STIMULATION OF HYDROCORTISONE-INDUCED RAT LIVER TRYPTOPHAN PYRROLASE BY TRIAMTERENE

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SUMMARY: Pretreatment with triamterene (2,4,7-triamino-6-phenylpteridine) results in significant increase in the level of endogenous holotryptophan pyrrolase over the induction caused by hydrocortisone alone. The pteridine perhaps results in a favorable stoichiometric ratio in vivo of apotryptophan pyrrolase and the heme coenzyme.

Triamterene (2,4,7-triamino-6-phenylpteridine) is a comparatively new synthetic drug, diuretic in effect and used in the treatments of edema associated with congestive heart symptoms, the nephrotic syndrome, cirrhosis, late pregnancy as well as steroid-induced edema (1,2,3). This substance belongs to a group called pteridines, the basic ring system of which perhaps occurs in all living organisms (4). Certain naturally occurring pteridines have been implicated in the biological oxidation of tryptophan and its derivative, kynurenine (5,6). As part of an experimental design based on stereochemical reasoning, to explore the effects of certain aromatic amino pteridines on the oxidative metabolism of tryptophan, it was of interest to study the nature of interaction between triamterene and hydrocortisone with regard to tryptophan pyrrolase activity in the rat liver.

EXPERIMENTAL

Intact albino male rats (the Sprague-Dawley line; body weights 200-225g.), lean and uniform with respect to vitality and morphology, were chosen for experimentation. When indicated by the treatment, hydrocortisone and triamterene were administered intraperitoneally at the rate of 25 mg/kg and 15 mg/kg body weights respectively. The steroid and the pteridine were dissolved in propylene glycol (Hartman-Leddon Company, Philadelphia).

Animals from different treatments were sacrificed at appropriate times and the livers removed for enzyme extraction and assay. First, the basal level of enzyme activity was established. The second and the third treatments consisted of injecting hydrocortisone at "zero" time and removing livers for enzyme extraction at the 4th and 8th hours respectively. Likewise, the 4-hour and 8-hour effects of triamterene were established in two separate treatments. The sixth treatment consisted of injecting hydrocortisone at "zero" time and triamterene 4 hours later and livers removed for enzyme extraction at the 8th hour beginning the administration of the steroid. The seventh treatment called for injection of triamterene at "zero" time and hydrocortisone 4 hours later, removing livers at the 8th hour beginning the administration of the pteridine.

The rat liver enzyme was prepared according to the method of Knox (7) and assayed as previously described (5), but triplicate reaction mixtures from each extract were submitted to two different ways of incubation: (i) at $37^{o}C$ for 2 hours immediately after centrifugation (referred to as "initial activity") and (ii) storage at $4^{o}C$ for 18 hours followed by incubation at $37^{o}C$ for 2 hours (referred to as "total activity"). The storage of the enzyme preparation with the substrate resulted in 2 to 4-fold increase in activity (Table 1). Two controls were run simultaneously for each reaction; (i) endogenous, containing all reaction components except L-tryptophan which was added after stopping enzyme activity with trichloroacetic acid and (ii) non-enzymatic, with all components except the enzyme preparation. Thus, each specific activity value was corrected for two controls. Enzyme activities reported in this paper are comparable to the active, endogenous holoenzyme (8), as distinct from that measured after the addition of exogenous cofactor or upon incubation with mitochondria (or microsomes) plus exogenous coenzyme.

RESULTS AND DISCUSSION

The 4-hour pretreatment with triamterene resulted in 285 percent increase in enzyme activity in the succeeding 4-hour effect of hydrocortisone (compare

9.258 μmoles with 3.249 μmoles, Table I). Strictly, the interaction between the steroid and the pteridine alone over the later 4-hour period resulted in 137 percent increase in activity (9.258 μmoles minus 3.904 μmoles) over the combined but separate effects of triamterene and hydrocortisone (0.655 μmoles plus 3.249 μmoles) of a comparable time span. However, when the injection of

TABLE I

Levels of Active, Endogenous Holoenzyme of Tryptophan Pyrrolase in the Rat Liver

TREATMENTS	Specific Activity-total (μmoles kynurenine/g. liver/2 hrs.)	% of treatment 7	Specific Activity-initial (μmoles kynurenine/g. liver/2 hrs.)	% of treatment 7
1. Basal Level	0.295 ± .02 (9)	3	0.063 ± .02 (9)	1.5
2. Hydrocortisone, 4 hour	3.249 ± .11 (9)	35	1.729 ± .04 (9)	42.2
3. Hydrocortisone, 8 hour	5.087 ± .08 (9)	55	2.282 ± .07 (9)	56
4. Triamterene, 4 hour	.220 ± .02 (9)	2.4	.053 ± .01 (9)	1.3
5. Triamterene, 8 hour	.655 ± .02 (9)	7.1	.173 ± 0 (9)	4.2
6. Hydrocortisone, "zero" time, triamterene 4 hours later, livers removed at the 8th hour	4.254 ± .18 (9)	46	1.236 ± .04 (9)	30
7. Triamterene, "zero" time, hydrocortisone 4 hours later, livers removed at the 8th hour	9.258 ± .16 (9)	100	4.07 ± .05 (9)	100

Figures in parenthesis indicate the total number of reactions performed with three different rats in each treatment. Significant differences in mean values at .05 level obtained by Factorial Analysis of Variance and the Duncan Multiple Range Test are indicated by "greater than" symbols in the two series of mean values: series $\longrightarrow$ 9.258 > 5.087 > 4.254 > 4.07 > 3.249 > 2.282 > 1.729 > 1.236 > 0.655 > 0.295 | 0.220 | 0.173; series $\longrightarrow$ 0.295 > 0.063 | 0.054. A bar between two values stands for non-significant difference.

hydrocortisone preceded triamterene by 4 hours, the level of activity was reduced by 17 percent (compare 5.087 μmoles plus 0.22 μmoles with 4.254 μmoles, Table I). Though this reduction was significant, according to statistical analysis, the low margin of difference may not allow one to make a positive argument in this case. Hydrocortisone effects more synthesis of the apoenzyme (9,10,11), whereas tryptophan stabilizes and prevents its degradation (12). The conjugation of apotryptophan pyrrolase and the coenzyme should increase the enzyme activity *in vivo* and in absence of an adequate amount of the coenzyme, the apoenzyme is likely to be degraded during metabolic turnover. A high rate of hormonal induction of the enzyme in conjunction with slow release and conjugation of the coenzyme *in vivo* should explain the lack of stimulation due to the 4-hour effect of triamterene following the administration of hydrocortisone.

The positive, significant triamterene-hydrocortisone interaction (treatment 7) reported in this paper is especially worthy of note. Such an effect may result from inhibition caused by triamterene of an enzyme or a reaction system with higher activity than tryptophan pyrrolase, both utilizing a common cofactor(s). Tryptophan pyrrolase is activated by hematin (13), ascorbic acid, H_2O_2 (14) and xanthin oxidase (15) and the roles of ascorbic acid and H_2O_2 have been ascribed to reduction of heme-iron to the ferrous state, the catalytic form of the enzyme. The stimulation caused by xanthin oxidase is indirect through production of H_2O_2 in the course of transforming hypoxanthin to uric acid (15). Triamterene, 1×10^{-4}M, when added in *in vitro* reactions, does neither stimulate nor inhibit xanthin oxidase or tryptophan pyrrolase (16). The heme moiety of tryptophan pyrrolase dissociates readily and this has been proposed as a major control in the holoenzyme $\rightleftharpoons$ apoenzyme + heme equilibrium (17). Inhibition by triamterene, then, of another reaction strongly competing for heme should produce better stoichiometry of the apo-and-the coenzyme of tryptophan pyrrolase resulting in the effect observed by us (treatment 7, Table I). One can conceive of mass action as well, with regard to coenzyme binding, by apotryptophan pyrrolase molecules.

However, the possibility of direct induction of more coenzyme through synthesis exerted at the genic, either transcriptional or translational, should not be ruled out. Such a mechanism should invoke the existence of a certain degree of repression for coenzyme synthesis and that triamterene suppresses the action of the "repressor".

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