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TRYPTOPHAN PYRROLASE IN THE LIVER OF GUINEA PIG: THE ABSENCE OF HYDROCORTISONE INDUCTION

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SUMMARY

The adaptive response of liver tryptophan pyrrolase (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) of guinea pig to hormone and substrate treatment was investigated and the results compared with similar studies on other species. Tryptophan treatment caused a marked increase in the tryptophan pyrrolase activity, and this increase was only slightly inhibited by actinomycin D. The administration of glucocorticoids increased the incorporation of [^{3}H]uridine into ribonucleic acid of guinea pig liver (and decreased that into ribonucleic acid of guinea pig thymocytes), but it failed to induce tryptophan pyrrolase. Two forms of tryptophan pyrrolase (oxidized and reduced forms) were demonstrated in the soluble fraction of liver homogenate from guinea pig.

INTRODUCTION

Tryptophan pyrrolase (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) from rat liver has been the object of many investigations dealing with the enzyme adaptation in mammals. Earlier reports have shown that the catalytic activity of rat liver tryptophan pyrrolase is regulated by its substrate L-tryptophan and glucocorticoids[1,2]. The separate mechanisms of rat liver tryptophan pyrrolase induction by hydrocortisone and tryptophan have been elucidated by SCHIMKE *et al.*[3]. The enzyme from tryptophan-treated rats is largely conjugated with its hematin prosthetic group although that from hydrocortisone-treated rats is mostly the apoenzyme[4].

There have been very few reports concerning the induction of tryptophan pyrrolase in species other than rat[5–8]. In this communication experiments on the induction* of tryptophan pyrrolase in the guinea pig liver will be described. The enzyme was readily induced by its substrate, but it was apparent that exogenous glucocorticoids do not induce this enzyme in the guinea pig liver. Two forms of tryptophan pyrrolase (the oxidized and the reduced holoenzymes) were demonstrated in the soluble fraction of liver homogenate from guinea pig.

* The term "induction" as used here means the accumulation of more enzyme in a cell as a consequence of an altered physiological state.

MATERIALS AND METHODS

Short-haired, English-type female and male guinea pigs weighing 350–600 g were used in the experiments. This strain can be divided into substrains one of which produces high and the other low amounts of hydroxylated cortisol derivatives[9]. It was not tested to which substrain our animals belonged. The guinea pigs were treated with 100 mg of L-tryptophan or 2.5 mg of hydrocortisone (as free alcohol, acetate or phosphate) per 100 g of body weight, all given intraperitoneally as suspensions in 0.9% saline solution. In some experiments either a watersoluble hydrocortisone sodium succinate or a more potent glucocorticoid, prednisolone sodium acetate, was used. The effects of a subcutaneous injection of hydrocortisone were also investigated. Unless stated otherwise, the animals were killed 5 h after treatment.

Soluble liver fractions were prepared at 5°, immediately after killing the animals. The animals were stunned, bled from the neck, and the livers were perfused *in situ* with cold 0.9% saline solution until they were blanched. The livers were weighed and homogenized using a glass–teflon tissue grinder in 0.14 M KCl containing 0.02 M sodium phosphate buffer, pH 7.0. The homogenate was centrifuged for 10 min at 10 000 × *g* and the sediment was discarded. The supernatant was centrifuged for 1 h at 105 000 × *g*. The upper part of the supernatant was withdrawn with a syringe avoiding contaminating it with the sediment. The activity of tryptophan pyrrolase was assayed according to the method of KNOX *et al.*[4] by measuring at 360 nm the accumulation of kynurenine. The enzyme formamidase (arylformylamine amidohydrolase EC 3.5.1.9) is present in an excess of 5000-fold in the guinea pig liver[10]. The assay mixture had a total volume of 3.0 ml, and contained 0.25 ml of 25% liver extract, 0.7 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.2 ml of 0.05 M L-tryptophan (Fluka) and distilled water. In some experiments 0.1 ml of 0.3 M freshly neutralized ascorbic acid and/or 0.2 ml of 20 μM hematin (Hemin, Mann Research Laboratories) were added into the assay mixture. The absorbance was measured in a Beckman DB constant recording spectrophotometer with a thermoregulated cell holder at 37° using an assay mixture without tryptophan as a reference. When calculating the results, corrections were made for the nonenzymatic increase of absorbance in a solution containing tryptophan and ascorbic acid (0.05 *A* units/h at 37°). The results are expressed as μmoles of kynurenine/h per g of liver during the linear phase of the reaction.

Preincubation of the enzyme solution for activation of the inactive tryptophan pyrrolase was carried out according to the method of KNOX *et al.*[4]. The soluble liver fraction with an equal volume of the homogenizing medium *plus* tryptophan and methemoglobin (Horse hemoglobin, Nutritional Biochemicals) was gently shaken in open test tubes at 37° for 1h. The final concentrations in the mixture were 12.5% liver extract, 2.5 mM tryptophan, 0.5 mg of methemoglobin per ml, and 30 mM ascorbic acid. Aliquots of the mixture were taken before and during the incubation period for the assay of the tryptophan pyrrolase activity by the standard assay.

As an additional method for determining kynurenine the Bratton–Marshall test was used[11,12].

For measuring the incorporation of [5-^{3}H]uridine into RNA small pieces of guinea pig liver were placed into 25-ml erlenmeyer flasks, where 0.9 ml of Krebs–Ringer phosphate medium (pH 7.6) supplemented with 1 mM D-glucose, was pipetted. For

measuring the incorporation of [5-^{3}H]uridine into RNA 0.1 ml of [5-^{3}H]uridine (29 C/mmole, 1 μC/0.1 ml, New England Nuclear) in 0.9% saline was added, and after a 60-min incubation in air at 37° the tissue pieces were removed with forceps. They were ground in 0.5 ml of cold water in all-glass homogenizer. Two samples of 0.1 ml were pipetted onto filter paper disks (Whatman No. 3 MM chromatography paper, 2.3 cm diameter). The radioactivity in RNA was measured with the method of MANS AND NOVELLI[13] omitting the hot trichloroacetic acid treatments. Radioactivity was measured in a liquid scintillation counter after which the disks were removed and washed with toluene, toluene–ethanol, and ethanol–ether and then subjected to a 60-min heat treatment at 90° in 5% trichloroacetic acid. After subsequent alcohol–ether and ether washes the disks were replaced in their original vials and recounted to give "non-RNA counts". These counts were usually very low and no substraction from the original counts was necessary. The total RNA content of the tissue was measured according to the method of MUNRO AND FLECK[14]. The results are expressed as counts/min per 100 μg of RNA/h.

The thymocytes were isolated from the thymus mechanically[15] and the incorporation of [5-^{3}H]uridine into RNA of the thymocytes *in vitro* was determined according to YOUNG[16].

RESULTS

The tryptophan pyrrolase activity in the soluble liver fraction from an untreated guinea pig was very low (0.43 $\pm$ 0.03 μmole/h per g liver, Mean $\pm$ S.E.). 10 mM ascorbic acid and 1.33 μM hematin did not have any effect on the activity, and preincubation of the soluble fraction in the presence of hemoglobin, ascorbic acid and

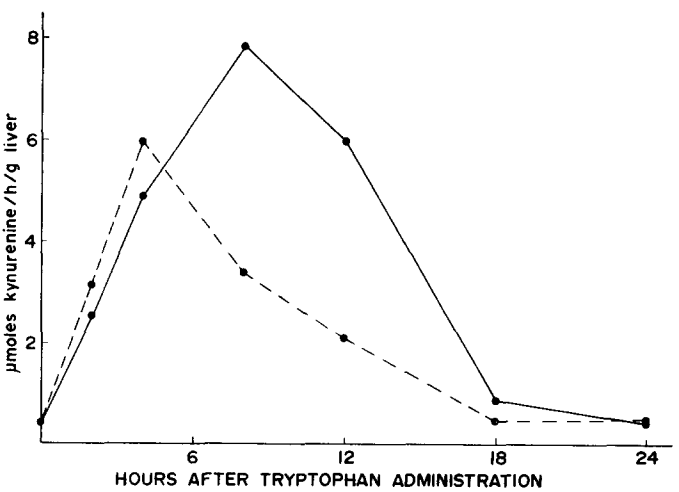

Fig. 1. Effect of tryptophan administration on the tryptophan pyrrolase activity. Guinea pigs were given 100 mg of L-tryptophan per 100 g of body weight intraperitoneally as suspension in 0.9% saline and killed at the indicated times thereafter. Every point represents the mean activity in the liver of three animals. The tryptophan pyrrolase activity in the soluble fraction of liver was assayed according to KNOX *et al.*[4] in the presence (———) and absence (— — —) of ascorbic acid.

tryptophan at 37° for 60 min totally abolished the activity. An identical activity (0.53 μmole/h per g liver) for tryptophan pyrrolase was delineated by measuring the activity of the total homogenate of guinea pig liver in oxygen atmosphere without any cofactors[2]. In these experiments kynurenine was determined by using the Bratton–Marshall test.

A marked increase in the tryptophan pyrrolase activity was demonstrated after tryptophan treatment (100 mg of L-tryptophan per 100 g in 1 ml of 0.9% saline intraperitoneally) of guinea pigs. The response of the enzyme activity to tryptophan administration at different times after treatment is shown in Fig. 1. The dash line

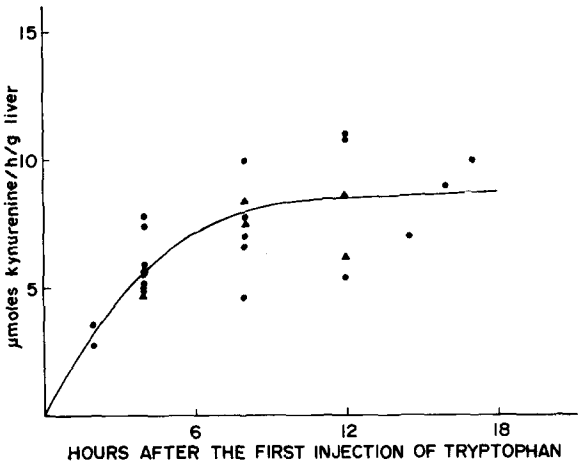

Fig. 2. Effects of repeated administrations of tryptophan and hydrocortisone on the tryptophan pyrrolase activity. Animals were given injections every 4 h as follows: 100 mg of L-tryptophan per 100 g intraperitoneally as suspension in 0.9% saline (●) or tryptophan *plus* 2.5 mg of hydrocortisone acetate per 100 g intraperitoneally as suspension in 0.9% saline (▲). Each point shows the activity in the soluble liver fraction of one animal. The tryptophan pyrrolase activity was assayed according to KNOX *et al.*[4] without any cofactors.

shows the activity without any cofactors. The activity reached its maximum at 4 h. The solid line represents the activity in the presence of ascorbic acid (10 mM) showing the maximum 4 h later. The basal level of the enzyme activity was reached at 18 h without cofactors (at 24 h with ascorbic acid). Fig. 2 shows the activity after the repeated injection of tryptophan (at 4-h intervals). There was a rapid increase of the activity during the first 4 h, which was followed by a slower increase. Ascorbic acid did not have any effect on the activity at any moment after the start of the tryptophan administration. The animals did not survive repeated doses of tryptophan, but died after three injections. The rise in the tryptophan pyrrolase activity after tryptophan administration was only slightly inhibited by actinomycin D (100 μg/100 g) (Table I) suggesting, that tryptophan-induced elevation of the enzyme activity does not require the renewal of cellular RNA.

The additions of hematin (1.33 μM) and ascorbic acid (10 mM) did not significantly increase the initial rate of the tryptophan pyrrolase reaction if a concentrated (25%) preparation was used. Dilution of the preparation revealed a need for ascorbic

TABLE I

EFFECT OF ACTINOMYCIN D ON THE INDUCTION OF TRYPTOPHAN PYRROLASE BY TRYPTOPHAN IN GUINEA PIG LIVER

L-Tryptophan (100 mg per 100 g of body weight) and actinomycin D (100 μg per 100 g of body weight) were administered simultaneously intraperitoneally to guinea pigs 5 h before sacrifice. Tryptophan pyrrolase in the soluble liver fraction was assayed according to KNOX *et al.*[4] with ascorbic acid in the incubation mixture. Each group consisted of 4–6 animals. Values are expressed as mean ± S.E.

Administered substances		*Tryptophan pyrrolase (μmoles kynurenine per h per g liver)*
Tryptophan	*Actinomycin D*	
−	−	0.43 ± 0.03
+	−	5.25 ± 0.17
+	+	3.65 ± 0.34

acid, which can be seen in Fig. 3. Hematin in concentrations of 0.083 to 2.67 μM did not activate the diluted enzyme solution.

If the soluble fraction (25% extract) was stored at 5° a marked inactivation of tryptophan pyrrolase was observed after two days. 10 mM ascorbic acid in the assay mixture restored partly the enzyme activity. Hematin (1.33 μM) (in the presence and absence of ascorbic acid) was without any effect on the activity of the stored enzyme. The effect of the concentration of ascorbic acid on the activation of the stored tryptophan pyrrolase is shown in Fig. 4. 3 mM ascorbic acid was necessary for the optimal activation.

Dialysis at 5° against distilled water for 18 h abolished totally the tryptophan pyrrolase activity. Hematin (1.33 μM) and ascorbic acid (10 mM) could not restore the activity of the dialysed enzyme solution.

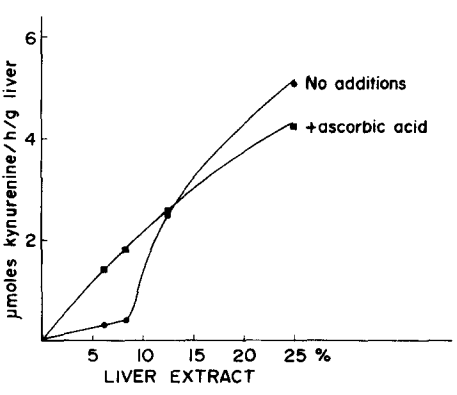

Fig. 3. Effect of the concentration of liver extract on the tryptophan pyrrolase activity. The concentrated enzyme solution (25%) from tryptophan-treated guinea pigs (sacrificed 5 h after tryptophan administration) was diluted with the homogenizing medium and kept at 5° for 30 min before the activity was assayed in the presence and absence of ascorbic acid.

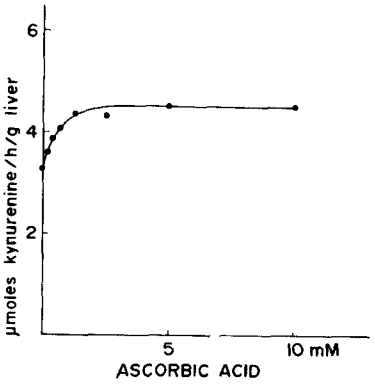

Fig. 4. Effect of the concentration of ascorbic acid on the tryptophan pyrrolase activity. The soluble liver fraction from tryptophan-treated guinea pigs (sacrificed 5 h after tryptophan administration) was stored for two days at 5° before the tryptophan pyrrolase activity was assayed in different concentrations of ascorbic acid.

When the tryptophan pyrrolase activity was assayed 4 to 12 h after the administration of hydrocortisone or prednisolone (2.5 mg/100 g in 1 ml of 0.9% saline solution intraperitonelly or subcutaneously), it was apparent that the enzyme was not induced by these hormones. The activity in the soluble fraction of the liver was always at the control level (less than 0.5 μmole/h per g liver), and was not dependent on cofactors (ascorbic acid and hematin). The preincubation of the enzyme solution at 37° for 60 min in the presence of tryptophan, ascorbic acid and methemoglobin inactivated the enzyme. Even a repeated injection of hydrocortisone (3 times at 4-h intervals) was without any effect on the activity. The administration of hydrocortisone and tryptophan together did not elicit any further increase in the tryptophan pyrrolase activity in comparison with that seen after tryptophan administration (Fig. 2).

Earlier studies have established that glucocorticoids augment the incorporation of radioactivity from labelled precursors into hepatic RNA in rat[17] but inhibit that

TABLE II

EFFECT OF PREDNISOLONE ON THE INCORPORATION OF [5-^{3}H]URIDINE INTO RNA OF GUINEA PIG LIVER AND THYMOCYTES

Animals were treated with prednisolone sodium acetate (2.5 mg per 100 g of body weight as suspension in 0.9% saline intraperitoneally) and sacrificed at the indicated times after hormone treatment. The incorporation of [5-^{3}H]uridine into RNA of liver and thymocytes *in vitro* was measured as explained in MATERIAL AND METHODS. The incubation times for liver and thymocytes were 1 h and 10 min, respectively. Values are expressed as mean ± S.E.

	Liver (counts/min per 100 μg of RNA)	*Thymocytes (counts/min per $1 \cdot 10^6$ cells)*
Control	687 ± 31	1215 ± 36
Prednisolone 4 h	1048 ± 124 ($P < 0.05$)	
10 h		552 ± 4

into RNA of thymocytes[18]. Table II shows that prednisolone does stimulate, although weakly, the incorporation of [5-^{3}H]uridine into RNA of guinea pig liver in 4 h after hormone treatment (2.5 mg per 100 g intraperitoneally). No significant increase of the incorporation could be demonstrated 4 h after hydrocortisone treatment. It was further seen that the incorporation of [5-^{3}H]uridine into RNA was markedly reduced in thymocytes isolated from guinea pig thymus 10 h after prednisolone treatment (Table II).

DISCUSSION

The induction of tryptophan pyrrolase by the glucocorticoids has been reported in the rat[2] and the mouse liver[5]. The failure of glucocorticoids to induce this enzyme in guinea pig liver is intriguing. SPIEGEL[6] has reported similar results with the frog liver tryptophan pyrrolase, and BROWN AND DODGEN[7] found that channel catfish liver tryptophan pyrrolase was not induced by either glucocorticoids or tryptophan. Both the guinea pig and the channel catfish were not able to survive repeated doses of tryptophan. JOHNSON AND DYER[8] obtained a reduction in tryptophan pyrrolase after administering tryptophan to the bovine. Aside from the unexpected regression after

tryptophan administration some of the treated bovine died. KNOX[19] has reported similar results in adrenalectomized rats, wheras intact rats, or adrenalectomized rats receiving hydrocortisone did not die. The failure of adrenalectomized rats to survive was attributed to a loss of the hormonal induction mechanism. Analogously, the failure of guinea pigs to survive repeated doses of tryptophan is an additional evidence supporting the absence of hormonal induction of the tryptophan pyrrolase. Although glucocorticoids were incapable of inducing the tryptophan pyrrolase, they did show other signs of glucocorticoid activity. Prednisolone treatment increased the incorporation of [5-^{3}H]uridine into RNA of the liver and decreased its incorporation into RNA of the thymocytes, which effects besides tryptophan pyrrolase induction are among the earliest signs of glucocorticoid action[17,18].

Two forms of tryptophan pyrrolase (oxidized and reduced forms) were found in the soluble fraction of guinea pig liver. The enzyme was not dependent on the presence of cofactors (ascorbic acid and hematin) in the incubation medium, when the soluble fraction was prepared from intact animals or from animals shortly after tryptophan treatment. However, ascorbic acid was necessary for attaining the maximal activity 8–18 h after tryptophan treatment. Dilution of the concentrated preparation and storage of the enzyme solution revealed a need for ascorbic acid, too.

We were not able to demonstrate the apoenzyme form of tryptophan pyrrolase in guinea pig liver. This form has been found in rat liver, and it is the most abundant form after hydrocortisone treatment. The preincubation of the enzyme solution according to KNOX *et al.*[4] inactivated the enzyme, and the addition of hematin into the incubation medium was without effect. None of the treatments (aging, dialysis and preincubation) caused unconjugation of the enzyme, which reflects poor reversibility of the conjugation.

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