INDUCTION OF LIVER TYROSINE AMINOTRANSFERASE BY THEOPHYLLINE AND ITS REPRESSION BY PHENTOLAMINE AND GLUCOSE IN ADRENALECTOMIZED RATS

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1. Introduction

Enhanced synthesis of rat liver tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) by cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is seen *in vivo* [1] and in organ cultures of fetal liver [2]. Glucagon, epinephrine and theophylline, which are known to elevate the intracellular level of cyclic AMP in liver [3], also induce tyrosine aminotransferase [2, 4]. However, the induction by epinephrine can be seen only in fetal liver [4]. The reason of the inability to induce the enzyme in adult rats is not clear.

On the other hand, a considerable attention has been focused on the adrenotropic receptor concept and the blocking agents [5, 6]. Recent report from our laboratory showed a repressive effect of the alpha-adrenergic blocking agent, phentolamine, on the induction of tyrosine aminotransferase by aromatic carboxylic acids [7].

In this paper, further evidence is presented which supports the existence of a specific adrenotropic receptor for the control of rat liver tyrosine aminotransferase.

2. Experimental

Male Wister rats were adrenalectomized 2 to 5 days before use and maintained on a laboratory chow and 0.9% NaCl as drinking water. The rats were fasted overnight before the experiment. Theophylline, l-epinephrine, N^6-2′-*O*-dibutyryl cyclic AMP (Boeringer, Mannheim), phentolamine (CIBA), propranolol (Sumitomo Kagaku, Osaka) or cycloheximide (Wako Chemical, Osaka) was injected intraperitoneally. D-Glucose was administered by stomach tube. All rats were killed between 1 to 3 p.m. to eliminate the effect of the diurnal variation of tyrosine aminotransferase [8]. Livers were homogenized in 0.15 M KCl-0.001 M EDTA with a loosely fitting Teflon pestle. Tyrosine aminotransferase of liver homogenate was assayed as described previously [7]. Enzyme activity is expressed as mμmoles *p*-hydroxyphenylpyruvate formed per min per mg of homogenate protein.

3. Results

Theophylline, a competitive inhibitor of cyclic 3′,5′-nucleotide phosphodiesterase [9], is an effective inducer of rat liver tyrosine aminotransferase, and a several fold increase of enzyme activity was seen within 2 hr after the intraperitoneal administration of theophylline (fig. 1). The increase of enzyme activity was inhibited by cycloheximide (table 1). These results suggest that the elevated activity is due to the enzyme formation *de novo*. Following a single injection of theophylline (10 mg/100 g, body weight), the enzyme activity was increased in an hour and the response was maximal by 2 to 3 hr (fig. 1). The rapid response of the enzyme has been also observed with cyclic AMP [1]. The dose of theophylline required for a maximum response of tyrosine aminotransferase 2 hr following a single injection was about 10 mg per 100 g body weight (fig. 2).

As seen in table 1, the induction by theophylline was inhibited by the alpha-adrenergic blocking agent,

Table 1
Effects of phentolamine, propranolol, epinephrine and glucose on the induction of tyrosine aminotransferase by theophylline, cyclic AMP or benzoate.

	Induction of tyrosine aminotransferase by			
	Control	Theophylline	Cyclic AMP	Benzoate
Period of induction (hr)	1	2	2	3
Additional treatment*				
None	15.8 ± 2.3 (9)	40.0 ± 5.2 (8)	59.5 ± 5.0 (3)	48.1 ± 6.3 (10)
Cycloheximide		11.7 ± 2.4 (4)		
Phentolamine	11.1 ± 1.7 (4)	22.5 ± 4.1 (4)**	56.8 ± 1.5 (3)	25.4 ± 2.6 (3)
Propranolol	17.7 ± 2.6 (4)	53.8 ± 5.2 (6)	54.2 ± 3.6 (3)	42.8 ± 3.6 (3)
Epinephrine	23.0 ± 1.4 (4)	31.6 ± 1.8 (4)		20.7 ± 3.7 (3)
Glucose	11.5 ± 2.0 (3)	15.2 ± 0.7 (4)	62.6 ± 1.2 (3)	14.9 ± 3.1 (4)
Epinephrine + propranolol	30.3 ± 3.1 (6)	40.6 ± 4.3 (4)		42.5 ± 3.7 (4)
Glucose + propranolol	14.2 ± 1.2 (3)	17.5 ± 1.7 (4)		12.7 ± 1.5 (3)

Adrenalectomized rats weighing about 150 g were given following treatments: theophylline, 15 mg; dibutyryl cyclic AMP, 4 mg; sodium benzoate, 40 mg; cycloheximide, 1 mg; phentolamine, 2 mg; propranolol, 0.7 mg; epinephrine, 100 μg; glucose, 0.5 g. Enzyme activity is expressed as mμmoles *p*-hydroxyphenylpyruvate formed/min/mg protein. Each value is the average ± standard deviation. Numbers in parentheses indicate number of rats used.

* This treatment was given 5 min before the administration of inducers.

** One mg of phentolamine was given. Above this dosage, rats often died within 2 hr.

phentolamine, which is also a potent inhibitor of induction of tyrosine aminotransferase by aromatic carboxylic acids [7]. On the other hand, the beta-adrenergic blocking agent, propranolol, somewhat enhanced the induction by theophylline up to the level of the induced activity by cyclic AMP (table 1, fig. 1).

The induction by theophylline was completely repressed by glucose. Epinephrine also had an inhibitory effect on the induction by theophylline, however, the effect was abolished by propranolol, which is capable of blocking epinephrine-induced rises in blood glucose [10]. The repressive effect of glucose on the induction by theophylline was not abolished by propranolol (table 1). From these results, it is suggested that the inhibitory effect of epinephrine on the induction by theophylline is due to the elevated level of blood glucose.

Administration of epinephrine alone did not increase the activity of tyrosine aminotransferase, as reported by Greengard [4]. However, when both epinephrine and propranolol were injected, a significant increase of enzyme activity was observed (table 1).

If intracellular cyclic AMP is a proximal inducer of tyrosine aminotransferase, the induction of this enzyme by cyclic AMP cannot be affected by the adrenergic blocking agents which act at the formation stage of cyclic AMP. As expected, phentolamine and propranolol and no effect on the induction of tyrosine aminotransferase by cyclic AMP (table 1). Glucose also showed no effect on the induction by cyclic AMP.

The effects of phentolamine, propranolol, epinephrine and glucose on the induction by sodium benzoate was essentially identical with those by theophylline (table 1). These results confirm the previous report [7] that the inducing mechanism of tyrosine aminotransferase by benzoate or tryptophan may include an accumulating process of cyclic AMP. There is a possibility that these substances might inhibit cyclic 3′,5′-nucleotide phosphodiesterase.

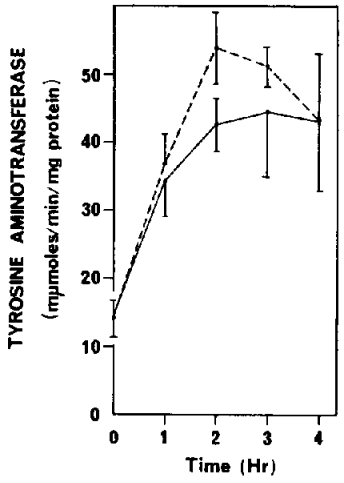

Fig. 1. Time-course of the induction of tyrosine aminotransferase by theophylline. A single dose (10 mg/100 g body weight) of theophylline was injected with (•- - - - - -•) or without (•———•) propranolol (0.5 mg/100 g body weight) and rats were killed after various time intervals as indicated. Each point is the average of 4 to 9 rats. Vertical lines show the standard deviations.

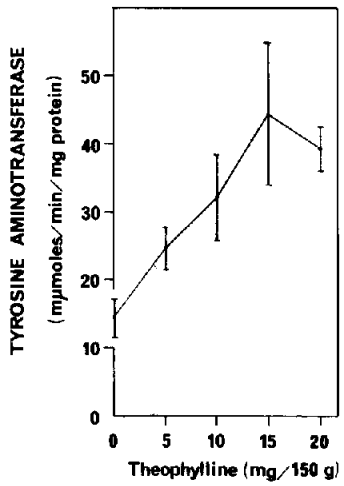

Fig. 2. Dose-response curve for the induction of tyrosine aminotransferase by theophylline. The indicated doses of theophylline were injected intraperitoneally and rats were killed 2 hours later. Each point is the average of 4 to 9 rats. Vertical lines show the standard deviations.

4. Discussion

Present results indicate the existence of a specific adrenotropic receptor for the control of liver tyrosine aminotransferase. From the inhibitory effect of alpha-adrenergic blocking agent phentolamine on the induction by theophylline, it can be suggested that the stimulation of alpha-adrenotropic receptor provokes the increased formation of tyrosine aminotransferase.

Furthermore, the receptor is apparently sensitive to glucose. Epinephrine, which elevates the level of circulating glucose by stimulating glycogenolysis in tissues [3], cannot induce the enzyme. But, if glycogenolytic action of epinephrine is blocked by an appropriate blocking agent, the inducing effect of epinephrine might be obvious as observed in fetal liver.

The phentolamine and glucose sensitive receptor might be located in liver, and the stimulation of the receptor could activate an adenyl cyclase and elevate the intracellular level of cyclic AMP in liver, which initiates the induction of tyrosine aminotransferase. To confirm this hypothesis, the correlation between the concentration of cyclic AMP and induction of tyrosine aminotransferase in liver must be examined.

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