During acute myocardial ischemia, catecholamines can have extremely deleterious effects. For example, catecholamine-mediated alterations in the cardiac action potential can lead to life-threatening arrhythmias; in addition, catecholamine-stimulated chronotropic and inotropic responses enhance myocardial oxygen consumption, which may promote extension of the zone of ischemia or infarction.

In previous studies with guinea pigs, we have shown that myocardial ischemia promotes an externalization of β-adrenergic receptors from a light vesicle, presumably intracellular fraction, where they are functionally uncoupled from adenylate cyclase, to the sarcolemma. This externalization increased the number of surface β-adrenergic receptors that were functional, as assessed by isoproterenol-stimulated adenylate cyclase activity. After chronic propranolol treatment, ischemia did not further alter receptor distribution. These results suggest that externalization of β-adrenergic receptors from a light vesicle fraction to the sarcolemma contributes to up-regulation of β-receptors that occur in response to both propranolol treatment and ischemia. Because propranolol-treated animals show blunting in externalization after myocardial ischemia, propranolol treatment and myocardial ischemia appear to access the same pool of intracellular β-adrenergic receptors. Depletion of this pool of receptors along with receptor blockade may thus contribute to the mechanism by which the drug is efficacious in preventing some adverse effects of ischemia. (Circulation Research 1986;60:108–112)

Materials and Methods

Minipump Implantation and Ischemia Induction

Male Hartley guinea pigs (400–500 g, 4–6 weeks old, Simonsen Co.) were given ketamine anesthesia (110 mg/kg, i.p.), and then Alzet Model 2001 osmotic minipumps were implanted subcutaneously. These minipumps were used to deliver propranolol at a rate of 0.15 mg/kg/hr or vehicle (10 mM HCl) for 7 days. At the time the animals were killed, we recorded heart rate via surface electrocardiographic leads, drew blood for propranolol levels (HPLC method, American Clinical Laboratories), and rapidly removed the heart. The left ventricle was then separated and washed in 50 mM ice-cold Tris-HCl (pH 7.4) and frozen in liquid
nitrogen for subsequent membrane preparation. In another group of animals, osmotic minipumps were implanted for 7 days prior to the induction of myocardial ischemia. These animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.), intubated, and ventilated with supplemental oxygen on a Harvard respirator. A left thoracotomy was performed, the pericardium was opened, and the proximal left anterior descending coronary artery was ligated with 6-0 prolene suture to create an anterior left ventricular region with intense cyanosis. An epicardial ECG lead was sutured over the area. ST-segment elevations confirmed the presence of ischemia. Ischemia was maintained for 60 minutes, at which time the animals were killed. Sham-operated animals were used as controls.

**Preparation of Sarcolemmal and Light Vesicle Membranes**

We isolated two distinct fractions from excised left ventricles: a purified sarcolemmal fraction in which β-adrenergic receptors are responsive to agonist, and a lighter, presumably intracellular membrane fraction of receptors. As previously described, the methods used are modifications of previously published techniques.8,9 Briefly, following extraction of contractile proteins with 750 mM NaCl, the samples were homogenized and the light vesicle fraction was obtained by centrifuging the supernatant of a 45,000g spin at 137,000g for 90 minutes. The pellet of the 45,000g spin was resuspended, washed several times, and the purified sarcolemmal membranes were obtained from high speed centrifugation of the supernatant of a 17,000g spin. We have previously shown that receptors in the light vesicle fraction are functionally uncoupled from the guanine nucleotide stimulatory G, protein and that this fraction has little adenylate cyclase activity.9 Protein was determined by the method of Lowry10 using bovine serum albumin as the protein standard.

**Radioligand Binding Assay**

Radioiodinated cyanopindolol ([125]IICYP) was prepared in our laboratory as described previously.12 Binding of β-adrenergic receptors to [125]IICYP was measured by incubating membranes in triplicate with 8–10 concentrations of radioligand (50–600 pmol) at 25°C for 1 hour. Binding was terminated by diluting samples to 10 ml with ice-cold buffer, filtering over Whatman GF/B filters that had been presoaked in 2% polyethyleneimine,13 and washing the filters with 10 ml of buffer. We used filters soaked in polyethyleneimine to minimize loss of receptors that might be present in small membrane fragments and therefore not be trapped in untreated glass fiber filters. Radioactivity retained on filters was determined using a γ-counter at 86% efficiency. Nonspecific binding for both sarcolemmal and light vesicle fractions was determined by [125]IICYP binding in the presence of 1 μM (-)-propranolol. The subtraction of nonspecific binding from total binding yielded specific binding that was routinely 80% of total binding for both membrane fractions.

**Adenylate Cyclase Assay**

Adenylate cyclase was assayed by the method of Salomon et al in a buffer containing 50 mM Tris-HCl, 10 mM MgCl₂, 0.3 mM cyclic adenosine monophosphate, 1 mM adenosine triphosphate (ATP), 100 μM guanosine triphosphate (GTP), 20 mM creatine phosphate, 50 μM creatine phosphokinase, and 800,000 counts/min [32P]ATP. The other compounds listed were used at the following concentrations: isoproterenol (100 μM), Mn²⁺ (10 μM), forskolin (100 μM), and Gpp(NH)p (10 μM).

**Data Analysis**

The equilibrium dissociation constant (Kₒ) and the maximal number of binding sites (B_max) for [125]IICYP were determined from Scatchard analysis9 of saturation binding isotherms. To determine statistical significance of differences in B_max values, we calculated paired two-tailed t tests.

**Results**

**Distribution of β-adrenergic Receptors in Sarcolemmal and Light Vesicle Membranes in Propranolol-Treated Guinea Pigs**

The heart rates in animals treated with propranolol were significantly lower than controls (187 ± 22 bpm vs. 264 ± 20 bpm, p < 0.001). Propranolol blood levels averaged 21 ng/ml (range of 12–46), with none detected in control animals. Figure 1 shows typical saturation isotherms for [125]IICYP binding in membranes prepared from control animals and from animals treated for 1 week with propranolol. Figure 1A shows that substantially more receptors are present in the sarcolemmal membranes of propranolol-treated animals than in controls. In contrast, propranolol treatment produces a decrease in receptor binding sites that can be recovered in the light vesicle fraction (Figure 1B). Figure 2 shows pooled data from 8 such experiments. After 1 week of propranolol treatment there was a 63% increase in sarcolemmal receptors and a 46% decrease in β-adrenergic receptors found in the light vesicle fraction (both differences p < 0.025). The dissociation constants (Kₒ), a measure of the affinity of the receptor for [125]IICYP, were similar for control and propranolol-treated groups and ranged between 0.08 nM and 0.16 nM. Thus, the up-regulation of β-adrenergic receptors previously observed in crude membrane preparations is associated with, and may be caused by, an externalization of β-adrenergic receptors to the plasma membrane with concomitant depletion of β-receptors from the light vesicle fractions.

In Table 1, myocardial adenylate cyclase activity is compared in control and propranolol-treated animals. Although adenylate cyclase activity stimulated by GTP, Gpp(NH)p, or forskolin in sarcolemmal membranes was not altered by propranolol treatment, isoproterenol-stimulated adenylate cyclase activity was greater in propranolol-treated animals (p < 0.05). Thus, the "new" sarcolemmal β-adrenergic receptors
that appeared during propranolol treatment were functionally active.

Effects of Ischemia on Control and Propranolol-Treated Animals

Figure 3 shows the effects of myocardial ischemia on the number of [\(^{125}\)I]ICYP binding sites in sarcolemmal membranes and light vesicle membranes of control and propranolol-treated animals. In animals with no pretreatment, 1 hour of ischemia increased the number of β-adrenergic receptors in the surface sarcolemmal membranes by 45% and decreased the number of receptors in the light vesicle fraction by 60%. As we have previously reported, this is evidence for an ischemia-induced redistribution or externalization of β-adrenergic receptors to the cell surface. Recovery of protein in the two fractions following ischemia was no different than control, and sarcolemmal fractions had similar adenylate cyclase and 5'-nucleotidase activity before and after ischemia. The distribution of β-adrenergic receptors in propranolol-treated animals with ischemia is presented in the right half of Figure 3. In propranolol-treated animals, ischemia led to no further significant change in the distribution of β-adrenergic receptors.

Discussion

Occupancy of β-adrenergic receptors by agonists promotes activation of adenylate cyclase and the intracellular accumulation of cAMP. In many cell types, brief (<30 minutes) exposure of cells to agonists leads to desensitization of cAMP generation and also to the movement of receptors ("internalization") from the plasma membrane. These receptors can be isolated in a light vesicular fraction that is deficient in plasma membrane markers. β-Adrenergic receptors in this

Table 1. Adenylate Cyclase Activity: Control vs. Propranolol Treatment in Sarcolemmal Membranes

<table>
<thead>
<tr>
<th>Animals</th>
<th>Mn(^{2+}) (10 mM)</th>
<th>Isoproterenol (100 μM) plus forskolin (100 μM)</th>
<th>Gpp(NH)p (10 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125 ± 10</td>
<td>360 ± 30</td>
<td>210 ± 10</td>
</tr>
<tr>
<td>Propranolol-treated</td>
<td>180 ± 12*</td>
<td>374 ± 26</td>
<td>190 ± 15</td>
</tr>
</tbody>
</table>

*p < 0.05.

Gpp(NH)p = guanylylimidodiphosphate.
light vesicle fraction are uncoupled from adenylate cyclase and appear to be able to recycle to the surface upon removal of agonist. We recently applied similar methods to examine β-adrenergic receptor translocation in myocardial ischemia, a setting in which a rapid increase in receptors occurs. We previously found, and the current results confirm, that ischemia in guinea pigs is associated with an externalization of β-adrenergic receptors from a light vesicle intracellular pool, where they are functionally uncoupled from adenylate cyclase, to the surface plasma membrane, where they are functional. The evidence that myocardial β-adrenergic receptors can move between two subcellular compartments provides a means by which the heart can modulate its ability to respond to neuronally released or circulating catecholamines.

β-Adrenergic antagonists, especially propranolol, have long been used in clinical practice in the treatment of angina, hypertension, and more recently in the treatment of myocardial infarction, both in the acute and chronic stages. In 1974, Alderman et al. reported the precipitous development of unstable angina and myocardial infarction, both in the acute and chronic stages. In 1974, Alderman et al. 20 reported the development of unstable angina and myocardial ischemia after abrupt withdrawal of propranolol in patients with angina. Studies with both experimental animals and humans have demonstrated that chronic treatment with propranolol can produce an up-regulation of both myocardial and lymphocyte β-adrenergic receptors. Although the mechanism of this up-regulation of β-receptors with propranolol treatment was unknown, it appeared that in many cases there was enhanced sensitivity of the cardiovascular system to the effects of catecholamines.

The present study demonstrates that propranolol treatment, like ischemia, externalizes β-adrenergic receptors. This translocation partially depletes the intracellular pool of β-adrenergic receptors and increases the number of surface β-adrenergic receptors that are functional, as assessed by isoproterenol-stimulated adenylate cyclase activity. Thus, the current results explain the previously reported “up-regulation” of cardiac β-adrenergic receptors in crude membrane preparations after propranolol treatment. Presumably other treatments that reduce exposure of receptors to agonists (e.g., neurotransmitter depletion and nerve lesions) would also promote receptor externalization.

We speculate that a continuous shuttling of β-adrenergic receptors occurs between the surface plasma membrane and the intracellular light vesicles. Externalization of β-adrenergic receptors by propranolol is probably due to a blockade of tonic internalization promoted by endogenous catecholamines. Alternatively, propranolol treatment might decrease synthesis of β-adrenergic receptors (thereby depleting intracellular receptors) or accelerate the translocation of receptors to the surface.

Despite the considerable experimental evidence suggesting that early administration of β-adrenergic antagonists may reduce the severity of cellular injury, as well as the likelihood of serious ventricular arrhythmias in the setting of myocardial ischemia, the mechanisms of the beneficial effect of β-blockers have been poorly understood. The present study demonstrates that after chronic propranolol treatment leading to up-regulation and externalization of β-receptors, ischemia does not further alter receptor distribution. Thus, propranolol treatment and ischemia appear to access and mobilize the same pool of intracellular β-adrenergic receptors. It is unclear whether depletion of this intracellular pool of β-adrenergic receptors by chronic propranolol treatment is a major mechanism by which this drug is clinically efficacious in preventing some deleterious effects of ischemia, but we believe that our results provide a means to help explain a protective role of propranolol treatment in preventing arrhythmias or sudden death associated with myocardial ischemia.

References
Propranolol treatment externalizes beta-adrenergic receptors in guinea pig myocardium and prevents further externalization by ischemia.
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