INHIBITORY EFFECT OF SOME MEMBRANE ACTIVE DRUGS ON RNA AND DNA SYNTHESIS IN CULTURED CHICK EMBRYO LIVER CELLS

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Summary: DL-Propranolol and oxprenolol, both beta-adrenergic receptor blocking agents with membrane active properties, were found to inhibit the incorporation of uridine and thymidine into RNA and DNA respectively in cultures of chick embryo liver cells. The membrane active compounds quinidine and lidocaine had similar effects. D-Propranolol, which has a very slight beta-blocking action only but has a membrane activity similar to DL-propranolol, showed inhibition like that of DL-propranolol. Pindolol and practolol, beta-adrenergic blocking agents nearly devoid of membrane activity, had no effect on incorporation. The inhibition is reversible and non-competitive. It seems to be related to the membrane active properties of the drugs examined.

In previous investigations we showed that some beta-adrenergic blocking agents with non-specific membrane effects and other membrane active compounds partially inhibit the induction of delta-aminolevulinate synthetase in rat liver and in cultures of chick embryo liver cells (1,2,3). In the latter system the same agents also partially inhibit the induction of tyrosine aminotransferase by dexamethazone (4). The inhibition of induction was found to be caused by a general inhibition of protein synthesis (5).

This study was undertaken in order to investigate the effects of some membrane active compounds on incorporation of uridine and thymidine into RNA and DNA respectively, in chick embryo liver cells in culture.

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Materials and Methods

Preparation of cell cultures: Cultures of seventeen-days-old chick embryo liver cells were prepared and incubated in 3 ml of Eagle's Minimal Essential Medium with 10% fetal calf serum at 37°C and 5% CO_2 as described by Granick (6). After a preliminary incubation of 24 hrs the medium containing non-adherent cells was discarded, replaced by fresh medium and incubation was continued for another 20 hrs. The experiments were carried out about one hr after an additional replacement of medium.

Determination of incorporation of (^{14}C)uridine into RNA: 1 μCi (^{14}C)uridine, (482 mCi/mmol) Radiochemical Centre, Amersham, England, was added to each culture together with the drugs to be tested. After 30 min incubation the medium was discarded and the petri dishes with adherent cells were rinsed with cold 0.32 M sucrose containing 3 mM $MgCl_2$ (7). The cells were scraped from the bottoms of the dishes and homogenized in the same medium. For protein determinations 0.02 ml of the homogenate were removed. Ice cold 10% trichloroacetic acid was added in equal volume to the homogenate and mixed. After centrifugation for 10 min at 5000x g the supernatant was removed; the precipitate was collected on a membrane filter (HAWP02500 0.45μm membrane filter, Millipore Corporation, Massachusetts) and washed three times with cold 5% trichloroacetic acid.

In each experiment control samples were heated for 20 min at 90°C to hydrolyze RNA after which they were centrifuged and treated as above. This permits a correction for acid insoluble uridine which is not incorporated into RNA (8). The correction amounted to less than 3% in all experiments.

Each filter was placed in a counting vial containing 10 ml scintillation fluid (PPO 11.2 g, dimethyl POPOP 15 mg, dioxane 375 ml, toluene 375 ml, methanol 225 ml and naphthalene 78 g). For determination of the acid soluble uridine, 0.1 ml of the supernatant was added to scintillation fluid as above. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, Model 544 with a correction unit for dpm. Results were calculated as dpm/mg protein.

Actinomycin D, 4 μg/ml and 8 μg/ml inhibited incorporation of (^{14}C)uridine into RNA by 88.5±0.3% and 95.7±0.6% respectively.

Determination of incorporation of (^{14}C)thymidine into DNA: This procedure was similar to that described for (^{14}C)uridine except for a few details. 1 μCi (2-^{14}C)thymidine, 60 mCi/mmol, (The Israel Atomic Energy Commission, Nuclear Research Centre, Negev) was added to cultures as described for (^{14}C)uridine. After 30 min incubation, cultures were rinsed with Na-phosphate-saline buffer, pH 7.4, instead of sucrose-$MgCl_2$ (7). In some experiments the trichloroacetic acid precipitate was solubilized in soluene-100 (Packard) instead of collected on filters. Both methods gave similar results. Addition of cytarabide (cytosine arabinoside), a specific inhibitor of DNA synthesis (9), 3 μg/ml medium, inhibited the incorporation of (^{14}C)thymidine into DNA by 90%. Results were calculated as dpm/μg DNA.

All the experiments were performed at the same time of the day to eliminate the possibility of diurnal variations in uptake and incorporation.

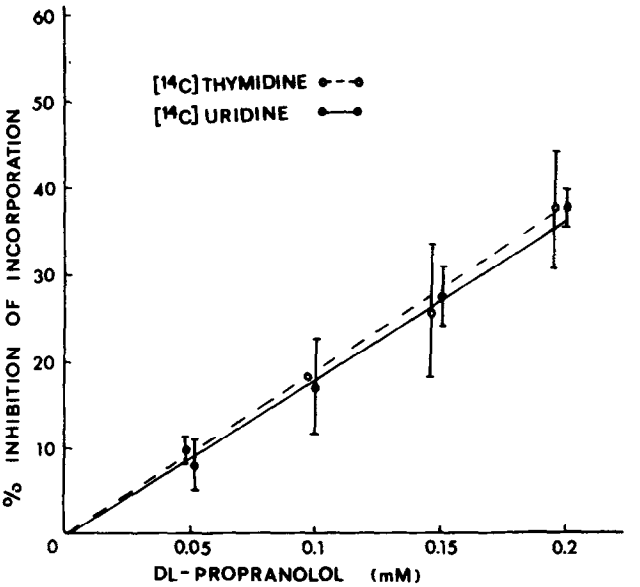

Fig. 1. The effect of DL-propranolol on incorporation of (^{14}C)uridine into RNA and of (^{14}C)thymidine into DNA. DL-Propranolol in various concentrations and 1 μCi (^{14}C)uridine or (^{14}C)thymidine were added to cultures of chick embryo liver cells. After 30 min incubation the incorporation of (^{14}C)uridine into RNA and of (^{14}C)thymidine into DNA was determined. The values of incorporation of (^{14}C)uridine and (^{14}C)thymidine obtained in the control cultures were 7004±489 dpm/mg protein and 1698±121 dpm/μg DNA respectively. These values were considered as 100% incorporation of (^{14}C)uridine or (^{14}C)thymidine into RNA and DNA respectively. Each value is the mean and standard deviation of 6-8 determinations.

Determination of protein: Proteins were determined by the method of Lowry et al. (10) with bovine serum albumin as standard.

Determination of DNA: DNA was determined fluorometrically using ethidium bromide, according to the method of Boer (11).

Each experiment was carried out in duplicate or triplicate 2-5 times.

Results

The presence of DL-propranolol in cultures of chick embryo liver cells causes an inhibition of incorporation of uridine and thymidine into RNA and DNA respectively, as shown in Figure 1. A similar reduction in (^{14}C)uridine and (^{14}C)thymidine after treatment with DL-propranolol could be detected in the acid soluble fraction (data

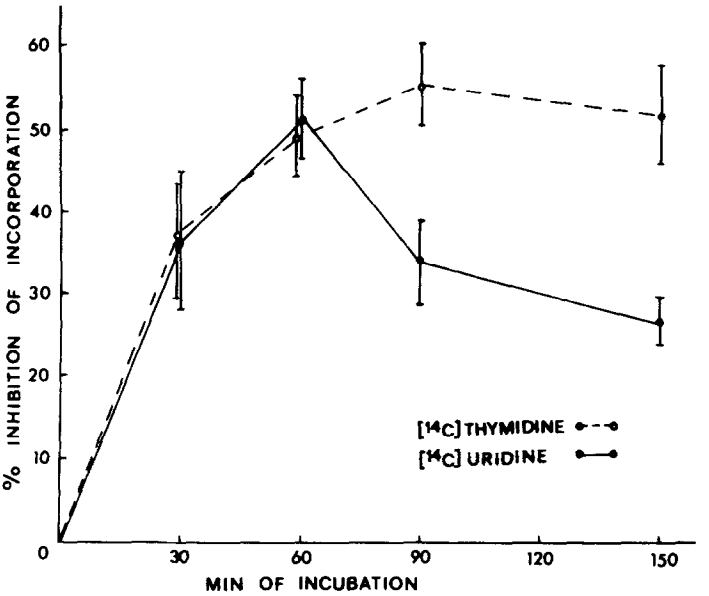

Fig. 2. The effect of duration of incorporation of DL-propranolol on incorporation of (^{14}C)uridine into RNA and (^{14}C)thymidine into DNA. Cultures were incubated for 0-240 min in the presence of DL-propranolol, 60 µg/ml medium. In simultaneously run experiments control cultures were incubated for 0-240 min in the presence of vehicle only. At the times indicated 1 µCi (^{14}C)uridine or (^{14}C)thymidine were added to cultures and incubation was continued for 30 min, after which incorporation was determined. Results are expressed as % inhibition of (^{14}C)uridine or (^{14}C)thymidine incorporation compared to control values. Each value is the mean and standard deviation of 6-8 determinations.

not shown). The inhibition is directly proportional to the concentration of propranolol up to a concentration of 0.2 mM. At higher concentrations the cells lose their adherence to the bottoms of the petri dishes and part of the cells incorporate Trypan blue, indicating loss of viability.

The inhibitory effect of propranolol is not maximally effective immediately; incubation for 60 min in the presence of propranolol increased the inhibition of incorporation from approximately 35% to approximately 50% for both uridine and thymidine as shown in Figure 2.

Table I. The effect of beta-adrenergic blocking agents and membrane active compounds on incorporation of (^{14}C)uridine into RNA and (^{14}C) thymidine into DNA

Drug	Concentration µg/ml medium	% inhibition of incorporation of (^{14}C)uridine	(^{14}C)thymidine
Beta-blockers with non-specific membrane effects			
DL-propranolol	60	37.2±2.2	37.3±6.8
oxprenolol (Trasicor)	150	30.0±0.0	30.0±2.1
	300	68.0±5.0	56.0±3.1
Beta-blockers without membrane effects			
pindolol (Visken)	30	0	0
practolol	150	0	0
Membrane active compounds without beta-blocking effects			
D-propranolol	60	39.6± 3.0	37.5± 0.5
quinidine sulfate	60	32.0± 0	45.0±12.3
	120	63.0± 1.0	77.2± 7.0
lidocaine	680	42.7±10.4	27.3± 5.3

Cultures were prepared as described in "Materials and Methods". (^{14}C)uridine or (^{14}C)thymidine 1 µCi were added to each culture together with the agent, the effect of which was examined. After 30 min incubation the medium was removed and incorporation of (^{14}C)uridine or (^{14}C)thymidine was determined. The control value was considered as 100% incorporation and results are expressed as % inhibition of incorporation of (^{14}C)uridine into RNA or (^{14}C)thymidine into DNA. Each value is the mean and standard deviation of 6-10 determinations.

Upon more prolonged incubation the inhibition of incorporation of uridine declined whereas that of thymidine remained at approximately the same level.

DL-Propranolol has both membrane active and beta-adrenergic receptor blocking properties. The concentrations required for

partially inhibiting the incorporation of uridine and thymidine into RNA and DNA are those at which the membrane stabilizing effect of propranolol becomes apparent. In order to further establish that the effects observed are related to the membrane effects and not to the beta-adrenoceptor blocking action a group of other substances with both or either of these properties were examined.

The results shown in Table I clearly indicate that the inhibitory effects of propranolol are related to its membrane active properties. Oxprenolol, which has both beta-adrenoceptor blocking and membrane active properties acts like DL-propranolol. Pindolol and practolol, both practically devoid of membrane effects, show no inhibition. The concentration of pindolol was chosen in accordance with its beta-blocking potency which is 5-10 times higher than that of DL-propranolol. D-Propranolol has the same membrane activity as DL-propranolol; D-propranolol, quinidine and lidocaine inhibited incorporation. The concentrations of quinidine and lidocaine were such that no cell damage was observed. Reversibility of the inhibition induced by DL-propranolol was obtained only after several changes of medium.

Lineweaver-Burk plots of the incorporation rates into acid-precipitable material against uridine concentrations establish that DL-propranolol, D-propranolol and quinidine sulfate act as non-competitive inhibitors with Ki values of 2.5×10^{-4}M, 3.3×10^{-4}M and 1.8×10^{-4}M respectively.

Km and Vmax values of the non-inhibited reaction are 0.5×10^{-5}M and 40 pmoles/mg protein/min as shown in Figure 3. Kinetics of thymidine incorporation are shown in Figure 4. Km and Vmax values are 0.1×10^{-5}M and 50 pmols/mg protein/min respectively. Similarly,

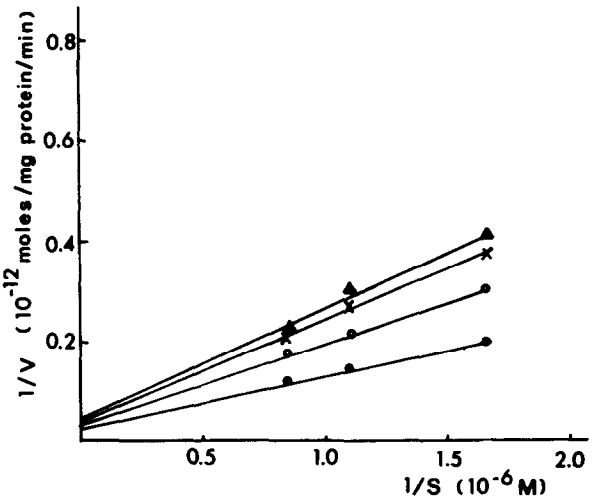

Fig. 3. Lineweaver-Burk plots of the incorporation of (^{14}C)uridine into acid precipitable material in the absence ●_____● or presence of either 0.2 mM (60 μg/ml) DL-propranolol X_____X, 0.2 mM D-propranolol O_____O, or 0.2 mM quinidine sulfate ▲ _____ ▲.

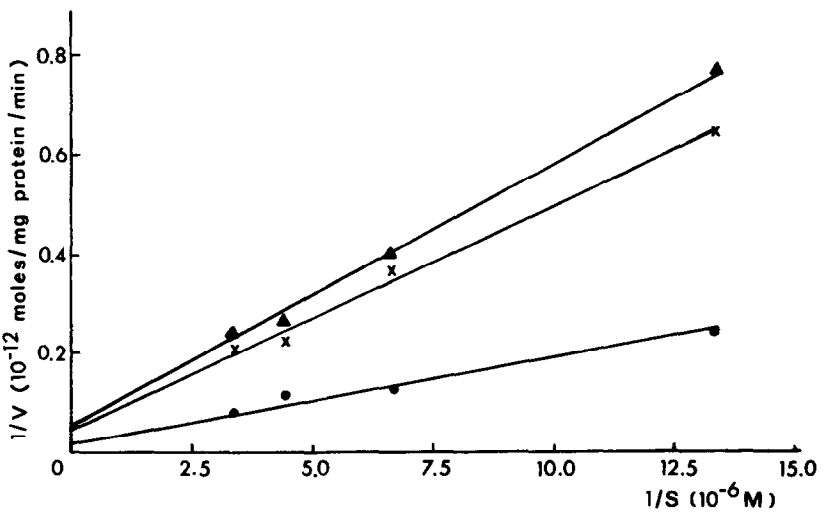

Fig. 4. Lineweaver-Burk plots of the incorporation of (^{14}C)thymidine into acid precipitable material in the absence ●_____● or presence of either 0.2 mM (60 μg/ml) DL-propranolol X_____X, or 0.2 mM quinidine sulfate ▲ _____ ▲.

DL-propranolol and quinidine inhibit this incorporation non-competitively with Ki values of 1.3×10^{-4}M and 8.5×10^{-5}M respectively.

Discussion

In previous investigations we showed that DL-propranolol and other membrane active drugs inhibit the incorporation of amino acids into proteins in cultures of chick embryo liver cells (5), probably caused by reduced transfer of amino acids across the cell membranes (12). A similar inhibition of incorporation of uridine and thymidine into RNA and DNA was observed in the present investigation. The acid-soluble fraction showed a numerically equal reduction after treatment of the cells with DL-propranolol. This would again tend to indicate that the inhibition of incorporation might be caused by a reduced transfer of the precursors of RNA and DNA into the cells.

If further investigations will confirm these tentative conclusions a new dimension will have to be added to the effects of high concentrations of some membrane active compounds on living cells. A reduction in transport of precursors of RNA and DNA of amino acids, and possibly of other basic compounds, may have far reaching effects on many aspects of cellular metabolism. Moreover, intracellular membranes may be similarly influenced by these agents in appropriate concentrations. Thus, oxidative phosphorylation by isolated mitochondria was shown to be inhibited by 10^{-3}M of DL-propranolol (13).

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