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LOWERING THE CONCENTRATION OF HIGH-AFFINITY BINDING SITES FOR CALCIUM IONS IN RAT HEART SARCOLEMMAL MEMBRANES BY THE BETA-BLOCKER PROPRANOLOL

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KEY WORDS: propranolol; sarcolemma; myocardium; glucocorticoids; catecholamines.

Injection of large doses of natural catecholamines or of the synthetic analog isoproterenol into rats causes a marked increase in accumulation of calcium ions by the heart muscle [1, 5]. This is one of the main causes of the cardiotoxic action of catecholamines [5]. Most effects of catecholamines are known to be mediated through their interaction with β-receptors on the cytoplasmic membrane and activation of the adenylate cyclase system. The β-blocker propranolol, when administed *in vivo*, partially inhibits the increase in calcium ion accumulation induced by catecholamines. However, it not only inhibits the binding of catecholamines by β-receptors by a competitive mechanism, but also evidently has side effects, the mechanism of which has not yet been adequately studied [4, 7].

The aim of the present investigation was to study the effect of propranolol *in vivo* on the accumulation of ^{45}Ca by heart tissue after injection of isoproterenol into intact rats or animals receiving repeated doses of hydrocortisone. Binding of calcium ions by preparations of sarcolemma from the hearts of intact rats also was investigated *in vitro* after addition of glucocorticoids and isoproterenol.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were kept on the standard laboratory diet. The rate of assimilation of calcium by myocardial tissue was determined by the method in [1]. The protein concentration was determined as in [6]. To obtain preparations of the sarcolemma the rats were killed, the heart tissue homogenized, and the homogenates fractionated by the method in [2]. The fraction of isolated sarcolemmal membranes was used on the same day to estimate ^{45}Ca binding [3]. The suspension of membranes with a protein concentration of 20-40 μg/ml was incubated with $^{45}CaCl_2$ in a concentration of about 1 μCi/mmole in the presence of nonradioactive $CaCl_2$ within the concentration range from 30 to 3000 μM at room temperature for 5 min.

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TABLE 1. Accumulation of ^{45}Ca in Rat Myocardial Tissue after Various Procedures *in vivo* (M ± m)

Experimental conditions	Number of animals	$\frac{^{45}Ca/g\ tissue}{^{45}Ca/ml\ serum}$ %
Intact animals	20	13,71±5,76
Propranolol	10	14,28±1,39
Isoproterenol	21	45,41±3,75
Isoproterenol + propranolol	10	28,64±2,40
Hydrocortisone + isoproterenol	32	79,35±5,77
Hydrocortisone + isoproterenol + propranolol	10	28,71±1,86

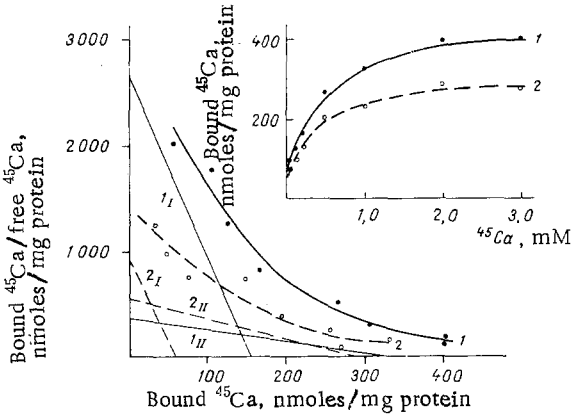

Fig. 1. Effect of propranolol *in vitro* on ^{45}Ca binding by sarcolemmal preparations from heart of intact rats. Bottom graph: Scatchard plot of data for binding with analysis of experimental curves as in [8]. Continuous line — incubation of membranes without propranolol, broken line — incubation with 10^{-6} M DL-propranolol hydrochloride. 1_{II}, 1_I, 2_I, 2_{II}) Rectilinear components for corresponding experimental curves. Calculated values of binding parameters without propranolol: K_I = 17,000 M^{-1}, N_I = 156 nmoles/mg protein, K_{II} = 1120 M^{-1}, N_{II} = 340 nmoles/mg protein; with propranolol: K_I = 21,000 M^{-1}, N_I = 1460 nmoles/mg protein, K_{II} = 1460 M^{-1}, N_{II} = 330 nmoles/mg protein. Top graph: binding of ^{45}Ca by membranes as a function of concentration of calcium added to incubation medium. Continuous line — without propranolol, broken line — with propranolol.

TABLE 2. Parameters of Binding of Calcium Ions by Sarcolemmal Preparations After Exposure to Various Agents *in vitro* (M ± m)

Experimental conditions	Number of animals	High-affinity binding		Low-affinity binding	
		N, nmoles/mg	K_{ass}, M^{-1}	N, nmoles/mg	K_{ass}, M^{-1}
Intact animals	12	120±8	15 000±1 300	390±27	1 510±29
Propranolol	6	54±6	24 500±5 900	300±26	1 200±190
Hydrocortisone	4	130±10	15 300±300	370±30	1 570±150
Propranolol + hydrocortisone	4	45±12	38 790±4 180	325±60	680±179
Propranolol + triamcinolone + acetonide	2	64	19 350	255	2 100

Legend. For each separate experiment the sarcolemmal fraction was isolated from a pool of 10-15 hearts. Propranolol concentration in medium 10^{-6} M, steroid concentration 10^{-8} M.

EXPERIMENTAL RESULTS

The level of radioactivity per gram myocardial tissue in intact animals killed 6 h after injection of ^{45}Ca was about 14% of its level per milliliter blood serum (Table 1). Isoproterenol sharply increased calcium accumulation in the heart muscle, but preliminary administration of hydrocortisone for 5 days potentiated the effect of isoproterenol considerably. These results agree qualitatively with data in the literature [5]. Similar results were obtained when adrenalin was used instead of isoproterenol [2]. Injection of DL-propranolol hydrochloride 30 min before ^{45}Ca caused no change in the basal level of accumulation of radioactivity in the heart, but depressed the stimulation of the process by isoproterenol considerably, although not completely. The potentiating action of hydrocortisone on the effect of isoproterenol was abolished in this case (Table 1). The results of binding of calcium ions by preparations of sarcolemma isolated from the hearts of intact rats are shown as Scatchard plots in Fig. 1 (bottom graph). The shape of the curve suggests the presence of at least two types of calcium binding sites. These sites can be characterized by graphic transformation of the experimental curve as in [8]. The existence of two types of binding sites for calcium ions on the sarcolemma was demonstrated previously [3].

It will be clear from Fig. 1 that the addition of DL-propranolol hydrochloride to the incubation medium in a concentration of 10^{-6} M led to marked changes in the saturation curve (the top graph) and the Scatchard plot (bottom curve). The number of high-affinity binding sites (N_I) was reduced by more than half, and this was accompanied by some increase in the apparent association constant (K_I). The binding parameters for low-affinity sites (N_{II} and K_{II}) showed little change. The mean values of parameters for binding of calcium ions by sarcolemmal preparations following addition of propranolol to the incubation medium, in the absence or presence of glucocorticoids, are given in Table 2. It follows from Table 2 that propranolol *in vitro* lowers the concentration of high-affinity sites for calcium binding by sarcolemmal membranes independently of glucocorticoids. The addition of hydrocortisone *in vitro* did not affect the calcium binding parameters.

The writers showed previously that a concentration of low-affinity binding sites on the sarcolemma isolated from hearts of adrenalectomized rats is much lower than in preparations from hearts of intact animals, and that injection of glucocorticoids into adrenalectomized rats restores these parameters to their normal level. In conjunction with the results of the present investigation this is evidence that integrity of the cell structure is essential for manifestation of the effect of glucocorticoids. Unlike the action of glucocorticoids, the effect of propranolol on calcium binding by membranes is clearly exhibited in a cell-free system. Evidently propranolol can thus influence the Ca-binding capacity of sarcolemmal membranes directly. In this connection its inhibitory action on catecholamine-stimulated calcium accumulation by the myocardium can be explained, at least partly, not by blocking of β-adrenoreceptors and changes in the intracellular cyclic AMP level connected with it, but by direct regulation of binding of calcium ions by the sarcolemma. This conclusion is confirmed by information on the ability of propranolol to inhibit platelet agreggation induced by ionophore A-23187 and to displace calcium from the surface of platelet membranes [9]. It was also shown previously [4] that propranolol can competitively inhibit calcium binding by

sarcolemmal membranes of the rat heart. However, the authors cited used a method of equilibrium dialysis to determine binding, and they found only one type of Ca-binding site.

The intracellular calcium pool, which participates directly in contraction, is regulated by binding of calcium ions by low-affinity sites on the surface of the sarcolemma [3]. Lowering the concentration of high-affinity calcium binding sites by propranolol may perhaps have some influence on effects of catecholamines, besides the classical blocking of β-receptors by this agent.

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EFFECT OF ETMOZINE ON PLATELET AGGREGATION

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KEY WORDS: platelet aggregation; etmozine; indomethacin.

Etmozine, a new Soviet antiarrhythmic drug of the phenothiazine series, has been used with success in the treatment of various forms of disturbance of the cardiac rhythm, especially in cases when the arrhythmia results from myocardial ischemia [4, 7, 12]. An increase in the aggregating activity of platelets is known to be one factor predisposing to the development of myocardial infarction and aggravating its course. Experiments *in vitro* have shown that phenothiazine derivatives (chlorpromazine, promethazine, nonachlazine) can interfere with the process of granulation of platelets and also exhibit a marked deaggregating action, i.e., cause aggregates already formed through induction by ADP to break up [1, 2, 5, 10]. It has also been shown that prostaglandins play an important role in aggregation processes [3, 11].

In this connection it was decided to study the ability of etmozine to interfere with processes leading to the development of platelet aggregation and also to discover whether its effects depend on possible interaction with the prostaglandin system.

EXPERIMENTAL METHOD

The aggregating properties of etmozine were studied *in vito* and *in vivo* by biological testing. The experiments *in vivo* were carreid out on platelet-enriched rabbit blood plasma. Platelet aggregation was determined by the method in [8], by recording changes in optical density graphically before and after addition of aggregation inducers to the incubation medium: ADP (10 μM) and arachidonic acid (100 μM). Etmozine was added to the plasma in a

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