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Epinephrine inhibits protein synthesis in isolated mouse hepatocytes through alpha adrenergic receptors in a calcium dependent way

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The inhibitory effect of epinephrine, glucagon and adenosine 3′, 5′ monophosphate (cAMP) on protein synthesis in liver slices has been first described by Pryor and Berthel [1, 2]. Observations on cAMP induced inhibition of protein synthesis have been reinforced [3, 4] and extended to cell free systems [3, 5, 6] and isolated hepatocytes [3], while epinephrine inhibition has also been confirmed in liver slices [3].

It is known that different catecholamine actions in the liver are mediated either by alpha or beta adrenergic mechanisms [7–11]. In isolated hepatocytes it has been demonstrated that the activation of glycogenolysis by catecholamines is mediated by alpha adrenergic receptors [8], and this process requires Ca^{2+} ions [10, 12, 13]. Unlike the effect of glucagon on glycogenolysis, the epinephrine provoked activation is independent from the simultaneous increase of the cAMP level [14] and occurs without the activation of cAMP dependent protein kinase [9].

The mode of action of epinephrine in protein synthesis of isolated hepatocytes has not been elucidated so far. Therefore, we investigated the inhibitory effect of epinephrine on protein synthesis in isolated hepatocytes and compared the effects of epinephrine and dibutyryl-cAMP (db-cAMP) in this experimental system in the presence and absence of Ca^{2+} in the medium. The effects of the alpha blocker phenoxybenzamine and the beta blocker propranolol were also examined on the epinephrine induced inhibition of protein synthesis.

Materials and methods

Male CFLP mice (20–25 g) fed *ad libitum* were used in the experiments. Isolated liver parenchymal cells were prepared with the collagenase perfusion method [15, 16] as described previously [17].

The isolated cells (5×10^6 cells/ml) were suspended and incubated in Krebs–Henseleit bicarbonate buffer [18] containing 2.5 mM Ca^{2+}, 8.5 mM glucose [19], 5 mM lactate, amino acids necessary for protein synthesis (1 mM each, except valine) and 1% albumin (dialysed before use) at 37°. To counteract the degradation of epinephrine 5 mg/ml ascorbate was added to the incubation mixture every 30 min. In a series of experiments calcium was omitted from the incubation medium. The calcium content of this medium was lower than 10^{-4}M determined by atomic absorption (Beckman model 1240). When Ca^{2+} was omitted from the incubation medium then 0.2 mM ethyleneglycol-bis(beta-amino ethyl ether) *N,N′*tetraacetic acid (EGTA) was added. The cells were stirred by bubbling with O_2:CO_2 (95:5 v/v). All glasses used were siliconized.

Viability of isolated hepatocytes was checked by the trypan blue exclusion test and was about 90%.

The incorporation experiments were started with the addition of labeled valine ([1-^{14}C]-1-Valine, sp. act. 65 TBq/mole, 18.5 KBq/ml) and stopped with ice cold 0.9% NaCl. Amino acid incorporation into protein was determined as described earlier [17]; the counting efficiency was about 60%.

The results are expressed in cpm/μg DNA. The DNA content of the samples was measured by the method of Burton [20]. Each experiment was carried out using hepatocytes from a single mouse liver.

Collagenase type II (179 U/mg) was purchased from Worthington Biochemical Corp. (Freehold, N.J.), epinephrine–HCl from BDH (Poole, U.K.), phenoxybenzamine from Smith Kline and French (Welwyn, U.K.), propranolol and db-cAMP from Sigma Chemical Co. (London, U.K.)

Results and discussion

Epinephrine inhibited amino acid incorporation into protein in isolated parenchymal liver cells (Fig. 1.). One μM

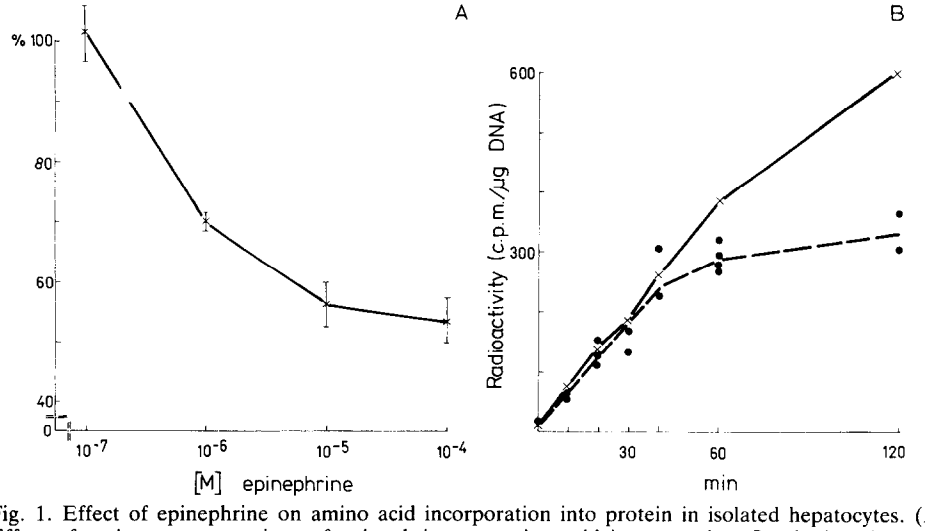

Fig. 1. Effect of epinephrine on amino acid incorporation into protein in isolated hepatocytes. (A) Effect of various concentrations of epinephrine on amino acid incorporation. Incubation time was 120 min. Vertical bars indicate ± S.E.M. (n = 5–11). (B) Time course of the inhibitory effect of epinephrine on amino acid incorporation. ×——×, control; ●- - - -●, 10 μM epinephrine.

Table 1. Comparison of the effects of epinephrine and db-cAMP on protein synthesis in isolated hepatocytes

Addition	Incorporation (%)	
	2.5 mM Ca^{2+}	0.2 mM EGTA
None	100	100*
10^{-5}M Epinephrine	57.8 ± 3.2 (11)†	106.4 ± 7.2 (7)
2×10^{-5}M Propranolol	93.2 ± 4.1 (4)	
2×10^{-5}M Propranolol + 10^{-5}M epinephrine	65.5 ± 7.1 (4)	
2×10^{-5}M Phenoxybenzamine	96.2 ± 2.0 (4)	
2×10^{-5}M Phenoxybenzamine + 10^{-5}M epinephrine	96.7 ± 5.1 (4)	
10^{-3}M db-cAMP	56.0 ± 6.2 (4)	55.5 ± 1.8 (4)
10^{-3}M db-cAMP + 10^{-5}M epinephrine	51.5 ± 1.3 (4)	

[^{14}C]Valine incorporation into protein was measured as described in Materials and methods. The incubation time was 120 min. EGTA (0.2 mM) was added when Ca^{2+} was omitted from the incubation medium of the hepatocytes.

* The control value measured in the absence of Ca^{2+} and in the presence of 0.2 mM EGTA was 51.5 ± 6.6% of the value measured in the presence of 2.5 mM Ca^{2+}.

† Mean ±S.E.M. (n = number of observations in different experiments).

epinephrine decreased the amino acid incorporation to 70% and 10 μM epinephrine to 55% of the control value after 120 min incubation (Fig. 1A). The inhibition was manifested only after a lag period of about 40 min (Fig. 1B). When the cells were preincubated with epinephrine for 30 min before the addition of the labeled amino acid an immediate inhibition of amino acid incorporation was observed without any lag period (data not shown).

No prevention of protein synthesis inhibition was observed in the presence of 20 μM propranolol, while 20 μM phenoxybenzamine reverted the inhibition caused by 10 μM epinephrine (Table 1). Thus, it is suggested that epinephrine exerts its inhibitory effect on protein synthesis through alpha adrenergic receptors.

db-cAMP has been found to decrease amino acid incorporation by 40–60% in isolated liver cells [3]. We compared the effect of db-cAMP and epinephrine on amino acid incorporation into protein in isolated hepatocytes in the presence of calcium ions and in calcium free medium (Table 1). Though omission of Ca^{2+} and the addition of 0.2 mM EGTA decreased amino acid incorporation to 51.5 ± 6.6% (mean ± S.E.M., number of observations = 12) of the value measured in the presence of 2.5 mM Ca^{2+}, percentual inhibition of protein synthesis caused by translation inhibitors was independent of calcium ion concentration (data not shown). Thus, amino acid incorporation into protein in the presence of 1 mM db-cAMP was 56% of the control value with Ca^{2+} and 55.5% in the absence of Ca^{2+}. The magnitude of the inhibition was similar to that observed in liver slices and cell free systems [1, 3–6]. On the other hand 10 μM epinephrine inhibited amino acid incorporation into protein in isolated cells only when calcium was present in the medium. The effects of 1 mM db-cAMP and 10 μM epinephrine were not additive: their combination did not enhance the inhibition beyond the effect caused by either of the agents in the presence of Ca^{2+} (Table 1).

It has been suggested that in the liver phosphorylation plays a dominant role in the inhibitory action of cAMP on protein synthesis [3, 5]. It has been evidenced in rabbit reticulocytes that the cAMP dependent protein kinase plays an important role in the translational control of eukaryotic cells by phosphorylation of the initiation factor eIF-2 phosphorylating enzyme, eIF-2 kinase (for review see [21]). More recently the phosphorylation of eIF-2 by cAMP independent protein kinases has been reported [22]. The importance of multiple (cAMP dependent and independent) phosphorylation mechanisms has been emphasized in the hormonal regulation of enzyme activities of phosphorylase kinase and glycogen synthase in glycogen metabolism of the liver (for review see [23]). eIF-2 has been shown to be phosphorylated by different protein kinases at the same sites in reticulocytes [24–27]. In isolated hepatocytes similar phosphorylation pattern of cytosolic proteins has been obtained after alpha adrenergic stimulus and glucagon treatment [28]. In experiments presented here no additivity has been found in the inhibition of protein synthesis on combined administration of db-cAMP and epinephrine suggesting that the target of these two agents is identical. Further, the calcium requirement of alpha adrenergic control in the regulation of hepatic glycogenolysis has been shown [10]. Recently another effect of epinephrine, the alpha adrenergic stimulation of oleate oxidation to CO_2 has been proved to be calcium dependent [29].

Our results suggest that similarly to the regulation of glycogenolysis in the liver epinephrine exerts its inhibitory effect on protein synthesis by a Ca^{2+} requiring mechanism mediated through alpha adrenergic receptors.

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Inhibitory action of macrocyclic polyamines on lipid peroxidation in rat liver microsomes

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It is well known that biomembranes are susceptible to lipid peroxidation which results in their disintegration and hence loss of function [1, 2]. In addition, microsomal lipid peroxidation has been found to be catalysed by NADPH–cytochrome *c* (P-450) reductase from the studies of the reconstituted systems containing purified NADPH–cytochrome *c* (P-450) reductase [3–5]. Since some of macrocyclic polyamines had a potent inhibitory effect on NADPH-supported lipid peroxidation in rat liver microsomes [6], we have investigated the reason why some macrocyclic polyamines are more effective than other polyamines in the inhibition of lipid peroxidation.

Rat liver microsomes were prepared as described previously [7]. Protein was determined by the method of Lowry *et al.* [8] using bovine serum albumin as a standard. Lipids used as substrates for lipid peroxidation were extracted from intact rat liver microsomes by the method of Folch *et al.* [9]. Lipid phosphorus was determined by the method of Bartlett [10]. The content of iron in microsomes was measured by atomic absorption spectrometry after the wet combustion of microsomes. NADPH–cytochrome *c* (P-450) reductase was purified from phenobarbital-treated rat liver microsomes by a minor modification of the method of Yasukochi and Masters [11]. NADPH–cytochrome *c* (P-450) reductase activity was measured by the method of Phillips and Langdon [12] using cytochrome *c* as an electron acceptor.

Microsomal lipid peroxidation was determined by thiobarbituric acid (TBA) method as described previously [7]. A reaction mixture for purified NADPH–cytochrome *c* (P-450) reductase mediated lipid peroxidation consisted of 0.25 M Tris–HCl (pH 6.8), 0.25 M NaCl, 2 mM ADP, 0.1 mM EDTA, 0.2 mM $Fe(NO_3)_3$, 1.0 mg of extracted microsomal lipids, 0.016 unit of NADPH–cytochrome *c* (P-450) reductase and 0.1 mM NADPH in a final volume of 1.0 ml. Malondialdehyde formed was calculated using a molar extinction coefficient for malondialdehyde–thiobarbituric acid complex of 156 $mM^{-1}cm^{-1}$ [13]. Although chelating agents such as EDTA have been demonstrated to inhibit microsomal lipid peroxidation [14], EDTA-chelated iron is required for the reconstituted system [3, 4]. Macrocyclic polyamines were prepared with a minor modification [15] of the method of Koyama and Yoshino [16] and Martin *et al.* [17]. The structure and abbreviations of macrocyclic polyamines are shown in Fig. 1. Since these synthetic polyamines do not have primary amino groups, they are probably more stable than naturally occurring polyamines *in vivo*.

Effects of macrocyclic polyamines and spermine on microsomal lipid peroxidation are shown in Fig. 2. An inhibitory effect was obtained with 2,3,3,3-, 2,3,4,3- and 3,3,3,4-cyclic polyamines and spermine. The maximum degree of inhibition was produced by the 2,3,3,3-cyclic polyamine.

The time course of NADPH-dependent microsomal lipid peroxidation in the presence or absence of 2,3,3,3-cyclic polyamine are shown in Fig. 3. Malondialdehyde was formed at the rate of 5.4 nmole/mg/min in the control mixture after a lag period. On the other hand, the rate of malondialdehyde formation in the presence of 0.1 mM and 0.2 mM of 2,3,3,3-cyclic polyamine were 1.57 and 0.83 nmole/mg/min, respectively. Furthermore, the inhibitory action of 2,3,3,3-cyclic polyamine was virtually independent of the incubation time. These results suggest that