CORTISOL ACTIVATION OF RAT LIVER GLUTAMATE ASPARTATE TRANSAMINASE *

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Kenney (1962) has recently suggested that adrenocorticoid dependent induction of rat liver alpha-ketoglutarate tyrosine transaminase involves a de novo enzyme synthesis. A similar mechanism is proposed by Segall et al. (1962) for the observed steroid induction of glutamate-pyruvate transaminase. With glutamate-aspartate transaminase (GOT), however, the action of steroids is not consistent; its inhibition in thymocites by cortisol has been reported by Blecher et al. (1957) and by Eischied and Kochackian (1954) in mice liver homogenates. On the other hand, Gavosto et al. (1957) observed that cortisol induced an increase in rat liver transaminase activity. Finally, Harding et al. (1960) pointed out the lack of activity of steroid hormones in rat liver homogenates as GOT inducers.

We studied the effect of some steroids on GOT activity employing small hormone doses in acute experiments in which the hormone was administered continuously by portal infusion. Measurements of enzymatic activity were carried out on homogenates of a series of liver biopsies during the perfusion.

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Methods. Albino male and female rats from a local colony weighing 150 to 200 gm and fed a purina diet were used. Cortisol, triamcinolone, progesterone, testosterone, estrone, estradiol and desoxicorticosterone acetate dissolved in propylene-glycol were suspended in 400 volumes of 0.9 % NaCl and perfused at a flow rate of 0.25 ml/min. The dose range assayed varied from 3.7 to 12.5 mcg/min. In some experiments 2 gm of a protein hydrolyzate (Difco, Casaminoacids) or 2 gm of a mixture of l-aspartic or l-glutamic with alpha-ketoglutaric acids were perfused with the hormone. 50 to 80 mg of liver tissue were removed at zero time and at 10 minutes intervals thereafter. Each liver fragment was homogeneized in a Potter blender with 20 volumes of ice cold Sorensen phosphate buffer at pH 7.6 and treated for 10 minutes with ultrasonic vibrations delivered by a Mullard Unit 20 kc, type 7685/2. GOT activity measurements were carried out in the supernatants obtained by centrifugation of the homogenates in the cold at 3000 rpm for 10 minutes. The method of Camaratta and Cohen (1951) as modified by Kun et al. (1960) was used. Under the conditions of the test, one Unit is equal to 0,001 micromole of oxaloacetic acid per minute. Protein was determined by the absorbancy at 280 mu using the Nessler modification of Lanni et al. (1950) for the nitrogen calibration curve.

Results and Discussion. The liver homogenates from 17 animals perfused with cortisol at the doses refered to above, showed a clear increase in GOT specific activity. Forty minutes after the infusion started, the average increase over the basal values was 50.8 ± 17.2 percent in non sonicated homogenates and 90.5 ± 33.9 percent in ultrasonic treated homogenates. The statistical significance applying a t test, gave a $p < 0.01$. Results on the effect of different perfused steroids on rat liver GOT activity are displayed in Fig 1 and Table 1. When cortisol infusion dose was below 3.7 mcg per minute no activation was

Table 1

EFFECT OF PERFUSED STEROIDS ON SPECIFIC ACTIVITY OF RAT LIVER GOT*

Steroid	Perfusion time in minutes 0	10	20	30	40	50	60
	(Non sonicated homogenates)						
Cortisol (12)**	28.8±5.2	28.8±5.7	29.5±5.4	33.2±6.6	43.4±7.6	46.8±8.3	47.2±9.1
	(Sonicated homogenates)						
Cortisol (8)**	33.6±6.0	24.1±6.2	34.5±6.1	45.2±9.3	64.1±14	65.4±14.1	64.8±14.8
Triamcinolone (5)**	24.28	24.22	24.35	26.65	29.43	31.33	32.14
Testosterone (2)**	30.30	31.06	31.25	32.00	31.26	31.78	30.75
Estrone (2)**	26.62	26.85	27.07	27.25	26.93	26.44	26.80
Progesterone (3)**	28.16	29.04	27.17	28.82	29.02	28.84	28.83
DOCA (2)**	31.75	31.25	31.86	33.06	33.88	32.67	31.25
NaCl 0.9% (2)**	25.55	25.78	25.69	26.02	26.08	25.97	25.90

* Values given in units of GOT activity per mg of protein nitrogen of homogenates. One unit of GOT equals 0.0011 micromole of oxaloacetic acid formed. In cortisol experiments, standard deviation values are given. For steroids other than cortisol, typical experiments in non sonicated homogenates, carried out in duplicate, are presented; sonication did not produce any change in GOT specific activity. All steroids were perfused at a dose of 12.5 mcg per minute.

** Figures in parentheses indicate number of experiments.

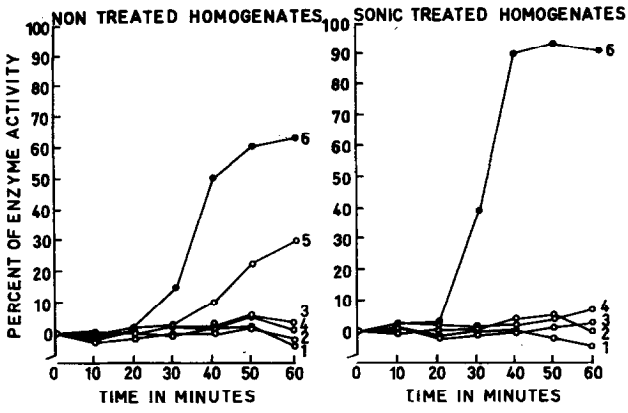

Figure 1. Activation of rat liver glutamic aspartic transaminase by perfused steroids.

Experimental values correspond to percent increase of specific enzymatic activity over homogenates at zero time prior to steroid infusion. All steroids were perfused at a dose of 12.5 mcg per minute.

Reaction mixture for GOT: l-aspartic acid 160 micromoles; alpha-ketoglutaric acid 80 micromoles; sodium tetraborate 200 micromoles; magnesium sulphate 1 micromole; pyridoxal 5' phosphate 20 mcg; tris buffer 0.2 M; 1: 20 rat liver homogenate 0.01 to 0.1 ml; final volume 3.0 ml, pH 8.3

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observed during the experimental period. No clearcut correlation between increasing amounts of perfused cortisol and a corresponding degree of enzyme activation was observed.

Triamcinolone, at doses similar to those of cortisol induced at least 30 percent GOT activation (Fig 1, Table 1). No transaminase activation with similar weight doses of estrone, estradiol, progesterone, desoxicorticosterone or testosterone was obtained. The addition of a protein hydrolyzate or GOT substrates along with the perfused steroids produced no effect.

The administration of 75 mg of ethionine in the perfusate did not suppress the cortisol induced GOT activation; complete suppression was obtained, however, by i.p. administration of ethionine 24 hours before cortisol perfusion.

The observation that 'latent' enzymes liberated by ultrasonic vibrations are more apt to be activated, suggests the possibility that the hormone is ex-

erting a direct effect on the enzymes originally located inside the mitochondria or in other subcellular particles or that the removal of positive inhibitors is more effective after disruption of these structures.

On the other hand, the effect of pretreatment with ethionine a known suppressor of protein synthesis on the GOT steroid induced activation, points to the possibility that the hormone may participate in the formation of apoenzyme, as has been previously postulated by Kenney (1962), Goldstein et al. (1962), Segall et al. (1962) and Barnabei and Sereni (1962) for other transaminases.

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