Biochemical Pharmacology, Vol. 26, pp. 867–870. Pergamon Press, 1977. Printed in Great Britain

THE EFFECT OF AGENTS BLOCKING ADRENERGIC β-RECEPTORS ON INCORPORATION OF AMINO ACIDS INTO PROTEIN IN TISSUE CULTURES OF CHICK EMBRYO LIVER CELLS*

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(*Received* 8 *September* 1976; *accepted* 14 *November* 1976)

Abstract—The effect of β-receptor blocking agents on [^{14}C]amino acid incorporation into protein in cultures of chick embryo liver cells was studied. DL-Propranolol, a β-blocker with non-specific membrane effects, caused a 40 per cent inhibition of incorporation of [^{14}C]amino acids into protein. The inhibition was concentration dependent and reversible. A similar inhibition was obtained by oxprenolol (Trasicor), which is also a β-receptor blocking agent with non-specific membrane effects, and by the membrane active compounds: D-propranolol, lidocaine and quinidine. Pindolol (Visken), and practolol, which are almost devoid of membrane activity, were ineffective. These data indicate that the inhibitory effect of DL-propranolol and oxprenolol on protein synthesis is caused by their non-specific membrane effects.

In previous investigations we have shown that DL-propranolol, an agent blocking adrenergic β-receptors with non-specific membrane effects [1], reduces significantly the induction of experimental porphyria in rats [2]. Further experiments carried out in tissue cultures of chick embryo liver cells showed an inhibitory effect of propranolol and some other β-blockers on induction of delta-aminolevulinate synthetase (EC 2.3.1.37) [3, 4], the rate limiting enzyme in porphyrin biosynthesis [5]. It was not clear whether this inhibition is caused by a specific effect of some β-receptor blocking agents on induction of delta-aminolevulinate synthetase or whether these agents non-specifically inhibit protein synthesis in general. We, therefore, examined the effect of DL-propranolol and other β-receptor blocking agents on amino-acid incorporation into protein in tissue cultures of chick embryo liver cells. The experiments showed that some of these agents reduce the incorporation of amino acids into proteins in this system.

MATERIALS AND METHODS

Preparation of cell cultures: Seventeen days old chick embryo liver cells were prepared and incubated in Eagle's Minimal Essential Medium with 10% fetal calf serum at 37° and 5% CO_2: 95% air, according to Granick [6]. Leucine concentration in the medium was 25.8 mg/l. Incubations were carried out in petri dishes, 5 cm diameter (Nunclon, Denmark). After a preliminary incubation of 24 hr the medium containing non-adherent cells was discarded, replaced by fresh medium and incubations were continued for another 24 hr.

Determination of incorporation of [^{14}C]leucine and [^{14}C]amino acids into trichloroacetic acid precipitable proteins: the culture medium was removed and fresh medium (3 ml) was added prior to labelling with [^{14}C]leucine. 1 μCi uniformly labelled [^{14}C]L-leucine 270–324 mCi/m-mole or [^{14}C]amino acids, high sp. act. mixture, both from the Radiochemical Centre, Amersham, England, were added to the cultures, together with DL-propranolol or other drugs. After 30 min incubation the medium was discarded and the petri dishes with adherent cells were rinsed twice with 0.05 M Tris-buffer pH 7.4. The cells were scraped from the bottoms of the dishes in 1 ml buffer and homogenized. For protein determination 0.02 ml homogenate were removed. Ice cold 10% TCA was added in equal vol to the homogenate and mixed. After centrifugation for 10 min at 5000 *g* the precipitate was washed three times with ice cold 5% TCA, transferred to a Millipore filter, 0.45 μm, (Millipore Corporation, Massachusetts) washed again with ice cold 5% TCA and dried. Each filter was placed in a counting vial containing 10 ml scintillation fluid composed of PPO 11.2 g, dimethyl POPOP 15 mg, dioxan 375 ml, toluene 375 ml, methanol 225 ml, and naphthalene 78 g. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, Model 544, with a correction unit for dis/min. Results were calculated as dis/min per mg protein.

In control experiments t-RNA was hydrolysed with 1 M NaOH, hot TCA (80°) or RNase. The difference in radioactivity between these samples and those treated with cold TCA was not significant.

Protein determination: Proteins were determined by the method of Lowry *et al.* [7] with bovine serum albumin as standard.

*This investigation was supported by a grant from Mrs. C. Ben-Noon in memory of her husband.

† Established Investigator of the Chief Scientist's Bureau, Ministry of Health, Israel.

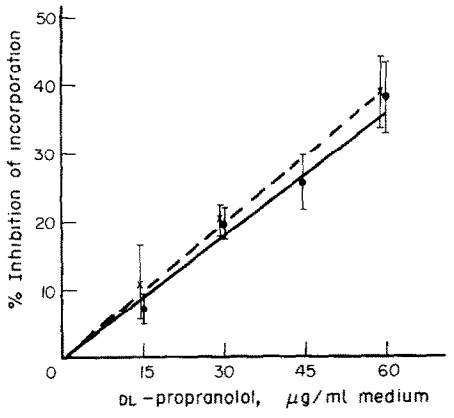

Fig. 1. The effect of DL-propranolol on incorporation of [^{14}C]leucine and of [^{14}C]amino acids into protein in chick embryo liver cells. DL-Propranolol in various concentrations and 1 μCi [^{14}C]leucine or [^{14}C]amino acids were added to cultures of chick embryo liver cells. After 30 min incubation the incorporation of [^{14}C]leucine or [^{14}C]amino acids into protein was determined (see Materials and Methods). The value of incorporation obtained in the control cultures was 8467 $\pm$ 656 dis/min/per mg protein. This value was considered as 100 per cent and results are expressed as per cent inhibition of [^{14}C]leucine or [^{14}C]amino acids incorporation into protein. Each value represents the mean and standard deviation of 6–8 determinations. ●——● Inhibition of incorporation of [^{14}C]leucine. ×——× Inhibition of incorporation of [^{14}C]amino acids.

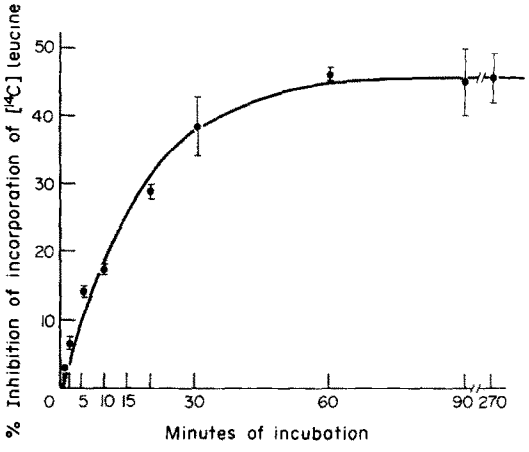

Fig. 2. The effect of time on inhibition of incorporation of [^{14}C]leucine into proteins caused by DL-propranolol in cultures of chick embryo liver cells. Cultures were incubated for 0–270 min in the presence of DL-propranolol, 60 μg/ml medium. In simultaneously run experiments control cultures were incubated for 0–270 min in the presence of vehicle only. At each time indicated incorporation of [^{14}C]leucine into protein of the cells was determined. Results are expressed as per cent inhibition of [^{14}C]leucine incorporation compared to control values. Each value is the mean and standard deviation of 6–8 determinations.

The addition of cycloheximide, 250 μg/ml medium, caused an inhibition of 99 per cent of [^{14}C]leucine incorporation in this system. Each experiment was carried out in duplicate or triplicate 2–5 times.

DL-Propranolol and practolol were donated by Abic, Ramat-Gan, Israel, and D-propranolol by Dr. H. A. Johnston, Imperial Chemical Industries, England. Pindolol was a generous gift from Dr. H. Friedli, Sandoz AG, Basel, Switzerland. Quinidine sulfate was obtained from Pharmaplantex, Netanya, Israel. Fetal calf serum was obtained from the Grand Island Biological Company, U.S.A. and Eagle's Minimal Essential Medium from the Laboratory of Public Health, Jaffa, Israel. All other reagents were of the highest purity commercially available.

RESULTS

DL-Propranolol inhibited to the same extent the incorporation of both [^{14}C]leucine and a mixture of [^{14}C]amino acids into trichloroacetic acid precipitable material in cultures of chick embryo liver cells; the inhibition was lineary related to the concentration of propranolol and reached 35–40 per cent in the presence of 60 μg propranolol per ml medium (Fig. 1). At higher concentrations of propranolol the cells lost their viability according to the trypan blue test and became detached from the bottoms of the culture dishes.

Figure 2 shows that the inhibition of leucine incorporation was dependent upon the duration of contact of the cells with propranolol. The curve relating per cent inhibition to duration of contact shows a steep slope during the first 30 min and a plateau after 60 min. Maximal inhibition of nearly 50 per cent was obtained after incubation of the cells in the presence of propranolol during approximately one hr after which no further increase in inhibition occurred.

The inhibition of incorporation of leucine into proteins was reversible, but the reversal required changes of the medium, as shown in Table 1.

Relief of the inhibition induced by propranolol could not be obtained without changing the medium several times, even when the incubation was continued for several hours after exchanging the propranolol containing medium with medium without propranolol.

Table 1. Reversibility of the effect of propranolol on incorporation of [^{14}C]leucine into protein*

Number of changes of medium	Min incubation after removal of propranolol	% decrease in incorporation of [^{14}C]leucine
0	0	38.5 $\pm$ 4.5
1	30	28.5 $\pm$ 4.5
2	60	23.0 $\pm$ 2.0
3	90	15.5 $\pm$ 0.5
4	120	0

* Cultures of chick embryo liver cells were incubated with and without DL-propranolol, 60 μg/ml medium. After 30 min the medium was removed, the adherent cells were rinsed and incubated for another 30 min with fresh medium not containing propranolol. Medium was exchanged up to four times after each 30 min of incubation. 1 μCi [^{14}C]leucine was added to the cultures at the beginning of the last 30 min incubation period. The per cent decrease was calculated on the basis of controls which were treated exactly like the propranolol experimental cultures but no propranolol was added to the medium during the first 30 min of incubation. The per cent decrease shows the mean and standard deviation of 4 separate experiments.

Table 2. The effect of β-receptor blocking agents and membrane active compounds on [^{14}C]leucine incorporation into protein in cultured chick embryo liver cells

Drug	Concentration μg/ml medium	% inhibition of [^{14}C]leucine incorporation
β-Receptor blocking agents with non-specific membrane effects		
1. DL-Propranolol	30	18.8 ± 12.2
	60	37.7 ± 7.5
2. Oxprenolol (Trasicor)	50	13.0 ± 1.0
	150	21.7 ± 6.7
	300	34.3 ± 11.3
β-Receptor blocking agents without membrane effects		
3. Pindolol (Visken)	15	0
	30	0
4. Practolol	75	4.8 ± 1.0
	150	3.7 ± 3.7
Membrane active compounds		
5. D-Propranolol	30	14.2 ± 6.3
	60	33.6 ± 13.2
6. Quinidine sulfate	30	12.5 ± 0.5
	60	27.2 ± 5.3
	120	60.3 ± 3.2
7. Lidocaine	50	5.5 ± 2.1
	340	9.5 ± 1.5
	680	16.0 ± 3.0
	1360	31.7 ± 1.0

Cultures were prepared as described in Materials and Methods. [^{14}C]leucine, 1 μCi, was added to each culture together with the agent the effect of which was examined. After 30 min incubation the medium was removed and [^{14}C]leucine incorporation was determined. The control value was considered as 100 per cent and results are expressed as per cent inhibition of [^{14}C]leucine incorporation into protein. Each value represents the mean and standard deviation of 4–8 determinations.

DL-Propranolol has both β-receptor blocking properties and a membrane stabilizing effect. In order to ascertain which of these activities caused the observed inhibition of protein synthesis some other drugs were examined.

Table 2 shows that the β-blockers DL-propranolol and oxprenolol caused virtually similar inhibitions of incorporation of L-leucine when given at high concentrations. Both these drugs also have membrane effects. Pindolol and practolol which are devoid of membrane activity were ineffective in concentrations related to their potency as β-receptor blocking agents.

D-Propranolol, the D-isomer of propranolol, has a very slight β-blocking effect but its membrane action is similar to that of DL-propranolol. Quinidine and lidocaine have a membrane stabilizing action but are structurally and functionally unrelated to β-receptor blocking agents. These three drugs also inhibit amino acid incorporation when added to the cultures. D-Propranolol was effective at a concentration similar to that of DL-propranolol. The effective concentrations of quinidine and lidocaine were determined experimentally. The high concentration of lidocaine required is in accordance with its lesser potency as compared to quinidine.

DISCUSSION

β-Receptor blocking agents with non-specific membrane activity have a multitude of effects, some of which are related to blockade of the β-receptors and others to their effects on membrane.

In previous investigations it was shown that DL-propranolol, a β-blocker drug with membrane effects, markedly decreased induction of the enzyme delta-aminolevulinate synthetase in rat liver *in vivo* and in tissue cultures of chick embryo liver cells. The drug had no effect on the basal activity of this enzyme [2–4].

This induction is thought to be the result of increased synthesis of the enzyme, either by increased translation or enhanced transcription [8–10]. In the present experiments, it is shown that the above inhibition caused by DL-propranolol is not specific for induction of delta-aminolevulinate synthetase but for protein synthesis in general. In cultures of chick embryo liver cells DL-propranolol, a β-blocker with non-specific membrane effects, partially inhibits the incorporation of [^{14}C]leucine and of [^{14}C]amino acids into proteins. This inhibition is reversible, though the technique required for complete reversal indicated that the drug has a high affinity for liver

cells. This is in accordance with other observations [11].

Examining a variety of other substances it was further shown that the membrane active compounds: D-propranolol, quinidine and lidocaine in appropriate concentrations also inhibit the incorporation of [^{14}C]leucine into proteins. Oxprenolol, a β-blocker drug with membrane effects, acted like DL-propranolol. On the other hand, pindolol and practolol, two drugs which are β-blockers practically devoid of membrane effects, did not influence the incorporation of [^{14}C]leucine into proteins. It seems, therefore, reasonable to assume that the membrane effects cause the inhibition of incorporation of amino acids into proteins in cultured chick embryo liver cells.

It has been pointed out that remarkably little information is available about the interaction between properties of biological membranes and protein synthesis [12]. Similarly, very little is known about the effect of membrane active drugs on protein synthesis. Local anesthetics are membrane active compounds. Their action is possibly mediated by an increase in the disorder of lipid molecules, an expansion of the lipid portion of the membrane, or conformational changes in proteins, intimately associated with lipids [13–15]. According to Vail *et al.* [16] the ability of these drugs to displace membrane bound Ca^{2+}, could induce changes in the organization of peripheral proteins involved in regulating the distribution of cell surface receptors. DL-Propranolol, a β-blocker agent with non-specific membrane activity, affects markedly the permeability and buffering capacity of mitochondrial membranes. These changes were interpreted to be the result of a change in the membrane charge distribution and of perturbation of the membrane structure [17]. It has also been shown that DL-propranolol affects sodium and potassium exchange [18, 19] and inhibits membrane ATPase [20].

Which of these membrane properties, or others, are related to inhibition of incorporation of amino acids into proteins in cultures of chick embryo liver cells is unclear. The effects observed might be caused by reduced uptake of amino acids by the cells. Further investigations are in progress to elucidate this inhibition.

Acknowledgements—Special thanks are expressed for the excellent technical assistance of Miss Yehudit Greenblat and Mrs. Orit Epstein.

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