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Induction of tyrosine aminotransferase in rat liver by epinephrine and theophylline

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TYROSINE aminotransferase (EC 2.6.1.5) is induced in liver of rats and in isolated, perfused rat liver by glycogon and by other hormones.[1–4] Glucagon stimulates adenyl cyclase, thereby increasing cyclic AMP concentrations. Cyclic AMP may mediate the induction of tyrosine aminotransferase by hormones, for dibutyryl cyclic AMP induces the enzyme in explants of liver from fetal rats.[5] Tyrosine aminotransferase in such cells is also increased by epinephrine, another stimulator of adenyl cyclase, and by theophylline, an inhibitor of phosphodiesterase.[5] We report here the effects of epinephrine and theophylline on tyrosine aminotransferase in liver of rats.

Male albino rats derived from the Wistar strain, weighing about 150 g each, were obtained from a local supplier. Some rats were bilaterally adrenalectomized 5 days before an experiment. All rats were given Purina Lab Chow and water (or saline in the case of adrenalectomized rats) *ad lib.*

L-Epinephrine bitartrate from Nutritional Biochemicals was used; it was injected s.c. at doses that are expressed as amount of free epinephrine. Theophylline anhydrous (Lilly) and hydrocortisone (Sigma) were injected i.p.

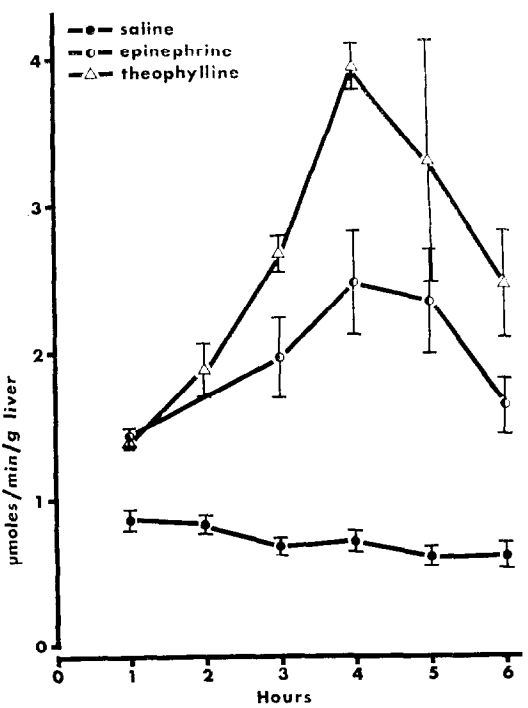

FIG. 1. Effect of epinephrine and theophylline on tyrosine aminotransferase in rat liver. Epinephrine (0·5 mg/kg) theophylline (75 mg/kg) or saline was injected at zero time, which was 9 a.m. There were five rats per group; means and standard errors are indicated.

Immediately after the rats were killed by decapitation, their livers were removed and frozen on dry ice; they were then stored frozen prior to analysis. Tyrosine aminotransferase activity in 100,000 *g* supernatant fractions from liver homogenates was assayed spectrophotometrically[6] in a Gilford multiple-sample absorbance recorder. Data are expressed in terms of micromoles of *p*-hydroxyphenylpyruvic acid formed. Corticosterone in plasma was measured fluorometrically.[7]

Figure 1 shows liver tyrosine aminotransferase activity at several times after administration of epinephrine or theophylline. Since there is a daily rhythm in liver tyrosine aminotransferase in rats,[8] it was necessary to include values for saline-treated controls at each time. As shown in Fig. 1, the period of the day chosen was one in which the liver enzyme remains relatively constant. Elevation of tyrosine aminotransferase by both epinephrine and theophylline was apparent 1 hr after drug administration. In this experiment, epinephrine caused a greater than 3-fold increase, whereas theophylline increased the enzyme by more than 5-fold. The maximum effect by both drugs was at 4 hr, and that time interval was used in further experiments.

Table 1, section A, contains dose–response data for epinephrine. In intact rats, epinephrine at a dose as low as 0·2 mg/kg significantly increased tyrosine aminotransferase. In this experiment, a maximum increase of 7-fold was obtained. Epinephrine did not induce the enzyme in adrenalectomized rats.

Table 1, section B, shows a dose–response study for theophylline. In intact rats, 25 mg/kg of theophylline caused a more than 2-fold rise in the enzyme, and the 150 mg/kg dose produced the maximum effect (a nearly 8-fold increase). In adrenalectomized rats, theophylline also stimulated tyrosine aminotransferase, but its effect was less than in intact rats.

TABLE 1. EFFECT OF EPINEPHRINE AND THEOPHYLLINE ON LIVER TYROSINE AMINOTRANSFERASE IN INTACT AND ADRENALECTOMIZED RATS*

	Dose of epinephrine (mg/kg)	Dose of theophylline (mg/kg)	Liver tyrosine aminotransferase (μmoles/min/g liver)	
			Intact rats	Adrenalectomized
A.	0		0·52 ± 0·06	0·44 ± 0·09
	0·2		0·83 ± 0·08†	
	0·5		2·15 ± 0·40†	0·58 ± 0·12
	1·0		3·57 ± 0·12†	0·44 ± 0·07
	1·5		3·54 ± 0·48†	0·51 ± 0·08
	2·0		3·04 ± 0·36†	0·54 ± 0·10
B.		0	0·51 ± 0·08	0·44 ± 0·09
		25	1·36 ± 0·29†	
		50	2·47 ± 0·45†	0·58 ± 0·07
		100	3·52 ± 0·43†	1·21 ± 0·23†
		150	3·90 ± 0·56†	1·50 ± 0·25†
		200	3·24 ± 0·14†	
C.	0	0	0·61 ± 0·15	0·54 ± 0·08
	1	0	3·06 ± 0·33†	0·66 ± 0·07
	0	150	2·79 ± 0·18†	0·95 ± 0·11†
	1	150	2·98 ± 0·20†	0·99 ± 0·07†

* Each value represents the mean and standard error for 5 rats per group. Drugs were injected 4 hr before the rats were killed at noon.
† Significantly different ($P < 0·05$) from the corresponding control group.

Since epinephrine and theophylline affect cyclic AMP by different mechanisms (stimulation of its formation and blockade of its destruction respectively), the combination of epinephrine and theophylline was studied (Table 1, section C). In intact rats, the effect of the combination was not significantly greater than that of either compound alone. Epinephrine did not significantly enhance the stimulation by theophylline in adrenalectomized rats.

Because epinephrine elevated tyrosine aminotransferase in intact but not in adrenalectomized rats, one might conclude that: (1) epinephrine acted by release of adrenal steroids, which in turn induced

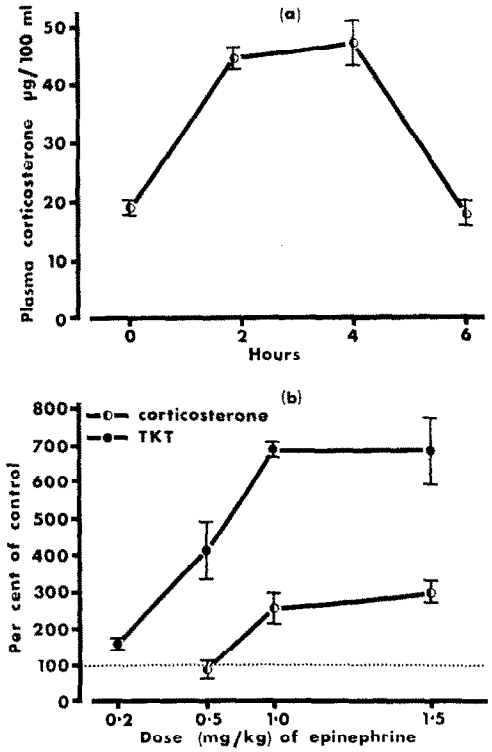

FIG. 2. Elevation of (a) plasma corticosterone and (b) liver tyrosine aminotransferase (TKT) and plasma corticosterone by epinephrine. Epinephrine (1·0 mg/kg) was injected (a) at zero time (9 a.m.) or (b) 4 hr before the rats were killed at noon. There were five rats per group; means with standard errors are shown.

the enzyme; or (2) epinephrine itself increased the enzyme, but required adrenal corticoids to act. Further experiments were designed to distinguish between these possibilities.

Injection of epinephrine into intact rats causes an increase in plasma corticosterone (Fig. 2a). The effects of various doses of epinephrine on plasma corticosterone and on liver tyrosine aminotransferase are shown in Fig. 2b. No elevation of plasma corticosterone was seen at 0·5 mg/kg, whereas that

TABLE 2. EFFECT OF EPINEPHRINE AND HYDROCORTISONE ON LIVER TYROSINE AMINOTRANSFERASE IN ADRENALECTOMIZED RATS*

Group	Liver tyrosine aminotransferase (μmoles/min/g liver)
Control	0·53 ± 0·07
Control + epinephrine	0·42 ± 0·03
Hydrocortisone-treated	2·42 ± 0·40
Hydrocortisone-treated + epinephrine	3·68 ± 0·37†

* Epinephrine (1·0 mg/kg) or hydrocortisone (25 mg/kg) or both were injected 4 hr before the rats were killed at noon. There were five rats per group; the values are means ± standard errors.

† Significantly different ($P < 0·05$) from group without epinephrine.

dose caused a 4-fold increase in liver tyrosine aminotransferase, and an even lower dose (0·2 mg/kg) significantly elevated the enzyme. These results show that the increase of liver tyrosine aminotransferase by epinephrine is not necessarily mediated by glucocorticoid release, i.e. that the first possibility is unlikely.

Direct evidence for the second suggested possiblity is in Table 2. Epinephrine given to adrenalectomized rats did not increase tyrosine aminotransferase, whereas epinephrine given to such rats after hydrocortisone treatment significantly elevated the enzyme.

Both epinephrine and theophylline induced tyrosine aminotransferase in intact rats. Adrenal corticoids may have exerted at least a permissive effect on the action of both drugs, for theophylline had a smaller effect in adrenalectomized rats, and epinephrine had no effect unless hydrocortisone was administered. Presumably there was an increased amount of enzyme protein after both drugs, although the experiments would not distinguish between an increased rate of synthesis of enzyme versus a decreased rate of degradation. Although there is no evidence that changes in cyclic AMP levels in liver mediated the effect of epinephrine or theophylline on tyrosine aminotransferase, recent studies[5, 9] make that possibility seem worth considering. Recently, Holt and Oliver[10] reported the induction of tyrosine aminotransferase by epinephrine in newborn (2-to 6-day-old) rats, and Reshef and Greengard[11] have published data very similar to those in Table 2.

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Modification of the toxic actions of *l*-tryptophan by pargyline and *p*-chlorophenylalanine

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IN THEIR extensive studies on the metabolism *in vivo* of numerous amino acids, Gullino *et al.*[1] observed that the *l*-form of tryptophan possessed the greatest toxicity in the rat. They also demonstrated that it was the only amino acid which exhibited a difference in toxicity between the *d*- and *l*-isomers, the latter being the more toxic form. These observations are of interest since a considerable amount of