Br. J. Pharmac. (1971), **43**, 39–52.

Regional and subcellular changes in the concentration of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the rat brain caused by hydrocortisone, DL-α-methyltryptophan, l-kynurenine and immobilization

G. CURZON AND A. R. GREEN*

Department of Chemical Pathology, Institute of Neurology, Queen Square, London, WC1

Summary

1. In agreement with previous findings on whole brain, the intraperitoneal injection of hydrocortisone, DL-α-methyltryptophan or L-kynurenine decreased the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in different regions of the rat brain.

2. Hydrocortisone caused similar decreases in the concentrations of both 5-HT and 5-HIAA, suggesting decreased 5-HT synthesis.

3. Changes in the concentration of 5-HIAA after hydrocortisone corresponded significantly to those after α-methyltryptophan. Changes in the concentration of 5-HT did not correspond, possibly due to falsely high 5-HT values because of interfering material derived from α-methyltryptophan.

4. In general, kynurenine caused larger decreases in the concentration of 5-HT than in the concentration of 5-HIAA.

5. In agreement with previous findings with whole brain, immobilization of rats for 5 h decreased the concentration of 5-HT and increased that of 5-HIAA in most brain regions.

6. The order of the percentage decreases in the concentrations of 5-HIAA 6 h after hydrocortisone injection was, in decreasing order: hypothalamus, striatum, cerebellum, mid-brain, pons + medulla and cortex. The percentage increases after immobilization for 5 h were in the reverse order.

7. The differences between the percentage decreases in the concentration of 5-HIAA after hydrocortisone and the percentage increases after immobilization were very similar in all regions except the hypothalamus. This is consistent with immobilization stress increasing the firing rate of 5-hydroxytryptaminergic neurones similarly in different regions.

8. During the first 3 h of immobilization the concentrations of 5-HIAA in the hypothalamus and in the rest of the brain increased approximately in parallel. Between 3 and 5 h, 5-HIAA returned to control concentrations in the hypothalamus while continuing to rise in the rest of the brain.

9. Relative changes in the concentration of 5-HT in particulate and supernatant fractions after the various treatments were comparable except 2 h

* Present address: Laboratory of Preclinical Pharmacology, National Institute of Mental Health, St. Elizabeth's Hospital, Washington D.C. 20032, USA.

after kynurenine injection when the concentration of 5-HT fell in the particulate but not in the supernatant fraction. The concentration of 5-HT did fall in the latter, though more slowly than in the former fraction, suggesting a concentration of amine synthesizing organelles in particulate material.

Introduction

A single intraperitoneal injection of hydrocortisone (5 mg/kg) causes a decrease in the concentration of 5-hydroxytryptamine (5-HT) and of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in rat brain, suggesting decreased 5-HT synthesis (Curzon & Green, 1968). The change in brain 5-HT metabolism may be a consequence of altered peripheral tryptophan metabolism since the liver enzyme tryptophan pyrrolase is induced by hydrocortisone (Knox & Auerbach, 1955) and administration of an inhibitor of this enzyme prevents the changes in brain amines. Furthermore α-methyltryptophan, which also increases pyrrolase activity, causes a decrease of brain 5-HT (Green & Curzon, 1968 ; Sourkes, Missala & Oravec, 1970). Certain tryptophan metabolites (Kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid), which are formed subsequent to pyrrolase action, have a similar effect on the concentration of 5-HT (Green & Curzon, 1970).

Immobilization also causes an increase in the activity of rat liver tryptophan pyrrolase (Nomura, 1965 ; Curzon & Green, 1969) presumably due to induction of the enzyme by corticosterone, since secretion of the latter increases upon immobilization (De Schaepdryver, Preziosi & Scapagnini, 1969). Previous work has indicated that during immobilization there is a decrease in the concentration of 5-HT in the brain apparently due to the pyrrolase change, and that brain 5-HT turnover also probably increases since the concentration of 5-HIAA in the brain rises (Curzon & Green, 1969). These changes have been confirmed (Nistico & Preziosi, 1969).

In the above work changes in whole brain only were investigated. In further investigations of these effects and their possible significance with respect to brain function, the changes in specific brain regions and in particulate and supernatant subcellular material have been determined.

Methods

Adult male Sprague-Dawley rats, 180–220 g (Animal Suppliers, London) were given an *ad libitum* diet of Oxo 41B pellets, and tap water and kept under controlled conditions as previously described (Curzon & Green, 1969). Animals were killed by stunning with a sharp blow across the thorax followed by decapitation. Animals were always killed at the same time of day (1600–1700 h) to minimize variations due to diurnal changes of 5-HT and pyrrolase. Immobilization procedures and subsequent killing (between 1600–1700 h) were as described by Curzon & Green, 1969. The following drugs were used: hydrocortisone sodium succinate (' Solu-Cortef ' Upjohn Ltd.), DL-α-methyltryptophan (Merck, Sharp & Dohme, Ltd.), and L-kynurenine sulphate (Calbiochem Ltd.). All drugs were dissolved in 0·9% w/v sodium chloride solution, and injected intraperitoneally in a volume of 5 ml/kg body weight. Concentrations of hydrocortisone

sodium succinate are quoted in terms of hydrocortisone. Control animals for the drug experiments were injected with 0·9% w/v sodium chloride solution, while those for the immobilization experiments were uninjected.

Regional 5-HT and 5-HIAA

After killing, the brain was rapidly removed, placed on a glass plate set in ice at −25° C and dissected by the procedure of Glowinski & Iversen (1966) except that the hippocampus was not separated from the mid-brain. Dissected material was stored at −25° C until use. The degree of reproducibility of the dissection technique is demonstrated in Table 1 which shows the mean weight and its standard deviation for each dissected region. 5-HT and 5-HIAA were measured in the same region of a single brain using *O*-phthalaldehyde as described by Curzon & Green (1970).

The effect of hydrocortisone and kynurenine on the concentrations of brain 5-HT and 5-HIAA were investigated at previously determined times of maximum 5-HT change. Thus concentrations were studied 6 h after hydrocortisone injection (Curzon & Green, 1968), 2 h after kynurenine injection (Green & Curzon, 1970) and 5 h after the start of immobilization (Curzon & Green, 1969). Time courses of changes after α-methyltryptophan injection were not investigated and animals were therefore killed 6 h after injection at which time appreciable 5-HT and 5-HIAA changes occur (Green & Curzon, 1968).

Subcellular distribution of 5-HT

Giarman & Schanberg (1959) observed that brain particles containing 5-HT tend to lose the amine during subcellular fractionation; they therefore developed a rapid, though crude, method for the separation of brain into particulate and supernatant fractions (Giarman, Freedman & Schanberg, 1964). A modification of this method was used in our investigation. The animal was killed and the brain removed and homogenized in 16 ml of cold (4° C) 0·25 M sucrose containing 0·002 M sodium ethylene diaminetetraacetic acid (EDTA) and 0·05 mM pargyline hydrochloride, using a Potter-Elvehjem type homogenizer with serrated Teflon pestle ('Uni-Form', Jencons Ltd.). The homogenizer volume was 25 ml, pestle clearance 0·0066–0·010 inches and homogenization was performed using five strokes at 500 r.p.m. All preparative procedures were carried out at 4° C. Homogenate (6 ml) was centrifuged at 100,000 *g* (average) for 20 min at 0° C using an MSE '50' ultra-centrifuge with a 3 × 10 ml swing out rotor. The supernatant was removed, adjusted to pH 10 by addition of NaOH (0·5N) and transferred to a 25 ml stoppered tube containing 8 ml of n-butanol, 3 g NaCl and 1 ml of 0·5 M borate buffer (pH 10). The pellet was resuspended in 5 ml 0·5 M borate buffer (pH 10) and transferred to a 25 ml stoppered tube containing 8 ml n-butanol

TABLE 1. *Reproducibility of dissection technique*

Brain region	Wt. (mg)
Cortex	762±58 (50)
Mid-brain	291±15 (49)
Pons+medulla	178±16 (49)
Cerebellum	236±20 (49)
Striatum	104±18 (48)
Hypothalamus	57± 9 (49)

No. of determinations shown in parentheses. Results expressed as mean ±1 S.D.

TABLE 2. *Concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in regions of rat brain*

	Cortex	Pons+medulla	Midbrain	Cerebellum	Striatum	Hypothalamus	Whole brain*
Injected with 0·9% sodium chloride solution							
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·52± 0·03 (11)	1·32± 0·08 (12)	1·04± 0·19 (11)	0·45± 0·08 (11)	1·67±0·30 (12)	2·64± 0.26 (12)	0·83
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wet wt.)	0·20± 0·03 (10)	0·60± 0·13 (8)	0·50± 0·04 (10)	0·21± 0·04 (9)	0·78± 0·21 (8)	1·09± 0·31 (10)	0·36
Ratio 5-HT/5-HIAA	2·60± 0·25 (8)	2·21± 0·38 (8)	2·08± 0·38 (8)	2·15± 0·44 (6)	2·14± 0·34 (8)	2·42± 0·28 (8)	2·30
Uninjected							
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·52± 0·03 (6)	1·24± 0·10 (6)	1·03± 0·10 (5)	0·39±0·08 (6)	1·36±0·23 (5)	2·56±0·22 (6)	0.79
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wet wt.)	0·20± 0·03 (7)	0·54± 0·04 (8)	0·51± 0·05 (7)	0·18± 0·01 (8)	0·69± 0·08 (6)	1·07±0·09 (6)	0·35
Ratio 5-HT/5-HIAA	2·60± 0·20 (5)	2·30± 0·35 (6)	2·01± 0·08 (6)	2·17± 0·18 (5)	1·97± 0.10 (4)	2·39± 0·27 (5)	2.25

* Calculated from the average concentration and weight of each region. Results expressed as mean ±1 S.D. Nos. of determinations shown in parentheses.

TABLE 3. *Effect of hydrocortisone (5 mg/kg) on the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in regions of rat brain*

	Cortex	Pons+medulla	Midbrain	Cerebellum	Striatum	Hypothalamus	Whole brain*
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·45± 0·03 (13)†	1·14± 0·13 (13)†	0·79±0·05 (11)†	0·32± 0·04 (12)†	1·37± 0·13 (12)†	2·07± 0·30 (13)†	0·68
Percentage decrease in 5-HT from control	12·9 ± 4·2 (11)	15·6 ± 8·4 (12)	15·5 ± 7·6 (10)	23·0 ± 8·2 (12)	22·6 ± 8·1 (12)	19·8 ± 8·4 (13)	18·0
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wrt wt.)	0·16± 0·01 (13)†	0·55± 0·12 (12)	0·42± 0·07 (10)†	0·17± 0·04 (12)‡	0·63± 0·05 (11)§	0·81± 0·25 (11)§	0·30
Percentage decrease in 5-HIAA from control	13·5 ± 6·2 (13)	14·2 ± 7·3 (12)	20·18±12·7 (10)	22·7 ± 8·3 (12)	25·7 ± 6·7 (11)	27·4 ± 7·0 (11)	16·7

Rats were killed 6 h after injection of hydrocortisone. * Calculated from average concentration and weight of each region. Results expressed as mean ±1 S.D. † Different from controls (Table 2) $P<0·001$. ‡ Different from controls (Table 2) $P<0·01$. § Different from controls (Table 2) $P<0·05$. Nos. of determinations shown in parentheses.

TABLE 4. *Effect of* DL-α-*methyltryptophan* (25 *mg/kg*) *on the concentrations of* 5-*hydroxytryptamine* (5-*HT*) *and* 5-*hydroxyindoleacetic acid* (5-*HIAA*) *in regions of rat brain*

	Cortex	Pons+medulla	Midbrain	Cerebellum	Striatum	Hypothalamus	Whole brain*
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·45± 0·06 (8)‡	1·14± 0·08 (8)†	0·88± 0·09 (8)§	0·33± 0·06 (8)†	1·38± 0·24 (6)	2·01± 0·20 (8)†	0·69
Percentage change in 5-HT from control	14·9 ±10·0 (6)	20·3 ±10·9 (6)	25·0 ±10·0 (6)	29·7 ± 9·7 (5)	17·3 ±10·6 (5)	24·3 ±12·0 (7)	16·9
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wet wt.)	0·17± 0·03 (8)‡	0·37± 0·08 (7)†	0·31± 0·07 (8)†	0·14± 0·05 (8)†	0·47± 0·09 (6)‡	0·62± 0·15 (8)†	0·25
Percentage change in 5-HIAA from control	23·0 ±18·2 (8)	26·2 ±13·5 (6)	39·2 ±10·3 (7)	21·0 ± 8·6 (4)	39·1 ±10·6 (6)	43·5 ±14·9 (8)	30·5

Rats were killed 6 h after injection of α-methyltryptophan. * Calculated from average concentration and weight of each region. Results expressed as mean ± 1 S.D. † Different from controls (Table 2) $P<0·001$. ‡ Different from controls (Table 2) $P<0·01$. § Different from controls (Table 2) $P<0·05$. Nos. of determinations shown in parentheses.

TABLE 5. *Effect of* L-*kynurenine* SO_4 (5 *mg/kg*) *on the concentrations of* 5-*hydroxytryptamine* (5-*HT*) *and* 5-*hydroxyindoleacetic acid* (5-*HIAA*) *in regions of rat brain*

	Cortex	Pons+medulla	Midbrain	Cerebellum	Striatum	Hypothalamus	Whole brain*
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·45± 0·07 (6)‡	0·92± 0·08 (5)†	0·81± 0·10 (6)‡	0·30± 0·08 (6)†	1·18± 0·20 (6)†	1·77± 0·17 (6)†	0·63
Percentage change in 5-HT from control	17·9 ± 6·8 (4)	25·7 ±14·5 (5)	22·0 ±11·2 (6)	32·3 ±18·7 (6)	16·0 ± 8·2 (4)	28·7 ± 5·7 (6)	23·8
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wet wt.)	0·19± 0·03 (6)	0·46 ±0·05 (5)§	0·41± 0·09 (6)‡	0·17± 0·02 (6)	0·55± 0·16 (6)§	0·88± 0·07 (6)	0·30
Percentage change in 5-HIAA from control	13·6 ± 3·5 (6)	16·8 ±11·8 (5)	20·0 ± 8·4 (6)	7·6 ± 9·4 (6)	21·2 ± 4·4 (4)	11·6 ± 6·2 (5)	16·7

Rats were killed 2 h after injection of L-kynurenine. *Calculated from average concentration and weight of each region. Results expressed as mean ± 1 S.D. † Different from controls (Table 2) $P<0·001$. ‡Different from controls (Table 2) $P<0·01$. §Different from controls (Table 2) $P<0·05$. Nos. of determinations shown in parentheses.

and 3 g NaCl. The tubes were shaken for 10 min, centrifuged at 3,000 r.p.m. for 3 min and 5 ml of the supernatant n-butanol phase pipetted into a 25 ml stoppered tube containing 7·5 ml n-heptane and 0·7 ml of 0·05 M phosphate buffer (pH 7). After shaking and centrifugation as before, the organic phase was discarded and 0·6 ml of the aqueous phase added to 0·05 ml of 0·1 M ninhydrin in a 10 × 25 mm test tube, heated for 30 min at 75° C, cooled at room temperature for 45 min and the fluorescence measured in micro-cuvettes in a Farrand spectro-photofluorometer, activation and fluorescence wave-lengths being 365 nm and 490 nm (both uncorrected) respectively. Activation and fluorescence spectra were essentially as described by Vanable (1963) for the 5-HT-ninhydrin complex.

Results are expressed as means ±1 standard deviation and significances calculated using Student's *t* test. The percentage changes from control concentrations have been calculated from the change in each experiment compared with results

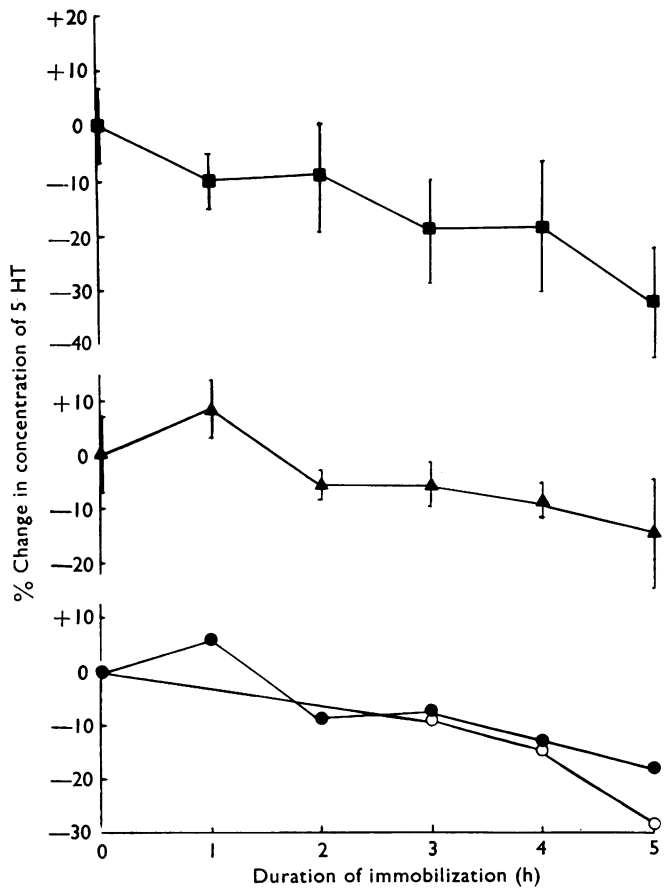

FIG. 1. Effect of immobilization on the concentration of 5-hydroxytryptamine (5-HT) in the hypothalamus and rest of the brain. Each point represents the mean of determinations on at least six rats ±1 S.D. (shown by vertical bars). (■), 5-HT in hypothalamus (control value = 1·98 ± 0·14 μg/g wet wt., eleven animals). (▲), 5-HT in rest of brain (control value = 0·56 ± 0·02 μg 5-HT/g wet wt., eleven animals). (●), 5-HT in whole brain calculated from above data; (○), 5-HT in whole brain determined directly (results from Curzon & Green, 1969).

for a control animal obtained at the same time. Results for whole brain have been calculated from the mean 5-HT or 5-HIAA concentration and mean weight of each region.

Results

Concentrations of 5-HT and 5-HIAA in brain regions of control rats

Concentrations of 5-HT and 5-HIAA in brain regions of rats injected with 0·9% sodium chloride solution were not significantly different from values obtained using uninjected rats (Table 2).

Effect of hydrocortisone on the concentrations of 5-HT and 5-HIAA in the rat brain

In agreement with earlier determinations on whole brain (Curzon & Green, 1968), 5 mg/kg hydrocortisone caused significant falls in the concentrations of both 5-HT and 5-HIAA in the regions of rat brain (Table 3) studied when compared with control values (Table 2), the only exception being in the case of 5-HIAA in the pons + medulla. In general the percentage falls of 5-HT were similar to those of 5-HIAA. There were moderate differences between the per-

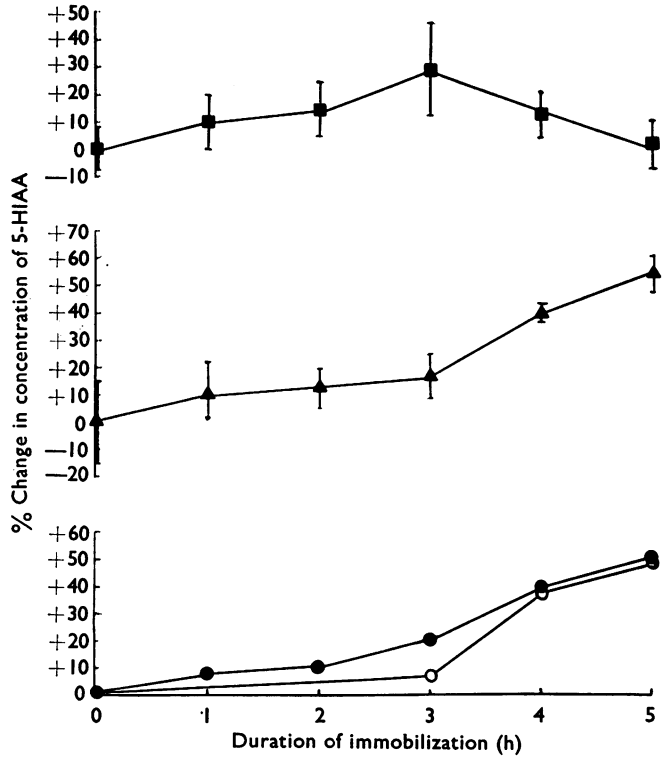

FIG. 2. Effect of immobilization on the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus and rest of the brain. Each point represents the mean of determinations on at least four rats ± 1 S.D. (shown by vertical bars). (■), 5-HIAA in hypothalamus (control value = 0·76 ± 0·06 μg/g wet wt., ten animals). (▲), 5-HIAA in rest of brain (control value = 0·34 ± 0·05 μg/g wet wt., nine animals. (●), 5-HIAA in whole brain calculated from above data, (○), 5-HIAA in whole brain determined directly (Curzon & Green, 1969).

centage changes in different regions. Concentrations in cortex, mid-brain and pons+medulla were less affected than in cerebellum, striatum and hypothalamus. Percentage changes in the concentrations of both 5-HT and 5-HIAA in the cortex and of 5-HIAA in the pons+medulla were significantly less than in each of the three latter regions.

Effect of DL-α-*methyltryptophan on the concentrations of 5-HT and 5-HIAA in rat brain*

Injection of α-methyltryptophan leads to prolonged increase of pyrrolase activity (Moran & Sourkes, 1963) largely by a direct effect on the enzyme (Civen & Knox, 1960), and also to a decrease in the concentration of brain 5-HT (Green & Curzon, 1968; Sourkes *et al.*, 1970).

The effects of α-methyltryptophan on the concentrations of 5-HT and 5-HIAA in regions of the brain 6 h after injection are shown in Table 4. Concentrations of both 5-HT and 5-HIAA were significantly decreased in all regions except in the case of 5-HT in the striatum. In agreement with Sourkes *et al.* (1970) the calculated change of 5-HIAA in whole brain was relatively larger than that of 5-HT and this was also found for all regions except the cerebellum. The percentage changes of 5-HIAA in different areas followed the same pattern as those after hydrocortisone injection except in the case of the cerebellum where changes were relatively small.

Effect of L-*kynurenine sulphate on the concentration of 5-HT and 5-HIAA in rat brain*

The concentration of tryptophan in the plasma of the rat falls only transiently after injection of hydrocortisone and the concentration of brain tryptophan does not change significantly (Green, Joseph & Curzon, 1970). Therefore, the fall in concentration of brain 5-HT after injection of hydrocortisone is probably not simply due to increased metabolism of tryptophan because of high pyrrolase activity resulting in a deficiency of tryptophan for 5-HT synthesis. As injection of L-kynurenine, L-3-hydroxykynurenine and 3-hydroxyanthranilic acid (metabolites formed subsequent to pyrrolase action on tryptophan) also caused a decrease in the concentration of whole brain 5-HT (Green & Curzon, 1970) the effect of L-kynurenine on concentrations of 5-HT and 5-HIAA in regions of rat brain was investigated. Two hours after injection of kynurenine the concentration of 5-HT was significantly decreased in all regions (Table 5).

The observation that the changes in 5-HIAA were smaller than those of 5-HT is in agreement with findings with whole brain (Green & Curzon, 1970). Decreases of 5-HIAA were smallest and those of 5-HT greatest in the hypothalamus and cerebellum.

Effect of immobilization for 5 h on the concentration of 5-HT and 5-HIAA in the brain

The concentration of 5-HT was significantly lower in all regions of the brain except the striatum and cerebellum after immobilization for 5 h (Table 6) than in control animals (Table 2). The concentration of 5-HIAA was significantly increased in all regions except the hypothalamus. Changes in the concentrations

TABLE 6. *Effect of immobilisation for 5 h on the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in regions of rat brain*

	Cortex	Pons+medulla	Midbrain	Cerebellum	Striatum	Hypothalamus	Whole brain*
5-Hydroxytryptamine (μg 5-HT/g brain; wet wt.)	0·43± 0·06 (5)‡	1·03± 0·12 (5)‡	0·84± 0·12 (4)‡	0·32± 0·05 (6)	1·53± 0·27 (6)	2·23± 0·19 (6)‡	0·68
Percentage decrease in 5-HT from control	17·2 ± 7·7 (5)	15·9 ± 7·3 (5)	19·3 ± 8·1 (4)	20·2± 9·5 (6)	−13·0 ± 4·9 (4)	15·0 ± 9·3 (6)	13·9
5-Hydroxyindoleacetic acid (μg 5-HIAA/g brain; wt wt.)	0·29± 0·04 (10)†	0·73± 0·11 (8)†	0·67± 0·15 (10)†	0·24± 0·02 (10)†	0·87± 0·13 (10)†	1·01± 0·11 (10)	0·46
Percentage increase in 5-HIAA from control	49·9 ±30·0 (10)	45·4± 14·6 (8)	43·0 ±19·9 (10)	37·7 ±24·2 (9)	30·0 ±17·0 (10)	− 4·3±4·5 (8)	31·5

*Calculated from the average concentration and weight in each region. Results expressed as mean ± 1 S.D. † Different from controls (Table 2) $P<0.001$. ‡ Different from controls (Table 2) $P<0.05$. Nos. of determinations shown in parentheses.

TABLE 7. *Effect of various treatments on concentrations of subcellular 5-hydroxytryptamine (5-HT)*

Treatment	Time after injection (h)	μg 5-HT/g brain supernatant	μg 5-HT/g brain Particulate	μg 5-HT/g brain total	Percentage 5-HT in supernatant
Saline	6	0·29±0·01 (13)	0·50±0·02 (13)	0·79±0·03 (13)	36·23±0·83 (13)
Hydrocortisone (5 mg/kg)	6	0·22±0·03 (9)†	0·43±0·04 (10)†	0·65±0·06 (9)†	33·77±3·52 (9)§
α-Methyltryptophan (25 mg/kg)	6	0·22±0·03 (10)†	0·37±0·02 (10)†	0·60±0·09 (10)†	37·20±2·48 (10)
L-kynurenine sulphate (5 mg/kg)	2	0·28±0·03 (8)	0·40±0·02 (8)†	0·67±0·04 (8)†	41·00±3·29 (8)†
Uninjected	—	0·29±0·02 (7)	0·50±0·03 (9)	0·80±0·02 (7)	35·71±1·38 (7)
Immobilized	5*	0·23±0·2 (6)†	0·40±0·09 (6)‡	0·64±0·03 (6)†	36·83±2·31 (6)

Results expressed as mean ± 1 S.D. * Duration of immobilization. † Different from respective control $P<0.001$. ‡ Different from respective control $P<0.01$. § Different from respective control $P<0.05$. Nos. of determinations shown in parentheses.

of 5-HT and 5-HIAA in the whole brain calculated from the concentrations found in different regions of the brain were in qualitative agreement with previous determinations on whole brain (Curzon & Green, 1969).

Effect of the duration of immobilization on the concentration of 5-HT and 5-HIAA in the hypothalamus

Because of the striking difference between the effects of immobilization for 5 h on the concentration of 5-HIAA in the hypothalamus and the concentrations in other regions, the concentrations of 5-HT and 5-HIAA in the hypothalamus and 'rest of brain' (comprising the whole brain minus hypothalamus) were determined after various periods of immobilization. Results for 5-HT are shown in Fig. 1 and for 5-HIAA in Fig. 2.

The concentration of 5-HT in the hypothalamus decreased from control levels after 1 h of immobilization and continued to fall when immobilization was prolonged to 5 hours. The percentage fall after immobilization for 5 h was greater

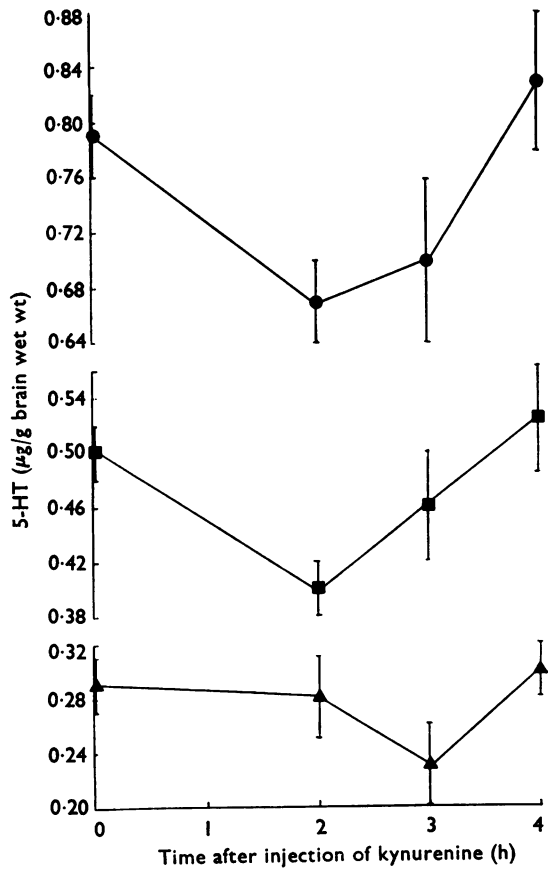

FIG. 3. Effect of L-kynurenine sulphate (5 mg/kg) on the concentration of 5-hydroxytryptmine (5-HT) in particulate and supernatant fractions of rat brain. Each point represents the mean of determinations on at least eight rats ± 1 S.D. (shown by vertical bars). (■), 5-HT in particulate fraction. (▲), 5-HT in supernatant fraction, (●), 5-HT in particulate + supernatant fractions calculated from above data.

than that found in the experiments indicated in Table 6. This difference is not readily explicable but might be related to quicker dissection as the 'rest of brain' was not subdivided. The concentration of 5-HT in the 'rest of brain' rose after immobilization for 1 h and then fell during the prolonged immobilization.

The concentration of 5-HIAA in the hypothalamus rose during immobilization for 3 h then fell reaching control values at 5 hours. The concentration of 5-HIAA in the 'rest of brain' rose slowly during the first 3 h of immobilization and then at a faster rate until 5 hours. Calculated changes in the concentrations of 5-HT and 5-HIAA for whole brain were comparable to previous findings (Curzon & Green, 1969).

Effects of hydrocortisone, DL-*α-methyltryptophan,* L-*kynurenine and immobilization on the concentration of 5-HT in the supernatant and particulate fractions from rat brain*

Hydrocortisone, α-methyltryptophan and immobilization all caused significant falls in the concentration of 5-HT in both the particulate and supernatant fractions of rat brain (Table 7). However, 2 h after injection of kynurenine, although there was a fall in the concentration of 5-HT in the particulate fraction, there was essentially no change in the supernatant fraction. Investigation of the time courses of changes in 5-HT after injection of L-kynurenine showed that while the particulate concentration of 5-HT was at a minimum 2 h after injection, the concentration of 5-HT in the supernatant decreased more slowly to a minimum concentration 3 h after injection (Fig. 3). The concentrations in both fractions had returned to normal 4 h after injection.

Discussion

Injection of hydrocortisone, α-methyltryptophan and L-kynurenine decreased the concentrations of 5-HT and 5-HIAA in regions of the brain in agreement with earlier findings with whole brain (Curzon & Green, 1968; Green & Curzon, 1968; Green & Curzon, 1970). Percentage decreases of 5-HT in the various regions of the brain after injection of hydrocortisone were generally similar to those of 5-HIAA in the same region as previously found for whole brain (Curzon & Green, 1968) suggesting the fall of 5-HT was due to decreased 5-HT synthesis. Although the percentage decreases of 5-HIAA in different regions after injection of hydrocortisone corresponded significantly to those after α-methyltryptophan injection, which might indicate some similarity in the mechanisms involved, there was no correspondence between the changes in 5-HT occurring after injection of the two drugs. Neither did the decreases in concentration of 5-HT after injection of α-methyltryptophan correlate with those of 5-HIAA. These anomalous findings with 5-HT after α-methyltryptophan are probably due to the formation of α-methyl-5-HT which can lead to falsely high values (Sourkes, 1971). In this case the decreases in concentration of 5-HIAA may reflect more closely the changes in the true concentration of 5-HT than do the apparent changes in 5-HT.

After injection of L-kynurenine changes in the concentration of 5-HIAA tended to be less than those of 5-HT, perhaps because the rapidity of its action resulted in the maximum effect on the concentration of 5-HIAA occurring later.

Previous work indicated that during immobilization, stress decreased synthesis of cerebral 5-HT due to adrenocortical changes and an increased turnover of

cerebral 5-HT are reflected in decreased brain 5-HT and increased 5-HIAA (Curzon & Green, 1969; Nistico & Preziosi, 1969). Similar changes were found in this study of brain regions after immobilization for 5 h; the exceptions were the striatum in which there was no significant change of 5-HT and the hypothalamus in which there was no change of 5-HIAA. Whilst the order of the percentage decreases in the concentration of 5-HIAA in brain regions 6 h after injection of hydrocortisone is, in decreasing order: hypothalamus, striatum, cerebellum, mid-brain, pons + medulla and cortex, the percentage increases after stress caused by immobilization for 5 h are in the reverse order.

Increases of 5-HIAA during immobilization are probably related to increased firing of 5-hydroxy tryptaminergic neurones (Aghajanian, Rosecrans & Sheard, 1967). The above relation between the effects of immobilization and hydrocortisone would occur if immobilization caused similar increases in the rates of firing of 5-hydroxy tryptaminergic neurones in different brain regions. Such similar increases would result in net percentage increases of 5-HIAA being smaller in those brain regions with larger opposing decreases in 5-HIAA due to adrenocortical changes. With the notable exception of the hypothalamus the differences between percentage decreases of 5-HIAA 6 h after injection of hydrocortisone (Table 3) and percentage increases after immobilization for 5 h (Table 6) are very similar: cortex, 63%; pons + medulla, 60%; mid-brain, 63%; cerebellum, 60%; striatum, 56% (but hypothalamus, 23%). These differences probably more closely reflect the increase of 5-HT breakdown to 5-HIAA than do the net 5-HIAA increases. The method used for adjusting the 5-HIAA increase after immobilization for 5 h for the concomitant decrease due to adrenocortical changes has some validity as the latter changes appear similar to those after injection of hydrocortisone insofar as they are reflected by similar increase in the activity of liver pyrrolase (Curzon & Green, 1969). Also the difference of 1 h between times of killing the animals in the immobilization and hydrocortisone experiments would have had negligible effect on concentrations of 5-HIAA (Curzon & Green, 1968, 1969).

Investigations of the negligible net change in 5-HIAA in the hypothalamus after immobilization for 5 h indicated that whilst the concentration of 5-HIAA in both the hypothalamus and the rest of brain rose until immobilization for 3 h, hypothalamic values fell to control levels by 5 h whilst those in the rest of the brain (as shown in Fig. 3) rose steeply after immobilization for 3 and 5 hours. It was found previously that when immobilization was prolonged for more than 6 h then the concentration of 5-HIAA in whole brain fell towards control values (Curzon & Green, 1969). Thus immobilization results in a peak in the concentration of 5-HIAA in the hypothalamus, which precedes the peak found for the brain as a whole. This finding may be compared with the suggestion of a rapid transient increase in the 5-hydroxytryptaminergic nerve activity in the hypothalamus and preoptic area but a delayed increase in the striatum on exposure of rats to high environmental temperature (Simmonds, 1970).

The apparent special response of the hypothalamus to stress situations is of interest in view of its neuroendocrine functions. It may also be of significance that after injection of hydrocortisone or α-methyltryptophan the mean fall in the concentration of 5-HIAA in the hypothalamus was greater than that in other areas. This might point to a particular sensitivity of 5-HT metabolism in the hypo-

thalamus to adrenocortical and/or pyrrolase changes in which could be relevant to suggested possible relations between such changes and mood (Curzon, 1969; Curzon & Bridges, 1970).

The initial rise in the concentrations of 5-HT in the rest of the brain during immobilization is in agreement with other workers who found moderate rises of brain 5-HT during short periods of stress. Thus immobilization stress for 1 h in rats (Nistico & Preziosi, 1969) or restraint stress for 2·5 h in mice (Welch & Welch, 1968) caused small increases in the concentration of 5-HT in the brain while Thierry, Fekete & Glowinski (1968) found an increased concentration of 5-HT in the rat brain after 3 h of intermittent foot shock. The decrease in concentration after immobilization for 3 or more hours agrees with our earlier work (Curzon & Green, 1969) and that of Corrodi, Fuxe & Hökfelt (1968) and Nistico & Preziosi (1969).

There were no striking differences between the relative changes in 5-HT in particulate and supernatant fractions after injection of hydrocortisone or α-methyl-tryptophan or after immobilization. However, 2 h after injection of L-kynurenine a significant fall in the concentration of 5-HT occurred only in the particulate fraction. The subsequent fall of 5-HT in the supernatant may indicate that the 5-HT of 5-HT synthesizing organelles is found only in the particulate fraction, so that decreased 5-HT synthesis is first manifest here and is only reflected later in the supernatant. These time dependent differences may be apparent after injection of L-kynurenine but not after hydrocortisone because of the greater time needed for an appreciable fall in the concentration of brain 5-HT in the latter case and thus the opportunity for differences to be obscured by equilibration of, or transport between, pools.

We thank Professor T. L. Sourkes for allowing us see his paper before publication, the Mental Health Research Fund for financial support and Merck, Sharp and Dohme Ltd. for supplies of α-methyltryptophan.

REFERENCES

AGHAGANIAN, G. K., ROSECRANS, J. A. & SHEARD, M. H. (1967). Serotonin: release in the forebrain by stimulation of midbrain raphe. *Science, N.Y.*, **156**, 402–403.

CIVEN, M. & KNOX, W. E. (1960). The specificity of tryptophan analogues as inducers, substrates, inhibitors and stabilizers of liver tryptophan pyrrolase. *J. biol. Chem.*, **235**, 1716–1718.

CORRODI, H., FUXE, K. & HÖKFELT, T. (1968). The effect of immobilization stress on the activity of central monoamine neurons. *Life Sci.*, **7**, 107–112.

CURZON, G. (1969). Tryptophan pyrrolase—a biochemical factor in depressive illness? *Br. J. Psychiat.*, **115**, 1367–1374.

CURZON, G. & BRIDGES, P. K. (1970). Tryptophan metabolism in depression. *J. Neurol. Neurosurg. Psychiat.*, **33**, 698–704.

CURZON, G. & GREEN, A. R. (1968). Effect of hydrocortisone on rat brain 5-hydroxytryptamine. *Life Sci.*, **7**, 657–663.

CURZON, G. & GREEN, A. R. (1969). Effects of immobilization on rat liver tryptophan pyrrolase and brain 5-hydroxytryptamine metabolism. *Br. J. Pharmac.*, **37**, 689–697.

CURZON, G. & GREEN, A. R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39**, 653–655.

DE SCHAEPDRYVER, A., PREZIOSI, P. & SCAPAGNINI, U. (1969). Brain monoamines and adrenocortical activation. *Br. J. Pharmac.*, **35**, 460–467.

GIARMAN, N. J. & SCHANBERG, S. M. (1959). The intracellular distribution of 5-hydroxytryptamine (5-HT, Serotonin) in the rat's brain. *Biochem. Pharmac.*, **1**, 301–306.

GIARMAN, N. J., FREEDMAN, D. X. & SCHANBERG, S. M. (1964). Drug induced changes in the subcellular distribution of serotonin in rat brain with special reference to the action of reserpine. *Progr. Brain Res.*, **8**, 72–80.

GLOWINSKI, J. & IVERSEN, L. L. (1966). Regional studies of catecholamines in the brain—I The disposition of (^{3}H) norepinephrine, (^{3}H) dopamine and (^{3}H) dopa in various regions of the brain. *J. Neurochem.*, **13**, 655–669.

GREEN, A. R. & CURZON, G. (1968). Decrease of 5-hydroxytryptamine in the brain provoked by hydrocortisone and its prevention by Allopurinol. *Nature, Lond.*, **220**, 1095–1097.

GREEN, A. R. & CURZON, G. (1970). The effect of tryptophan metabolites on brain 5-hydroxytryptamine metabolism. *Biochem. Pharmac.*, **19**, 2061–2068.

GREEN, A. R., JOSEPH, M. H. & CURZON, G. (1970). Oral contraceptives, depression and amino acid metabolism. *Lancet*, **1**, 1288.

KNOX, W. E. & AUERBACH, V. H. (1955). Hormonal control of tryptophan peroxidase in the rat. *J. biol. Chem.*, **214**, 307–313.

MORAN, J. F. & SOURKES, T. L. (1963). Induction of tryptophan pyrrolase by α-methyltryptophan and its metabolic significance in vivo. *J. biol. Chem.*, **238**, 3006–3008.

NISTICO, G. & PREZIOSI, P. (1969). Brain and liver tryptophan pathways and adrenocortical activation during restraint stress. *Pharmac. Res. Comm.*, **1**, 363–368.

NOMURA, J. (1965). Effect of stress and psychotropic drugs on rat liver tryptophan pyrrolase. *Endocrinology*, **76**, 1190–1194.

SIMMONDS, M. A. (1970). Effect of environmental temperature on the turnover of 5-hydroxytryptamine in various areas of rat brain. *J. Physiol., Lond.*, **211**, 93–108.

SOURKES, T. L. (1971). Alpha-methyltryptophan and its actions on tryptophan metabolism. *Fedn. Proc.*, **30**, 897–903.

SOURKES, T. L., MISSALA, K. & ORAVEC, M. (1970). Decrease of cerebral serotonin and 5-hydroxyindoleacetic acid caused by (−)-αmethyltryptophan. *J. Neurochem.*, **17**, 111–115.

THIERRY, A. M., FEKETE, M. & GLOWINSKI, J. (1968). Effects of stress on the metabolism of noradrenaline, dopamine and serotonin in the central nervous system of the rat (II) Modifications of serotonin metabolism. *Eur. J. Pharmac.*, **4**, 384–389.

VANABLE, J. W. (1963). A ninhydrin reaction giving a sensitive quantitative fluorescence assay for 5-hydroxytryptamine. *Anal. Biochem.*, **6**, 393–403.

WELCH, B. L. & WELCH, A. S. (1968). Differential activation by restraint stress of a mechanism to conserve brain catecholamines and serotonin in mice differing in excitability. *Nature, Lond.*, **218**, 575–577.

(*Received January* 13, 1971)