Relationship between β-Adrenergic Receptor Numbers and Physiological Responses during Experimental Canine Myocardial Ischemia

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SUMMARY. In the present study, we evaluated the physiological responsiveness of the increased numbers of β-adrenergic receptors in ischemic canine myocardium to in vivo stimulation by (−)-isoproterenol and epinephrine. After 1 hour of temporary proximal left anterior descending coronary artery occlusion and during a 15-minute period of reflow, dogs received (−)-isoproterenol intravenously at a rate sufficient to increase their heart rates 20-40 beats/min. Following the infusion of isoproterenol, myocardial tissue was obtained from the LV ischemic and nonischemic regions for measurement of β-adrenergic receptor numbers, cyclic AMP content, and phosphorylase b to a conversion. β-Adrenergic receptor numbers were significantly increased in the left ventricular (LV) ischemic tissue. The administration of (−)-isoproterenol was associated with significant increases in cyclic adenosine monophosphate content and phosphorylase b to a conversion in the LV ischemic tissue. Also, the administration of (−)-epinephrine significantly increased the phosphorylase b to a conversion in ischemic tissue over the nonischemic tissue and this conversion was blocked by pretreatment with (±)-propranolol. These data suggest that, in this experimental model, the increased numbers of β-adrenergic receptors in canine LV ischemic tissue are capable of translating physiological responses when they are activated with an appropriate agonist in vivo. (Circ Res 50: 735-741, 1982)

Our earlier studies have demonstrated that experimental myocardial ischemia produced in dogs by proximal left anterior descending coronary artery (LAD) ligation for 1 hour is accompanied by increases in the number of (−)-[3H]dihydroalprenolol binding sites ("β-adrenergic receptors") without changing their dissociation constants in ischemic left ventricular (LV) tissue (Mukherjee et al., 1979). The increase in β-adrenergic receptor numbers persists for at least 8 hours and is accompanied by marked decreases in norepinephrine content in the same tissue (Mukherjee et al., 1979). In contrast, in the same experimental model, 1 hour of proximal LAD ligation does not result in a significant change in the number of [3H]quinuclidinyl benzilate binding sites ("muscarinic cholinergic receptors") or their dissociation constants (Mukherjee et al., 1979). The present study evaluated the ability of the β-adrenergic receptors in LV ischemic tissue to translate physiological responses following in vivo stimulation with an appropriate agonist. In these studies, isoproterenol was administered intravenously following a 1-hour period of proximal LAD coronary artery ligation and during a 15-minute reflow period and the formation of cyclic adenosine monophosphate and conversion of phosphorylase b to a in the LV ischemic and nonischemic tissues were measured.

Experimental Procedures

Materials

Glycogen (G-8876), α-α-glucose-1,6-diphosphate tetracyclohexylammonium salt (G-5875), glucose-6-phosphate dehydrogenase (G-6378), phosphoglucomutase (P-7502), adenosine monophosphate (A-1752), bovine serum albumin (A-7638), calf thymus DNA (D-1501), adenosine triphosphate (A-6144), and (±)-propranolol HCl (P-0884) were obtained from Sigma Chemical Co. (−)-[3H]Dihydroalprenolol, specific activity, 48-55 Ci/mmol, was obtained from New England Nuclear. Cyclic AMP radioimmunoassay kits were obtained from Beckton Dickinson.

Methods

Mongrel dogs (25-35 kg) of either sex were used. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), intubated, and ventilated with room air on a Harvard respirator (Harvard Apparatus Co.). A left thoracotomy was performed, the pericardium opened, and the proximal LAD isolated. The proximal LAD was ligated for 1 hour so as to create an anterior LV region with intense cyanosis (Mukherjee et al., 1979). We have previously shown that the cyanotic region is an area of lactate accumulation and high energy phosphate depletion, epicardial S-T segment elevation (Karlsson et al., 1973) and a region of segmental functional alterations (Roan et al., 1979), and thus should
be considered to be the ischemic region. Following 1 hour of ischemia, the LAD ligation was released and (-)-isoproterenol was infused intravenously at a rate sufficient to increase heart rate 20-40 beats/min [0.31 ± 0.06 (±) μg/kg per min] during a 15-minute reflow period. In six dogs, (-)-epinephrine (0.09 μg/kg per min) infused for 7 minutes following 1 hour ligation, and in six dogs, (±)-propranolol (1 mg/kg) was administered 13 minutes before epinephrine was infused. After 7- to 15-minute reflow periods, biopsies from the LV ischemic and nonischemic tissues were obtained according to the procedure of Skinner et al., using a biopsy needle with the capability of in situ freezing of the tissue by circulating liquid-nitrogen-cooled alcohol through the needle (Skinner et al., 1978).

The frozen tissue was removed from the heart, transferred in liquid nitrogen, and stored at −80°C until analyzed for cyclic AMP content. The hearts were removed and the left ventricle divided rapidly into the LV ischemic and control zones. Tissue was frozen in liquid nitrogen and stored at −80°C until analyzed further.

Cardiac Membrane Preparation

Cardiac membranes were prepared by three methods. Crude membranes were prepared as described previously (Mukherjee et al., 1979). The other methods were as follows: Ten grams of heart tissue were minced finely in 15 ml of ice-cold buffer A (0.25 M sucrose and 20 mM MOPS-Tris, pH 7.4). The minced tissue was homogenized in a small Waring blender (Eberbach Corp.) for 5 seconds. The homogenate was centrifuged at 1,000 g (max) for 10 minutes at 4°C. The pellet was resuspended in 15 ml of buffer A and centrifuged again at 1,000 g (max) for 10 minutes at 4°C. The above step was repeated once more. This procedure of centrifugation and resuspension helps to remove blood adherent to the tissue. The pellet was suspended in 15 ml of buffer A and homogenized in a Polytron PT 10 (setting at 7) for three 30-second bursts with cooling in between and while homogenizing. The homogenate was centrifuged at 10,000 g (max) for 10 minutes at 4°C. The pellet was discarded and the supernatant centrifuged at 48,000 g (max) for 30 minutes at 4°C. The pellet was washed twice by resuspension and centrifugation and finally suspended in the desired volume (usually 1-2 mg protein/ml) with buffer B (160 mM NaCl + 20 mM MOPS-Tris, pH 7.4).

In two experiments, sarcolemmal-enriched fractions were prepared from 25 g of LV ischemic and nonischemic tissues pooled from four dogs by the method of Reeves and Sutko (1980). Briefly, the tissues were homogenized and membranes were prepared in 0.3 M sucrose, 10 mM MOPS-Tris, and 0.5 mM EDTA-Tris, pH 7.4. Membranes (in 10 ml of buffer) were layered on 27 ml of 0.6 M sucrose, 10 mM MOPS-Tris and 0.5 mM EDTA-Tris, pH 7.4 and spun at 25,000 g (max) for 90 minutes with a SW27 rotor. The interphase at 0.3 and 0.6 M sucrose was aspirated and diluted to 40 ml with 160 mM NaCl and 20 mM MOPS-Tris, pH 7.4 (buffer B) and spun at 46,000 g (max) for 30 minutes. The pellet was suspended in 20 ml of the buffer B and spun at 8,000 g (max) for 10 minutes, the pellet was discarded, and the supernatant spun at 48,000 g (max) for 30 minutes. The pellet was finally suspended in buffer B at a concentration of approximately 1 mg protein/ml.

β-Receptor Assay in Cardiac Membranes

The membrane preparations were assayed for (-)[3H] dihydroalprenolol (DHA) binding, according to the method of Williams et al. (1972), which has been described in an earlier paper (Mukherjee et al., 1979). In brief, membranes were incubated at 37°C for 10 minutes with (-)[3H] DHA (0.3-10 nM for microsomal- and sarcolemmal-enriched fractions and 0.2-20 nM for crude preparations) and in the presence and absence of 10 μM (±)-propranolol. Following 10 minutes of incubation at 37°C, the membranes were diluted and filtered through GF/C filters. The filters were washed, dried, and counted. Specific binding was determined by subtracting nonspecific from total binding. Using the direct-binding methods, the number and affinity of DHA binding sites in membranes were analyzed according to Scatchard fitting the regression lines by the method of least squares. Eight to nine data points were obtained for the Scatchard analysis. Each determination was done in duplicate. Specific binding for DHA was 40-50% of total binding for the membranes isolated by the first method and 70-90% of total binding for the membranes isolated by the second and third methods.

Phosphorylase (1,4-α-α Glucan-Orthophosphate α-Glucosyl Transferase, EC 2.4.1.1) assay

The phosphorylase assay was performed in the 4,000 g supernatant fraction of either 15 mM β-mercaptoethanol homogenate or 0.02 M NaF, 0.001 M EDTA, 0.05 M Tris-HCl (pH 6.8), which homogenate by the coupled enzyme technique according to the procedure of Helmreich and Cori (1964) as described in Boehringer Mannheim's biochemical information catalogue. The assay mixture contained glycerol, 2 mg; potassium phosphate buffer (pH 6.8), 50 mM; EDTA-Na, 0.1 mM; MgCl2, 13 mM; glucose-1,6-diphosphate, 4 mM; NADP, 0.36 mM; sucrose, 2.5 mM; Tris-HCl, pH 7.4, 10 mM; phosphoglucomutase, 5 units; and glucose-6-phosphate dehydrogenase, 1 unit, in a total volume of 1 ml made with distilled water. The rate of NADP reduction was monitored in a kinetic Beckman J25 recording spectrophotometer at a wave length of 340 nm at 25°C. The reaction was performed with and without 2 μM AMP (Madsen and Cori, 1957). The results are expressed as -AMP/+AMP; an increase in the ratio indicates increased activation (b to a) of phosphorylase (Madsen and Cori, 1957).

5'-Nucleotidase Assay

5'-Nucleotidase (5'-ribonucleoside phosphohydrolase, EC 3.1.3.5) was assayed by the method of Gentry and Olsson (1975), using 0.045 μCi of [3H]-AMP and unlabeled AMP to give a final AMP concentration of 50 μM. The reaction was carried at pH 7.4 at 37°C for 10 minutes with 20 μg membrane protein per ml.

Cyclic AMP Determination

The cyclic AMP content was measured in trichloroacetic acid (TCA) extracts of frozen biopsies using the radioimmunoassay procedure of Steiner et al. (1972), as supplied by Beckton-Dickinson Immunodiagnostics. Recovery of [3H]-cyclic AMP in the TCA extract was about 80-90%. Values were not corrected for recovery, since recovery was the same with ischemic or nonischemic tissues. The sensitivity of the assay kits is 0.05-0.10 pmol cyclic AMP per tube.

Protein Determination

Protein was determined in the membrane fractions by the method of Lowry et al. (1953) using bovine serum albumin (Sigma Chemical Co.) as a standard.

Deoxyribonucleic Acid Determination

DNA was determined in the homogenate using the diphenylamine reagent according to the procedure of
Schneider (1959) using highly polymerized calf thymus DNA as a standard.

Statistical Analysis

The data were evaluated for statistical significance using Student's group t-test. The results were considered to be statistically significant when $P < 0.05$ with a two-tailed $T$ test.

Results

Proximal LAD ligation for 1 hour, followed by 15 minutes of reflow during which (-)-isoproterenol was infused, resulted in a significant increase in protein content (mg/g wet weight tissue) in the 40,000 g (max) pellet obtained from the LV ischemic tissue (Table 1). However, the DNA content of the LV ischemic and nonischemic tissues did not change in the same homogenates (Table 1). Therefore, we have normalized $\beta$-adrenergic receptor numbers in LV ischemic and nonischemic tissue by expressing them per mg DNA.

Proximal LAD ligation for 1 hour followed by 15 minutes of reflow with or without the administration of (-)-isoproterenol resulted in a significant increase in $\beta$-adrenergic receptor numbers in LV ischemic tissue as compared to nonischemic tissue (Fig. 1 and Tables 1 and 3). Similar increases in $\beta$-adrenergic receptor numbers in LV ischemic tissue were observed in our earlier studies with 1 hour of permanent LAD occlusion (Mukherjee et al., 1979).

No significant differences in the $\beta$-adrenergic receptor dissociation constants ($K_{DB}$) were noted between LV ischemic and nonischemic tissues in either the present study or in our earlier measurements (Mukherjee et al., 1979) (Tables 1-3). To ascertain that the changes in $\beta$-adrenergic receptor number in LV ischemic tissue are specific ones, 5'-nucleotidase was assayed in membranes from ischemic and nonischemic tissue following 1 hour of LAD ligation. The results in Table 2 indicate that there is no difference in enzyme activity between LV ischemic and nonischemic tissue, indicating that the changes observed in $\beta$-receptor number are relatively specific ones. Our earlier studies demonstrated that there was no change in muscarinic receptor numbers between LV ischemic and nonischemic tissue (Mukherjee et al., 1979).

In order to be certain that further purification of sarcolemmal membranes would not alter the conclusion that $\beta$-adrenergic receptor numbers increased in LV ischemic tissue, additional studies were performed. In six additional canine hearts with proximal LAD occlusions for 1 hour, $\beta$-adrenergic receptors were measured in microsomal fractions. Table 2 dem-

![Figure 1](http://circres.ahajournals.org/contentfigures/123/7/737/F1.large.jpg)

**Figure 1.** The percentage increases above the nonischemic tissue in $\beta$-adrenergic receptors (BAR), cyclic AMP content and phosphorylase activity in LV ischemic tissue with and without the administration of (-) isoproterenol during 15 minutes of reflow following one hour of proximal LAD ligation are shown. $N$ = number of experiments in each group. Vertical bars represent the standard errors of the mean. The data demonstrate that there was a significant increase in all three parameters in LV ischemic tissue when (-)-isoproterenol was administered during reflow. However, ischemia and reflow without (-)-isoproterenol was associated with an increase in LV ischemic region BAR numbers, but no change in cyclic AMP or phosphorylase contents in the same tissue. See text for experimental details.

### Table 1

| Membrane Protein, DNA Content, (-)-[^{3}H]-DHA Binding, Phosphorylase Activity (-AMP/+AMP), and Cyclic AMP Content of LV Ischemic and Nonischemic Tissue after 1 Hour of Proximal LAD Ligation and 15 Minutes of Reflow with (-)-Isoproterenol Infusion |
|-----------------|----------------|-----------------|-----------------|
|                  | mg/g wet wt tissue | No. of (-)-[^{3}H]-DHA binding sites (fmol/mg DNA) | $K_{D}$ of (-)-[^{3}H]-DHA (nM) | Cyclic AMP (pmol/mg wet wt tissue) | Phosphorylase (-AMP/+AMP) |
| Nonischemic LV tissue | 2.41 ± 0.20 | 2.11 ± 0.29 | 114 ± 21 | 7.81 ± 0.86 | 0.54 ± 0.07 | 0.41 ± 0.04 |
| Ischemic LV tissue | 3.58 ± 0.37 | 2.02 ± 0.26 | 202 ± 37 | 8.23 ± 0.98 | 0.92 ± 0.20 | 0.58 ± 0.04 |
|                  |                  |                  |                  |                  |                  |                  |
|                  |                  |                  |                  |                  |                  |                  |
|                  |                  |                  |                  |                  |                  |                  |
|                  |                  |                  |                  |                  |                  |                  |

Results are expressed as mean ± SEM; $n$ = number of animals used.

* DNA was measured in total homogenate.
Demonstrates that receptor numbers increased significantly in membranes isolated from the LV ischemic tissue (221 ± 27 in LV nonischemic vs. 369 ± 32 fmol (—)-[3H]-DHA per mg protein in LV ischemic tissue). In two experiments, sarcolemmal enriched fractions were prepared by sucrose density gradient centrifugations. Table 2 demonstrates that beta adrenergic receptor numbers were still increased in LV ischemic tissues. Thus, these data indicate that the changes in LV ischemic tissue receptor numbers in crude membrane preparations are not due to artifactual alterations in membrane protein.

The relationships between the increased β-adrenergic receptor numbers and the physiological responses to stimulation with (—)-isoproterenol were also evaluated. Table 1 demonstrates that the administration of (—)-isoproterenol in vivo in a dosage sufficient to increase heart rates by 20-40 beats/min was associated with a significant increase in cyclic AMP content in LV ischemic tissue compared to the nonischemic tissue in the same animals. There was also a significant increase in the ratio of −AMP/+AMP activity of phosphorylase in the LV ischemic tissue following the administration of isoproterenol (Table 1). This indicates that there was an increased transformation of β to a phosphorylase in the LV ischemic tissue associated with the administration of isoproterenol during the reflow period (Table 1).

Table 4 demonstrates that (—)-epinephrine infusion during reflow significantly increased phosphorylase activation in LV ischemic tissue compared to the nonischemic tissue. However, when (±)-propranolol was administered before (—)-epinephrine infusion, phosphorylase activation was inhibited, indicating that the activation was probably mediated through cyclic AMP and activation of the β-adrenergic receptors.

In three other canine hearts with proximal LAD occlusions for 15 minutes and the administration of similar amounts of (—)-isoproterenol during a reflow period of 15 minutes, β-adrenergic receptor numbers, cyclic AMP content, and phosphorylase activity were also measured. No significant increase in β-adrenergic receptor numbers was observed in LV ischemic tissue in these dogs (LV nonischemic 109 ± 9 vs. LV ischemic tissue 118 ± 9 fmol/mg DNA). Similar results were also found in our earlier experiments with 15-minute periods of fixed LAD occlusion (Mukherjee et al., 1979). No significant differences in cyclic AMP content in LV ischemic and nonischemic tissues (LV nonischemic 0.62 ± 0.21 vs. LV ischemic 0.60 ± 0.06 pmol/mg tissue) were found. Similarly, there was no significant difference in phosphorylase (−AMP/+AMP) activity between LV nonischemic (0.46 ± 0.04) and ischemic (0.45 ± 0.04) tissues. Thus, these data suggest that the administration of (—)-isoproterenol

### Table 2

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<tr>
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<th>Specific Binding and Dissociation Constant of (—)-[3H]-Dihydroalprenolol in Crude, Microsomal, and Sucrose Density Gradient-Enriched Sarcolemmal Fraction and 5'-Nucleotidase Activity from LV Ischemic and Nonischemic Tissues in Canine Myocardium with 1 Hour of Proximal LAD Ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Receptor numbers (fmol (—)-[3H]-DHA bound per mg protein)</td>
</tr>
<tr>
<td>LV nonischemic tissue</td>
<td>101 ± 18</td>
</tr>
<tr>
<td>LV ischemic tissue</td>
<td>163 ± 18</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n = number of animals used.

* 5'-Nucleotidase activity is assayed in microsomal fraction and is expressed as nmol/mg protein per 10 min at 37°C. 1 = crude membrane preparation; 2 = microsomal fraction; 3 = 0.6 and 0.3 M sucrose density gradient interphase (enriched sarcolemmal fraction).

### Table 3

(-)-[3H] DHA Binding, cyclic AMP Content, and Phosphorylase Activity (−AMP/+AMP) of LV Ischemic and Nonischemic Tissues after 1 Hour of Proximal LAD Ligation and 15 Minutes of Reflow without (—)-Isoproterenol Infusion

<table>
<thead>
<tr>
<th></th>
<th>No. of (—)-[3H]-DHA binding sites (fmol/mg DNA)</th>
<th>K_D of (—)-[3H]-DHA (nmol/mg wet wt tissue)</th>
<th>CyclicAMP (nmol/mg wet wt tissue)</th>
<th>Phosphorylase (−AMP/+AMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemic LV tissue (n = 3)</td>
<td>106 ± 14</td>
<td>7.43 ± 1.21</td>
<td>0.44 ± 0.04</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Ischemic tissue (n = 3)</td>
<td>172 ± 21</td>
<td>8.20 ± 1.21</td>
<td>0.34 ± 0.01</td>
<td>0.29 ± 0.05</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n = number of animals used.
TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic tissue</th>
<th>Ischemic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)-Epinephrine</td>
<td>0.51 ± 0.04</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>(±)-Propranolol and epinephrine</td>
<td>0.30 ± 0.02</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
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<tr>
<td>NS</td>
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</table>

Results are expressed as mean ± sem; n = number of animals used.

Discussion

The data obtained in this study indicate that proximal LAD ligation for 1 hour followed by 15 minutes reflow with or without the administration of (−)-isoproterenol results in a significant increase in β-adrenergic receptor numbers. Similar increases in β-adrenergic receptor numbers were observed in our earlier studies with 1 hour of permanent LAD occlusion (Mukherjee et al., 1979). No significant differences in the β-adrenergic receptor dissociation constants were noted between LV ischemic and nonischemic tissues; however, the present study or in our earlier measurements (Mukherjee et al., 1979). We have also evaluated the effect of sarcolemmal membrane enrichment on this alteration in β-adrenergic receptor numbers in LV ischemic tissue. The data in Table 2 show that the increase in β-adrenergic receptors in LV ischemic tissue persists even when the receptors are measured in sarcolemmal membrane-enriched fractions.

It should also be emphasized that, in our earlier studies, both β-adrenergic and muscarinic cholinergic receptor numbers and, in the present study, 5'-nucleotidase, were measured in the same membrane fractions isolated from the LV tissues following 1 hour of fixed proximal LAD ligation. Since there was no change in muscarinic cholinergic receptor numbers and in 5'-nucleotidase activity in the same animals in which β-adrenergic receptors were increased, it does not appear that artificial alterations in protein content are a logical explanation for the increase in β-adrenergic receptor numbers in LV ischemic tissue.

In the present study, we evaluated whether the in vivo administration of appropriate agonists can stimulate the increased numbers of β-adrenergic receptors in LV ischemic tissue and translate this stimulation into the increased formation of cyclic AMP (Robison et al., 1971) and phosphorylase b to a conversion. To test this possibility, we administered either (−)-isoproterenol or (−)-epinephrine in doses sufficient to increase heart rate by 20-40 beats/min during a 7- to 15-minute reflow period after 1 hour of LAD occlusion. Table 1 demonstrates that the administration of (−)-isoproterenol was associated with a significant increase in cyclic AMP content in LV ischemic compared to nonischemic tissue. The administration of (−)-isoproterenol during reflow following 1 hour of LAD occlusion resulted in an increase in LV ischemic region cyclic AMP content from 0.34 ± 0.01 to 0.92 ± 0.20 (Tables 1 and 3). Since cyclic AMP has been expressed as pmol per mg wet wt of tissue, the increase in cyclic AMP content in the LV ischemic tissue is almost certainly an underestimation of its actual value since there is an approximately 28% increase in water content in ischemic tissue after 40 minutes of myocardial ischemia followed by 15 minutes reflow (Kloner et al., 1974). This should increase the wet weight of the LV ischemic tissue and decrease the cyclic AMP content per mg protein.

Table 3 shows that the cyclic AMP content in LV nonischemic tissue is 0.44 pmol/mg wet weight in dogs after 1 hour of myocardial ischemia followed by 15 minutes reflow without the administration of (−)-isoproterenol. Cyclic AMP content in LV nonischemic tissue increased to 0.54 pmol/mg wet weight...
tissue in a different group of dogs receiving this relatively small dose of (-)-isoproterenol under the same experimental circumstances (Table I). In additional studies in our laboratory, we have found that—in eight anesthetized dogs with permanent proximal LAD ligation for 1 hour—cyclic AMP content in LV nonischemic tissue was 0.40 ± 0.04 (n = 8) pmol/mg wet weight of tissue. Similar concentrations of cyclic AMP (0.4–0.7 pmol/mg wet weight of tissue) have also been observed by others in the myocardium of different animal species (Wollenberger et al., 1969; Podzuwiet et al., 1978; Ahumada et al., 1979).

The best understood direct effect of catecholamines in cardiac metabolism is the activation of the sequence of events that begins with the formation of cyclic AMP and ends with the transformation of phosphorylase b to its activated state (Mayer, 1974). Thus, phosphorylase (−AMP/+AMP) was assayed in the 40,000 g (max) supernatant in tissue obtained from LV ischemic and nonischemic regions using the same experimental models. Tables I and 4 demonstrate that there was a significant increase in the phosphorylase activity in the LV ischemic tissue in dogs that received either isoproterenol or epinephrine during the 7- to 15-minute reflow period. In dogs that received (-)-isoproterenol during reflow, phosphorylase (−AMP/+AMP) increased from 0.27 to 0.41 in LV nonischemic tissue and from 0.29 to 0.58 in LV ischemic tissue. Thus, β-agonists increase phosphorylase activation in LV nonischemic as well as ischemic tissue, but to a greater extent in LV ischemic tissue. This increase in phosphorylase activity was inhibited by (±)-propranolol (Table 4). An increase in phosphorylase activity has also been observed in hyperthyroid rat hearts when they are perfused with (-)-isoproterenol. β-Adrenergic receptor numbers in the hearts of these animals were increased by 50% (Hess et al., 1980).

In three additional canine hearts with proximal LAD occlusions for 1 hour and reflow for 15 minutes without (-)-isoproterenol, β-adrenergic receptors, cyclic AMP content, and phosphorylase activity were also measured. Table 2 demonstrates that there was an increase in β-adrenergic receptors in LV ischemic tissue without an increase in either cyclic AMP content or phosphorylase (−AMP/+AMP) activity in the LV ischemic tissue. In a preliminary communication, Drummond and Sordahl (1980) have reported a decrease in cyclic AMP content in canine LV ischemic tissue following permanent circumflex coronary artery ligation. Administration of (-)-isoproterenol during a reflow period after 15 minutes of proximal LAD occlusion failed to increase cyclic AMP content or phosphorylase b to a conversion. Thus, it appears that the increased formation of cyclic AMP and conversion of phosphorylase b to a in LV ischemic tissue require that β-adrenergic receptor numbers be increased and that an appropriate agonist be administered during reflow after coronary artery occlusion.

Our earlier studies (Mukherjee et al., 1979) and other studies (Russell et al., 1961) have shown a significant decrease in LV ischemic tissue norepinephrine content of approximately 30–50% following 1 hour of proximal LAD ligation in the canine model. This may be an important factor responsible for the lack of increase in cyclic AMP content and phosphorylase activation observed in LV ischemic tissue following 1 hour of fixed LAD occlusion or 1 hour of temporary occlusion and 15 minutes of reflow in the absence of (-)-isoproterenol.

It might be argued that catecholamines released into the circulation during 1 hour of temporary occlusion could be taken up in the myocardium during reflow and thus increase the tissue cyclic AMP level and activate phosphorylase. However, it seems likely that catecholamines released from the myocardium are metabolized rapidly and are also taken up by other noncardiac tissues, thus diminishing the concentration that might be exposed to the LV ischemic region β-adrenergic receptors. Whether stress or other physiological reasons for increased catecholamine release result in a greater concentration of endogenous catecholamines reaching the LV ischemic region will have to be determined in future studies.

It is well known that there is increased ischemic region Ca++ uptake following 40–60 minutes of myocardial ischemia followed by reflow (Peng et al., 1977). Ca++ can activate phosphorylase kinase by increasing its catalytic activity (Krebs, 1972). This, in turn, activates phosphorylase (Mayer, 1974). Since phosphorylase was not activated during reflow in the present study without the administration of (-)-isoproterenol, it appears that increased calcium uptake per se is not responsible for the increased LV ischemic region cyclic AMP content or phosphorylase b to a conversion.

In our earlier studies with fixed LAD occlusion for 1 hour, the protein concentrations in the 40,000 g (max) pellet were not significantly changed in LV ischemic as compared to nonischemic tissue (Mukherjee et al., 1979). The significant increase in protein concentration with no change in DNA concentration during reflow may thus be due to hemorrhage which results in the sequestration of erythrocytes in the ischemic area during reflow (Willerson et al., 1977). Earlier studies by us (Willerson et al., 1977) and by Kloner et al. (1974) have shown that, with reflow following 40 minutes of myocardial ischemia, intracellular and interstitial edema develop, hemorrhage occurs, and cell membranes may be disrupted. These results indicate that altered vascular and cell membrane permeability occurs with reflow following temporary coronary artery occlusion lasting at least 40–60 minutes. Therefore, we have expressed β-adrenergic receptor numbers in the LV ischemic and nonischemic tissues in terms of DNA content rather than normalizing them for protein content.

In summary, our previous study (Mukherjee et al., 1979) and the present one identify a significant increase in β-adrenergic receptor numbers in LV ischemic tissue following fixed LAD occlusion for 1 hour and temporary occlusion for the same period followed by reflow. When these increased β-adrenergic receptors are stimulated by the appropriate dose
of (−)-isoproterenol in vivo, a biological response occurs as indicated by an increase in cyclic AMP content and phosphorylase activation (b to a) in LV ischemic tissue. Although this activation sequence indicates that the increased β-adrenergic receptors have the ability to translate biological responses when they are activated by an appropriate agonist in vivo, alterations distal to the receptor-cyclase complex may also contribute to the changes observed in these studies.

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