the rate of accumulation after blockade of metabolic degradation is taken as an approximation of the rate of synthesis[28], our results indicate that 5-HT synthesis was in fact markedly increased by the administration of HCA. This interpretation is supported by the recent work of Millard *et al.*[21] which also showed an increase of the synthetic rate of brain 5-HT in rats after the administration of corticosterone.

While a single injection of HCA appeared to have no effect on tryptophan hydroxylase activity and 5-HTP decarboxylase activity, brain tryptophan levels were significantly elevated. Several recent studies have presented evidence in support of the contention that brain tryptophan hydroxylase *in vivo* is unsaturated with its substrate and that endogenous tryptophan levels are therefore also rate-limiting in the synthesis of 5-HT. Grahame-Smith[8] reported an increase in rat brain 5-HT within 60 min after injection of tryptophan. The magnitude of the increase of 5-HT was shown to be directly proportional to the amount of tryptophan administered over a wide range of non-physiological concentrations. Fernstrom and Wurtman[6] reported a similar effect produced by small changes in blood tryptophan levels. By causing the blood level of tryptophan in rats to fluctuate within a physiological range of values, they demonstrated parallel fluctuations in brain 5-HT levels, suggesting a direct relationship between tryptophan and 5-HT concentrations under the physiological condition. Similarly, the effect of HCA in increasing brain 5-HT synthesis as shown in this study and also by Millard *et al.*[21] could, at least in part, be a result of the marked elevation of brain tryptophan levels, especially considering the fact that the activity of the rate-limiting synthesizing enzyme for 5-HT remained unchanged.

The proposed mechanism for the action of the glucocorticoid in the regulation of the 5-HT system through tryptophan is by no means unique to the hormone. Several drugs, including systemically administered reserpine, D-amphetamine, lithium and cesium chloride, and intraventricularly administered dibutyryl cyclic AMP, as well as high environmental temperature and electroconvulsive shock, have been reported to increase the rate of synthesis of 5-HT in mammalian brain[24–26]. Each of these treatments resulted in a concurrent elevation of brain tryptophan levels. Environmental stress is known to stimulate glucocorticoid output and to modify 5-HT synthesis[27]. Ongoing experiments in our laboratory suggest that a similar sequence of events including the elevation of brain tryptophan levels may also exist in the glucocorticoid-mediated action of stress.

Although glucocorticoids have a variety of biochemical actions on several target tissues, the effect of HCA in rapidly elevating tissue tryptophan levels appears to be unique to the brain. Most remarkably, the elevated tryptophan levels appear to occur mainly in the nerve endings. Therefore, the rapid elevation of tryptophan levels specifically in the nerve endings cannot be accounted for by a general proteolytic effect of the hormone. Although the elevated tryptophan levels were measured in the general population of synaptosomes, the elevation is correlated with the observed increase in 5-HT synthesis. This is further supported by the observation that the levels of tyrosine, recently implicated in the regulation of catecholamine synthesis[30], remained unchanged in the nerve endings following HCA treatment. A direct action of the hormone on the nerve endings in modifying tryptophan transport is demonstrated in the *in vitro* synaptosomal system. When HCA or corticosterone was added to the suspended synaptosomes incubated *in vitro*, significant increases in the amounts of [^{3}H]tryptophan taken up by the synaptosomes were observed. HCA concentration as low as 10^{-7} *M* produced a significant increase in the synaptosomal uptake of [^{3}H]tryptophan. Physiologically, the concentrations of glucocorticoids in blood have been reported to range from 10 to 30 μg% (2.8–8.4 $\times$ 10^{-7} *M*), depending on environmental input[31]. Thus, the increase of synaptosomal uptake of tryptophan by HCA demonstrated *in vitro* may be related to physiological regulation *in vivo*. This effect was apparently specific to the glucocorticoids, since androgenic and progesterone-like steroids were found to be ineffective.

In several recent studies, McEwen *et al.* have demonstrated that the nuclei of CNS neurons are target sites for glucocorticoid action[18–20]. This hormone is apparently transported by a cytoplasmic binding protein into the nucleus where it may modify macromolecular synthesis. In the present study, we have presented evidence that the nerve endings of CNS neurons may also be target sites for glucocorticoid action, and that the hormone may thus rapidly regulate neurotransmitter metabolism through means other than modification of protein synthesis. In fact, this is the first demonstration that any hormone may have a direct action on presynaptic sites. Details of the molecular events of the glucocorticoid action on nerve terminals are current subjects of investigation in our laboratory.

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