A TWO-STEP MECHANISM FOR THE REGULATION OF TRYPTOPHAN PYRROLASE

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Allopurinol, (4-hydroxypyrazolo[3,4-d]pyrimidine) an inhibitor of xanthine oxidase, prevents the corticoid-stimulated increase in tryptophan pyrrolase activity but does not impair the hormone-mediated synthesis of this enzyme. We conclude that the regulatory mechanism occurs via two steps: 1) the synthesis of inactive tryptophan pyrrolase molecules; and 2) the activation of these molecules by xanthine oxidase.

It is well known that a higher activity of tryptophan pyrrolase can be induced in rat liver by injection of corticoids (1). Moreover, this increase in activity is attributed to synthesis of tryptophan pyrrolase de novo (2). Allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine), the competitive inhibitor of xanthine oxidase (3), has been demonstrated to inhibit tryptophan pyrrolase activity in crude liver preparations either when added during the assay (4) or when injected into control rats (5). This compound has been shown to prevent the activation of tryptophan pyrrolase by purines in vitro (4) suggesting the involvement of xanthine oxidase in this process. Preliminary experiments performed in our laboratory demonstrated that injection of allopurinol with hydrocortisone is followed by a decreased activity of tryptophan pyrrolase when compared with the enzyme activity in animals injected with the inducer only (6,7). A possible explanation for these results would be that allopurinol

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does not interfere with the induction mechanism but rather inhibits the conversion by xanthine oxidase of the inactive tryptophan pyrrolase to the active enzyme. If this hypothesis is correct, the administration of allopurinol should lead to an accumulation of an inactive tryptophan pyrrolase.

EXPERIMENTAL METHODS

Tryptophan pyrrolase activity in the homogenates or particulate free supernatants was assayed according to different methods as mentioned in the legends. Several liver tryptophan pyrrolase preparations were obtained either by the method of O. Greengard and P. Feigelson (8), or by the method of R. T. Schimke, et al. (9). These preparations were tested in the absence and presence of allopurinol (0.33 mM) and hemoglobin (3×10^{-3} mM) in an assay including 0.2 M sodium phosphate buffer pH 7.0, 0.03 M tryptophan and sufficient 0.14 M KCl for a total volume of 3.0 ml. The appearance of formylkynurenine was followed at 321 mμ.

Antibodies were produced against the purified tryptophan pyrrolase prepared according to Schimke, et al. (9). Rabbits were given toe pad injections once a week for 5 weeks of 0.25 mg tryptophan pyrrolase, administered in 0.25 ml buffer plus 0.25 ml Freund's adjuvant 1:8, complete:incomplete. At the end of the sixth week the blood was withdrawn by cardiac puncture. The globulin fraction was isolated from the sera by column chromatography on a DE-52 column equilibrated and eluted by 0.01 M sodium phosphate buffer pH 7.4. The pooled globulin fraction was concentrated by ultrafiltration.

RESULTS AND DISCUSSION

Figure 1 shows that when adult male rats (Holtzman albino strain) were injected with both hydrocortisone and allopurinol (4-hydroxypyrazolo[3,4-d] pyrimidine) the activity of tryptophan pyrrolase in whole liver homogenates determined by the method which measures the active holoenzyme (10) is substantially lower in comparison with animals injected with the inducer only. A delayed injection of allopurinol after hydrocortisone as shown in Figure 1 also inhibits the tryptophan pyrrolase even though the action of allopurinol in one case was

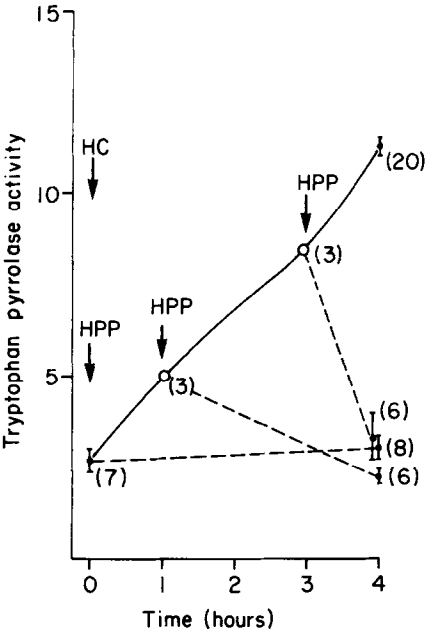

Fig. 1. Effect of injection allopurinol on tryptophan pyrrolase activity induced by hydrocortisone. Male albino rats were injected intraperitoneally with hydrocortisone (HC) 50 mg/kg at zero time. Allopurinol (HPP) 20 mg/kg in 0.1 N sodium bicarbonate, was injected at time indicated by the arrows, i.e. at zero time or 1 or 3 hours after administration of hydrocortisone. Four hours after the administration of hydrocortisone the animals were sacrificed and the livers were homogenized in a buffer containing tryptophan (4). Tryptophan pyrrolase activity in the homogenates was assayed after the original method of Knox (10), which does not employ addition of ascorbic acid or hemoglobin into the assay medium. The activity is expressed as micromoles of kynurenine formed per gram of liver (wet weight ± S.E.) per hour. The numbers in parentheses represent the number of animals used.

limited to one hour, suggesting that tryptophan pyrrolase undergoes a rather fast inactivation and activation process in vivo. These results show that if the activation process is impaired by administration of the inhibitor of xanthine oxidase, less of the active holoenzyme is found.

A lower activity of tryptophan pyrrolase after simultaneous injection of allopurinol and hydrocortisone is also found when the activity is measured in particulate free supernatants. (Table I - Column A). These were subjected to further analysis in order to determine if this treatment (i.e. injection of allopurinol plus hydrocortisone) causes the accumulation of an inactive enzyme during the hormonal induction. When the particulate free preparations from

Table I

Accumulation of inactive holoenzyme during corticoid induction of tryptophan pyrrolase in rats injected with allopurinol

Treatment	Tryptophan pyrrolase activity: Active Enzyme non-dialyzed A	Active Enzyme dialyzed B	Inactive Enzyme B-A
Hydrocortisone	10.6 ± 1.5 (7)	9.10 ± 1.2 (6)	
Hydrocortisone plus allopurinol	6.1 ± 0.07 (7)	11.5 ± 1.3 (6)	5.4

Rats were injected with hydrocortisone plus allopurinol as described in Fig. 1. Particulate free supernatants were prepared from liver homogenates (4) 4 hours after administration of the compounds, and tryptophan pyrrolase was assayed in the presence of hypoxanthine (0.33 mM) as described earlier (4). Enzyme activity was tested before and after dialysis (18 hr) against three changes of buffer containing L-tryptophan (4). The activity of tryptophan pyrrolase ± S.E. is expressed as in Fig. 1. The numbers in parentheses represent the number of animals used. The difference in activity in non-dialyzed samples from hydrocortisone and hydrocortisone plus allopurinol-treated rats is statistically significant ($P < 0.01$).

livers of hydrocortisone and allopurinol injected animals were extensively dialyzed to remove allopurinol, the activity of tryptophan pyrrolase recovered in the dialyzed samples was the same or higher than that in the rats injected with the corticoid only (Table I - Column B). Therefore, the difference in the activity before and after dialysis is a measure of the amount of inactive holoenzyme accumulated after injection of allopurinol.

Comparison of the activity of tryptophan pyrrolase in particulate free supernatants prepared from rats injected with hydrocortisone and hydrocortisone plus allopurinol shows that when the activity is measured by the assay employing both ascorbic acid and hemoglobin, no inhibition of tryptophan pyrrolase can be observed (Table II - Column A). These results confirm the recent observations (6,7) that the inhibition of tryptophan pyrrolase can be seen only when this enzyme is assayed in the absence of ascorbic acid. This suggests that the inhibitory action of allopurinol on tryptophan pyrrolase activity is not due to a direct effect of allopurinol on tryptophan pyrrolase, but rather due to the

Table II

Effect of addition of antitryptophan pyrrolase on the activity of liver tryptophan pyrrolase from hydrocortisone and hydrocortisone plus allopurinol-treated animals.

Exp.	Treatment	Tryptophan pyrrolase activity		Activity inhibited by antibodies	Percent Inhibition
		A	B		
		Control	Antitryptophan pyrrolase	A - B	
	Hydrocortisone	18.6	4.9	13.7	78
1	Hydrocortisone plus allopurinol	23.9	7.6	14.3	68
	Hydrocortisone	32.8	13.7	9.0	28
2	Hydrocortisone plus allopurinol	30.5	11.6	8.8	30

Particulate free (105,000 xg) liver supernatants (4) obtained from rats (pooled sample from 3 animals) 4 hours after injection of hydrocortisone or hydrocortisone plus allopurinol (for doses see Fig. 1) were assayed for tryptophan pyrrolase activity using the assay mixture of Knox, et al. (11) which contains ascorbic acid and hemoglobin, without preincubation. The assay was performed after addition of purified antitryptophan pyrrolase or control rabbit globulin fraction. Immunoelectrophoresis on cellulose-acetate strips showed one precipitation band for the antitryptophan pyrrolase (12). Two different antibody preparations and high speed supernatants were used in experiments 1 and 2. The particulate free supernatant diluted 1:3 with 0.14 M KCl was exposed to an equal volume of antitryptophan for 14 hours at 4° C before assaying. Tryptophan pyrrolase activity is expressed as described in the legend to Figure 1.

inhibition of xanthine oxidase. Apparently activation of tryptophan pyrrolase by ascorbic acid bypasses the endogenous activating process involving xanthine oxidase.

Additional evidence that the induction mechanism is not impaired by allopurinol was demonstrated by the fact that livers from animals treated with allopurinol plus the inducer contain no less immunologically reactive material. This is demonstrated in Table II where the inhibition of tryptophan pyrrolase activity is obtained by treating the particulate free supernatant with antibodies prepared against a purified preparation of tryptophan pyrrolase.

An attempt was then made to determine if the allopurinol directly inhibits

tryptophan pyrrolase activity. When several batches of tryptophan pyrrolase were purified by two different procedures of purification, the activity of these preparations was not inhibited by addition of allopurinol (12). This offers substantial evidence that the tryptophan pyrrolase molecules are not directly affected *in vitro* by allopurinol.

The results prove that the induction mechanism is not impaired by allopurinol. On the contrary, in most of the experiments in which dialyzed particulate free extracts were assayed in the presence of hypoxanthine (Table I - Column B), or non-dialyzed particulate free extracts were assayed in the presence of ascorbic acid plus hemoglobin (Table II - Experiment 1, Column A), a somewhat higher activity was observed in animals treated by allopurinol. This suggests a possible effect of allopurinol on the induction of tryptophan pyrrolase via the adrenal-pituitary system. Furthermore, in particulate free extracts, the higher tryptophan pyrrolase activity corresponded to a lower inhibition by antitryptophan pyrrolase (Table II). The results suggest that since allopurinol does not affect the induction mechanism nor the tryptophan pyrrolase directly, it apparently causes a dissociation between two events which must occur in order to realize a higher tryptophan pyrrolase activity after the injection of corticoids, i.e. between the formation of an inactive enzyme caused by the corticoid and the activation of this form of tryptophan pyrrolase to the active enzyme through the action of xanthine oxidase. When the activation step is inhibited, inactive tryptophan pyrrolase is accumulated in the liver. This activation by xanthine oxidase indicates a role of this enzyme in metabolism previously unknown.

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