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PROPRANOLOL-INDUCED STRUCTURAL CHANGES IN HUMAN ERYTHROCYTE GHOST MEMBRANES

A SPIN LABEL STUDY

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Abstract—The effect of propranolol on the structure of human erythrocyte membranes was studied using a spin labeling technique. Changes in electron spin resonance spectra of spin labeled membrane proteins were detected at concentrations of the drug corresponding to its antihemolytic effect on intact erythrocytes. The character of spectral changes suggests that propranolol-induced alterations in organization of membrane proteins are connected mainly with perturbation of protein sites located on membrane surface. Propranolol also produces a decrease in order parameter of membrane lipids. The disordering effect is, however, small and detectable only at relatively high concentrations of the drug.

In recent years a great deal of studies has been carried out to gain insight into the interaction of various drugs with biological membranes. It has been shown that some drugs interact specifically with membrane protein sites, whereas others perturb the physical state of membrane lipids. In the present work we report on the interaction of the widely used drug, propranolol, with human erythrocyte membranes. Propranolol is well known mainly as a specific competetive antagonist of adrenergic beta receptors [1]. In recent years, however, more general membrane perturbing action of the drug has been demonstrated. Such membrane action of propranolol, which is not stereoselective and unrelated to beta adrenergic receptors, has been suggested to contribute substantially to local anaesthetic [2–4], antipsychotic [5], anticonvulsant [6] and antiarrhythmic [4, 7] effects of the drug.

Propranolol has been shown to perturb the functional state of various membrane systems [2–4, 7–13]. We explore here drug-induced structural changes using human erythrocyte ghost membranes as a model system. On the basis of results obtained using amino and sulfhydryl group-specific reagents it has been suggested that both membrane lipids and proteins may be perturbed upon interaction with the drug [12]. The molecular aspects of propranolol-induced alterations in membrane structure are, however, still far from being clearly understood. The knowledge of these aspects could contribute to a better understanding of some pharmacological properties of the drug. Moreover, the detailed knowledge of the molecular mechanism of propranolol–membrane interaction may be of special interest in connection with its membrane stabilizing effect [10, 11]. Many other drugs, such as anesthetics and tranquillisers, also act as membrane stabilizers [10, 14] and it is possible that all these compounds perturb structural components of biological membranes in a similar manner.

The spin labeling technique employed in the present study has been extensively used in investigations of drug–membrane interactions [15–17]. It provides information about the physical state of membrane lipids and conformation of membrane proteins.

MATERIALS AND METHODS

Spin labels used in this study: 4-*N*-maleimido-2,2,6,6-tetramethylpiperidine-1-oxyl (structure I in Fig. 1) and methyl ester of 5-keto palmitic acid (structure II in Fig. 1) were a generous gift from Dr. K. Gwozdzinski (Department of Biophysics, University of Lodz). DL-Propranolol hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO)

Human erythrocyte ghost membranes were prepared by the method of Dodge, Mitchell and Hanahan [18]. For protein spin labeling, membrane ghosts were incubated at 4° overnight with maleimide spin label employing a 1:25 weight ratio of spin label to total membrane protein. The excess label was removed by washing 4–5 times in 10 mM phosphate buffer, pH 7.4. For labeling of membrane lipid core spin probe II was dissolved in chloroform in a conical flask. After evaporation of the solvent membrane suspension was added, and spin probe was incorporated into lipidic part of the membrane by gentle shaking at room temperature for about 20 min. Spin labeled membranes were incubated with appropriate amounts of propranolol for 30 min at room temperature. Electron spin resonance (ESR) spectra were recorded with a Jeol-PE-1X or a SE/X-28 (Wroclaw Technical University) X-band spectrometer at room temperature.

RESULTS

The maleimide spin label I is well known to bind mainly to sulfhydryl groups of membrane proteins

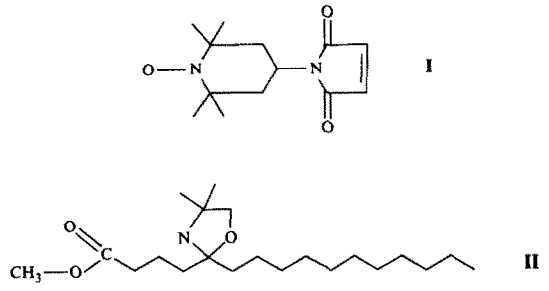

Fig. 1. The structure of spin labels used to study the propranolol–membrane interaction. I—4-(*N*-maleimido)-2,2,6,6-tetramethylpiperidine-1-oxyl; II—methyl ester of 5-keto palmitic acid.

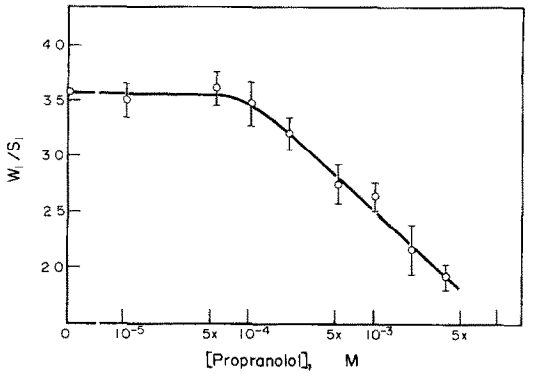

Fig. 3. The effect of propranolol on the mobility parameter W_I/S_I derived from spectra of erythrocyte membrane labeled with maleimide spin label I.

although a very small fraction of binding sites may also be amino groups [19–21].

A typical ESR spectrum of maleimide spin label attached to erythrocyte membrane is shown in Fig. 2a. The relatively complicated shape of the spectrum is due to the overlapping of at least two spectra and, as such, it reflects at least two different classes of -SH group sites in erythrocyte membrane [15–17, 19–23]. Lines denoted as *S* arise from spin labels of severely restricted motion whereas peaks *W* are due to spin labels possesing relatively high motional freedom. Because of the overlapping of the central parts of the spectra arising from two classes of spin labels it is difficult to characterize quantitatively the mobility of weakly immobilized labels in terms of rotational correlation time. However, the widths of *W*-lines can be used as a qualitative measure of the mobility of this class of spin labels. The mobility of strongly immobilized spin labels can be estimated based on the distance between the outermost hyperfine extrema [24].

Figures 2b–2c demonstrate the effect of increasing concentrations of propranolol on the spectra of maleimide spin labeled erythrocyte membranes. The line widths of signals due to weakly immobilized labels and the distance between outer hyperfine extrema due to strongly immobilized labels are essentially equal in control and drug treated samples. This indicates that the above discussed two classes of spin labeled membrane protein sulfhydryl groups have essentially the same mobility in the presence and absence of propranolol. In contrast to unchanged mobility of both classes of spin label binding sites, significant changes in relative amplitudes of signals due to weakly and strongly immobilized labels may be observed. The W_I/S_I ratio (as defined in Fig. 2a) of spectral amplitudes of low field lines of labels attached to weakly immobilized -SH groups to those attached to strongly immobilized -SH groups has been widely used to characterize this kind of spectral change [15–17, 21–23]. It is considered as a measure of relative population of each class of sulhydryl sites. The decrease in W_I/S_I ratio, as demonstrated quantitatively in Fig. 3, indicates that propranolol causes a conversion of weakly immobilized -SH sites to strongly immobilized ones.

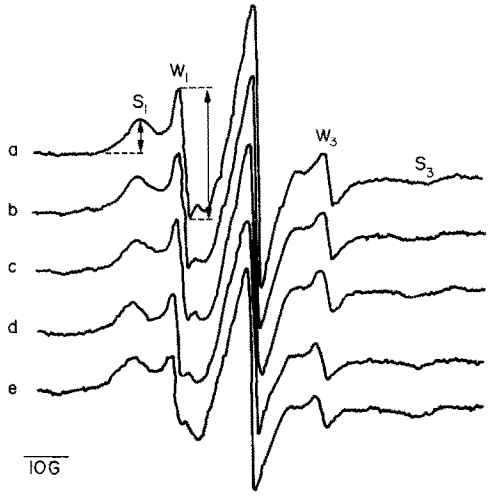

Fig. 2. Electron spin resonance spectra of erythrocyte ghost membranes (2–2.5 mg protein/ml) labeled with maleimide spin label I. (a) No drug, (b) 0.2 mM propranolol, (c) 0.5 mM propranolol; (d) 1.0 mM propranolol and (e) 4.0 mM propranolol.

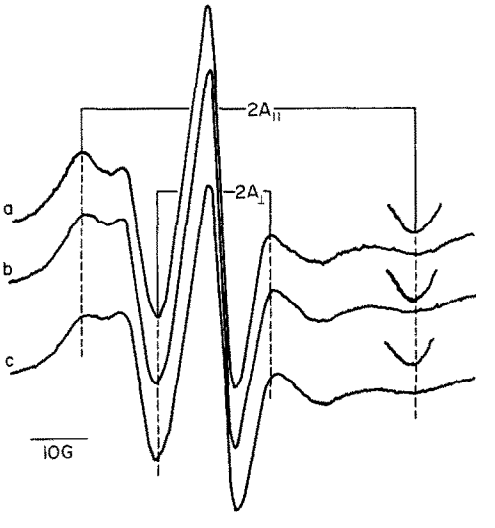

Fig. 4. Electron spin resonance spectra of erythrocyte ghost membranes (5–6 mg protein/ml) labeled with lipidic spin probe II (1×10^{-4} M). (a) No drug, (b) 2.0 mM propranolol and (c) 7.5 mM propranolol.

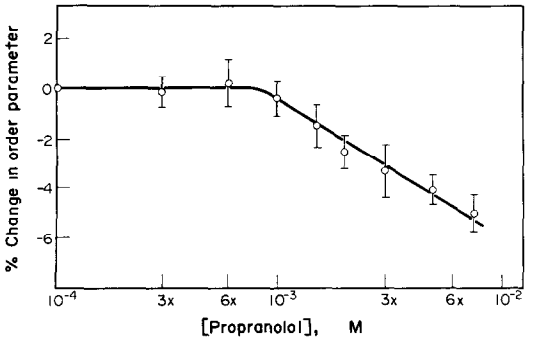

Fig. 5. The effect of propranolol on order parameter change of spin labeled erythrocyte ghost membranes. The mean order parameter for control membranes is 0.638 ± 0.007.

Spin probe II monitors the physical state of lipid core of the membrane [25]. ESR spectrum of spin label II incorporated into human erythrocyte ghost membranes is demonstrated in Fig. 4a. Spectra of this type, characteristic for highly anisotropic motion, may be analysed using formalism of order parameter. The order parameter, S, is a measure of molecular order in the lipid bilayer. It is obtained from the anisotropic hyperfine splittings $A_{\parallel}$ and $A_{\perp}$, measured as shown in Fig. 4a, by using the equation [25]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - \frac{1}{2}(A_{xx} + A_{yy})} \times \frac{a}{a'}$$

where A_{xx}, A_{yy} and A_{zz} are the components of hyperfine splitting tensor obtained from single crystal spectra [26]; $a' = 1/3\ (A_{\parallel} + 2\ A_{\perp})$; and $a = 1/3\ (A_{xx} + A_{yy} + A_{zz})$.

Addition of propranolol at concentrations up to approx. 1 mM did not produce significant changes in ESR spectra of spin probe II in erythrocyte membranes. At higher drug concentrations a small decrease in 2 $A_{\parallel}$ distance between outermost lines and increase in 2 $A_{\perp}$ distance between inner lines was detected (Figs. 4b–4c). Such spectral changes indicate some decrease in molecular order of membrane lipids, as demonstrated quantitatively in Fig. 5.

DISCUSSION

The results obtained using maleimide spin label demonstrate that the action of propranolol resulted in a significant perturbation in membrane protein conformation. This perturbation manifests itself as a change in the ratio of two subpopulations of -SH groups. Previous studies have shown that weakly immobilized sulfhydryl group sites are exposed to the polar medium on the membrane surface [21]. The strongly immobilized component of the spectrum, by contrast, is probably associated with labels located in the more hydrophobic lipoprotein matrix of the membrane [20]. The character of spectral changes, i.e. conversion of weakly immobilized -SH group sites to strongly immobilized ones, would indicate, therefore, that propranolol-induced alterations in membrane protein organization are connected mainly with perturbation of protein sites located in the polar environment on the membrane surface.

Changes in the W_1/S_1 ratio of the heights of peaks due to weakly and strongly immobilized labels are relatively marked. It is, however, to emphasise that the W_1/S_1 ratio is only a rough parameter. Due to very different widths of spectral W and S lines, the ratio of peak heights is not a true measure of the relative population of two -SH group classes. Exact values of the mobile and immobile fractions content may be extracted using the procedure based on computer simulations of superposition of mobile and immobile ESR spectra [27]. Employing this method it was estimated that the fraction of weakly immobilized labels amounts to approx. 13 and 9.5% in the control sample and at a propranolol concentration of 1 mM, respectively. The so estimated values and the character of spectral changes indicate that conformational alterations in membrane proteins are confined to relatively small portions of protein sites located on the membrane surface.

Structural changes in membrane proteins were detected at concentrations of propranolol corresponding to its antihemolytic effect on intact erythrocytes [10, 12, 28]. It is worthwhile to note that propranolol-induced structural changes are qualitatively very similar to those observed in the presence of phenothiazines [15, 16], acting similarily like propranolol as membrane stabilizing agents. On the other hand, some antitumor drugs have been shown to produce entirely different changes in ESR spectra of maleimide spin labeled membrane proteins, i.e. they caused conversion of strongly immobilized labels to weakly immobilized ones [17].

Although caution should be exercised in attempting to extrapolate from drug-induced effects in isolated erythrocyte membranes to those in intact cells, it seems very likely that sulfhydryl groups involved in conformational changes in membrane proteins are at least partly responsible for the antihemolytic effect of propranolol on red cells. The importance of membrane proteins in propranolol-induced membrane stabilization is also supported by the recent observation that, in contrast to red cells, liposomes prepared from erythrocyte membrane lipids are not protected by propranolol against hypotonic lysis [28].

Moreover, it is possible that membrane protein conformational changes may play a significant role in Ca^{2+}-dependent selective rapid K^+-transport induced by propranolol in red cells [9] and in other effects of the drug on functioning of biological membranes.

The disordering of erythrocyte membrane lipids was observed at higher concentrations of propranolol than alterations in membrane protein conformation. It would suggest the predominant interaction of propranolol with protein components of the membrane. Drug–lipid interaction may be, however, also of considerable importance. Results obtained using model phospholipid membranes of different composition show that propranolol interacts preferentially with acidic phospholipids [29]. This class of phospholipids comprises, however, only a small fraction of total erythrocyte membrane lipids [30]. Using

the spin labeling technique we were able to observe only an overall effect of propranolol on erythrocyte membrane fluidity. Therefore, at low concentrations of the drug, perturbation in the physical state of relatively small fractions of lipids might have negligible effects on ESR spectra.

It is interesting to compare the disordering effect of propranolol on erythrocyte membranes with the recently reported ordering effect of the drug on phosphatidylserine and phosphatidic acid bilayers [29]. The opposite effect of propranolol on the fluidity of cholesterol-rich erythrocyte membranes and cholesterol-free phospholipid bilayers may be attributed to the proposed modulating role of cholesterol [31]. The present results appear to confirm the observation of Pang and Miller [31] that some anesthetic-like molecules may exert either a disordering or a condensing effect on biological and model membranes, depending on their cholesterol content.

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