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THE EFFECT OF PROPRANOLOL ON THE OSMOTIC FRAGILITY OF RED CELLS AND LIPOSOMES AND THE INFLUENCE OF THE DRUG ON GLYCEROL TRANSPORT ACROSS THE MEMBRANE OF RED CELLS*

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Abstract—Propranolol has been found to have different effects on the stability of red-cell membranes and model phospholipid membranes (liposomes). Its effect on red-cell membranes is biphasic. At lower concentrations the drug protects the cells from hypotonic haemolysis, whereas at high concentrations it promotes haemolysis. In contrast to red cells, liposomses were destabilized by propranolol. The lytic effect on lipid membranes was observed within the same concentration range of the drug at which it exerts an antihaemolytic effect on red cells. Propranolol has been shown also to perturb glycerol transport in red cells. It stimulates passive diffusion of glycerol into bovine red cells and inhibits facilitated diffusion in human red cells. The experimental results suggest that although both membrane lipids and proteins are involved in drug–membrane interaction, the crucial role in propranolol-induced red-cell membrane stabilization is played by the protein component.

Propranolol is known mainly as a beta-adrenergic blocker. It has been demonstrated, however, that in some tissues the major fraction of the drug may have binding sites which are not stereoselective and are unrelated to beta-adrenergic receptors [1, 2]. This nonstereoselective binding of propranolol may lead to more general membrane perturbations. It seems to account for the local anaesthetic action of propranolol [3, 4] and, as suggested [5], it may also substantially contribute to other pharmacological properties of the drug, such as its antipsychotic, anticonvulsant and antiarrhythmic effects.

Several studies on propranolol–membrane interaction have been carried out using red-cell membranes as a model system. These investigations were focussed mainly on the effects of the drug on cation permeability [6–9], activity of membrane bound enzymes [5] and membrane stabilization, as shown by the protection of intact red cells against hypotonic haemolysis [5, 9–11].

In the present paper we explore in more detail the molecular mechanisms of membrane stabilization, comparing the effects of propranolol on the osmotic fragility of red-cell membranes and model phospholipid membranes (liposomes). We discuss also the effect of propranolol on the transport of nonelectrolyte-glycerol. The elucidation of the antihaemolytic effect of propranolol and other drugs is important since a correlation appears to exist between the antihaemolytic properties of some drugs and their ability to perturb the functional state of excitable tissues [12].

MATERIALS AND METHODS

Chromatographically pure egg yolk phosphatidylcholine was prepared by the method of Singleton *et al.* [13]. Red-cell lipids were extracted from haemoglobin-free ghosts by the method of Folch *et al.* [14]. D,L-Propranolol hydrochloride, dicetylphosphate, cholesterol, 2,2′-dinitro-5,5′-dithiobenzoic acid (DTNB) and reduced glutathione were from Sigma. Sephadex G-25 was from Pharmacia.

Heparinized human blood and citrated bovine blood was used shortly after collection. Red cells were washed three times in isotonic solution (0.9% NaCl/5 mM phosphate buffer, pH 7.2) and finally suspended at 50% PCV. The procedure used for studying the effect of propranolol on osmotic fragility of human red cells involved an initial 15-min incubation at room temperature of 0.2 ml of stock cell suspension in 2 ml of isotonic buffer containing various concentrations of propranolol. Subsequently 0.2 ml of each sample was rapidly mixed with 2 ml of hypotonic solution (0.36% NaCl/5 mM phosphate buffer, pH 7.2) containing propranolol of the same concentration as in previous medium. After 10 min incubation period cells were centrifuged and the extent of haemolysis (expressed as a per cent of total haemolysis in distilled water) was determined from the absorbance of supernatants at 540 nm.

The effect of propranolol on glycerol transport across red-cell membranes was tested using a glycerol haemolysis time method. The rate of lysis was followed according to a previously described procedure

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by measuring the decrease in turbidity of cell suspension in glycerol solution [15, 16]. Briefly, 0.05 ml aliquots of stock cell suspension were added to 1 ml portions of isotonic phosphate buffer containing propranolol at appropriate concentrations. After 15 min incubation at room temperature, 0.1 ml of such prepared suspension was quickly injected into cuvettes containing 2 ml of buffered 300 mM glycerol solution and propranolol of the same concentration as in incubation medium. The suspension was rapidly mixed and the rate of lysis was followed spectrophotometrically at 625 nm. Glycerol lysis times were defined as times required for a decrease in optical density of red-cell suspension to 50 per cent of its initial value [16].

Multilamellar liposomes were prepared and their osmotic fragility was determined as described by Alhanaty & Livne [17]. In brief, for liposome preparation, about 20 mg of lipids were taken to dryness in a round-bottomed flask. The dried films were then vortexed with 1 ml of suspension containing 100 μmoles DTNB in 0.1M Tris–HCl buffer, pH 8.0. Untrapped marker (DTNB) was removed by Sephadex G-25 chromatography. In order to determine the osmotic fragility of these liposomes, 0.04 ml aliquots of gel filtered liposome suspension were rapidly mixed with 2 ml of distilled water containing propranolol at the desired concentration. The per cent of marker released from liposomes was determined by measuring the absorbance at 420 nm of reaction product between released DTNB and added reduced glutathione. The readings were corrected for light scattering by liposome suspension. The total amount of the trapped marker was determined after complete liposome disruption by Triton X-100.

RESULTS AND DISCUSSION

The effect of propranolol on the osmotic fragility of human red cells was studied at a final NaCl concentration of 0.45%. In the absence of the drug, about 70 per cent lysis was observed. After addition of propranolol to the test solution a biphasic effect of the drug on red-cell stability was observed (Fig. 1).

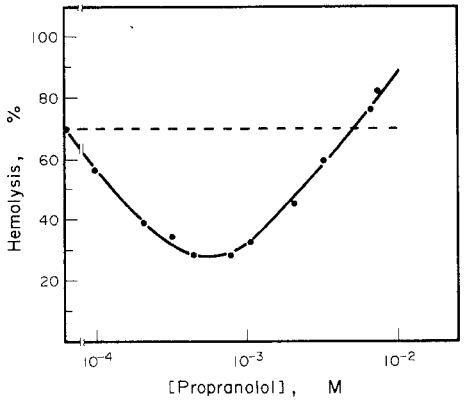

Fig. 1. Effect of propranolol on hypotonic haemolysis of human red cells. Each point represents mean of 6–8 separate experiments.

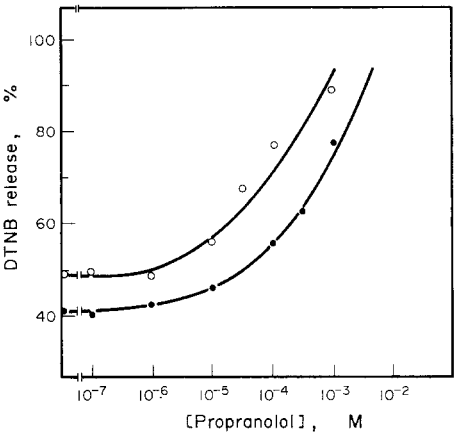

Fig. 2. Effect of propranolol on the release of DTNB from liposomes made of red-cell lipids (open symbols) and phosphatidylcholine : cholesterol : dicetylphosphate (1 : 1 : 0.1) (closed symbols). Each point represents mean of 5–8 experiments.

At relatively low concentrations propranolol protected red cells against osmotic lysis whereas at higher concentrations of the drug its protective effect was diminished and finally it was reversed by the lytic action. Such biphasic effect is typical for a number of other molecules of pharmacological importance (anaesthetics, tranquilizers) [12]. To examine the possible role of the lipid bilayer in propranolol-induced membrane stabilization and destabilization we tested the effect of the drug on osmotic fragility of liposomes. Multilamellar liposomes, like red cells, behave as osmometers and their usefulness in exploring mechanisms of membrane action of antihaemolytic compounds has been previously demonstrated [17]. The osmotic fragility of liposomes may be conveniently studied by following the release of trapped marker upon rupture of lipid membranes in hypotonic solution. DTNB appears to be very convenient marker for such studies because its low permeability across lipid bilayer means that the amount of marker released from intact liposomes is negligible compared with that released as a result of osmotic rupture of the membranes [17].

When liposomes prepared from phosphatidylcholine: cholestrol: dicetylphosphate (1 : 1 : 0.1) mixture with trapped DTNB and 0.1M Tris buffer were rapidly injected into distilled water, about 42 per cent of trapped marker was released as a result of this osmotic shock. Liposomes prepared from red-cell membrane lipids were slightly less stable, releasing about 50 per cent of DTNB. With propranolol present, liposomes, in contrast to red cells, became more fragile as reflected in increased marker release (Fig. 2). Membranes prepared from phosphatidylcholine: cholesterol: dicetylphosphate mixture and from red-cell lipid extract showed a similar response to propranolol. The lytic effect of propranolol on lipid membranes was observed at the same concentration range of the drug at which its antihaemolytic effect on intact red cells was found. Comparison of the effects of propranolol on red cell membranes

and on model lipid membranes allowed the conclusion that factors other than direct drug–lipid interactions are involved in the antihaemolytic action of the drug. Apparently membrane proteins are required for the antihaemolytic effect of propranolol. Membrane stabilization may result from direct interaction of propranolol with membrane protein and/or from drug-induced perturbation in lipid–protein interaction.

The biphasic effect of propranolol on the stability of red cells may be explained by assuming that at lower drug concentrations its interaction with protein or lipoprotein plays a dominating role, leading to membrane stabilization. At higher concentrations lytic interaction of the drug with membrane lipids promotes hypotonic haemolysis in red cells.

It may be worth noting that, from among other compounds so far tested in this respect, phenothiazine derivatives behaved similarly to propranolol, i.e. they did not stabilize lipid membranes against osmotic shock. On the other hand, fatty acids and hashish components, cannabidiol and Δ^1-tetrahydrocannabinol stabilize both red cells and liposomes, suggesting that direct lipidic interactions are involved in the antihaemolytic effects of these substances [17].

The effect of propranolol on glycerol transport across red-cell membranes was studied using a turbidimetric method for measuring glycerol lysis times. Although this technique is too crude for permeability coefficients determination, it is useful in comparative studies [16]. It was decided to test the influence of propranolol on glycerol transport across the membranes of both human and bovine red cells since glycerol translocation in the cells of these two species represents different types of nonelectrolyte transport. It is considered as a facilitated diffusion in human red cells and passive diffusion in bovine red cells [18].

Comparison of the results, presented in Figs. 3 and 4, shows that the effect of propranolol on the above mentioned two types of nonelectrolyte transport is opposite. The transport of glycerol is accelerated in bovine cells (Fig. 3) and inhibited in human cells (Fig. 4) as reflected in haemolysis time changes. Such an effect of propranolol on nonelectrolyte transport, i.e. the stimulation of simple passive diffusion and inhibition of facilitated diffusion is very similar to that observed for ethanol and other anaesthetics [19].

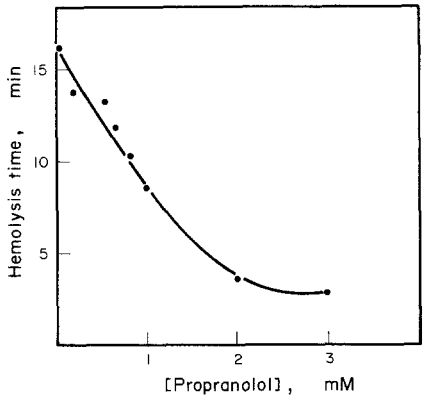

Fig. 3. Effect of propranolol on glycerol haemolysis time of bovine red cells. Each point represents mean of 6–8 experiments.

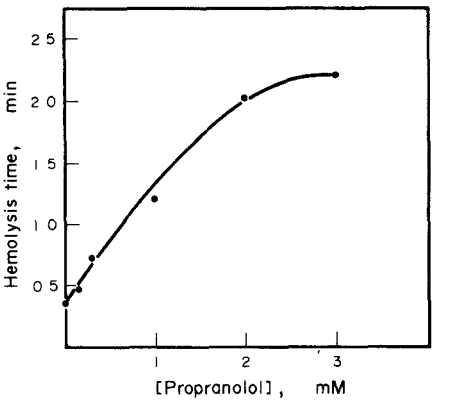

Fig. 4. Effect of propranolol on glycerol haemolysis time of human red cells. Each point represents mean of 8–12 experiments.

Passive diffusion of glycerol into bovine red cells seems to be controlled mainly by the physical state of membrane lipids whereas some proteins are certainly involved in facilitated diffusion in human red cells. The present data would therefore indicate that both membrane proteins and lipids are perturbed upon interaction with propranolol. This complies with the results of previous structural studies [5].

In conclusion, the effect of propranolol on the properties of red cell membranes resembles the general pattern shared by wide class of anaesthetics, i.e. a biphasic effect on osmotic fragility, stimulation of glycerol passive transport in bovine cells and inhibition of facilitated diffusion in human cells. Although both membrane lipids and proteins seem to be involved in drug–membrane interaction, it appears that a crucial role in propranolol-induced red-cell membrane stabilization is played by the protein component of the membrane.

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