INHIBITORY EFFECT OF MEMBRANE ACTIVE COMPOUNDS ON INDUCTION OF TYROSINE AMINOTRANSFERASE IN CHICK EMBRYO LIVER CELLS IN CULTURE

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1. Introduction

In the course of investigations on the effect of beta-adrenergic receptor blocking agents in experimental porphyria we observed an inhibitory action of DL-propranolol and other membrane active compounds on the induced activity of delta-aminolevulinate synthetase (δ-ALAS) (EC 3.2.1.37) both in vivo and in vitro [1–3]. Beta-adrenergic receptor blocking agents compete with catecholamines for activation of beta-adrenergic receptors. Some of these agents also possess non-specific membrane effects such as changes in depolarisation and/or repolarisation of the cellular membrane, local anesthetic and anti-arhythmic properties, etc. Other drugs, which have no beta-receptor blocking effects, including quinidine and lydocain, have non-specific membrane activity and are regarded as membrane active compounds. The various membrane effects of these drugs and of beta-receptor blockers are, at least partially, dissimilar.

In order to determine whether the inhibitory effect of DL-propanolol and other membrane active compounds is specific for induction of δ-ALAS, we examined their effect on induction of another inducible enzyme, tyrosine aminotransferase (TAT) (EC 2.6.1.5). TAT is a soluble enzyme, the activity of which is increased by administration of steroid hormones with glucocorticoid activity, both in rat liver [4] and in hepatoma cells in culture [5,6]. Dexamethasone is known to be a potent inducer in cultured hepatoma cells. It raises TAT activity 4–8-fold in this system [7,8]. Our experiments were carried out in cultures of chick embryo liver cells.

2. Materials and methods

Preparation and premilinary incubation of cultures of chick embryo liver cells in Eagle's essential minimal medium with 10% fetal calf serum were carried out as described by Granick [9].

Since fetal calf serum may contain hormones or other substances capable of affecting enzyme activity the actual experiments were performed in essential minimal medium without addition of serum. Serum free medium containing the inducer and the experimental drugs was added to the cultures 18 h before determination of the activity of TAT.

The fraction containing the enzyme was prepared from the monolayer cultures by the method of Whitlock et al. [7] with slight modifications: after discarding the culture medium containing inducer and test substances, the monolayer was washed twice with 3 ml 0.05 M phosphate-buffered saline (pH 7.4). Cells from 3 petri dishes (9 cm diameter each) were scraped off and collected in 5 ml cold phosphate-buffered saline. After centrifugation for 10 min, 3000 × *g*, at 4°C, the pellet was homogenized in 1 ml of 0.2 M potassium phosphate buffer, pH 7.6. Aliquots of 0.01 ml of the homogenate were removed for protein determination according to Lowry et al. [10]. After a second centrifugation as above, TAT activity was determined in 0.2 ml of the supernatant according to Diamondstone [11]. In short, the fraction of supernatant containing the soluble enzyme is incubated in the presence of tyrosine and α-ketoglutarate, forming *p*-hydroxy-phenylpyruvate. At a basic pH the latter is converted into *p*-hydroxy benzaldehyde,

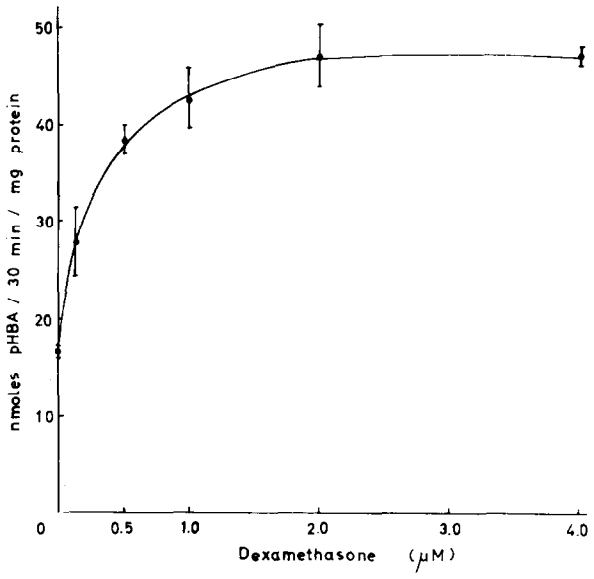

Fig.1. The effect of dexamethasone on activity of tyrosine aminotransferase in cultured chick embryo liver cells. Chick embryo liver cells were incubated in serum free medium in the presence of various concentrations of dexamethasone. After 18 h incubation at 37°C the activity of tyrosine aminotransferase was determined (See Materials and methods). Values are expressed as nmoles *p*-hydroxybenzaldehyde produced /30 min/mg protein.

which is measured spectrophotometrically. TAT activity is expressed as nmoles *p*-hydroxybenzaldehyde produced during 30 min incubation at 37°C/mg protein.

Dexamethasone, 2×10^{-6} M was found to be the optimal concentration in this system as shown in fig.1.

3. Results

Results are summarized in table 1. DL-propranolol and oxoprenolol, both beta-adrenergic blocking agents

Table 1
The effect of beta-adrenergic blocking agents and membrane active compounds on basal and induced activity of tyrosine aminotransferase in chick embryo liver cells in culture

Drug	Concentration (μg/ml)	% Decrease in activity of tyrosine aminotransferase	
		non-induced cells	induced cells
Beta-adrenergic blocking agents with non-specific membrane effects			
DL-propranolol	60	2.2 ± 2.2	69 ± 9.5
Oxprenolol (Trasicor)	150		36.5 ± 9.6
	300	5.5 ± 1.5	67.6 ± 11.1
Beta-adrenergic blocking agents without membrane effects			
Pindolol (Visken)	30	2.8 ± 2.7	5.9 ± 5.9
Practolol	150	3.3 ± 2.0	6.4 ± 5.1
Membrane active compounds			
D-propranolol	60	12.4 ± 11.9	69.5 ± 0.5
Quinidine sulfate	50	6.0 ± 4.0	67.1 ± 3.9
Lydocain	500	2.5 ± 2.4	54.4 ± 12.3

Cultures were prepared as described in Materials and methods. Cells were incubated for 18 h with or without dexamethasone 2×10^{-6} M, in the absence (controls) or presence of various drugs and the activity of TAT was determined. The values obtained in control experiments, i.e., with or without dexamethasone but without addition of the various drugs examined, were considered as 100%. Average activity of TAT in non-induced cells (without dexamethasone) was 15.7 ± 6.7 nmol *p*HBA/30 min/mg protein versus 38.5 ± 9.9 nmol *p*HBA/30 min/mg protein in induced cells. The effect of drugs on the activity of TAT was calculated as percent decrease in the enzyme activity when compared to the appropriate controls which were always performed simultaneously.

with non-specific membrane effects, inhibited induced TAT activity by 65–70%. Practolol and pindolol, both beta-adrenergic blocking agents devoid of membrane properties, did not affect induced TAT activity. Membrane active compounds, D-propranolol, quinidine and lydocain reduced the induced activity by 50–70%.

The concentration of all drugs was chosen according to their potency as beta-adrenergic blocking or membrane active compounds, compared to DL-propranolol and quinidine. All the drugs tested did not significantly affect the activity of the non-induced enzyme.

4. Discussion

The induction of TAT is thought to be under post-transcriptional control [12]. Increased TAT activity is apparently the result of increased synthesis of tyrosine aminotransferase and not of any alteration in enzyme activity or decay [13,14].

The induction of δ-ALAS is also thought to be caused by increased synthesis of the enzyme [15,16]. According to our results the membrane-active compounds examined partially inhibit induction of tyrosine aminotransferase and of δ-ALAS [2,3]. These observations may be explained by assuming an inhibitory effect of membrane-active compounds on protein synthesis. Further investigations to test this hypothesis are in progress in our laboratory.

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