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Effects of immobilization on rat liver tryptophan pyrrolase and brain 5-hydroxytryptamine metabolism

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1. Rat liver tryptophan pyrrolase increased on immobilization. The concentration of 5-hydroxyindoleacetic acid in the brain also rose and that of 5-hydroxytryptamine fell.

2. When adrenalectomized rats were immobilized pyrrolase activity did not rise and brain 5-hydroxytryptamine concentration fell to a lesser extent but the 5-hydroxyindoleacetic acid concentration rose as in intact animals.

3. When intact rats were injected with the pyrrolase inhibitor Allopurinol both the increase of pyrrolase and the fall of 5-hydroxytryptamine on immobilization were less prominent but the concentration of 5-hydroxyindoleacetic acid rose as before. Allopurinol did not affect the changes in immobilized adrenalectomized rats.

4. Immobilization thus appears to cause (*a*) decreased brain 5-hydroxytryptamine synthesis resulting from pyrrolase induction and (*b*) increased 5-hydroxytryptamine breakdown by a more direct effect on the brain. Results of experiments on rats injected with lysergic acid diethylamide, and with α-methyltryptophan or probenecid are consistent with the above interpretation.

5. The 5-hydroxytryptamine and 5-hydroxyindoleacetic acid changes were maximal after 5–6 hours' immobilization and became less on more prolonged immobilization, which suggests regulatory changes.

A single intraperitoneal injection of hydrocortisone 5 mg/kg causes a fall in the concentrations of both 5-hydroxytryptamine (5-HT) and of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the rat brain, suggesting decreased synthesis of brain 5-HT (Curzon & Green, 1968). Scapagnini, Preziosi & De Schaepdryver (1969) have recently reported a comparable decrease in 5-HT after injection of betamethasone, a synthetic glucocorticoid. The changes after hydrocortisone injection appeared to be due to an increase in the liver enzyme tryptophan pyrrolase, which it induces (Knox & Auerbach, 1955), because after injection of Allopurinol, which decreases pyrrolase activity, hydrocortisone no longer caused a decrease of brain 5-HT (Green & Curzon, 1968). This effect of exogenous hydrocortisone would be of little physiological significance, however, if the pituitary-adrenal system could not cause similar changes. A study was therefore made of brain 5-HT changes resulting from immobilization stress. Changes in brain 5-HT and 5-HIAA and liver

tryptophan pyrrolase activity were determined over an 8 hr period of immobilization stress. The effect of immobilization has also been studied after administration of various drugs, and also on adrenalectomized rats.

Methods

Male Sprague-Dawley rats (180–230 g) were used. Intact rats were supplied by Animal Supplies, London N.W.3, and adrenalectomized rats by Scientific Product Farms, Ash, Canterbury, Kent.

Animals were fed an *ad libitum* diet of Oxo 41B pellets. Drinking water was replaced by physiological saline for adrenalectomized rats. Rats were kept in the laboratory in a housing which was acoustically lagged, having regular air changes and a controlled temperature of 25° ± 1° C. Light was automatically controlled on a 06.00–18.00 hr light–dark cycle and animals were always killed at the same time of day (15.30–17.30 hr) to minimize variations due to diurnal changes in 5-HT (Scheving, Harrison, Gordon & Pauly, 1968) and in tryptophan pyrrolase (Hardeland & Rensing, 1968). Animals were removed from the housing for about 10 min for immobilization, which was done by passing their legs through holes in a wire grid and fastening front and hind pairs of legs together with adhesive tape. As the immobilized animals were unable to obtain food and water during the experiment, these were also withheld from the control animals. After various periods of immobilization, rats were stunned by a blow across the back of the neck while still immobilized, and decapitated. The following drugs were used: Allopurinol (Burroughs Wellcome Ltd.), D(+)-lysergic acid diethylamide (LSD) (Sandoz Ltd.), Probenecid and α-methyl tryptophan (Merck, Sharp & Dohme Ltd.) and chlorpromazine (May & Baker Ltd.). Drugs were injected intraperitoneally in a volume of 5 ml./kg, dissolved or suspended in 0·9% saline. 0·01% methyl cellulose was added to the saline to assist suspension of Allopurinol (Becking & Johnson, 1967). Probenecid was moistened with Tween 80 and triturated before suspending in saline. Control animals were injected with saline or the relevant suspension medium.

Analytical methods

Pyrrolase was determined essentially as described by Knox & Auerbach (1955) with the addition of 2×10^{-6} M haematin to the reaction mixture (Kevitz & Wagner, 1965). Due to an arithmetical error, values reported earlier (Green & Curzon, 1968) must be doubled to correspond with those quoted here. 5-HT was determined by the method of Snyder, Axelrod & Zweig (1965). 5-HIAA was determined after 0·4 N perchloric acid deproteinization (Roos, 1962) and butyl acetate extraction followed by washing with 0·1 N hydrochloric acid saturated with sodium chloride and back extraction into 0·1 M phosphate buffer pH 7, as described by Giacalone & Valzelli (1966). The gel which formed in the buffer was broken by rapid shaking and recentrifugation and the fluorescence of the clear solution read on a Farrand spectrophotofluorimeter (activation = 310 mμ, fluorescence = 550 mμ, both wavelengths uncorrected) after bringing to 3 N with concentrated HCl. Ascorbic acid (0·01%) was added to the phosphate buffer to stabilize the fluorescence but not at earlier stages, since this caused high blanks and was not necessary for adequate recovery of added 5-HIAA (80–85%). Statistical significances of results were calculated as described by Moore (1957).

Results

Effect of duration of immobilization on brain 5-HT and 5-HIAA and liver pyrrolase

Results are shown in Fig. 1. Liver pyrrolase was increased after immobilization for 3 hr or more ; pyrrolase changes after a single dose of hydrocortisone are also shown. Brain 5-HT decreased, reaching a minimum after 5 hr immobilization, but returned towards control values after more prolonged immobilization. There was no significant change in the concentration of 5-HIAA in the brain for the first 3 hr of immobilization, after which time it rose to a peak at 6 hr immobilization and then fell towards control values.

Effect of Allopurinol

Previously, hydrocortisone-induced increases of pyrrolase activity, comparable with those obtained by immobilization, were found to cause an apparent decrease in

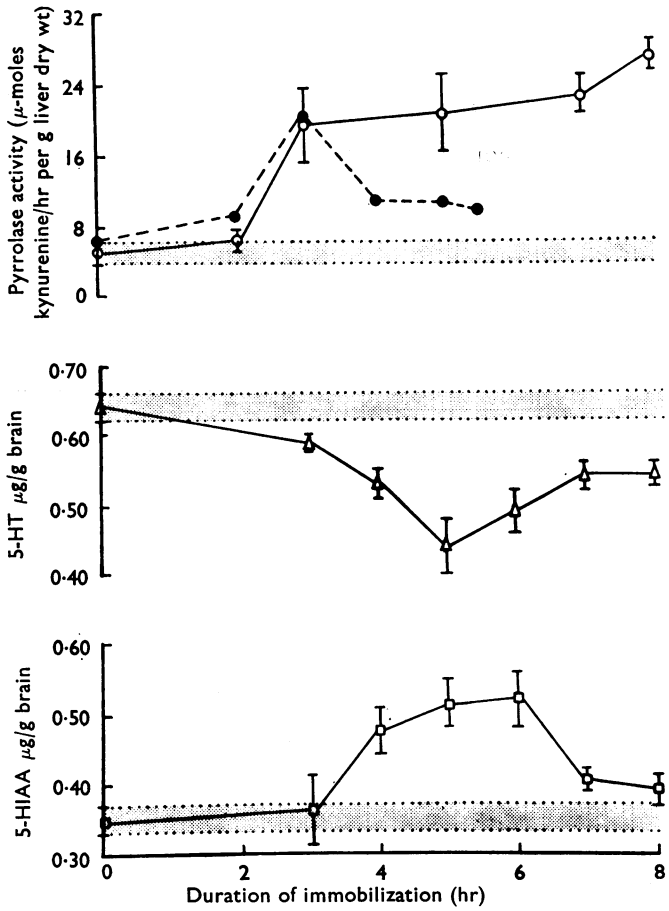

FIG. 1. Effect of duration of immobilization on rat liver pyrrolase and brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). ○, Pyrrolase ; △, 5-HT ; □, 5-HIAA ; ●, pyrrolase after a single intraperitoneal injection of hydrocortisone 5 mg/kg to non-immobilized rats. Points represent means (±S.D.) of determinations on at least four rats. Shaded areas indicate mean (±S.D.) of determinations on uninjected control animals.

brain 5-HT synthesis (Green & Curzon, 1968), indicated by a decrease of both 5-HT and 5-HIAA in this tissue. During immobilization, brain 5-HT decreased but 5-HIAA increased, pointing to increased 5-HT breakdown.

A coexistent pyrrolase dependent decrease of 5-HT synthesis during immobilization, however, is indicated by the finding (Table 1) that the reduction in 5-HT, but not the increase in 5-HIAA, was in part prevented by Allopurinol, which is a pyrrolase inhibitor (Becking & Johnson, 1967; Chytil, 1968; Green & Curzon, 1968).

Although the rise of pyrrolase activity after 5 hr immobilization was only partly prevented by Allopurinol, the rise after 3 hr immobilization was completely prevented (Table 2).

Effect of adrenalectomy

Adrenalectomized animals showed changes in the concentrations of brain 5-HT and 5-HIAA when immobilized which were comparable with those in intact immobilized Allopurinol treated rats (Table 3). In the adrenalectomized animals, pyrrolase did not change significantly and Allopurinol did not influence 5-HT or 5-HIAA.

Effect of lysergic acid diethylamide

Since LSD appears to reduce the turnover rate of brain 5-HT by depressing the activity of 5-HT neurones (Anden, Corrodi, Füxe & Hökfelt, 1968) it might also prevent the increased turnover found after immobilization stress, but leave the effects

TABLE 1. *Effect of 5 hr immobilization and Allopurinol on liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)*

Treatment	Injected	Pyrrolase activity (μ-moles kynurenine/ hr per g liver dry wt.)	μg/g brain wet wt. 5-HT	5-HIAA
Control	—	5·52±0·98 (11)	0·64±0·02 (10)	0·35±0·02 (8)
Immobilized (5 hr)	Saline	21·20±9·06 (7)*	0·47±0·04 (8)*	0·51±0·04 (7)*
Immobilized (5 hr)	Allopurinol (20 mg/kg)	13·80±6·42 (9)	0·56±0·03 (10)*†	0·47±0·04 (8)*

No. of animals are shown in parentheses. Allopurinol was injected 10 min after immobilization was initiated.

* Different from control, $P<0·01$.
† Different from immobilized, saline treated, $P<0·01$.
Results expressed as mean±S.D.

TABLE 2. *Effect of 3 hr immobilization and Allopurinol on liver tryptophan pyrrolase activity*

Treatment	Injected	Pyrrolase activity μ-moles kynurenine/hr per g liver dry wt.
Control	—	5·17±1·73 (4)
Immobilized (3 hr)	—	19·52±4·00 (4)*
Immobilized (3 hr)	Allopurinol (20 mg/kg)	4·09±0·97 (4)

No. of animals are shown in parentheses. Allopurinol injected 10 min after immobilization was initiated.

* Different from control, $P<0·001$. Results given as mean±S.D.

TABLE 3. *Effect of immobilization on pyrrolase, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid of adrenalectomized rats*

Treatment	Injected (i.p.)	Pyrrolase activity (μ-moles kynurenine/ hr per g liver dry wt.)	μg/g brain (wet wt.) 5-HT	5-HIAA
Control	Saline	5·60±1·84 (9)	0·71±0·03 (9)	0·36±0·01 (6)
Immobilized (5 hr)	Saline	6·96±3·76 (4)	0·65±0·02 (4)*	0·56±0·04 (4)*
Immobilized (5 hr)	Allopurinol (20 mg/kg)	3·08±0·40 (4)	0·64±0·02 (4)	0·55±0·03 (4)*

No. of animals are shown in parentheses. * Different from control, $P<0.01$. Results expressed as mean±S.D.

TABLE 4. *Effect of lysergic acid diethylamide (LSD) on 5-hydroxyindoleacetic acid levels after immobilization*

Treatment	Injected (i.p.)	μg 5-HIAA/g brain (wet wt.)
Immobilized (5 hr)	LSD (250 μg/kg×5)	0·33±0·04 (4)
Immobilized (5 hr)	Saline	0·56±0·02 (4)
Control	LSD (250 μg/kg×5)	0·26±0·01 (4)
Control	Saline	0·34±0·01 (5)

No. of animals are shown in parentheses. Rats were injected with saline or LSD at −10 min, 1 hr, 2 hr, 3 hr, 4 hr after immobilization and killed at 5 hr. Results expressed as mean±S.D.

TABLE 5. *Effect of probenecid on 5-hydroxyindoleacetic acid levels after immobilization*

Treatment	Injected (i.p.)	No. of hours of immobilization	μg 5-HIAA/g brain (wet wt.)
Control	Saline*	—	0·34±0·37 (2)
Control	Probenecid*	—	1·35±0·14 (4)
Immobilized	Saline*	3	0·44±0·01 (3)
Immobilized	Probenecid*	3	1·48±0·09 (4)
Control	Saline†	—	0·35±0·02 (8)
Control	Probenecid†	—	1·69±0·12 (4)
Immobilized	Saline†	5	0·51±0·04 (7)
Immobilized	Probenecid†	5	2·25±0·30 (4)

* Injected at +10 min and 2 hr (200 mg/kg). † Injected at +10 min, 2 and 4 hr (200 mg/kg). No. of animals are shown in parentheses. Results expressed as mean±S.D.

TABLE 6. *Effect of α-methyl tryptophan on pyrrolase and 5-hydroxytryptamine*

Injected i.p.	Time of death after injection (hr)	Pyrrolase activity (μ-moles kynurenine/ g liver dry wt.)	μg 5-HT/g brain (wet wt.)
Intact rats			
Saline	6	7·86±1·00 (4)	0·63±0·01 (6)
α-Methyl tryptophan (25 mg/kg)	6	44·46±1·74 (4)*	0·43±0·01 (6)*
Saline	16	4·76±1·2 (4)	0·66±0·02 (4)
α-Methyl tryptophan (25 mg/kg)	16	42·10±7·0 (4)*	0·64±0·02 (4)
Adrenalectomized rats			
Saline	6	5·60±1·84 (9)	0·71±0·03 (9)
α-Methyl tryptophan (25 mg/kg)	6	28·46±1·32 (4)*	0·54±0·01 (4)†

Number of animals are shown in parentheses. * Different from respective control, $P<0.001$. † Different from respective control, $P<0.01$. Results expressed as mean±S.D.

of increased pyrrolase intact. When five consecutive 250 μg/kg doses of LSD were given to immobilized rats the concentration of 5-HIAA in the brain only rose slightly above the concentrations seen in LSD treated, non-immobilized animals. An attempt to prevent completely the increase by administration of a single 500 μg/kg dose of LSD immediately before immobilization had to be abandoned because of strong cholinergic-like effects, which occurred within 1 hr, similar to those observed with toxic doses of tremorine, namely tremor, copious salivation and bloody lachrimation (Trautner & Gershon, 1959). Salivation was observed in some immobilized animals given LSD 250 μg/kg 5 times, but none of the control animals given LSD salivated. Aghajanian & Weiss (1968), who gave 500 μg/kg to non-immobilized rats, reported no ill effects.

Effect of probenecid

Because of the slow appearance of the 5-HIAA changes, probenecid was injected to block efflux of 5-HIAA from the brain (Neff & Tozer, 1968) and to determine whether a change in 5-HIAA transport out of the brain during immobilization was obscuring an increased 5-HIAA formation. The results (Table 5) indicate that this is unlikely, for probenecid raised 5-HIAA concentrations in approximately the same proportion in both controls and in rats after 3 or 5 hr immobilization. The rather high probenecid dose given caused lethargy and occasional convulsive movements.

Effect of α-methyl tryptophan

The 5-HT and 5-HIAA changes on immobilization decreased when it was prolonged, although pyrrolase activity remained high. Therefore, the effect on brain 5-HT of the prolonged high pyrrolase levels induced by α-methyl tryptophan (Moran & Sourkes, 1963) was investigated. As described previously (Green & Curzon, 1968), a fall in the concentration of brain 5-HT was found 6 hr after α-methyl tryptophan injection. Sixteen hours after injection, however, 5-HT returned to control values even though pyrrolase activity was still high (Table 6). Adrenal activity is not a requisite for the pyrrolase increase after α-methyl tryptophan injection (Civen & Knox, 1960). A reduction in brain 5-HT 6 hr after injection occurred in both intact and in adrenalectomized rats (Table 6).

Discussion

In agreement with Nomura (1965) immobilization was found to cause an increase of rat liver tryptophan pyrrolase activity. This presumably results from pyrrolase induction by adrenocortical hormones, the secretion of which is increased in response to immobilization stress (De Schaepdryver, Preziosi & Scapagnini, 1969). Pyrrolase activity was elevated to a value comparable with that obtained 3 hr after injection of 5 mg/kg hydrocortisone, but the duration of the increase was more prolonged. Both hydrocortisone injection (Curzon & Green, 1968) and immobilization led to a fall in the concentration of 5-HT in the brain. However, while hydrocortisone injection also caused a reduction in brain 5-HIAA (Curzon & Green, 1968), indicating that the fall in the concentration of 5-HT was due to decreased synthesis, immobilization caused brain 5-HIAA to increase, suggesting increased 5-HT breakdown. The fall in the concentration of 5-HT after hydrocortisone injection was completely prevented by Allopurinol (Green & Curzon, 1968), which

inhibits a mechanism of pyrrolase activation (Chytil, 1968). While Allopurinol only prevented part of the pyrrolase rise upon 5 hr immobilization, it completely prevented the rise on 3 hr immobilization, and previous experiments with hydrocortisone (Green & Curzon, 1968) showed a lag of not less than 2 hr between pyrrolase changes and consequent 5-HT changes. The fall in the concentration of 5-HT after 5 hr immobilization, though not prevented by Allopurinol, was significantly diminished, suggesting that a decreased 5-HT synthesis occurs as well as the increased breakdown shown by the 5-HIAA increase.

Immobilization of adrenalectomized animals caused a much smaller reduction of brain 5-HT than that in intact animals, and a considerable increase in 5-HIAA. These changes were comparable with those found in Allopurinol-treated immobilized intact rats and show that the increased 5-HT breakdown, unlike the decreased 5-HT synthesis upon immobilization, is not mediated by the adrenal. The evidence that brain 5-HT synthesis during a stress situation may be decreased through a mechanism involving the adrenal, enhances the physiological and pathological significance of the previous finding of decreased 5-HT synthesis after hydrocortisone injection (Curzon, 1969).

The changes of 5-HT and 5-HIAA were not apparent until more than 3 hr immobilization. The time lag in the alteration of the 5-HT concentrations is explicable in so far as the changes are partly due to increased pyrrolase activity which did not rise until more than 2 hr after the start of immobilization. If the 5-HIAA increase is due to a stress-provoked increased activity of 5-HT neurones, however, then the time lag before the concentration of 5-HIAA changes seems remarkable. Increases in the concentration of 5-HIAA in the brain on heat stress (Aghajanian & Weiss, 1968) or direct electrical stimulation of 5-HT neurones (Aghajanian, Rosecrans & Sheard, 1967) are both rapid. A possible explanation for the time lag observed during immobilization is an altered transport of 5-HIAA from the brain. This is not supported by the results of experiments in which active transport out of the brain was prevented by probenecid. The findings suggest that during stress, brain 5-HT may be influenced by mechanisms causing (1) decreased synthesis related to increased pyrrolase activity and (2) centrally initiated increased breakdown. Because during prolonged immobilization both 5-HT and 5-HIAA concentrations returned towards control values, there may be regulatory processes opposing these two mechanisms.

Increased brain 5-HT breakdown under stress conditions as indicated by increased 5-HIAA concentrations has also been found by Bliss, Ailion & Zwanziger (1968) using rats and foot shock and by Welch & Welch (1968a) using immobilization of mice. A number of authors have shown moderately increased concentrations of brain 5-HT after short periods of stress (3–6 hr) instead of the decrease in 5-HT reported here. Thus, Welch & Welch (1968b) and De Schaepdryver *et al.* (1969) found immobilization to result in increased concentrations of brain 5-HT in mice and rats respectively, and Thierry, Fekete & Glowinski (1968) found an increase in the concentration of 5-HT in the rat brain after foot shock. On the other hand, Corrodi, Füxe & Hökfelt (1968) found a reduction in brain 5-HT in the rat after immobilization, Eleftheriou & Church (1968) found a lower concentration of brain 5-HT in the mouse after exposure to aggression and defeat and Rosecrans (1968) found that acute oscillation stress caused a decreased 5-HT synthesis in rat brain. As the fall in the concentration of 5-HT in the brain after hydrocortisone injection

or during immobilization is dependent on a pyrrolase increase, it is not likely to be apparent until after the period required for pyrrolase activity to rise. In agreement with the finding that the 5-HT and 5-HIAA changes decreased when immobilization was prolonged, it has often been noted that changes in the concentration of these substances in the brain on acute stress disappear or are reversed when stress is prolonged (Rosecrans & De Feo, 1965 ; Corrodi *et al.*, 1968 ; Eleftheriou & Church, 1968). Somewhat similarly, a reduction in the concentration of 5-HT in the brain resulted from acute but not from chronic hydrocortisone injection (Curzon & Green, 1968) and from acute but not from chronic high pyrrolase activity due to α-methyl tryptophan. These regulatory changes may be a consequence of the development of increased 5-HT synthesizing capability in response to low 5-HT concentrations caused by neuronal activity and by decreased synthesis through pyrrolase action. Such an increased capacity ensues when brain 5-HT decreases as a result of reserpine action (Ebadi, Russell & McCoy, 1968 ; Gal, Heater & Millard, 1968). Thierry *et al.* (1968) found increased brain 5-HT synthesis to result from foot shock.

The work described in this paper demonstrates that a specific stress in a specific environment causes a complex alteration of brain 5-HT metabolism which is time dependent. As immobilization appears to lead to both processes resulting in decreased 5-HT synthesis and processes resulting in increased synthesis, it is hardly surprising that different studies in which the effects of immobilization or other stresses were examined after only one time interval should lead to qualitatively different findings.

Therefore it is important in investigations of this type to use stresses of various durations. It is also relevant that changes in brain amine metabolism during stress may be dependent upon previous conditions under which animals have been kept (Welch & Welch, 1968b).

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