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LIVER AND BRAIN TRYPTOPHAN METABOLISM FOLLOWING HYDROCORTISONE ADMINISTRATION TO RATS AND GERBILS

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1 Liver tryptophan pyrrolase activity is low in the mongolian gerbil (*Meriones unguiculatus*) and is not induced by hydrocortisone (5 mg/kg). In contrast, there is measurable activity in the rat liver and this is induced by hydrocortisone. *In vivo* measurements confirmed the absence of induction in gerbils but suggested that they were able to metabolize tryptophan. However, no detectable pyrrolase activity was found in any other tissues either before or after hydrocortisone.

2 In agreement with previous observations hydrocortisone decreased rat brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) 6 h after administration. Brain tryptophan concentrations were also decreased at this time. In contrast, hydrocortisone did not alter gerbil brain 5-HT, 5-HIAA or tryptophan. α-Methyltryptophan activated hepatic tryptophan pyrrolase and decreased brain 5-HT and 5-HIAA in both animals.

3 Results suggest that the decrease in rat brain 5-HT and 5-HIAA following hydrocortisone may be associated with the rise in liver tryptophan pyrrolase and that the brain amine changes are mediated through the decrease in brain tryptophan concentration.

Introduction

Intraperitoneal injection of hydrocortisone (5 mg/kg) into rats decreases the concentrations of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brain, suggesting decreased 5-HT synthesis (Curzon & Green, 1968; Yuwiler, Wetterberg & Geller, 1971). Corticosterone and the synthetic glucocorticoid betamethasone produce comparable changes (Scapagnini, Preziosi & DeSchaepdryver, 1969). The change in brain 5-HT metabolism may be a consequence of altered peripheral tryptophan metabolism, because hepatic tryptophan pyrrolase activity is induced by corticosteroids (Knox & Auerbach, 1955), and injection of the pyrrolase inhibitor, allopurinol, prevents the brain amine changes (Green & Curzon, 1968). α-Methyltryptophan also increases pyrrolase activity and decreases rat brain 5-HT and 5-HIAA (Green & Curzon, 1968; Sourkes, Missala & Oravec, 1970).

It has recently been reported that pyrrolase activity is low in the mongolian gerbil (*Meriones unguiculatus*), and that it is not induced by hydrocortisone (Baughman & Franz, 1971). We have now investigated the differences in hepatic tryptophan metabolism in rats and gerbils, both before and after hydrocortisone. The effect of hydrocortisone on 5-HT metabolism in both rat and gerbil brain has also been examined.

Methods

Adult male gerbils 50-90 g (MRC Laboratory Animals Centre, Carshalton, Surrey, and Canadian Breeding Farm and Laboratory, St Constant, Quebec, Canada) and male Sprague-Dawley rats 140-160 g (Carworth-Europe, Alconbury, Huntingdon and Canadian Breeding Farm and Laboratory) were used. They were fed an *ad libitum* diet of small animal 41B pellets and tap water. They were killed by decapitation at the same time of day (14 h 30 min-16 h 30 min) to minimize the effects of possible diurnal variations of pyrrolase or 5-HT. The following drugs were used: hydrocortisone sodium succinate (Solu-Cortef, Upjohn Co.), DL-α-methyltryptophan (Upjohn Co., Kalamazoo, Mo., U.S.A.), L-kynurenine sulphate (Sigma Chemical Co) and DL-[ring-2-^{14}C]-tryptophan

(2 mCi/mmol; I.C.N. Research Products, Montreal, Quebec, Canada). All drugs were dissolved in 0.9% w/v NaCl solution (saline) and injected intraperitoneally. The hydrocortisone dose is quoted as free steroid. Control animals were injected with isotonic saline.

Analytical methods

Pyrrolase activity was measured *in vitro* in supernatants (16,000 *g* for 1 h) from 25% homogenates after preincubation of the soluble liver fraction with methaemoglobin, tryptophan and freshly neutralized ascorbic acid (Knox, Piras & Tokuyama, 1966), or thermal activation, when the soluble liver fraction was incubated at 55°C for 5 min, chilled, centrifuged and the supernatant used (Schutz & Feigelson, 1972). Measurement of pyrrolase activity in liver homogenates was essentially by the method of Knox & Auerbach (1955) in which whole liver homogenates are incubated in the presence of 0.003 M tryptophan, except that 2×10^{-6} M haematin was added to the reaction mixture (Kevitz & Wagner, 1965). Pyrrolase activity *in vivo* was determined by the method of Madras & Sourkes (1968) as modified by Young, Oravec & Sourkes (1974). Rats were injected with hydrocortisone (50 mg/kg) 5 h before DL-[ring-2-^{14}C]-tryptophan (2.5 μmol/kg). The animals were then placed in glass metabolism cages, without food or water, through which CO_2-free air was drawn. Labelled CO_2 was trapped by bubbling the expired gas through ethyleneglycol monomethyl ether-ethanolomine (2 : 1). Aliquots of this solution were removed, mixed with scintillant and counted by scintillation spectrometry.

Brain 5-HT and 5-HIAA were measured by the method of Curzon & Green (1970) and brain tryptophan by that of Denkla & Dewey (1967).

Results

In vitro *activity of tryptophan pyrrolase in rat and gerbil liver after hydrocortisone*

Rat liver pyrrolase activity in whole liver homogenates was essentially the same as previously reported (Green & Curzon, 1968) and the rise in activity 3 h after hydrocortisone (5 mg/kg) was also as previously seen (Table 1). However, in the gerbil, hepatic pyrrolase activity was only just detectable (Table 1) and the value is probably not significant (see discussion section). Furthermore, hydrocortisone administration did not cause a statistically significant increase in pyrrolase activity 3 h after administration. Since enzyme induction in the gerbil might have occurred at a different time from that in the rat, pyrrolase activity was also measured in groups of 4 gerbils at 2, 4, 5 and 6 h after injection. However, no significant increase in activity was found at any time after hydrocortisone administration.

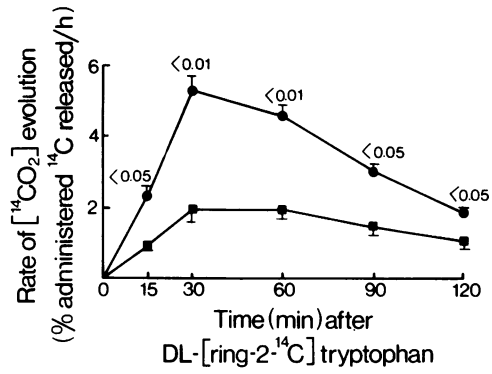

Figure 1 Effect of hydrocortisone (50 mg/kg) on [$^{14}CO_2$] release in the rat following injection of DL-[ring-2-^{14}C] tryptophan (2.5 μmol/kg). Each point is mean of 6 animals. Vertical bars indicate s.e. mean. Rats were injected with hydrocortisone 50 mg/kg (●) or saline (■) 4 h before injection of DL-[ring-2-^{14}C] tryptophan (2.5 μmol/kg). [$^{14}CO_2$] evolution was measured as described in methods section. Significance compared to control shown for each point.

The low activity in gerbil liver might have been due to inadequacies in the method used. Therefore we tried, but failed, to detect significant activity in gerbil liver supernatants after preincubation with methaemoglobin according to Knox *et al.* (1966) and after thermal activation, by the method of Schutz & Feigelson (1972). Furthermore, addition of an equal volume of gerbil homogenate to a rat liver homogenate had no effect on either conjugation or activity of the rat pyrrolase as measured by the method of Knox *et al.* (1966).

In vivo *activity of tryptophan pyrrolase in rat and gerbil liver after hydrocortisone*

The rate of tryptophan degradation was measured in both rats and gerbils by the *in vivo* techniques of Madras & Sourkes (1968). Rats showed an increase in [$^{14}CO_2$] evolution following hydrocortisone (Figure 1). In agreement with the *in vitro* results, there was no statistically significant increase in [$^{14}CO_2$] excretion after hydrocortisone in the gerbil even at a dose of 50 mg/kg. However, the radioactive tryptophan was metabolized by the gerbil suggesting pyrrolase activity *in vivo* (Figure 2).

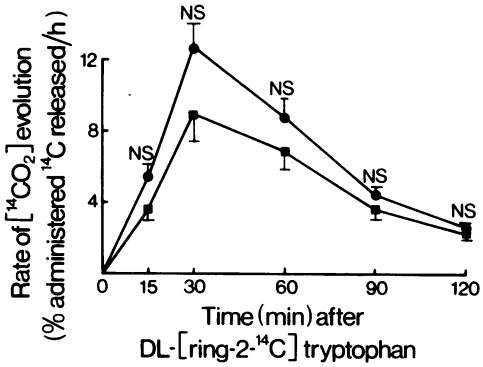

Figure 2 Effect of hydrocortisone (50 mg/kg) on [$^{14}CO_2$] release in the gerbil following injection of DL-[ring-2-^{14}C] tryptophan (2.5 μmol/kg). Each point shows mean of 9 animals. Vertical bars indicate s.e. mean. Gerbils were injected with hydrocortisone 50 mg/kg (•) or saline (▪) 4 h before injection of DL-[ring-2-^{14}C] tryptophan (2.5 μmol/kg). [$^{14}CO_2$] evolution was measured as described in methods section. NS—not significantly different from saline-injected control.

Examination of other gerbil tissues for pyrrolase activity

To determine whether the radioactive tryptophan was being degraded by tissues other than the liver, the *in vitro* assay of Knox *et al.* (1966) was used to see whether there was detectable pyrrolase activity in other tissues. No significant pyrrolase activity was found in gerbil intestine, kidney, spleen, heart or brain either before or after hydrocortisone treatment.

Effect of hydrocortisone on gerbil and rat brain 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and tryptophan

In agreement with previous observations (Green & Curzon, 1968), rat brain 5-HT and 5-HIAA decreased 6 h after hydrocortisone (5 mg/kg) (Table 1). We also found, in agreement with Curzon & Knott (personal communication), that brain tryptophan concentrations were decreased at this time (Table 1).

Table 1 Effect of hydrocortisone and α-methyltryptophan on activity of gerbil and rat liver tryptophan pyrrolase and brain tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations

Injected (i.p.)	*Pyrrolase activity (μmol kynurenine h^{-1} g^{-1} liver (dry wt))*	*Brain tryptophan (μg/g brain (wet wt))*	*Brain 5-HT (μg/g brain (wet wt))*	*Brain 5-HIAA (μg/g brain (wet wt))*
Gerbils				
Saline	1.86 ± 0.86 (6)	6.07 ± 1.04 (6)	0.57 ± 0.03 (9)	0.44 ± 0.02 (9)
Hydrocortisone (5 mg/kg)	3.32 ± 0.54 (6)	6.97 ± 0.20 (6)	0.55 ± 0.04 (12)	0.45 ± 0.05 (12)
DL-α-methyl tryptophan (25 mg/kg)	13.51 ± 0.99* (6)	ND	0.50 ± 0.01** (6)	0.30 ± 0.02* (6)
Kynurenine (5 mg/kg)	ND	5.80 ± 1.06 (6)	0.56 ± 0.03 (4)	0.44 ± 0.02 (4)
Rats				
Saline	6.02 ± 0.85 (6)	6.81 ± 0.24 (10)	0.52 ± 0.02 (10)	0.36 ± 0.02 (10)
Hydrocortisone (5 mg/kg)	20.02 ± 3.42* (6)	4.03 ± 0.27** (6)	0.41 ± 0.02* (6)	0.23 ± 0.03* (6)
DL-α-methyl tryptophan (25 mg/kg)	44.46 ± 1.74* (4)	ND	0.44 ± 0.02** (6)	0.23 ± 0.03* (6)
Kynurenine (5 mg/kg)	ND	3.45 ± 0.68** (6)	0.41 ± 0.03* (6)	0.25 ± 0.03* (6)

Pyrrolase activity measured 3 h after injection. 5-HT, 5-HIAA and tryptophan measured 6 h after injection, except kynurenine where brain tryptophan results are shown 1 h after injection and 5-HT and 5-HIAA 2 h after injection.

* Different from saline treated $P < 0.001$. ** Different from saline-treated, $P < 0.01$. ND Not determined.

In contrast, hydrocortisone injection at the same dose elicited in the gerbil neither a change in 5-HT and 5-HIAA, nor a decrease of brain tryptophan (Table 1).

Effect of α-methyltryptophan on liver pyrrolase, brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in rats and gerbils

Injection of α-methyltryptophan into rats activates tryptophan pyrrolase (Sourkes & Townsend, 1955). The increase in the enzyme activity following tryptophan and α-methyltryptophan occurs in adrenalectomized rats (Civen & Knox, 1960) and is thus not mediated by glucocorticoids. However, unlike tryptophan, α-methyltryptophan is not a substrate for the enzyme (Sourkes & Townsend, 1955). Baughman & Franz (1971) reported that gerbil pyrrolase activity was increased by injection of the substrate tryptophan. The effect of the analogue α-methyltryptophan was therefore examined. α-Methyltryptophan (25 mg/kg) increased pyrrolase activity as measured in whole liver homogenates from both rats and gerbils. It also produced a decrease in brain 5-HT and 5-HIAA in both rodents (Table 1).

Effect of L-kynurenine on rat and gerbil brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid

During an investigation into the possible mechanism of the proposed pyrrolase-5-HT association it was found that the product of pyrrolase activity, L-kynurenine, injected in a dose of 5 mg/kg, decreased brain 5-HT concentrations 2 h after injection (Green & Curzon, 1970). This was confirmed in the current study (Table 1). This compound did not influence gerbil brain 5-HT and 5-HIAA concentrations 2 h after injection (Table 1), nor was any change noted 1 h or 3 h after injection.

Although brain tryptophan concentrations were not decreased in the rat or gerbil brain 2 h after kynurenine injection (rat: 6.70 ± 0.56 μg/g ($n = 6$); gerbil: 6.13 ± 0.76 μg/g ($n = 6$)), a decrease in concentration was found in the rat brain 1 h after injection. However, concentrations were unaltered in the gerbil (Table 1).

Discussion

Baughman & Franz (1971) found no significant pyrrolase activity in gerbil liver *in vitro*. In the present study measurable values were found. However, the results of Baughman & Franz (1971) are expressed as μmol kynurenine formed h^{-1} g^{-1} liver (wet wt), whereas those of this study are expressed as μmol kynurenine formed h^{-1} g^{-1} liver (dry wt). If our results are divided by the wet wt/dry wt ratio for liver of 3.45 (Woods & Krebs, 1971) this gives a value for pyrrolase activity of 0.5 μmol kynurenine formed h^{-1} g^{-1} liver (wet wt) which is in excellent agreement with the results of Baughman & Franz (1971). Franz & Knox (1967) have discussed the criteria for definite enzymic activity and concluded that anything less than 1.0 μmol kynurenine formed h^{-1} g^{-1} liver (wet wt) is not indicative of true enzyme activity, due to the limitations of the spectrophotometric method.

In rat liver, tryptophan pyrrolase exists in at least three forms, the inactive apoenzyme (Greengard & Feigelson, 1962) and the oxidized and reduced holoenzyme (Tanaka & Knox, 1959; Tokuyama & Knox, 1964). While addition of tryptophan and the co-factor haematin activates the enzyme in whole homogenates this only occurs after a time lag (Knox & Ogata, 1965). We therefore also attempted to measure pyrrolase activity in cell supernatants following either preincubation with methaemoglobin (Knox *et al.*, 1966) or thermal activation (Schutz & Feigelson, 1972), both techniques being designed to ensure that all enzyme present is converted to the active reduced holoenzyme before assay. Even with these assay systems no significant enzyme activity was found in the gerbil. It must be remembered however that none of these assay methods can demonstrate the absence of enzyme activity, merely that its activity is below an ascribed amount.

No detectable enzyme activity was found in any gerbil tissues examined. Furthermore, gerbil liver homogenates had no effect on either conjugation or activity of rat liver homogenates, suggesting the absence of an endogenous inhibitor. Clearly this cannot be ruled out since a gerbil liver inhibitor would not necessarily inhibit rat pyrrolase. Nevertheless, this absence of enzyme activity when measured *in vitro* made the *in vivo* results surprising. Results clearly suggested that the gerbils degraded tryptophan more rapidly than rats, indicating pyrrolase activity.

There are two main possibilities to account for this discrepancy. The first is that tryptophan is metabolized in the gerbil by a different degradative route not involving pyrrolase. However, if this is the case it is surprising that formamidase activity in the liver is normal (Baughman & Franz, 1971). The second and more likely situation is that the *in vitro* assay is not reflecting the *in vivo* condition, a situation that has been observed with other enzyme systems (see review by Youdim & Woods, 1975).

Although the discrepancy between the *in vivo*

and *in vitro* assays is not explained, they are in agreement on the lack of effect of hydrocortisone in the gerbil, unlike the rat. The *in vitro* assay shows no significant increase in enzymic activity and the *in vivo* assay demonstrates that there is no increase in the rate of tryptophan degradation after hydrocortisone. It is unlikely that the lack of induction is due to a more rapid turnover, or excretion, of hydrocortisone in gerbils, since doses as high as 500 mg/kg have no effect on pyrrolase (Baughman & Franz, 1971).

In confirmation of previous findings, injection of hydrocortisone (5 mg/kg) induced rat hepatic pyrrolase activity and decreased brain 5-HT and 5-HIAA. In contrast, the same dose of hydrocortisone, which in gerbils does not induce pyrrolase, also has no effect on brain 5-HT metabolism in these animals. This suggests an association between pyrrolase activity and brain 5-HT metabolism and is consistent with the observation that the pyrrolase rise and the brain 5-hydroxyindole decrease following hydrocortisone both diminish with increasing age (Green & Curzon, 1975). The decrease in rat brain 5-HT concentrations in younger rats following hydrocortisone presumably results from the fall in brain tryptophan concentration (Table 1). It seems reasonable to assume that this decrease of brain tryptophan is due to the increase in tryptophan pyrrolase activity, especially as the gerbil shows neither an enzyme rise nor a brain tryptophan decrease. In a previous communication we were unable to find a significant fall in brain tryptophan (Green, Joseph & Curzon, 1970). However, measurements were then made 3 h after hydrocortisone, not at 6 h, which is when the major 5-HT decrease has occurred (Curzon & Green, 1968).

Activation of gerbil pyrrolase after α-methyltryptophan is of the same magnitude as that observed in the rat (7-fold) and is consistent with the finding that gerbil pyrrolase activity can be increased by substrate (Baughman & Franz, 1971). Furthermore, this compound caused a decrease in gerbil brain 5-HT and 5-HIAA as it does in rats (Table 1; Green & Curzon, 1968; Sourkes *et al.*, 1970). The larger decrease of 5-HIAA than 5-HT has been reported previously (Sourkes *et al.*, 1970; Curzon & Green, 1971) and is due to the formation of α-methyl-5-HT which produces falsely high values (Roberge, Missala & Sourkes, 1971; 1972).

It is probable that α-methyltryptophan decreases brain 5-HT because of the increase of pyrrolase activity since there is a large decrease of both plasma and brain tryptophan following administration of this compound when pyrrolase activity is high (Sourkes *et al.*, 1970).

In the rat, kynurenine injection produced a significant decrease in brain 5-HT and 5-HIAA concentrations. This is probably because it competes with tryptophan for uptake into the brain, since kynurenine decreases accumulation of labelled tryptophan in brain slices (Green & Curzon, 1970; Kiely & Sourkes, 1972) and decreases rat brain tryptophan 1 h following injection. Thus the finding that this compound had no effect on brain tryptophan concentrations and did not decrease brain 5-HT or 5-HIAA concentrations in the gerbil again indicates differences in tryptophan metabolism between these two rodents. It is possible that accumulation of tryptophan by the gerbil brain is by a different mechanism from that in the rat. However, Leklem, Woodford & Brown (1969), working with the cat (another mammal in which pyrrolase is not subject to hormonal induction), recovered very little of an injected dose of kynurenine in the urine. They suggested that this was because the compound was rapidly conjugated and therefore was undetectable with their assay methods. This might also be true in the gerbil, since the conjugated compound would presumably not affect cerebral amino acid transport.

While it is not clear what the physiological reasons are for the differences in tryptophan metabolism in rat and gerbil our results add weight to the hypothesis that increased pyrrolase activity is associated with a decrease in brain 5-HT synthesis and suggest that this decrease in 5-HT synthesis is the result of a decrease in brain tryptophan concentrations.

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