Effect of Melittin on the Contractility of Rat Papillary Muscle

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Melittin, a phospholipase A_2 activator, induces two-phase changes in the contractility of isolated rat papillary muscle. The first phase is characterized by an increase in the force of muscle contraction during the first 5-15 min of perfusion, the increase being the greatest at 0.4 μg/ml melittin. This phase is abolished by 10 μM indomethacin. In the second phase, dose-dependent inhibition of muscle contraction reaches the maximum after 30-45 min of perfusion and is abolished by the lipoxygenase inhibitor nordihydroguaiaretic acid (10 μM).

Key Words: *papillary muscle; melittin; phospholipase A_2; lipoxygenases; cyclooxygenases*

One of the general mechanisms by which pathological processes of various origin arise (allergic, hypoxic, ischemic, and others) is degradation of the plasma membrane phospholipids as a result of phospholipase activation. Phospholipase A_2 (PLA_2), an enzyme found in most animal cells, catalyzes deacylation of phosphoglycerols in the second position and the formation of free fatty acids and lysophospholipids. This is the position in which unsaturated fatty acids, including arachidonic (AA) and linoleic acids, occur. These fatty acids are accumulated in ischemic myocardium [1]. Lysophospholipids, which are also products of phospholipid-mediated reactions, exhibit detergent activity and may impair electrophysiological properties of heart tissues [2]. The effect of phospholipids on myocardial contractility has been studied indirectly by evaluating the effects of exogenous lysolipids and fatty acids on the myocardium [11] or during myocardial ischemia-reperfusion, when many processes leading to membrane damage are activated [9,14].

In this study we examined the effect of melittin, an activator of intracellular PLA_2 [10], on the contractility of rat papillary muscle (PM).

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MATERIALS AND METHODS

Mechanical activity of PM isolated from the right cardiac ventricle of Wistar rats was measured. Papillary muscle segments 1-1.5 mm in diameter and 3-5 mm in length were placed in a thermostated chamber (28°C) and perfused with solution containing 125 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.05 mM $MgCl_2$, 24 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, and 5 mM glucose (pH 7.2). The perfusate was bubbled with a 95% O_2/5% CO_2 mixture. The PM preparation was then stimulated with suprathreshold current pulses at a frequency of 0.3 sec^{-1}. One end of the muscle was attached to a force sensor and the other to the lever of precision ergometer. After a 40-min stabilization period, during which isometric contractions of the muscle became steady, PM was stretched to the maximum physiological length, L_{max} (the length at which the force of isometric contraction is maximal), and of the mechanical activity parameters were recorded in the isometric mode. Melittin, indomethacin, and nordihydroguaiaretic acid (NDGA) were from Sigma.

RESULTS

Preliminary tests showed that a 3-h perfusion of rat PM after a 40-min stabilization period induced no

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significant changes in the force of isometric contractions in response to electric stimulation. The intracellular phospholipase activator melittin elicited a two-phase effect on isometric contractions of rat PM. During the first phase, after a latent period the force of isometric contractions reached the maximum on the 10th-15th min of perfusion. During the second phase the contraction force decreased, reaching the maximum on the 40th-45th min of perfusion (Fig. 1, *a*). Both the increase and decrease in contraction force were dose-dependent, although in different ways. Thus, the intensity of stimulation increased as the melittin concentration in the perfusate was raised from 0.1 to 0.4 μg/ml and progressively decreased at higher melittin concentrations so that the first phase virtually disappeared at >1 μg/ml melittin. By contrast, during the second phase the reduction in the force of isometric contractions was always directly proportional to melittin concentration in the perfusate (Table 1).

Activation of PLA_2 during myocardial ischemia-reperfusion [9] results in formation of lysophospholipids and unsaturated fatty acids, including AA, in cardiac tissues. The formation of free AA is a rate-limiting reaction in the reaction cascade whereby eicosanoids are produced [5]. It has been shown that cardiomyocytes are capable of synthesizing both cyclooxygenase products (prostanoids) and lipoxygenase products (hydroxy acids, hydroperoxy acids, leukotrienes, and lipoxines) and that the synthesis of eicosanoids is increased during hypoxia and reoxygenation [6,8]. With this in mind we tested the cyclooxygenase inhibitor indomethacin (10 μM) and the lipoxygenase inhibitor NDGA (10 μM) for their ability to modulate the effect of melittin on PM contractility. Perfusion of rat PM with indomethacin for 5 min before the addition of melittin abolished its effect in the first phase, i.e., the enhancement of PM contractility, shortened the lag period after melittin inhibited PM contractility (from the 10th min to the 15th min of perfusion), and slightly enhanced the inhibitory effect of melittin on PM contractility on the 40th-45th min (Fig. 1, *a*, *b*). It was reported that prostaglandin E_1 increases the contractility of dog PM, this presumably adrenergic effect being weaker when β-receptors were blocked [4]. A similar inotropic effect of prostaglandin E_1 was observed in a culture of rat cardiomyocytes [13]. Published data and our findings suggest that during short perfusion AA produced in cardiomyocytes is metabolized by the cyclooxygenase pathway to form prostaglandins, including prostaglandin E_1. On the other hand, one of the main prostaglandins formed in the heart, prostacyclin, exerts no pronounced inotropic effect on isolated rat cardiomyocytes or cat PM [3]. These findings

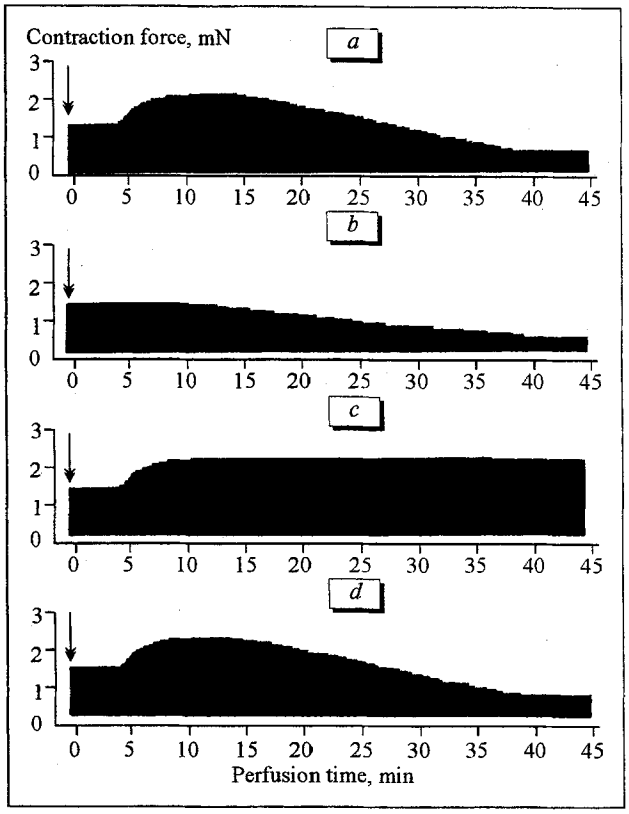

Fig. 1. The effects of melittin (0.4 μg/ml) on the contraction force of rat papillary muscle: *a*) perfusion with melittin alone; *b*, *c*, *d*) perfusion with melittin after perfusion with 10 μM indomethacin, 10 μM NDGA, and 10 mM tocopherol, respectively. The arrows indicate the addition of melittin.

completely rule out the possibility of prostaglandin-independent mechanism enhancing the contractility of rat PM during a short-term perfusion with melittin.

When the addition of melittin was preceded by perfusion of PM with the lipoxygenase inhibitor NDGA, the second phase of melittin action, i.e., inhibition of muscle contractility, disappeared (Fig. 1, *c*): PM contractility was stimulated throughout the perfusion period (up to 60 min). Since lipoxygenase inhibitors, including NDGA, exhibit antioxidant activity, we estimated the influence of preliminary perfusion with the antioxidant tocopherol (10 mM) on the effect of melittin. Tocopherol altered neither the magnitude nor the phases of this effect when PM was perfused with melittin (Fig. 1, *d*).

The abrogation of the inhibitory effect of melittin by NDGA suggests that this effect was due to formation of unidentified lipoxygenase metabolites. Previously, we showed that incubation of isolated cardiomyocytes with a mixture of lipoxygenases and AA (or linoleic acid) markedly increases the intracellular calcium content with subsequent irreversible damage to these cells, this effect being blocked by the lipoxygenase inhibitor NDGA [7]. From this

TABLE 1. Contraction Force of Rat Papillary Muscle (F_{max}, %) at Different Melittin Concentrations in the Perfusate (n=3-4)

Melittin concentration, μg/ml	Phase 1, perfusion for 10-15 min	Phase 2, perfusion for 35-40 min
0.1	107±4	95±7
0.3	119±5*	63±5*
0.4	140±7**	37±4*
0.5	113±4*	23±4*
1.0	104±5	15±3*

Note. F_{max} before the addition of melittin to perfusate is taken as 100% (baseline). $p<0.05$: *relative to baseline; **relative to the F_{max} at melittin concentrations of 0.3 and 0.5 μg/ml.

finding we concluded that prolonged activation of intracellular PLA_2 and the resultant rise of free AA activate intracellular lipoxygenases, inducing accumulation of the fatty acid metabolites. Many of these metabolites are cardiotoxic [12] and are formed by the lipoxygenase pathway. Blockade of cyclooxygenases by indomethacin potentiated the inhibitory effect of melittin (Fig. 1), probably because relatively large amounts of the released unsaturated fatty acids were metabolized by the lipoxygenase pathway.

Thus, cyclooxygenase inhibition by indomethacin aggravates the pathological process, whereas inhibition of lipoxygenase activity or leukotriene synthesis markedly reduces coronary constriction and calcium-induced contracture of cardiac myofibrils.

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