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VARIATIONS BY EPINEPHRINE OF HEPATIC AND SERUM AMINOTRANSFERASES AND LACTATE DEHYDROGENASE IN THE RAT

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ABSTRACT

Incubation of rat hepatocytes with epinephrine inhibited alanine aminotransferase (ALT) (80%) and aspartate aminotransferase (AST) (53%) activities with no effect on lactate dehydrogenase (LDH) activity. Injection of epinephrine caused a progressive increase with time in hepatic LDH activity, being 52% at 24 h. Preinjection with propranolol eliminated the hormone effect and caused further inhibition (28%) of the enzyme activity. Liver ALT activity in ephinephrine-treated animals decreased by 37% at 24 h, after which it rose again to the control levels at 48 h. Propranolol raised epinephrine-induced enzyme inhibition up to 53%. Phentolamine, however, abolished the inhibitory effect. The activity of hepatic AST was not affected by epinephrine. Plasma ALT and LDH activity increased in epinephrine injected rats by 55% and 75%, respectively, but AST activity did not change. Propranolol alone stimulated serum ALT (41%) and AST (21%) activities. The data suggest that epinephrine exerts its effects on these enzymes through α-receptor activation and/or via the cell lesion resulting in the change of intra- and extracellular enzyme levels.

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Keywords: Epinephrine, aminotransferase, lactate dehydrogenase

INTRODUCTION

Hormonal regulation of hepatic enzymes has been reported by several investigators.[1-3] Epinephrine decreases the activity of phosphatidate phosphohydrolase in rat hepatocytes via β-adrenoceptoractivation.[4] *In vivo* studies have revealed that the activities of hepatic aldolase and aminotransferases are increased by epinephrine.[5] Epinephrine regulates glucose 6-phosphate dehydrogenase activity in rat liver but has no effect on the brain enzyme.[6] James and McCornac[7] have shown that epinephrine stimulates liver pyruvate dehydrogenase activity and that glucagon increases its effect. Injection of epinephrine in adrenalectomized rats changes the activities of hepatic enzyme.[8] This hormone also exerts stimulatory effect on cytochrome oxidase activity in liver, brain, and kidney, suggesting its involvement in mitochondrial enzymeregulation.[9]

In the present article *in vivo* and *in vitro* effects of epinephrine on liver and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities were studied and the possible mechanism by which these enzymes are affected by the hormone is discussed.

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MATERIALS AND METHODS

Reagents: Nicotinamide adenine dinucleotide (reduced form), sodium pyrurate, propranolol, phentolamine, and epinephrine were purchased from Sigma Chemical Co. (London branch). All other chemicals were of reagent grade.

Animals: Wistar male rats were obtained from Pasteur Institute (Tehran). The rats having free access to food and water were maintained as described before.[10]

Hepatocyte preparation and incubation: The liver was perfused *in situ* using Ca^{2+} free Hank's solution and the hepatocytes were isolated according to Wange *et al.*[11] Cell viability was assessed with trypan blue staining, generally exceeded 90%. Incubation was done in Krebs-Hensleit bicarbonate buffer (pH 7.4) under an atomosphere of 95% O_2 and 5% CO_2 (vol/vol) as described previously.[4] The incubation mixture contained 9×10^6 cells/ml and the indicated amounts of epinephrine (see figure legends) in a total volume of 10 ml. Pre-incubation before adding hormone was performed (10 min.) for all experiments. Incubation was terminated by separating the cells from the medium by centrifugation at 600 g for 2 minutes. The cells were washed (three times) with 10 volumes of saline and resuspended in 4 volumes of 100 mM Tris-HCl buffer (pH 7.8) containing 0.15 M KCl. The mixture was homogenized for 5 minutes on ice and centrifuged at 10,000 g for 30 minutes. The enzyme activity was measured in the supernatant fluid.

In vivo studies: Rats (220-250 g) were selected in groups of 12, from which 6 rats were injected intraperitoneally with epinephrine and/or the α- or β-blockers (see the legends) and 6 rats with saline as controls. When epinephrine and the blockers were used together, the blocker was injected 30 minutes prior to epinephrine injection. After the indicated time, each rat was anesthetized with diethyleter, the liver was perfused with saline to remove the blood and homogenized as described above. The enzyme activities were measured in the supernatant. For serum preparation each rat was sacrificed by decapitation, the blood collected and the serum was separated.

Enzyme assay: Lactate dehydrogenase activity was measured in 80 mM Tris-HCl buffer (pH 7.8) containing 0.2 mM NADH and 1.6 mM pyruvate (sodium salt) according to Vassault.[12] One enzyme unit was taken as the amount of enzyme required for production of 1 μmole NAD^+ per minute. The reaction was monitored by measuring the change in absorbance at 340 nm at 30°C using a Perkin-Elmer spectrophotometer model 551 S.

The activities of hepatic aspartate and alanine aminotransferases were measured as described by Reitman and Frankel using a commercial kit (Roche).[13]

Protein determination: Protein concentration was measured by the method of Lowry, *et al.*[14] Student's t-test was used for the statistical analyses.

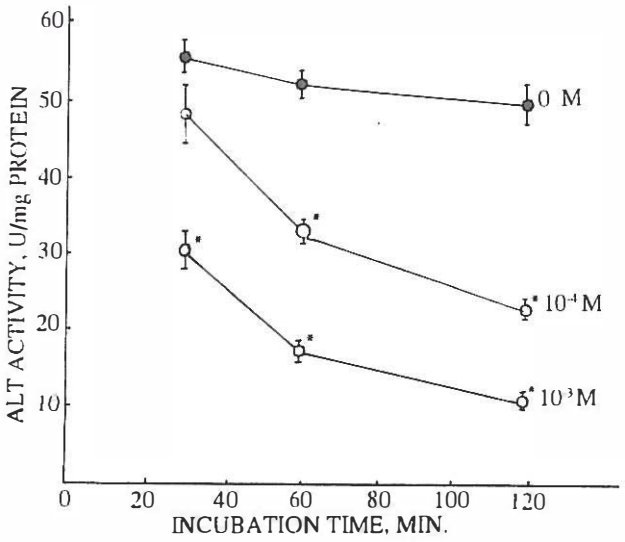

Fig. 1. Time course of the changes in ALT activity following incubations with epinephrine. Hepatocytes (9×10^6 cells) were incubated with epinephrine (10^{-3} - 10^{-4} M) at 37°C. Each point represents mean ± SE of four independent experiments. ○ = Experimental; ● = control; * = significantly different from controls ($P < 0.005$). For details see text.

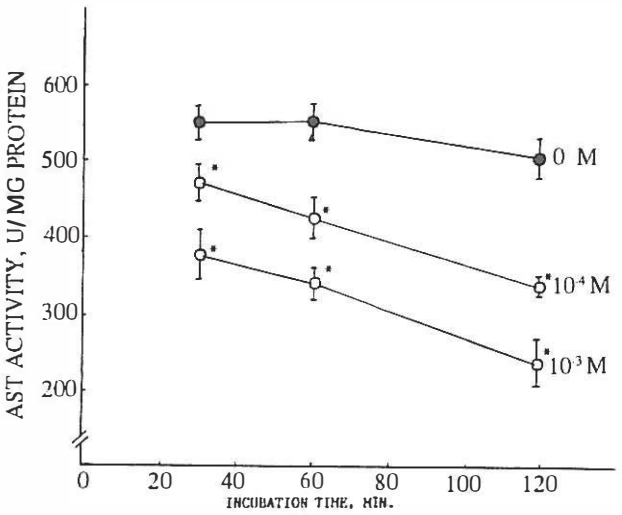

Fig. 2. Time course of the changes in AST activity following incubation of rat liver cells with epinephrine. Experimental conditions and symbols as in Fig. 1.

RESULTS

In vitro studies: Incubation of hepatocytes with epinephrine at concentrations of 10^{-4} to 10^{-3} M caused a progressive inhibition of ALT activity (Fig. 1). At the

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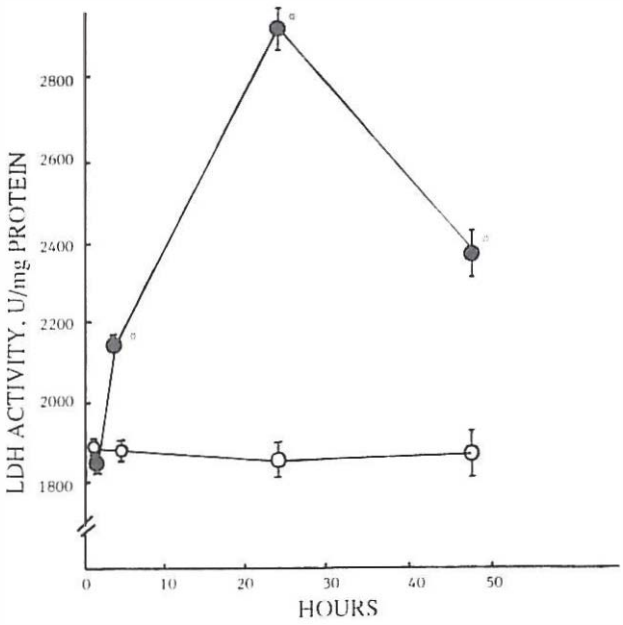

Fig. 3. In vivo effect of a single dose of epinephrine on hepatic LDH activity. Each point represents mean ± SE of 6 rats. O = control; ● = experimental; *= significantly different from control ($P < 0.005$). For detail see text.

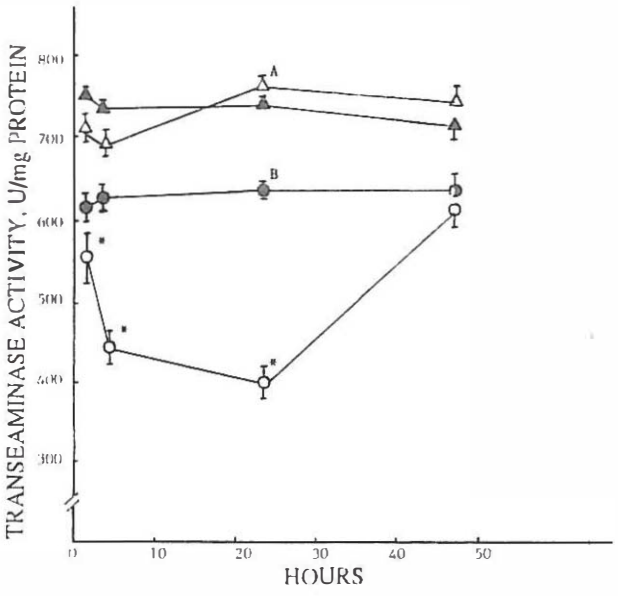

Fig. 4. In vivo effect of a single dose of epinephrine on liver transaminases activities. Each point represents mean ± SE of 6 rats. A: AST activity; △ = Experimental; ▲ = control. B: ALT activity; ○ = Experimental; ● = control. *= significantly different from control ($P < 0.005$). For details see text.

hormone concentration of 10^{-3} M the enzyme inhibition reached 46% and 80% after 30 and 120 minutes incubation, respectively. Similar inhibitory effect of epinephrine was demonstrated for AST activity (Fig. 2). The maximum inhibition obtained by the hormone (10^{-3} M) was 53% for 120 minutes incubation time. Incubation of hepatocytes in the presence or absence of epinephrine (10^{-3} - 10^{-4} m) caused a decline of about 38% in LDH activity within 120 minutes, suggesting no significant inhibitory effect of the hormone on the enzyme activity.

In vivo studies: The effects of epinephrine injection on hepatic activities of ALT, AST and LDH are shown in Figs. 3-4. Epinephrine (500 µg/kg) caused a progressive increase with time in LDH activity during the first 24 h (52%) after which it declined but remained above the control levels at 48 h. (Fig. 3). The activity of ALT in epinephrine-treated rats decreased by about 29% four hours after injection and reached a maximum decrease of 37% at 24 h but rose again to the control levels within the second 24 h (Fig. 4). The activity of hepatic AST, however, did not change significantly by epinephrine within the 48 h studied (Fig. 4).

In other sets of experiments the *in vivo* effects of β-blocker, propranolol, and α-blocker, phentolamine on epinephrine-induced changes in the enzyme activities were investigated in both liver and serum (Tables I, II). The data presented in Table I show that epinephrine decreases hepatic ALT activity by 37% and epinephrine and propranolol raises this inhibition up to 53%. Phentolamine, however, abolishes the inhibitory effect on AST activity but injection of epinephrine together with propranolol decreases the enzyme activity by about 22%. Neither propranolol nor phentolamine alone affected the activities of hepatic ALT and AST. The activity of LDH, however, was inhibited by propranolol. The activity of liver LDH increased by 53% in epinephrine-treated rats. Injection of epinephrine plus propranolol, however, not only eliminated the stimulatory effect of the hormone on LDH activity but also inhibited the enzyme activity (28%).

The variations in the serum enzymes following epinephrine treatment are shown in Table II. Epinephrine increased serum ALT activity by 55%. Propranolol alone also stimulated ALT activity (41%) and when injected together with epinephrine its synergistic effect raised the enzyme activity up to 108%. Serum AST activity was not affected by epinephrine but propranolol increased the activity by 21%. Injection of epinephrine plus propranolol elevated serum AST activity by 79%. Phentolamine alone did not change serum AST activity but its administration together with epinephrine slightly decreased the enzyme activity (16%). Serum LDH activity rose about 25% by epinephrine. Neither propranolol nor phentolamine significantly changed the enzyme activtity.

DISCUSSION

We have previously reported the possible regulatory

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Table I. The effect of epinephrine in the presence and absence of α- and β- blockers on liver enzymes

Injected	Enzyme activity Units/mg Protein ALT	AST	LDH
None	610 ± 19	724 ± 18	21244 ± 521
Epinephrine (500 μg/kg)	382 ± 9*	737 ± 22	32593 ± 587*
Propranolol (200 μg/kg)	596 ± 17	802 ± 25	17214 ± 391*
Epinephrine (500 μg/kg) + Propranolol (200 μg/kg)	255 ± 16*	565 ± 18*	15340 ± 306*
Phentolamine (200 μg/kg)	630 ± 19	740 ± 14	20478 ± 360
Phentolamine (500 μg/kg) + Epinephrine (500 μg/kg)	623 ± 18	732 ± 16	32680 ± 506*

Rats were injected with the indicated reagents and after 24 hr the enzyme activity was measured in liver as described in Methods. Data are mean ± S.D. of 6 rats.
*Significantly different from controls ($P < 0.005$).

Table II. The effect of epinephrine in the presence and absence of α- and β-blocker on serum enzyme

	Enzyme activity Units/mg Protein ALT	AST	LDH
None	75 ± 3.6	6.6 ± 0.27	39.8 ± 2.4
Epinephrine (500 μg/kg)	116 ± 6.2*	7.17 ± 0.23	50 ± 2.95*
Epinephrine (500 μg/kg) + Propranolol (200 μg/kg)	156 ± 4.0	11.8 ± 0.4*	40.6 ± 2.6
Propranolol (200 μg/kg)	106 ± 5.5*	8 ± 0.2*	37.1 ± 2.1
Epinephrine (500 μg/kg) + Phentolamine (500 μg/kg)	76 ± 3.5	5.5 ± 0.13	50.3 ± 2.68*
Phentolamine (500 μg/kg)	75.5 ± 3.2	6.1 ± 0.15	42 ± 1.8

Rats were injected with the indicated reagents and after 24 hr the enzyme activities were measured in the serum as described in Methods. Data are mean ± S.D. of 6 rats.
*Significantly different from controls ($P < 0.005$).

roles of catecholamines on hepatic enzymes.[4,10,15] Both *in vivo* and *in vitro* studies showed that epinephrine inhibits hepatic ALT activity. There are several explanations for the mechanism hy which epinephrine inhibits this enzyme. First, the inhibition may he mediated through the change in intracellular concentrations of Ca^{++} or cAMP which in turn are involved in phosphorylation-dephosphorylation processes. The activity of phosphatidate phosphohydrolase in rat hepatocytes has been shown to be inhibited by epinephrine via β-receptor activation.[4] It is also prohable that epinephrine-induced liver cell injury[16,17] increases the soluble enzyme leakage causing a decrease in intracellular enzyme activity with a simultanous elevation of its plasma levels. Vincent[18] hasreported that the agents affecting liver cells increase the activities ofplasma transauminases. Further work is necessary to prove these suggestions. The results obtained from the *in vivo* studies (Tahle I-II) also show that epinephrine decreases hepatic ASTactivity with an increase in its plasma levels. The change in the enzyme activity is aholished by α-blocker, phentolamine, suggesting that the effect is mediated via α-receptor activation. Lee, *et al.*[19] have shown that in rabbits norepinephrine induces lesion in the liver increasing serum ALT levels but pretreatment with α-blocker, prazosin, inhibits theeffect. They suggested

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that this may be the result of hepatic ischemia and necrosis brought about by α-adrenoceptor activation.

The present finding that serum aminotransferases induced by epinephrine is further stimulated by β-blocker, propranolol, may also be the result of inshemic property of propranolol.[20]

The elevated hepatic LDH activity of epinephrine-injected rats (Fig. 3) probably results from an increase in protein synthesis consistent with other studies[21,22] or from the increased concentration of intracellular cAMP which is involved in activation of certain protein kinases.[23]

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