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MODULATION OF LIPOLYTIC ACTIVITY IN ISOLATED CANINE CARDIAC SARCOLEMMA BY ISOPROTERENOL AND PROPRANOLOL

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Summary: Cardiac sarcolemmal preparations isolated from dog were tested for membrane-associated phospholipase A and lipoprotein lipase activities. The sarcolemma hydrolyzed 1-acyl 2-^{14}C-linoleoyl 3-glycerophosphorylethanolamine at pH 7.0 to form predominantly ^{14}C-lyso PE with 5 mM EDTA and ^{14}C-free fatty acid with 5 mM Ca^{2+} suggesting the presence of both phospholipases A_1 and A_2 and/or lysophospholipase activities in these preparations. Sarcolemmal PLA activity was stimulated 300% by 10^{-5} to 10^{-6} M dl-isoproterenol; this stimulation was blocked by 10^{-4} M dl-propranolol. Lipoprotein lipase activity associated with the sarcolemmal fraction was enhanced 10-fold by 10^{-5} M dl-isoproterenol; stimulation was blocked by dl-propranolol. Thus, the activities of membrane-bound lipolytic enzymes appear to be modulated by β-adrenergic agents in canine cardiac sarcolemma and could affect lipid dependent enzymes and/or membrane permeability.

INTRODUCTION

Pharmacologic doses of isoproterenol elevate serum levels of free fatty acids (1) while large or repeated doses with experimental animals lead to damage and necrosis of the myocardium that are indistinguishable from myocardial infarction (2-4). The increased lipolysis in adipose tissue from isoproterenol administration is thought to be mediated by β-adrenergic receptors and cAMP, but little is known concerning the direct effects of β-adrenergic agonists on sarcolemmal lipolysis and membrane function in heart. We have recently described a procedure for the isolation of hamster and canine cardiac sarcolemma (5) and have characterized phospholipid hydrolyzing enzymes in these systems (6). In this report we describe the modulation of sarcolemmal lipolytic activities by isoproterenol and propranolol.

MATERIALS AND METHODS

Sarcolemmal (SL) membranes, enriched 5 to 8 fold in $(Na^+ + K^+)$-Mg^{2+} ATPase activity, were isolated from canine myocardium as previously described (5). Protein was determined by the method of Lowry (7) using BSA as a standard. Phospholipase A activity (PLA) was measured using 1-acyl 2-^{14}C-linoleoyl 3-glycerophosphorylethanolamine (PE) substrate (8). Reaction mixtures in a total volume of 1.0 ml contained 25-100 µg SL protein, EDTA or $CaCl_2$ as indicated, freshly prepared dl-isoproterenol

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100 µmoles Tris-Maleate pH 7.0 and 50 nmoles of 1-acyl 2-^{14}C-linoleoyl PE (9,000 cpm added as an aqueous ultrasonic suspension). Reaction mixtures were incubated for 2 - 4 hours at 37°C and activity was linear with time and protein under the conditions used. Reactions were stopped and the products isolated as previously described (8). PLA activity is expressed as nmoles of product (LPE + FFA) formed/hr or as per cent of control. Lipoprotein lipase (LPL) activity was measured using Intralipid as a stabilizing agent (9). The substrate consisted of 2 vol. of 20% w/v BSA, 1 vol of 0.7 M Tris-HCl buffer, 0.5 vol rat serum, 1 vol of water, 0.5 vol Intralipid and sufficient 1-^{14}C-triolein dispersed by sonication to yield 50,000 cpm per incubation. The Intralipid was preincubated with serum for 30 min at 37°C prior to addition of the above components and then sonicated. Reaction mixtures in total volume of 1.0 ml contained 0.5 ml substrate, 25-100 µg sarcolemmal protein, 100 nmoles Tris-HCl, pH 8.1, 0.1% Triton X-100 and the indicated concentrations of freshly prepared dl-isoproterenol and dl-propranolol. Reaction mixtures were incubated for 3 hrs, the reaction was stopped and lipids were extracted and separated as previously described (9). The only radioactive product formed was 1-^{14}C-oleate. Lipoprotein lipase activity is calculated as total cpm FFA divided by the total cpm lipid and is expressed as per cent of control. All values are corrected for non-enzymatic hydrolysis.

RESULTS

The hydrolysis of PE by sarcolemmal PLAs as a function of Ca^{2+} and EDTA concentration is shown in Figure 1. In the absence of added Ca^{2+} or EDTA moderate hydrolysis of PE occurs and equal quantities of FFA and LPE, are formed. In the presence of EDTA the predominant product is ^{14}C-LPE indicating the action of a PLA specific for the 1-position. By contrast, in the presence of 5 mM calcium, ^{14}C-FFA is the major product and could be formed by the action of PLA_2 or the concerted action of PLA_1 and lyso-PLA. Hydrolysis of PE with increasing protein

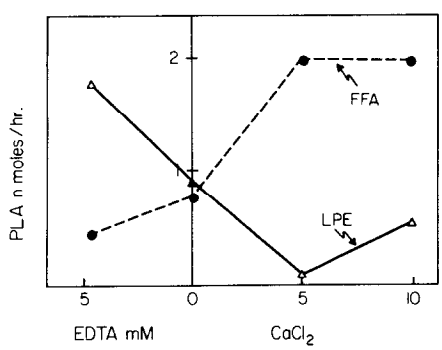
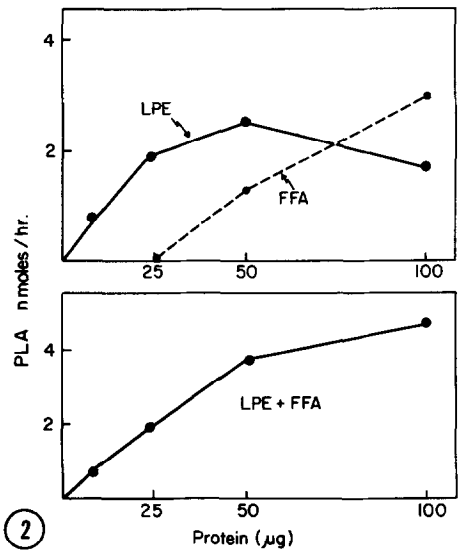


Figure 1 Hydrolysis of PE as a function of Ca^{2+} or EDTA. Standard reaction mixtures, as described in Methods, contained 100 µg sarcolemmal (SL) protein, EDTA or $CaCl_2$ as indicated, and were incubated for 2 hrs at 37°C. PLA activity is expressed as nmoles product formed/hr; LPE (——) refers to lysoglycerophosphorylethanolamine and FFA (---), ^{14}C-free fatty acid. All values are corrected for non-enzymatic hydrolysis which was less than 3% in all experiments.



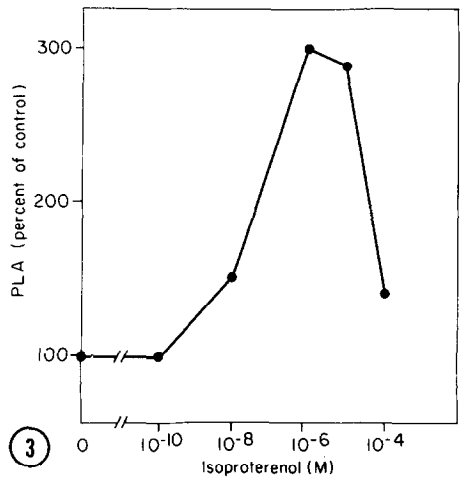


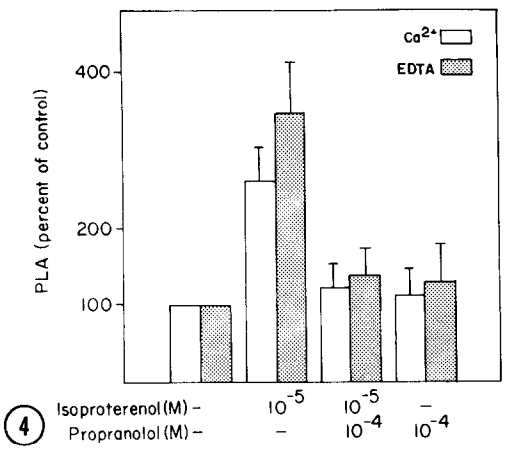
Figure 2 Hydrolysis of PE as a function of protein concentration. Standard reaction mixtures contained 25-100 μg SL protein, and were incubated for 2 hrs at 37°C. PLA activity is expressed as nmoles of FFA or LPE formed/hr (Figure 2A) and as nmoles total product (FFA + LPE) formed/hr (Figure 2B).

Figure 3 Effect of dl-isoproterenol on hydrolysis of PE. Standard reaction mixtures contained 50 μg of SL protein, 5 mM $CaCl_2$, the indicated concentrations of dl-isoproterenol and were incubated for 4 hrs at 37°C. PLA activity is expressed as percent of control (control = 1.0 nmole product formed/hr= 100%).

(Figure 2) demonstrates that lysophospholipid accumulates initially and then decreases concomitantly with the appearance of ^{14}C-FFA suggesting sequential degradation: PE → LPE → FFA. Thus, when hydrolysis is expressed as total product formed (LPE + FFA) per μg protein (Figure 2) a nearly linear relationship is noted.

Since previous reports suggested that β-adrenergic agonists increased endogenous phospholipid turnover in perfused cardiac tissue (10), we examined the effect of dl-isoproterenol on the endogenous PLAs of SL. Figure 3 illustrates that hydrolysis of PE by the SL fraction was stimulated 300% by 10^{-5} to 10^{-6}M dl-isoproterenol in the presence of Ca^{2+}. Interestingly, concentrations of isoproterenol greater than 10^{-4} M and less than 10^{-8} M had little effect on enzymatic activity. A similar dose-response with isoproterenol was noted in the presence of EDTA (not shown).

As shown in Figure 4 the stimulation of PLA by dl-isoproterenol (10^{-5}M) with EDTA or Ca^{2+} was totally blocked by the addition of 10^{-4} M



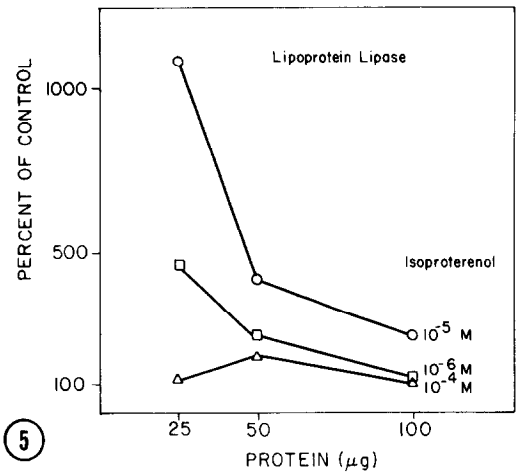


Figure 4 Hydrolysis of PE as a function of dl-isoproterenol and dl-propranolol. Standard reaction mixtures contained 50 μg SL protein, 5mM Ca^{2+} or EDTA, the indicated concentration of dl-isoproterenol and/or dl propranolol, and were incubated for 4 hrs at 37°C. PLA activity is expressed as percent of control ± % S.D. (control = 1.4 ± 0.4.nmoles product formed/hr= 100%).

Figure 5 Effect of isoproterenol on sarcolemmal LPL activity. Standard reaction mixtures, as described in Methods, containing the indicated protein and dl-isoproternol concentrations were incubated for 3 hrs at 37°C. LPL activity is calculated as cpm ^{14}C FFA/cpm total lipid and expressed as percent of control (control = 300 nmoles of oleate formed/hr/mg = 100%).

dl-propranolol which itself had no effect on the hydrolysis of PE. In homogenates of PMN leukocytes dl-isoproterenol (10^{-5}M) enhanced PLAs activity by 270%; in homogenates of alveolar macrophages the stimulation was 400%; both activities were blocked by dl-propranolol (10^{-4}M) (not shown). Since lipoprotein-lipase (LPL) is associated with cardiac membrane fractions and is stimulated by adrenergic agents in other organ systems (11) we tested the effect of isoproterenol on sarcolemmal hydrolysis of a ^{14}C-triolein-Intralipid substate. Figure 5 shows that serum dependent LPL activity was stimulated maximally by 10^{-5} M isoproterenol and was unaffected by 10^{-4}M isoproterenol. Interestingly, the degree of stimulation was inversely related to content of protein. The product formed from glycerol 1-^{14}C-trioleate was almost entirely ^{14}C-FFA with little or no mono-and di-glyceride accumulation. Again this stimulation was blocked by 10^{-5} M dl-propranolol; regardless of content of protein no stimulation was evident when the concentration of isoproterenol was less than 10^{-7}M (not shown).

DISCUSSION

Many studies have demonstrated that administration of large or repeated doses of isoproterenol to rats causes cardiac pathology which resembles ischemic necrosis (2,3). Only recently have reports focused upon early events in this injury process. Boutet et al. (12) have demonstrated that intracellular diffusion of horseradish peroxidase was due to permeability alterations of the sarcolemma in rat hearts perfused with norepinephrine or isoproterenol. Marshall et al. (13) concluded that rapid ion permeability alterations resulting in hyperpolarization occur at the sarcolemma of the myometrium in response to isoproterenol (4×10^{-7} M). These studies suggest that alterations of sarcolemmal permeability may be an early event in catecholamine-induced cardiac cell injury. We believe that catecholamine-stimulated lipolysis could be an important mechanism by which the cardiac cell can modify its sarcolemmal lipid composition resulting in deleterious alterations of membrane permeability and/or membrane enzymatic systems. Our results demonstrate that PLA and LPL of canine sarcolemmal membranes and particulate PLAs of PMN leukocetes and macrophages are stimulated by the β-adrenergic agonist, isoproterenol; the stimulation is blocked by the β-antagonist, propranolol. Why stimulation of these sarcolemmal lipolytic activities was not evident at higher concentrations of isoproterenol (10^{-4}M) is unclear, although Furchgott (14) has observed similar effects with high doses of catecholamines and proposed that the inhibition was due to receptor desensitization and/or autoinhibition. These data with isolated cardiac membranes confirm observations previously made in perfused organs and whole animal studies that adrenergic as well as cholinergic agonists can stimulate membrane phospholipid turnover, particularly inositol phospholipids, in pineal, parotid and submaxillary glands (15-17), as well as cerebral cortex (18) and smooth muscle cells (19,20); however the effect of these agonists on phospholipid metabolism varies considerably with tissue and species. In the above mentioned studies, it should be noted that endogenous phospholipid turnover was measured in whole cells or perfused organs, whereas our observations with isolated membranes infer an increased expression of enzymic activity toward exogenously added lipid. Unlike the well-studied control of lipolysis in adipose tissue, the mechanism by which phospholipid and triglyceride metabolism in heart is modulated by adrenergic agents is poorly understood. As suggested by Christian et al. (21) and Lech (22), the similarity between the effects of lipolytic stimulants and inhibitors in tissues may be similar. Thus, cAMP may act as an intracellular mes-

senger to modulate lipolysis in heart and membrane-associated lipolytic activities in heart may be modulated by a cascade mechanism involving β-receptors, cAMP, and phosphorylation.

We have previously demonstrated the association of phospholipase A_2 activities with cardiac sarcolemma in hamster and dog (6). The generation of ^{14}C-FFA from 1-acyl 2- ^{14}C-linoleoyl PE with increasing time or protein (Figure 2) indicates the combined action of phospholipase A_1 and lysophospholipase activities, enzymes previously described in canine sarcolemmal preparations (23). This report also demonstrates that this membrane fraction contains lipoprotein lipase activity. Thus, cardiac SL may contain an isoproterenol-responsive lipolytic enzyme system with broad substrate specificity. This enzyme system may contribute to the normal metabolism of lipids in cell membranes and circulating lipoproteins (24). Whether these lipolytic enzymes mediate catecholamine-induced cell injury or participate in ischemic injury of the sarcolemma are important questions for future studies.

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