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Research Article

We have recently demonstrated enhanced alpha-adrenergic responsiveness assessed electrophysiologically in ischemic and reperfused myocardium. This study was performed to determine whether ischemia alters alpha 1-adrenergic receptor number (Bmax) of affinity (KD) based on [3H]prazosin binding. Within 30 min after occlusion, Bmax increased in ischemic regions to 207% of control to 27 +/- 2 fmol/mg protein, with the increase persisting (+ 141% of control) during early reperfusion (2 min), before returning to control base-line values (13 +/- 1.6) after 15 min of reperfusion. KD was not altered at any interval studied. Beta receptor number of ([3H]dihydroalprenolol) and Na+-K+ ATPase activity were comparable in control compared to ischemic myocardium although beta-receptor Bmax and KD in both regions decreased during early reperfusion. Thus, the enhanced alpha-adrenergic responsivity previously recognized with ischemia and reperfusion is correlated with an increase in alpha 1-adrenergic receptors.

Increased α-Adrenergic Receptors in Ischemic Cat Myocardium

A POTENTIAL MEDIATOR OF ELECTROPHYSIOLOGICAL DERANGEMENTS

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ABSTRACT We have recently demonstrated enhanced α-adrenergic responsiveness assessed electrophysiologically in ischemic and reperfused myocardium. This study was performed to determine whether ischemia alters α_1-adrenergic receptor number (B_{max}) or affinity (K_D) based on [^{3}H]prazosin binding. Within 30 min after occlusion, B_{max} increased in ischemic regions to 207% of control to 27±4 fmol/mg protein, with the increase persisting (+141% of control) during early reperfusion (2 min), before returning to control base-line values (13±1.6) after 15 min of reperfusion. K_D was not altered at any interval studied. Beta receptor number ([^{3}H]dihydroalprenolol) and Na^+-K^+ ATPase activity were comparable in control compared to ischemic myocardium although β-receptor B_{max} and K_D in both regions decreased during early reperfusion. Thus, the enhanced α-adrenergic responsivity previously recognized with ischemia and reperfusion is correlated with an increase in α_1-adrenergic receptors.

INTRODUCTION

Recently we have found that α-adrenergic blockade with either phentolamine or prazosin (but not β-adrenergic blockade) abolishes ventricular fibrillation induced by either coronary occlusion or subsequent reperfusion and that the enhanced idioventricular rate associated with reperfusion is abolished by phentolamine but not propranolol (1). In addition, efferent left stellate nerve stimulation during early reperfusion increases the idioventricular rate, a response abolished by phentolamine but not by propranolol (1). Thus, α-adrenergic stimulation contributes to the dysrhythmia. The present study was performed to detect alterations in α_1-adrenergic receptors in ischemic and reperfused myocardium using [^{3}H]prazosin, a radioligand specific for α_1-receptor sites (2) and to compare their time-course with the previously reported enhanced α-adrenergic responsivity.

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METHODS

Animal preparation. Adult cats were anesthetized initially with intramuscular ketamine (12.5 mg/kg) followed by intravenous α-choloralose (75 mg/kg). The surgical procedures, control of temperature and ventilation, isolation of the proximal portion of the left anterior descending coronary artery, and recording of systemic arterial pressures and surface electrocardiograms were identical to those previously described (1, 3). The left anterior descending coronary artery was ligated for 35 min, and then released to allow reperfusion. This procedure results in a readily visible cyanotic area over the left ventricular region comprising 30–40% of total left ventricular weight. The vessel was isolated but not ligated in sham-operated controls. After selected intervals of occlusion or reperfusion, the heart was excised rapidly, ventricular myocardium quickly divided into central ischemic and a central control zone, and the tissue was placed in homogenization buffer at 0–4°C (0.25 M sucrose, 5 mM Tris base, 1 mM $MgCl_2$ at pH 7.4), dissected free of great vessels and fibrous tissue, and weighed.

Membrane preparation. Due to the large amount of protein required for receptor binding assays and Scatchard analysis, segments from five to seven hearts were combined for the preparation of the membrane fraction. Initial experiments showed that freezing of hearts prior to homogenization did not alter the receptor analysis. The pooled tissues from either central ischemic or central normal zones were placed in a Waring blender (Waring Products Div., New Hartford, Conn.) with a large volume (300–500 cm^3) of homogenization buffer, homogenized with a Cole-Parmer rotary pestle, (Cole-Parmer Instrument Co., Chicago, Ill.), and centrifuged at 1,000 *g* for 10 min. The supernate was removed, centrifuged at 39,000 *g* for 10 min, and the membrane pellets resuspended in cold incubation buffer (50 mM Tris base, 10 mM $MgCl_2$, pH 7.4, at 4°C); recentrifugation was repeated two times at 39,000 *g*. After the final resuspension, the preparation was

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filtered through a single layer of cheesecloth and protein concentration adjusted to 1 mg/ml (4).

Radioligands. [^{3}H]prazosin (sp act, 33 Ci/mmol, Radiochemical Center, Amersham, England) was indistinguishable from unlabeled prazosin in a solvent system containing ethyl acetate/methanol/diethylamine (80:20:1, by vol) and ether/isopropylamine (95:5, by vol). Thin-layer chromatography before and after incubation with control and ischemic tissue homogenates provided complete recovery of the intact ligand. [^{3}H]dihydroalprenolol, (DHA,[1] sp act, 46 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Stock solutions of both compounds were made by dissolving the radioligands in 6% ethanol with dilution with deionized water.

Binding assay. For determination of α-adrenergic receptors, membrane preparations (800 μl) were incubated in triplicate for 20 min at 25°C with selected concentrations (0.5–5 nM) of [^{3}H]prazosin (100 μl) with and without the unlabeled antagonist (100 μl), either prazosin (1 μM) or phentolamine (10 μM). Unlabeled prazosin (Pfizer Chemicals Div., New York) was utilized without purification (88% pure as base). After incubation for 20 min, the entire mixture was filtered under vacuum through Whatman GF/C glass fiber discs (Whatman, Inc., Clifton, N. J.). The filters were washed immediately with 15 ml of incubation buffer (25°C). In selected experiments β-receptor number and affinity were determined with [^{3}H]DHA. Incubations with membranes were performed for 18 min at 25°C in a total volume of 900 μl and terminated by vacuum filtration. The GF/C filters were washed with 10 ml of incubation buffer (4°C) prior to scintillation spectrometry. Specific binding of tritiated prazosin was defined as the radioactivity bound to the membrane preparation, which could be displaced by either prazosin (1 μM) or phentolamine (10 μM) and ranged from 80–90% of total binding after subtraction of filter blanks. In control tissue, at 0.5 nM, total disintegrations per minute after subtraction of filter blanks was ~200 with 170 disintegrations per minute associated with specific binding. At 5 nM [^{3}H]-prazosin, total binding disintegrations per minute rose to 700 with 600 disintegrations per minute specific binding. The number of specific counts increased proportionally in ischemic tissue by 30 min. For [^{3}H]DHA, specific binding was defined as net radioactivity displaced by propranolol (1 μM) and ranged from 60 to 70% of total binding. For each experiment, binding was determined in triplicate for each incubation concentration with and without cold antagonist. A minimum of six concentrations were utilized for each Scatchard analysis. Displacement of ligand by selected adrenergic agonists was performed in solutions containing 50 μM EDTA. EDTA alone did not alter specific binding of either [^{3}H]prazosin or [^{3}H]-DHA. In the stereospecificity studies using catecholamines, care was taken to minimize oxidation of these compounds by light, metals, or vortexing.

Sodium-potassium dependent ATPase assay. Enzyme activity was measured conventionally (5) in preparations from central ischemic or normal regions pooled from three cats for each assay.

RESULTS

Binding properties of α-adrenergic receptors. [^{3}H]-prazosin binding was rapid, with half-maximal binding at 5 min at 25°C and maximal binding at 12 min. It was maximal and saturable at 5 nM and completely reversible with the addition of either 1 μM prazosin or 10 μM phentolamine. Specific binding was linear with increases in protein concentration from 0.4 to 2.0 mg/ml. Hill plots yielded coefficients of 0.98 to 1.01 indicating the absence of cooperative interactions. Adrenergic agonists competed with [^{3}H]prazosin for binding in order of their expected specificity and stereospecificity (Fig. 1). The apparent reason for the reduced potency of L-epinephrine compared to L-norepinephrine may be related to species differences or the fact that norepinephrine appears to be slightly more potent on α_1-adrenergic receptors and epinephrine more potent on α_2-adrenergic receptors (6). Yohimbine, a specific α_2-antagonist competed for specific [^{3}H]prazosin binding sites far less effectively (effective concentration $[EC]_{20}$ =8 μM; EC_{50} = 9 mM) than did prazosin (EC_{20} = 8 nM; EC_{50} = 0.3 μM), indicating that binding of [^{3}H]prazosin was specific to α_1- rather than α_2-adrenergic receptors.

Influence of ischemia and reperfusion. Sham-operated controls demonstrated no regional differences in either total receptor number (B_{max}) or affinity (K_D) (Fig. 2). Similarly, after 5 or 15 min of ischemia no significant changes were evident in α_1-adrenergic receptors in ischemic compared to control tissue. However, within 30 min after the onset of ischemia, α_1-adrenergic receptors increased significantly in ischemic tissue, with no apparent alteration in receptor affinity (Fig. 2). As shown in the Scatchard plot in Fig. 3, the increase was nearly twofold. 2 min after reperfusion performed

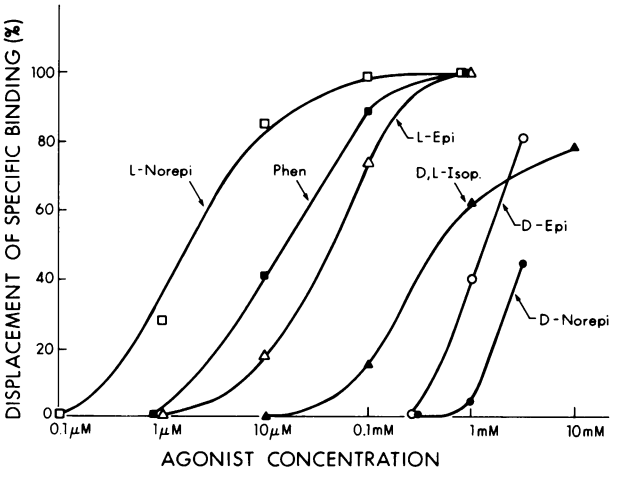

FIGURE 1 Stereospecificity of specific [^{3}H]prazosin binding. The percent displacement of specific binding is shown on the ordinate and each curve represents the displacement induced by a different agonist with increasing concentration on the abscissa. Norepi = norepinephrine; Epi = epinephrine; Phen = phenylephrine; and Isop = isoproterenol. EC_{50}; L-Norepi = 3 μM, D-Norepi = 5 mM, L-Epi = 70 μM, D-Epi = 2 mM, Phen = 30 μM, D, L-Isop = 0.7 mM.

[1] *Abbreviations used in this paper*: B_{max}, total receptor number; DHA, dihydroalprenolol; EC, effective concentration; K_D, dissociation constant.

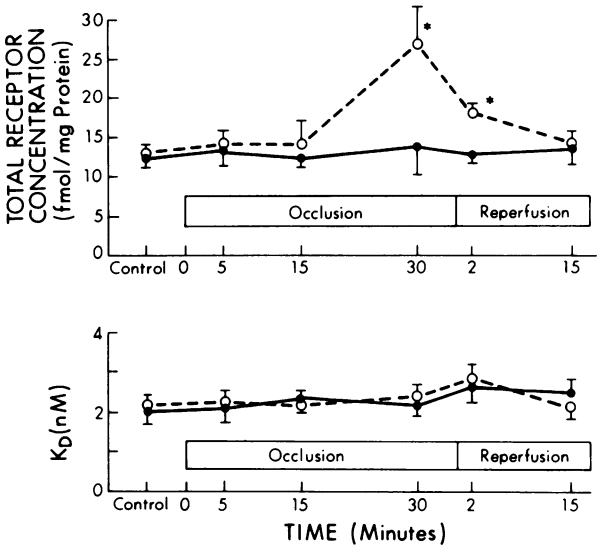

FIGURE 2 Summary data illustrating the mean values for total α_1-adrenergic receptor number (B_{max}, top) and affinity (K_D, bottom) at selected intervals (horizontal axis) before ischemia (control); 5, 15, and 30 min after ischemia; and 2 and 15 min after reperfusion. Data are means±SEM for control (●) and ischemic (○) regions. The * ($P < 0.01$) indicates a significant difference between control and ischemic regions at the same interval. The means at each point represent at least five complete Scatchard curves derived from at least three hearts per Scatchard. The total number of animals that were the source for these data is 102.

35 min following coronary occlusion, B_{max} remained significantly ($P < 0.01$) elevated at 2.0 min (by 141% of control) compared to control, again with no alteration in K_D. Within 15 min after the onset of reperfusion, the increase in B_{max} in the reperfused region had abated. The increase in specific binding after 30 min of ischemia and 2 min of reperfusion demonstrated stereospecific displacement identical to that in control tissue (Fig. 1). The recovery of protein per gram wet weight of tissue was constant in control and ischemic zones at all intervals (range = 1.31 to 1.43 mg/g wet weight). The reasons for the apparent discrepancy between the EC_{20} for cold prazosin displacement of [^{3}H]prazosin binding (8 nM) and the calculated K_D from the Scatchard analysis (2 nM) are not clear. One possible explanation may be that displacement was assessed using [^{3}H]-prazosin binding at the higher concentration range (5 nM) for maximal sensitivity whereas the calculated K_D were assessed using the entire 0.5–5-nM range. In addition, a 12% variation between the displacement value and K_D would be expected based on the 88% purity of the cold prazosin compared to the purified [^{3}H]prazosin.

Influence of ischemia and reperfusion on β-adrenergic receptor number and affinity. We found that [^{3}H]DHA binding was saturable, reversible, stereospecific, and no cooperative binding was apparent. After 30 min of ischemia no significant differences were seen between control and ischemic regions in either B_{max} (96.8±6.8 fmol/mg protein vs. 100.4±5.4) or K_D (4.8±0.6 nM vs. 4.6±0.3). At 2 min of reperfusion, there also was no significant difference between B_{max} (84.6 ±4.0 vs. 79.4±3.8) or K_D (3.4±0.3 vs. 3.1±0.4) in control vs. ischemic regions.

Influence of ischemia and reperfusion on Na^+-K^+ ATPase activity. To determine whether the increase in α_1-adrenergic receptors in ischemic (30 min) and reperfused myocardium (2 min) was secondary to nonspecific destruction of the sarcolemma, Na^+-K^+ATPase activity was assayed. Neither ouabain or Mg^{2+} inhibited Na^+-K^+ATPase activity were depressed at either interval (Fig. 4), although a small, though statistically significant, increase in the ouabain-inhibited activity was evident in the ischemic region 30 min after occlusion.

DISCUSSION

We have previously demonstrated important deleterious electrophysiological influences of α-adrenergic stimulation in ischemic and in reperfused myocardium (1). The present results indicate that α_1-adrenergic

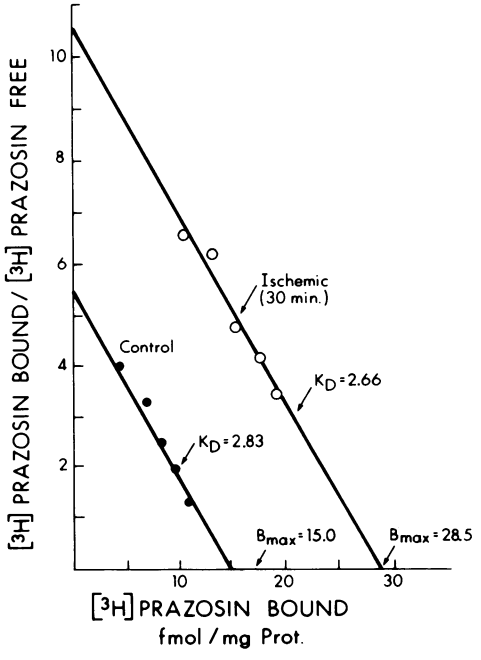

FIGURE 3 Representative Scatchard curves obtained from tissue from three animals subjected to 30 min of ischemia illustrating the nearly twofold increase in total α_1-adrenergic receptor number in ischemic (○) vs. control (●) regions.

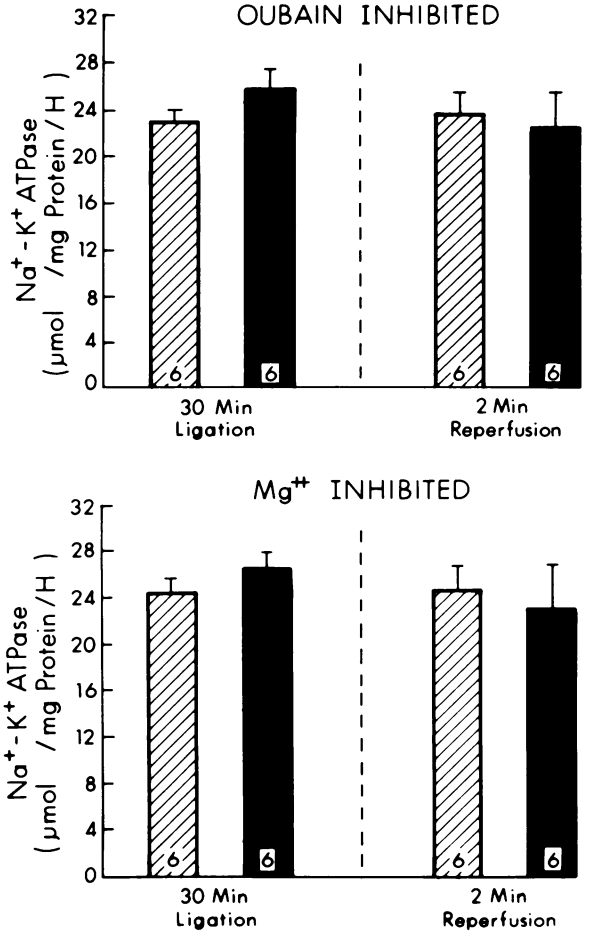

FIGURE 4 Summary data illustrating the influence of 30 min of myocardial ischemia or 35 min of ischemia followed by 2 min of reperfusion on ouabain inhibited (top, 30 min, $P < 0.005$; 2 min, NS) and Mg^{2+} inhibited (bottom, 30 min, NS; 2 min, NS) Na^+-K^+ ATPase. Numbers within histograms indicate the number of determinations made, each representing analysis of pooled tissue from three animals. ▨, control zone; ■, ischemic zone.

receptors increase nearly twofold in ischemic tissue within 30 min, with no apparent alteration in receptor affinity. These changes correlate with the enhanced electrophysiological responsivity. The specificity of the increase in α_1-adrenergic receptors is supported by: (*a*) the increase being confined to ischemic regions; (*b*) its reversibility with sustained reperfusion of 15-min duration; (*c*) the lack of alteration of affinity of the receptors; (*d*) no differential change in β-adrenergic receptor number or affinity in ischemic tissue compared to control; and (*e*) the lack of depression of activity of Na^+-K^+ ATPase at the same intervals in which α_1-adrenergic receptor number was increased.

Mukherjee et al. (7) have recently demonstrated an increase in β-adrenergic receptor number in ischemic myocardium 1 h after coronary ligation, although no significant change was seen after 30 min in agreement with our findings. Thus, the increase in α_1-adrenergic receptors 30 min after the onset of ischemia is not associated with corresponding alterations in β-adrenergic receptors. On the other hand, the sustained increase in α_1-adrenergic receptors 2 min after the onset of reperfusion was associated with a significant decrease in β-adrenergic receptors in both control and ischemic regions. This may explain our previous observations (8) of a rapid reduction in cyclic AMP in both reperfused and control regions within 1 min of reperfusion.

In our previous study (1), reperfusion of the ischemic region was associated with an enhanced idioventricular rate that was mediated by α-adrenergic mechanisms. Indeed, the enhanced responsivity to α-adrenergic input assessed by either regional infusion of methoxamine or efferent left stellate nerve stimulation was apparent within 2 min after the onset of reperfusion and dissipated within 15 min of reperfusion, the same time-course as that observed for increased α_1-adrenergic receptors.

α_1-adrenergic blockade protects against ventricular fibrillation induced by coronary occlusion (1). The apparent early deleterious influence of α_1-adrenergic stimulation does not appear to be mediated by a net early increase in α_1-adrenergic receptors. However, the present results do not exclude the possibility that very early after ischemia, regional increases (undetectable in pooled samples) in α_1-adrenergic receptors occur within ischemic tissue.

The mechanisms responsible for the increase in α_1-adrenergic receptors during ischemia as well as early after reperfusion are not yet known. Alterations in membrane phospholipids (9) in ischemic tissue may influence the relative number of adrenergic receptors. In frog erythrocytes, phospholipase A_2 decreases [^{3}H]-DHA binding and isoproterenol-stimulated adenylate cyclase activity (10). Methylation of membrane phospholipids also alters β-receptor number apparently because of differential uncovering of receptors in a more fluid membrane environment, although the effect on α_1-adrenergic receptors has not yet been determined. Regardless of the mechanisms ultimately found to be responsible, the increase in α-adrenergic receptors appears to be responsible for the increased responsivity to and deleterious influence of, α-adrenergic stimulation 30 min after ischemia and early after the onset of reperfusion.

ACKNOWLEDGMENT

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