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EFFECTS OF HYDROCORTISONE AND IMMOBILIZATION ON TRYPTOPHAN METABOLISM IN BRAIN AND LIVER OF RATS OF DIFFERENT AGES

A. RICHARD GREEN* and GERALD CURZON†

*MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE, England, and †Department of Neurochemistry, Institute of Neurology, National Hospital, Queen Square, London WC1 3BG, England

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Abstract—Hydrocortisone sodium succinate (5 mg/kg i.p.) led to a larger absolute increase in hepatic tryptophan pyrrolase activity in younger (26, 46 and 66 days) than older (107 and 225 days) rats. Activity in control animals was lower in the older rats and there was no obvious correlation between percentage change and age. Responses of male and females were comparable. Consistent with previous findings brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) decreased 6 hr after hydrocortisone only at ages when a large absolute increase of pyrrolase activity occurred (26, 46 and 66 days). Brain 5-HT and 5-HIAA levels both before and after hydrocortisone were comparable in males and females. Immobilization led to pyrrolase induction and brain 5-HT changes in 66-day but not in 225-day old rats. Studies with different hydrocortisone preparations showed that the soluble succinate salt caused a more rapid increase of pyrrolase activity than the insoluble acetate salt or free base and a more marked brain 5-HT decrease. Results are discussed in relation to the suggested association between liver tryptophan pyrrolase activity and the subsequent changes in brain 5-HT and 5-HIAA.

We have previously shown that the increased activity of the first enzyme on the kynurenine pathway of tryptophan metabolism, liver tryptophan pyrrolase (L-tryptophan, H_2O_2 oxidoreductase EC 1.11.14) after injection of hydrocortisone sodium succinate (5 mg/kg i.p.) into adult male rats (150–200 g) is followed by a decrease in the concentration of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brain [1, 2]. These findings were confirmed [3] and Scapagnini, Preziosi and De Schaepdryver [4] observed similar 5-HT changes after injection of corticosterone or betamethasone although Benkert and Matussek [5] were unable to show the amine changes after hydrocortisone acetate. The decrease in brain 5-hydroxyindole content is suggested to be associated with the increase in hepatic pyrrolase activity, since inhibition of pyrrolase prevents the brain amine changes [2].

Since pyrrolase induction by tryptophan is age-dependent [6, 7] and oestrogens increase both pyrrolase activity [8] and plasma levels of corticosteroids [9], the effect of hydrocortisone on pyrrolase induction and brain 5-hydroxyindoles has been examined in male and female rats of different ages. The effect of immobilization on rats of different ages has also been studied as this increases adrenocortical secretion [10] and pyrrolase activity [11, 12] and is followed by decreased brain 5-HT concentrations [12, 13].

METHODS

Sprague–Dawley rats (Carworth-Europe, Alconbury, Huntingdon) were obtained at least 3 days before experimentation and kept in controlled conditions as described previously [12]. Animals were always killed at the same time of day to avoid variation due to diurnal changes of 5-HT [14] and tryptophan pyrrolase [15]. Immobilization procedures were as previously described [12].

Rats of the following age ranges in days were used (approximate age used in text shown in brackets: 26 ± 2 (26); 46 ± 2 (46); 66 ± 2 (66); 107 ± 2 (107); 225 ± 15 (225). All rats were virgins except the 225-day old animals which were ex-breeders. Body weights are shown in Table 1.

Drugs were injected i.p. dissolved or suspended in saline. Doses of hydrocortisone sodium succinate (Solu-cortef, Upjohn, Crawley, Sussex) hydrocortisone acetate (Boots, Nottingham) and hydrocortisone (British Drug Houses, Poole, Hants.) are quoted in terms of free base.

Whole brain 5-HT and 5-HIAA were determined by the method of Curzon and Green [16] and tryptophan pyrrolase activity by the method of Knox and Auerbach [17] with the addition of 2×10^{-6} M haematin to the reaction mixture [18]. Pyrrolase activity is expressed in terms of units activity/g liver (μmoles kynurenine formed/hr/g liver (dry wt)).

Table 1. Body weights of Carworth-Europe CFY rats of different ages

Age (days)	Body weights (g) Male	Female
26	80 ± 8 (18)	75 ± 10 (24)
46	240 ± 15 (31)	180 ± 15 (28)
66	364 ± 20 (31)	236 ± 16 (29)
107	472 ± 37 (28)	299 ± 21 (19)
225	718 ± 74 (15)	396 ± 26 (15)

Results given ± 1 S.D. Number of animals on which determinations were made shown in brackets. Age ranges given in Methods.

RESULTS

Effect of different hydrocortisone preparations on pyrrolase activity and brain 5-HT. In our previous work hydrocortisone sodium succinate was used. However, Benkert and Matussek [5] found no 5-HT decrease after injection of hydrocortisone acetate. A preliminary comparison was therefore made of different hydrocortisone preparations. In this part of the study animals were obtained from Animal Suppliers Ltd. (London) as in our earlier studies [1, 2]. These animals were male Sprague–Dawley (160–200 g) and had a higher steady-state concentration of brain 5-HT than those used in the rest of this investigation.

Hydrocortisone sodium succinate is soluble in aqueous media, while the acetate salt, like hydrocortisone itself, is normally almost insoluble and is injected as a suspension. Presumably a consequence of this is that both the acetate and base are absorbed more slowly than the succinate. Consistent with this is the finding that succinate caused a well defined peak of pyrrolase activity 3 hr after injection (Ref. 2; Table 2), whereas the increase in activity 3 hr after injection of the acetate or base was less pronounced and activity continued to rise over the next 2 hr (Table 2).

These differences in the speed of pyrrolase induction may well explain the observed differences in 5-HT changes following injection of the succinate or acetate. Hydrocortisone sodium succinate causes a 5-HT decrease 6 hr after injection which is still apparent at 8 hr (Ref. 1; Table 2). The acetate salt, on the other hand, led to a less pronounced, though statistically significant decrease at 6 hr and 8 hr (Table 2). It is probable also that variations in particle size of acetate preparations would affect the changes. The soluble succinate salt was therefore used in all subsequent experiments.

Induction of tryptophan pyrrolase in male and female rats of different ages. The increase in pyrrolase activity following injection of hydrocortisone (5 mg/kg) as the sodium succinate was studied in both male and female rats of different ages. The results (Fig. 1) show a marked age-dependence of pyrrolase induction, the

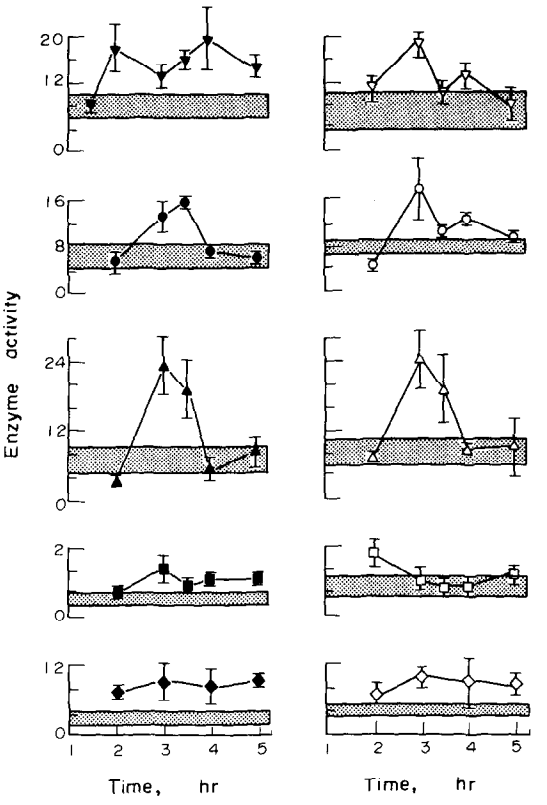

Fig. 1. Effect of hydrocortisone sodium succinate on liver tryptophan pyrrolase activity in male and female rats of different ages. Rats were injected with hydrocortisone sodium succinate (5 mg/kg) and pyrrolase activity measured at varying times after injection but always at the same time of day. Ordinate: Tryptophan pyrrolase activity expressed as μMoles kynurenine formed per hr/g liver (dry wt). Abscissa: Time in hours after hydrocortisone injection. Results show mean ± I.S.D. (bars) on a group of six animals. Shaded area: mean ± 1 S.D. on a group of saline-injected animals killed at same time of day. Closed symbols: males, open symbols: females. 26 day (▼▽) 46 day (●○) 66 day (▲△) 107 day (■□) 225 day (◆◇).

Table 2. Effect of various hydrocortisone preparations on rat liver pyrrolase and brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)

	Pyrrolase activity [μmoles kynurenine formed/hr/g liver (dry wt)]		Brain 5-hydroxytryptamine [μg 5-HT/g brain (wet wt)]	
Time of observation (hr)	3	5	6	8
Injected				
Saline	4·6 ± 0·6 (4)	4·2 ± 1·0 (5)	0·64 ± 0·03 (6)	0·65 ± 0·01 (4)
Hydrocortisone sodium succinate (5 mg/kg)	22·4 ± 1·1 (5)*	11·4 ± 1·4 (8)	0·46 ± 0·02 (4)*	0·50 ± 0·02 (4)*
Hydrocortisone acetate (5 mg/kg)	12·7 ± 3·3 (8)†	29·8 ± 6·8 (6)*	0·54 ± 0·01 (4)*	0·56 ± 0·04 (9)*
Hydrocortisone (5 mg/kg)	9·1 ± 1·9 (3)†	20·8 ± 6·9 (6)*	N.D.	N.D.

All drugs injected at zero time. Liver pyrrolase activity and brain 5-HT concentrations measured at times after injection shown as "time of observation". Animals used in this part of the study were obtained from Animal Suppliers, London. (See Results section.)

Results expressed as mean ± 1 S.D. No. of animals on which observations were made shown in brackets.

* Different from control $P < 0{\cdot}001$.

† Different from control $P < 0{\cdot}01$.

N.D. Not Determined.

Table 3. Effect of hydrocortisone on brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in rats of different ages

Age (days)	5-hydroxytryptamine [μg 5-HT/g brain (wet wt)] Saline	Hydrocortisone	5-hydroxyindoleacetic acid [μg 5-HIAA/g brain (wet wt)] Saline	Hydrocortisone
26	0·45 ± 0·01 (16)	0·37 ± 0·01 (8)*	0·45 ± 0·01 (14)	0·29 ± 0·01 (8)*
46	0·44 ± 0·01 (13)	0·37 ± 0·02 (8)*	0·45 ± 0·02 (13)	0·35 ± 0·01 (8)*
66	0·42 ± 0·01 (13)	0·36 ± 0·01 (8)*	0·41 ± 0·01 (6)	0·34 ± 0·01 (8)*
107	0·38 ± 0·01 (15)	0·42 ± 0·01 (8)	0·40 ± 0·02 (12)	0·40 ± 0·02 (8)
225	0·39 ± 0·01 (9)	0·37 ± 0·02 (10)	0·38 ± 0·01 (9)	0·39 ± 0·02 (10)

* Different from age matched saline injected control $P < 0·001$.
Results expressed as mean ± 1 S.D.
Animals supplied by Carworth-Europe (see Results).

absolute increase being less in the 107- and 225-day old animals than in younger rats. As enzyme activity of older (107 and 225 days) saline-injected control rats was also lower than that of the younger rats there was no obvious relationship between age and percentage increase of activity. The responses of male and female rats were comparable. Peak activity occurred between 3 and 4 hr at all ages studied. The 26-day old animals (both male and female) were exceptional, as they showed a biphasic response with peaks at 2 and 4 hr (male) and 3 and 4 hr (female) after hydrocortisone injection.

Effect of age on the decrease of rat brain 5-HT and 5-HIAA after hydrocortisone injection. No significant differences were found between 5-hydroxyindole concentrations or changes in male and female rats of the same age and the results have therefore been combined. In agreement with earlier findings [1, 2], a significant fall in the concentration of brain 5-HT and 5-HIAA was seen in young rats (26, 46 and 66 days) 6 hr after hydrocortisone (5 mg/kg). In 107- and 225-day-old animals there was no decrease in 5-HT or 5-HIAA concentrations following hydrocortisone (Table 3).

Effect of immobilization of 66- and 225-day-old female rats on liver pyrrolase activity and brain 5-HT concentrations. Immobilization caused increased pyrrolase activity and decreased brain 5-HT in young (150–200 g) male rats [12, 13]. As the pyrrolase and 5-HT changes following hydrocortisone were dependent on age, it was of interest to investigate the effect of age on these changes during immobilization. Five hours after the start of immobilization of 66-day-old female rats, there was a marked increase on pyrrolase activity and decrease of brain 5-HT. The 225-day-old rats did not exhibit either change (Table 4).

DISCUSSION

Previous studies of age-dependence of pyrrolase induction have been mainly on substrate induction [6, 7]. It was found that in 1-month-old animals activity increased faster, reached maximum activity earlier and declined more rapidly than in 3–12-month rats. Such a relationship was not apparent in the present study after hydrocortisone. Instead, hydrocortisone induced pyrrolase at similar times in all animals studied, but although it produced a large induction in young animals the response in older (107 and 225 days) rats was either small or absent (Fig. 1). Gregerman [19] found similar induction changes following hydrocortisone to 12- and 24-month rats to these seen in the current study of 107- and 225-day animals but he did not study younger animals. Adelman [20] has suggested that enzyme induction may in some instances be a biochemical function of ageing with inducibility decreasing as age increases. Our results are consistent with this hypothesis.

Our findings show that the brain 5-HT decrease following hydrocortisone injection occurs only in younger animals. This may be related to the finding that large increases of pyrrolase activity (measured *in vitro*) were found only in younger animals. A relationship between liver pyrrolase and brain 5-HT changes is consistent with the finding that hydrocortisone produces neither a pyrrolase increase nor 5-HT decrease in the gerbil [21]. The decrease in brain 5-HT in younger animals following hydrocortisone is presumably a consequence of the decreased brain tryptophan concentrations in these animals [21, 22] which was not found in gerbils [21].

Immobilization of 66-day rats produced a pyrrolase increase and brain 5-HT decrease similar to those pre-

Table 4. Effect of immobilization on 65 and 240 day rat liver tryptophan pyrrolase and brain 5-hydroxytryptamine

Treatment	Age (days)	Pyrrolase activity [μ moles kynurenine/hr/g liver (dry wt)]	5-hydroxytryptamine [μg 5-HT/g brain (wet wt)]
Control	66	8·6 ± 2·4 (6)	0·44 ± 0·04 (5)
Immobilized	66	17·2 ± 1·8 (4)*	0·34 ± 0·02 (4)*
Control	225	4·0 ± 0·77 (4)	0·39 ± 0·02 (4)
Immobilized	225	6·1 ± 2·7 (5)	0·43 ± 0·04 (4)

* Different from age matched control. $P < 0·001$.
Number of animals on which determinations were done shown in brackets.
Results expressed as mean ± 1 S.D.
Animals supplied by Carworth-Europe (see Results).

viously reported [10, 11]. However, in agreement with the findings with hydrocortisone, there was only a small pyrrolase rise and no brain 5-HT decrease in 225-day rats.

Unlike Benkert and Matussek [5], we found a decrease of 5-HT 6 hr after hydrocortisone acetate injection. However, the decrease was smaller than that following hydrocortisone sodium succinate. Possibly the rapid increase in pyrrolase activity following injection of the succinate salt leads to a more marked decrease of brain tryptophan, which is reflected in the greater decrease in brain 5-HT concentration.

The results presented here, together with previous observations [2, 21] add to the evidence that the increase in hepatic tryptophan pyrrolase following hydrocortisone injection is associated with a decrease of brain 5-HT and 5-HIAA. However, various factors influence this association, such as age and the hydrocortisone salt injected.

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