INTERACTION OF β-ADRENERGIC DRUGS WITH MYOCARDIAL PLASMA MEMBRANES

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The initiating (trigger) stage of the adrenergic response is interaction between drugs of this group and adrenergic receptors (AR) [4]. Since AR are membrane functions, functional and structural changes in both receptor and nonreceptor parts of the membrane predetermine the development of the adrenergic process [1].

The aim of this investigation was to study conformational changes in specific binding sites on plasma membranes.

EXPERIMENTAL METHOD

Experiments were carried out on an enriched fraction of plasma membrane obtained from the guinea pig myocardium [10]. Heat effects (Q) of interaction of adrenergic ligands with plasma membranes were recorded directly by the method of microcalorimetric titration [2] on a DAK-1-1m differential isothermic microcalorimeter (of "Calvet" type) at 289±0.001°K.

To study structural conversions in the lipid fraction of the membranes under the influence of adrenomimetics and adrenoblockers, the method of EPR spectroscopy was used, and with involvement of spin-labeled and polar parts of the fatty acid $C_{17}H_{35}COO-C$⟨⟩$N{\cdot}O$.

The concentration of the spin label in the samples was $2 \cdot 10^{-5}$ M, and the concentrations of the adrenomimetics and adrenoblockers was 10^{-6}–10^{-7} M (Fig. 1).

The β-adrenomimetic isoproterenol and the β-adrenoblockers propranolol and alprenolol [6] were used.

EXPERIMENTAL RESULTS

Binding of the β-adrenoreceptor (BAR) agonist isoproterenol with the plasma membranes was characterized by a fall in both entropy ($\Delta S = -104.2 \pm 8.2$ J/mole·°K) and enthalpy ($\Delta H = -71.0 \pm 1.2$ kJ/mole). The negative change of the enthalpy of this process indicates binding of isoproterenol with specific receptor binding sites. This direction of the change in the entropy factor is evidence of transformation of the receptor macromolecule into a conformational state thermodynamically different from the previous conformation, and of stabilization of one of its conformation forms.

The trend of the thermodynamic parameters of interaction of BAR antagonists was in a different direction. Interaction of propranolol and alprenolol was characterized by lowering of enthalpy ($\Delta H = -35.3 \pm 0.7$ kJ/mole and $\Delta H = -29.0 \pm 2.6$ kJ/mole for propranolol and alprenolol respectively) and by an increase of entropy ($\Delta S = +85.3 \pm 4.5$ J mole·°K and $\Delta S = +77.7 \pm 5.9$ J mole·°K for propranolol and alprenolol respectively). The fall of enthalpy is evidence of binding of the β-adrenoblockers with specific membrane binding sites, but the considerable positive entropy factor indicates the difficulty of the conformational changes induced.

The reciprocal character of the changes in the thermodynamic parameters during binding of β-adrenomimetics and β-adrenoblockers can be explained by the different effects of these drugs on the nonreceptor components of the plasma membranes and, in particular, on lipids [1, 10]. It was shown by the spin labels

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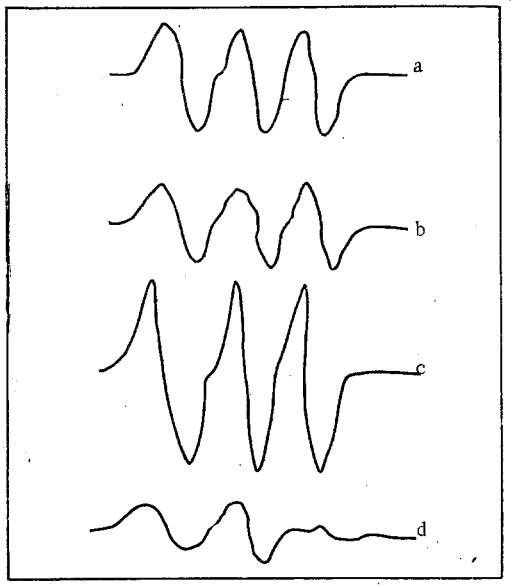

Fig. 1. EPR spectra of lipid spin label during interaction of adrenergic drugs with plasma membrane of guinea pig myocardium: a) control spectrum of label dissolved in alcohol, b) spectrum of label on insertion into membrane, c) spectrum of label during interaction between isoproterenol and membrane, d) spectrum of label during interaction of of propranolol with membrane.

method that during interaction of isoproterenol with plasma membranes the amplitude of the EFR spectrum increased, whereas during interaction of propranolol and alprenolol with the membranes it decreased.

Various suggestions have been put forward regarding the structure of BAR, starting from the general idea of their protein nature [7, 11, 12]. It has also been shown that AR function as components of a macromolecular complex with adenylate cyclase and with one or more guanidine nucleotide-sensitive (G) proteins [5, 9]. The character of interaction of components of the β-adrenergic complex depends essentially on the other membrane structures and, in particular, on lipids. Lipids, as basic components of cell membranes, are the medium in which receptor reactions take place, and on their state depend both processess of coupling of BAR with G-protein and adenylate cyclase [8] and the ultimate pharmacological effect. The important role of lipids in the mechanism of the β-adrenergic reaction is confirmed by the fact that on treatment of membranes with lipid-binding agents or with phospholipase A_2, β-adrenergic reactions either are not manifested at all or they are considerably depressed. The fact that phospholipases prevent binding of β-adrenergic agonists to a greater degree than proteolytic enzymes [3] also is evidence that at least some of the BAR are located in the lipophilic zone of the membrane.

On the basis of the results of these experiments and data in the literature the following hypothesis can thus be submitted. The initial stage of interaction of β-adrenomimetics and β-adrenoblockers with specific membrane binding sites is characterized by opposite trends of the thermodynamic parameters. The fall of entropy and enthalpy during interaction with isoproterenol reflect the initial reaction of interaction with BAR and subsequent agonist-specific isomerization of the receptor into a confirmational form which activates adenylate cyclase. Propranolol and alprenolol do not change the conformation of BAR and thus they participate only in the initial binding reaction, which is characterized by a fall of enthalpy and a rise of entropy of the process of interaction of β-adrenoblockers with specific membrane binding sites.

The opposite trend of the conformational changes of BAR induced by adrenomimetics and adrenoblockers can be explained by the different character of their effect on mobility of the membrane lipids, i.e., on the viscosity of the plasma membranes. During interaction of isoproterenol with the protein receptor molecule a decrease in viscosity of the membrane is observed. This facilitates an increase in mobility of the BAR, G-protein, and adenylate cyclase molecules and increases the probability of coupling between the components of the β-adrenergic complex. BAR antagonists propranolol and alprenolol interact not only with the protein receptor

molecule, but also with polar groups of membrane lipids, with a consequent increase in viscosity of the membrane and its stabilization. Coupling of BAR, G-protein, and adenylate cyclase does not take place under those conditions.

On the basis of the foregoing facts BAR can be regarded as a lipoprotein membrane complex, and the difference between the action of adrenomimetics and adrenoblockers can be explained by the different character of the influence of these substances on the lipid components of the β-adrenoreceptor complex.

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SOME CHARACTERISTICS OF SEROTONIN BINDING BY CELLS OF IMMUNOCOMPETENT TISSUES AND BY SYNAPTOSOMES

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The writers showed previously that cells of immunocompetent tissues have affinity for the biogenic amine, serotonin (5-HT), which has some of the basis features of receptor interaction: high specificity of binding (nonspecific binding for blood leukocytes and peritoneal cells was 5.8 and 4.5% respectively), reversibility, saturation, and the presence of temperature of pH optima. The localization of 5-HT-binding structures both on the membrane surface and inside the cell [4, 11] was determined with the aid of imipramine, which inhibits the passage of predominantly 5-HT through the plasma membrane [18, 19]. There is also information in the literature on the character of 5-HT binding by the coarse membrane fraction obtained from blood leukocytes [2]. As later investigations showed, activity of 5-HT binding is changed by immunization [5].

In the investigation described below relations between surface and intracellular adsorption of 5-HT by cells of immune and nonimmune animals were compared in order to determine which type of adsorption is functionally linked with immunogenesis, with the aim of using the information thus obtained to shed light on the effector mechanisms of serotoninergic immunomodulation and subsequently to use it for deliberate intervention in immunogenesis.

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