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Effect of epinephrine on the *in vitro* biosynthesis of fibrinogen

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Abstract. In an *in vitro* rat liver slice incubation system, the synthesis of fibrinogen, when measured by immunoprecipitation technique was stimulated in the presence of epinephrine. An increase in poly $(A)^+$ RNA content of the liver slice was also observed after epinephrine treatment. This *in vitro* experiment demonstrated that epinephrine stimulated *de novo* synthesis of fibrinogen by acting directly on the liver.

Keywords. Epinephrine; rat liver slice; fibrinogen; poly $(A)^+$ RNA; stimulation; *de novo* synthesis.

Introduction

The plasma fibrinogen level is elevated during certain pathophysiological conditions (Chatterjee *et al.,* 1978; Kampschmidt and Upcharch, 1974). At the molecular level no defined physiological substance or mediator has been identified to explain the elevation of fibrinogen level. However, a nonphysiological substance, turpin oil after administration to rats showed higher plasma level of fibrinogen (Bouma *et al.,* 1975). The administration of biogenic amines to rats also elevated the level of plasma fibrinogen (Sur *et al.,* 1979). Inhibitors of monoamine oxidase and metabolites of amines failed to show such stimulatory response (Sarkar *et al.,* 1984). A *de novo* synthesis of fibrinogen was suggested for this elevation of plasma fibrinogen level since higher incorporation of [^{14}C]-amino acid into plasma fibrinogen was abolished by actinomycin D (McKenzy *et al.,* 1966; McKenzy and Fowler, 1968; Roy *et al.,* 1985a). The liver, being the site of fibrinogen synthesis (Mann *et al.,* 1951; Miller *et al.,* 1951, 1963), was investigated for the incorporation of [^{14}C]-orotic acid into polysomal RNA, the level of DNA-dependent RNA polymerase activity and also for the content of fibrinogen-specific functional mRNA from the animals treated with amines and untreated one. Each of these parameters showed distinct elevation for amine treated animals only (Roy *et al.,* 1985a, b). All these *in vivo* studies led to the suggestion that the amine itself is responsible for the stimulatory synthesis of fibrinogen.

To answer the question whether the biogenic amine acts directly on the liver, an isolated rat liver slice incubation system was designed as an *in vitro* fibrinogen synthesising system. The present study demonstrated that the effect of the amine was direct on the liver and specific for stimulated synthesis of fibrinogen.

Materials and methods

L-Epinephrine, α-amino caproic acid, aptroteinin, amino acids were purchased from

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Sigma Chemical Co., St. Louis, Missouri, USA, Oligo (dT)-cellulose was from Collaborative Research, Lexington, Massacusetts, USA and casamino acid was procured from Difco Laboratories, USA. [^{14}C]-Chlorella hydrolysate and [^{32}P] (carrier free inorganic phosphate) were obtained from Bhabha Atomic Research Centre, Bombay. All other reagents used were of analytical grade.

Experimental procedure

Incubation of liver slices: Livers from rats (Wister albino, IICB inbred) pretreated with reserpine (Vijayalakshmi *et al.,* 1978) 24 h prior to sacrifice were perfused for making slices of 0·3 mm thick (Ballard and Oliver, 1963). About 0·2 g of this was primed with epinephrine by 30 min incubation at 37°C in 2 ml of Kreb's Ringer bicarbonate buffer solution having 20 μg/ml epinephrine. Then the slices were washed with the same buffer twice and the washings were discarded. This was then grouped into experimental and control. Two ml of Kreb's Ringer bicarbonate buffer solution having trasylol (500 KIU/ml), α-amino caproic acid (1 mM), [^{14}C]-chlorella hydrolysate (20 μCi) and amino acid mixture at half the concentration used by Morgan (1969) was used for both the sets. Experimental samples received 20 μg per ml of epinephrine, while controls received only vehicle. The slices were incubated at 37°C in an atmosphere of air and CO_2 (95 and 5%). The medium from each flask was collected after specified period of incubation, clarified and used for estimation of radiolabelled fibrinogen content by immunoprecipitation technique. For measuring poly $(A)^+$ RNA synthesis, the liver slices were labelled with ^{32}Pi (100 μCi/5 ml medium).

Immunoprecipitation of fibrinogen: The monospecific antifibrinogen antibody was prepared by immunoaffinity chromatography (Bouma and Fuller, 1975). Elution was effective with 3 M ammonium thiocyanate, which was readily eliminated from the eluate by quick dialysis and the antibody was stored at –20°C until use. The incubation medium collected and clarified as stated above was treated for 10 min with formalinised heat killed *Staphylococcus aureous* (Cown-1 strain) bacterial suspension (10%) for its pre-cleaning (Martial *et al.,* 1977). The soup, after separation of bacteria was incubated with 3-fold excess of antibody (with respect to the approximate fibrinogen content of the soup) for 3 h at room temperature and then at 4°C overnight. The immune complex so developed was then estimated after Kessler (1975). Unlabelled pure rat fibrinogen was added in excess (100 μg) to the clarified and precleaned incubation medium prior subjecting it to immunoprecipitation and then the radioactivity in the immune complex was estimated.

Protein was estimated using bovine serum albumin as standard (Lowry *et al.,* 1951).

Poly (A)+ RNA preparation and determination: Approximately 1g of the incubated slices from [^{32}P] containing incubation system was used in each batch for RNA preparation. Polysomal RNA was prepared according to Palmiter (1974) and then subjected to fractionation over oligo (dT)-cellulose affinity column into bound and unbound fractions (Aviv and Leader 1972; Nickerson and Fuller 1981). The content of RNA was measured following the procedure of Ceriotti (1955) using yeast RNA as standard.

Results and discussion

In this *in vitro* incubation system, the incorporation of [^{14}C]-Chlorella protein hydrolysate into TCA precipitable material increased linearly up to 4h of incubation. But [^{32}P] incorporation into TCA insoluble form showed a linear increase only up to 2h of incubation. Hence incubation medium for estimation of fibrinogen content was collected after 2h of incubation and liver slices for RNA preparation was taken after 1 h of incubation.

The synthesis of fibrinogen both in the presence and absence of epinephrine in the liver slice incubation systems was determined by immunoprecipitation method using incubation medium. The labelled fibrinogen obtained after the liver slice incubation with epinephrine was nearly 62% higher than that obtained from controls (table 1). Table 1 further indicated that total protein synthesis, was however, slightly reduced in the presence of epinephrine. The *in vitro* synthesised fibrinogen was displaced in a quantitative manner when unlabelled pure rat fibrinogen was added to the incubation medium prior to the addition of anti-fibrinogen antibody for immunoprecipitation. In the presence of excess unlabelled fibrinogen the radioactivity in the immune-complex was nearly identical to the background value (data not shown). Therefore, this *in vitro* experiments demonstrated that the amine acted directly on the liver to give the stimulated synthesis of fibrinogen. The effect of amine is apparently specific at least for fibrinogen. Epinephrine, at a dose of 20 μg/ml stimulated maximally the incorporation of radioactivity into fibrinogen (figure 1).

Table 1. *In vitro* effect of L-epinephrine on fibrinogen synthesis.

	cpm/mg of protein (extracellular)		
	Total protein	Immuno-precipitation	Stimulation (%)
Control	18,049 ± 62	296 ± 35	—
L-Epinephrine (20 μg/ml)	14,823 ± 34	480 ± 28*	62

Values are mean ± SE of 6 determinations.
*P <0·01 *vs* control.
The immunoprecipitate was washed with buffer A (150 mM NaCl, 5 mM EDTA, 50 mM Tris. HCl pH 7·4 and 0·02% NaN_3). This was then heated with 200μl of 3% SDS, 10% glycerol at 90°C for 5 min. Then the centrifugation separated the supernatant containing labelled antigen, 50 μl aliquot of this was used for radioactivity determination.

Poly (A)$^+$ RNA synthesis

The polysomal RNA synthesis of the liver slices for both the control and epinephrine containing incubation systems was estimated and the results are shown in table 2.

The Poly (A)$^+$ RNA isolated from epinephrine-treated liver slices showed 37% higher incorporation of [^{32}P] than the controls. The oligo (dT)-cellulose unbound fraction of polysomal RNA as well as total polysomal RNA showed only a marginal increase in amine treatment compared to controls. It is possible that the increased

Table 2. Effect of L-epinephrine on [^{32}P] incorporation in polysomal RNA, oligo (dT)-cellulose bound and unbound fractions of polysomal RNA (*in vitro*).

	Polysomal RNA cpm/mg of RNA Mean ± SE	Unbound		Bound	
		cpm/mg of RNA	Stimulation (%)	cpm/mg of RNA	Stimulation (%)
Control	4192 ± 24	3806 ± 41	—	1054 ± 25	—
L-Epinephrine	4402 ± 56	4075 ± 92	—	1450 ± 63	37·5

Each value represents the mean ± SE of 6 determinations; liver slice incubation was described in materials and methods. The experimental details are given in text.

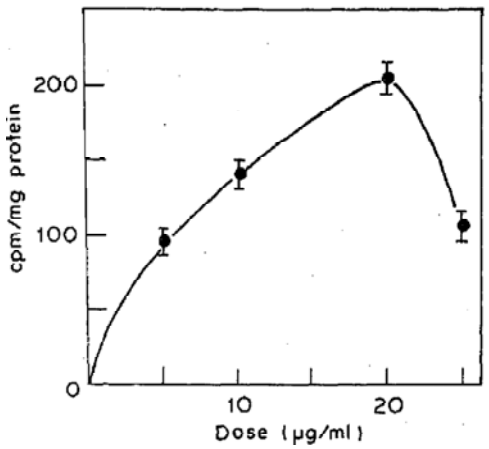

Figure 1. Dose response curves of L-epinephrine on fibrinogen synthesis in rat Oliver slice system.

poly $(A)^+$ mRNA synthesis in epinephrine treatment may include fibrinogen mRNA as well.

However, it may be pointed out that the extent of stimulation for both the fibrinogen and poly $(A)^+$ RNA was considerably low in this *in vitro* system compared to *in vivo* effects. Such lower value might arise from the loss of organisational integrity of liver, and certain cellular damage during its slicing. In conclusion, this *in vitro* study demonstrated that direct action of epinephrine on the liver is responsible for stimulated synthesis of fibrinogen.

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