STUDY OF THE ROLE OF TRANSMEMBRANE IONIC CURRENTS IN THE MECHANISM OF ACTION OF β-ADRENOBLOCKERS

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Existing methods of seeking new β-adrenoblockers can be divided conventionally into two groups: physiological and biochemical. The methods of group 1 are based mainly on determination of the effectiveness of β-adrenoblockers in inhibiting the effects of β-adrenomimetics [10, 11]. By means of biochemical methods the β-adrenoblocking properties of substances are determined by measuring adsorption of the isotope-labeled substance on cell membranes [5] or changes in the concentration of an intracellular mediator [14].

In this investigation interaction of the β-agonist isoproterenol and of certain β-adrenoblockers with β-adrenoreceptors was studied by observing the effect on the transmembrane calcium current of myocardial cells: Meanwhile the effect of β-adrenoblockers on spontaneous calcium, sodium, and potassium currents was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on atrial trabeculae of the frog (Rana ridibunda) 0.1-0.15 mm in diameter. The current or voltage was clamped by a four-electrode circuit using a double sucrose gap [6]. The width of the test gap was 0.2 mm.

Stimulation of the preparation by an assigned program, recording of the action potential and ionic currents, and calculation of the parameters of the action potential, current–voltage characteristics, and other parameters of the ionic channels and display of the data were carried out under automated conditions by means of an SM-3 computer linked with the apparatus through a KAMAK module [1]. Ringer's solution of the following composition (in mM) was used: NaCl 110, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 4.8; pH 7.6-7.8. The sucrose solution was first deionized on ion-exchange columns. The sucrose concentration was 5.5 mM. Its specific resistance after deionization was 2-3 MΩ. The composition of the ion-exchange resin was: anion-exchange resin AV-17-8 and cation-exchange resin KU-22-8 in the ratio of 1.5 : 1 respectively. The experiments were done at room temperature (18-20°C).

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TABLE 1. Decrease in Spontaneous Calcium and Sodium Currents under the Influence of β-Adrenoblockers (in %)

Concentration of substance, M	$2 \cdot 10^{-6}$		10^{-5}		10^{-4}	
	I Ca	I Na	I Ca	I Na	I Ca	I Na
Propranolol	21±4	*	46±3	32±6	94±5	72±8
Oxprenolol	20±2	—	27±4	21±3	55±7	51±4
Pindolol	—	—	24±2	—	40±9	—
Atenolol	—	—	25±3	—	28±7	—
Labetalol	22±5	—	35±7	—	43±8	19±7

Legend. * No effect.

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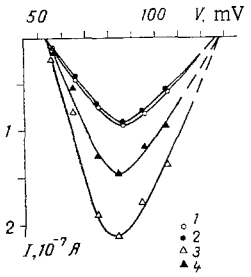

Fig. 1

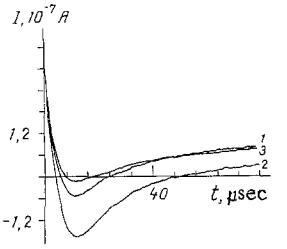

Fig. 2

Fig. 1. Changes in current–voltage characteristics of calcium current (I_{Ca}) under the influence of propranolol and isoproterenol. 1) Normal; 2) propranolol, $2 \cdot 10^{-7}$, 8 min; 3) isoproterenol, $4 \cdot 10^{-6}$ M), 6 min; 4) isoproterenol + propranolol, 8 min.

Fig. 2. Effect of propranolol on increase in calcium current (I_{Ca}) induced by isoproterenol. 1) Normal; 2) isoproterenol, $4 \cdot 10^{-6}$ M, 6 min; 3) isoproterenol, $4 \cdot 10^{-6}$ M + propranolol, $2 \cdot 10^{-7}$ M, 8 min.

Isoproterenol (from Germed, East Germany) was used as specific agonist of β-adrenoblockers. Of the β-adrenoblockers we chose unselective β-adrenoblockers propranolol (ICI, England), oxprenolol (Gedeon Richter, Hungary), pindodol (Sandoz, Switzerland); the cardioselective β-adrenoblocker atenolol (ICI, England), and the β-, α-adrenoblocker labetanol (Allen and Hanbury, England).

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that in small doses propranolol (10^{-7}–10^{-6} M), oxprenolol (10^{-7}–10^{-6} M), pindolol (10^{-8}–$2 \cdot 10^{-6}$ M), atenolol (10^{-6} to $5 \cdot 10^{-6}$ M), and labetanol (10^{-7}–10^{-6} M) did not affect the spontaneous slow inward calcium current or fast inward sodium and outward potassium currents. Isoproterenol ($4 \cdot 10^{-6}$ to $8 \cdot 10^{-6}$ M) caused a dose-dependent increase in the calcium current, which reached a maximum after 2-3 min and thereafter remained virtually unchanged for 15-18 min after addition of the drug. The β-adrenoblockers tested inhibited the increase in calcium current in response to addition of isoproterenol, and with respect to activity they were arranged in the following order: pindolol > oxprenolol ≥ propranolol = labetalol > atenolol (Figs. 1 and 2). A similar relationship for β-adrenoblocking activity in general was found when traditional methods were used also to assess activity of the preparations and, in particular, when their influence on the positive chronotropic effect of isoproterenol was studied in experiments on cats and dogs [7]. The exception was the position of atenolol after labetalol with respect to activity, possibly due to the fact that, unlike the mammalian heart, the frog's heart contains mainly β_2-adrenoreceptors [3], whereas atenolol is a selective β_1-adrenoblocker.

In high concentrations ($2 \cdot 10^{-6}$ to 10^{-4} M) the β-adrenoblockers began to exhibit an inhibitory action on the spontaneous slow inward calcium current, but some of them (propranolol, oxprenolol, and labetalol) also inhibited the fast inward sodium current (Table 1). Incidentally, β-adrenoblockers began to act on the calcium current in lower concentrations than on the sodium current. No correlation could be found between the action of β-adrenoblockers on the spontaneous calcium or sodium current and β-adrenoblocking activity.

One of the main side effects of β-adrenoblockers is provocation of heart failure as a result of depression of contractile activity of the heart [13]. This can be explained not only by blockage of the β-adrenoreceptors of the heart, but also by the nonspecific membrane-stabilizing (quinidine-like) action of β-adrenoblockers, expressed as a decrease in steepness of the rising phase of the action potential [4, 13]. It is now known that the rising phase of the action potential of myocardial cells is formed chiefly by sodium ions [12, 15]. Theoretical analysis of interaction between the various intracellular components does not rule out a possible connection between the reduction of the sodium current and the fall of contractile activity in accordance with the following scheme: reduction of sodium current – reduction of the intracellular sodium ion concentration – increased rate of outflow of sodium ions from the cells by the sodium–calcium exchange pump in exchange for the inflow of deficient sodium ions – decrease in the intracellular calcium ion concentration – decrease in contractility of the heart [2].

As regards the mechanisms of inhibition of the currents induced by β-adrenoblockers, in the case of the calcium current this may be connected with their action on calcium channels or on adenylate cyclase. Their

action on the sodium current can be explained (by analogy with the action of catecholamines and their derivatives) by their nonspecific binding with membrane lipids [8].

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IONIC DEPENDENCE OF GABA-POTENTIATING EFFECTS OF BENZODIAZEPINE TRANQUILIZERS AND HARMAN

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Postsynaptic responses of central neurons evoked by gamma-aminobutyric acid (GABA) are potentiated by benzodiazepine tranquilizers [1, 8, 12] and by harman, an endogenous ligand of benzodiazepine receptors [5]. The GABA-potentiating action of chlordiazepoxide and harman is similarly dependent on chloride concentration in the medium [1, 5], but the GABA-potentiating action of chlordiazepoxide is exhibited over the concentration range 10^{-7}-10^{-5} M. With a further increase in the harman concentration the GABA-potentiating effect weakens as the depolarizing action characteristic of harman, which is connected with blockade of the electrically excitable K^+ channels [5], develops.

Considering that the slow outward potassium and inward calcium electrically excitable currents in spinal ganglionic neurons of young rats have a similar time course [6] and the work pattern of receptor-linked ionic channels of chemically excitable membranes, besides other factors, depends essentially on the intracellular Ca^{++} ion concentration [3], it was decided to investigate whether a causal connection exists between the ability of harman to block electrically excitable K^+-channels and its GABA-potentiating activity. With this aim, the dependence of the GABA-potentiating effects of harman and chlordiazepoxide on the external Ca^{++} ion concentration and their changes under the influence of blockers of electrically excitable K^+- and Ca^{++}-ionic channels were compared.

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