EFFECT OF α_1-ADRENERGIC STIMULATION AND LITHIUM ON CALCIUM SENSITIVITY OF HYPERPERMEABLE RAT MYOCARDIAL FIBERS

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The action of α_1-adrenomimetics on membrane receptors of the myocardium stimulates hydrolysis of plasmalemmal phosphatidylinositol-4,5-diphosphate and the formation of 1,2-diacylglycerol (DG) and inositol-1,4,5-triphosphate (ITP) [1]. DG activates protein kinase C (PKC) [10], which, by phosphorylating contractile proteins [8], may perhaps modulate calcium sensitivity of the myofibrils (CSM) of the heart. Data on the effect of ITP on CSM are contradictory [3, 15]. The action of an increase in the inositol phosphate concentration during inhibition of inositol monophosphatase, on CSM likewise is not clear.

The aim of this investigation was to study the effect of the $\alpha_1$adrenomimetic phenylephrine (PHE) on calcium sensitivity of hyperpermeable fibers of the rat heart, treated with saponin. During skinning with saponin the integrity of the sarcolemma is disturbed, but fragments of it are preserved [5], so that it is possible to study processes linked with transmission of the signal from the receptors. We also tested the action of PHE on cardiac fibers in the presence of 10 mM LiCl, a classical inhibitor of inositol monophosphatase [1, 9].

EXPERIMENTAL METHOD

Male Wistar rats were anesthetized (1.6 g/kg urethane) and the heart was quickly removed and placed in Krebs' solution at 0-4°C. Bundles of fibers 0.2-0.3 mm in diameter were isolated from the subendocardium of the left ventricle and incubated in relaxing solution (see below), containing 50 μg/ml saponin or 1% Triton X-100, for 1 h. The ends of the skinned fibers were held with clips; one of the clips was connected to a force transducer (FT 03, "Grass") and amplifier (model 13421202, "Gould"), and the signal was recorded on an automatic writer ("Dinear"). The held fiber was stretched until force appeared, and thereafter for a further 20% of its length. The fibers were activated for 2 min by the maximal concentration of Ca^{2+} (pCa 4.5), then relaxed in calcium-free solution, and the curve of isometric tension as a function of pCa was plotted using solutions with different Ca^{2+} concentrations (pCa from 9.0 to 4.5). The basic solutions (activating and relaxing) contained 3 mM free Mg^{2+}, 5 mM MgATP, 15 mM phosphocreatine, 20 mM imidazole, 10 mM EGTA, and 0.5 mM dithiothreitol; pH 7.0. The ionic strength was adjusted to 0.16 M by the addition of potassium propionate. The activating solution contained calcium in the form of Ca-EGTA, to obtain pCa = 4.5. Media with intermediate values of pCa were obtained by mixing the basic solutions in the necessary proportions. The total EGTA concentration was always 10 mM. Addition of the solutions was calculated by means of a set of equations [6]. Tests were carried out at room temperature (22-23°C). All experiments with saponin-skinned fibers were conducted in the presence of propranolol to block β-receptors. During analysis of the curves, pCa_{50} was calculated: the concentration of Ca^{2+} at which isometric contraction of half the maximal value was developed. To test the presence of parts of the sarcolemma in the saponin-skinned fibers, activity of ouabain-sensitive ATPase was determined after homogenization in the cold in medium containing 50 mM Tris-HCl, 5 mM inorganic phosphate, and 5 mM $MgCl_2$, by a combined enzymic method [13] in the presence of 10 mM Na azide and traces of Ca^{2+}.

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TABLE 1. Effect of PHE (10^{-6} M) and Lithium (10 mM) on Calcium Sensitivity of Hyperpermeable Rat Myocardial Fibers (M ± m, n = 8-14)

	Control	Preactivation in absence of PHE	Preactivation in presence of PHE	LiCl	PHE + LiCl
pCa_{50}	5,43±0,03	5,37±0,02	5,32±0,02*	5,27±0,03**	5,24±0,02**
In presence of parazocin (10^{-6} M):	5,41±0,03	—	5,39±0,02	5,38±0,03	5,36±0,02

Legend. All measurements made in presence of 10^{-6} M propranolol. *$p < 0.01$, **$p < 0.001$ compared with control.

EXPERIMENTAL RESULTS

Fibers treated with saponin showed high activity of ouabain-sensitive ATPase: in unskinned fibers ATPase activity was 0.07 ± 0.01 μmole/min/mg protein, or 44% of total ATPase activity, whereas in saponin-treated fibers it was 0.16 ± 0.02 μmole/min/mg protein (37%). This indicates that a large part of the sarcolemma was preserved after skinning under the above conditions.

Data on the calcium sensitivity of the hyperpermeable fibers following exposure to various conditions are given in Table 1.

PHE (10^{-6} M) lowered CSM, and the effect was much greater if PHE were present in the medium during preliminary activation by maximal calcium concentration. Consequently, the action of PHE on CSM depends on the Ca^{2+} ion concentration. This conclusion is confirmed by the more gradual slope of the pCa – force curve obtained in experiments in which PHE was not present during preliminary incubation in solution with pCa = 4.5. The measure of steepness was Hille's coefficient (H), calculated by the equation:

$$T \text{ (relative tension)} = [Ca]^H/(K + [Ca]^H),$$

where [Ca] is the Ca^{2+} concentration and K the binding constant with Ca^{2+}, which in these experiments was significantly lower (3.05 ± 0.21) than in experiments in which PHE was present during preliminary calcium activation (3.70 ± 0.20; $p < 0.05$). In the first case, successive reduction of CSM evidently developed during measurement of force as the Ca^{2+} concentration was increased. The results indicate involvement of calcium-dependent processes in the mechanism of the change in CSM. Both phospholipase C, catalyzing α_1-stimulated hydrolysis of phosphatidylinositol diphosphate [2], and also PKC are known to be activated by calcium [4, 14]. It can therefore be postulated that these enzymes are involved in the lowering of CSM under the influence of PHE.

LiCl (10 mM) considerably reduced CSM, but addition of 10^{-6} M PHE led to some increase in this effect. The action of PHE in medium containing Li^+ ions was weaker than in medium without Li^+. This may be due to accumulation of inositol phosphates under the influence of Li^+ [9].

Prazocin (10^{-6} M), an α_1-receptor blocker, abolished the effect of PHE in both the absence and the presence of Li^+. Consequently, the observed action of PHE is mediated through stimulation of α_1-receptors.

To test whether lithium acts directly on myofibrils, we studied the action of 10 mM LiCl on fibers in which all membrane structures had been removed by skinning with Triton X-100. For comparison, KCl in an equimolar concentration was used. LiCl shifted the pCa – relative tension curve by 0.08 ± 0.01 pCa unit ($p < 0.01$) toward higher Ca^{2+} concentrations, whereas KCl did not affect CSM. Thus Li+ evidently has a direct specific action on myofibrils, lowering their sensitivity to Ca^{2+}.

The experimental results showed that the CSM of the heart is reduced during stimulation of α_1-adrenoreceptors. However, the action of PHE on cardiomyocytes with a damaged sarcolemma in [12] led to an increase in CSM. During incubation with PHE the cells were in a relaxed state, and the Ca^{2+} ion concentration in the cytoplasm was probably insufficient to activate the mechanism of the negative action of PHE on CSM. In addition, in experiments on skinned fibers, as a result of an increase in permeability of the sarcolemma, certain substances soluble in the cytoplasm and involved

in the mechanism of increased calcium sensitivity may have been lost. Processes leading both to an increase and to a decrease in CSM evidently take place in the living cell under the influence of PHE. These data can be explained by the opposite effects of α_1-adrenomimetics on inotropic function of the myocardium discovered previously [7, 11].

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