Identification of $\alpha_1$-Adrenergic Receptors on Sarcolemma From Normal Subjects and Patients With Idiopathic Dilated Cardiomyopathy: Characteristics and Linkage to GTP-Binding Protein

Tarcisio Vago, Maurizio Bevilacqua, Guido Norbiato, Gabriella Baldi, E. Chebat, Pierluigi Bertora, Giorgio Baroldi, and Roberto Accinni

Discontinuous density sucrose gradient centrifugation was used to isolate membrane vesicles from the left ventricle of three normal subjects (one prospective organ donor and two traffic victims whose hearts were obtained 1 hour after death) and nine patients undergoing cardiac transplantation as a consequence of idiopathic dilated cardiomyopathy. Sarcolemma-enriched subcellular fractions, detected in the interface between 8.55% and 25% sucrose, were identified by the increased activity of Na$^+$,K$^+$-ATPase and by enrichment in $\beta$-adrenergic receptor density. The density of $\beta$-adrenergic receptors was lower in vesicles from diseased hearts (610±71 fmol/mg protein) than in vesicles from normal hearts (1,410±226 fmol/mg protein; $p<0.01$). $\alpha_1$-Adrenergic receptors were identified in these membrane vesicles by $[^3H]$prazosin binding. Specific binding of $[^3H]$prazosin was about 50% of the total binding at 1 nM, and $\alpha_1$-adrenergic binding sites were saturable at approximately 3 nM. Scatchard analysis revealed 58±5 fmol/mg protein ($K_D=0.90±0.08$ nM) in pathological hearts and 30±5 fmol/mg protein ($K_D=0.90±0.03$ nM) in normal hearts ($p<0.01$). The displacement curve of (-)-norepinephrine in membrane vesicles from normal hearts delineated one subpopulation of $\alpha_1$-adrenergic receptors; the addition of 0.1 mM GTP did not cause right shift. In membrane vesicles from diseased heart, the displacement curve of (-)-norepinephrine disclosed two subpopulations of $\alpha_1$-adrenergic receptors. A right shift that occurred after addition of GTP showed that in this case $\alpha_1$-adrenergic receptors were functionally coupled with GTP-binding protein. This study demonstrated the presence of $\alpha_1$-adrenergic receptors in sarcolemma-enriched subcellular fractions from both normal and idiopathic dilated cardiomyopathic hearts. Only $\alpha_1$-adrenergic receptors from pathological hearts were coupled to GTP-binding protein. (Circulation Research 1989;64:474–481)

The presence of $\alpha$-adrenergic receptors in the heart of many mammals has been well described.$^1$ In rat cardiomyocyte, $\alpha_1$-adrenergic receptors are coupled to a guanine nucleotide–binding protein, and activation of $\alpha_1$-adrenergic receptors is associated with changes in intracellular cyclic AMP (cAMP) content and in an altered phospholipid turnover.$^1$–$^5$ Skomedal et al$^6$ showed that norepinephrine increases contractility in human auricula by an $\alpha_2$-mediated adrenergic mechanism. However, methoxamine, a pure $\alpha_1$-adrenergic agonist, fails to increase contractility in normal human hearts.$^7$ Finally, $\alpha_1$-adrenergic receptors were identified with a radioiodinated ligand in human left ventricle,$^8$ but their characteristics were not reported.

The aim of this study was to characterize the human heart $\alpha_1$-adrenergic receptors by radioligand techniques. Since in preliminary experiments we did not find evidence of any specific $\alpha_1$-adrenergic receptor binding on the crude homogenate, we decided to investigate $\alpha_1$-adrenergic receptors on sarcolemma-enriched membrane vesicles from the left ventricle of normal subjects and of patients with
idiopathic dilated cardiomyopathy who were undergoing myocardial transplantation.

**Materials and Methods**

Chemicals used in this study included (+)-norepinephrine (Sterling-Winthrop, Rensselaer, New York); prazosin [7-methoxy-3H] (specific activity 80.9 or 82.0 Ci/mmol) and [3H]dihydroalprenolol (DHA) (specific activity 90.2 or 99.9 Ci/mmol) (New England Nuclear, Boston); and (-)-norepinephrine, EGTA, Tris-HCl, and alamethicin (Sigma Chemical, St. Louis).

**Preparation of Cardiac Membrane Vesicles**

All procedures were performed at 4° C.

Fifteen to 20 grams of the left ventricle (anterior inferior wall adjacent to apex) was obtained from nine male patients aged 28–53 years undergoing myocardial transplantation as a consequence of idiopathic congestive cardiomyopathy and from three normal subjects aged 27, 35, and 41 years, one a prospective organ donor and two traffic victims whose hearts were obtained at autopsy 1 hour after death. Excluded were patients with cardiomyopathic syndrome resulting from coronary artery disease.10 Medications taken by the patients included diuretics, digoxin, and vasodilators (nitrates and angiotensin-converting enzyme inhibitors). No recipient heart had been exposed to β-adrenergic or α1-adrenergic agonists or antagonists within 2 weeks of operation.

The tissues were freed of connective and adipose tissue and trimmed. They then were homogenized in a buffer containing 8.55% sucrose, 100 mM KCl, 25 mM Tris-HCl, 2 mM MgCl2, and 1 mM EGTA (pH 7.6), by use of a VirTis tissue disruptor (model 45, Gardiner, New York) twice at maximum speed for 10 seconds. The homogenate was filtered on a cheesecloth and centrifuged at 600g for 5 minutes. The pellet was discarded and the supernatant was centrifuged in the same buffer at 48,000g for 35 minutes. The pellet was resuspended in the same medium and recentrifuged at 48,000g for 35 minutes. This crude homogenate was resuspended in buffer containing 8.55% sucrose, 25 mM Tris-HCl, 2 mM MgCl2, and 1 mM EGTA (pH 7.6) by a Potter Elvehjem glass grinder (Vismarca, Milano, Italy) and then newly homogenized by a Polytron PT20 (Kinetica, Lucerne, Switzerland) (setting 8, five times for 7 seconds). The homogenate was centrifuged at 13,000g for 15 minutes, the pellet was discarded, and the supernatant was suspended in buffer without KCl and centrifuged at 160,000g for 45 minutes. This crude homogenate was resuspended in the same buffer (8.55% sucrose) and centrifuged at 160,000g for 90 minutes on a discontinuous sucrose gradient (25%, 30%, 35%, and 40%). The white rims that became evident at the interfaces between sucrose steps were saved. Fraction A was collected at the 8.55–25% interface, fraction B at the 25–30% interface, fraction C at the 30–35% interface, and fraction D at the 35–40% interface. These fractions were diluted with a buffer containing 50 mM Tris-HCl and 10 mM MgCl2 (pH 7.5) and washed twice at 160,000g for 60 minutes. The membranes to be used for binding experiments were resuspended in 50 mM Tris-HCl, 10 mM MgCl2, and 1 mM EGTA (pH 7.5), whereas the membranes to be used in adenosine triphosphatase (ATPase) assays were resuspended in the same buffer without EGTA. The membranes were immediately used or stored in liquid nitrogen.

**ATPase Assay**

Na⁺,K⁺-ATPase activity was assessed in a medium containing 20 μg/ml of vesicle protein, 3 mM MgCl2, 50 mM DL-histidine, 1 mM Tris-EGTA, 100 mM NaCl, 10 mM KCl, 5 mM NaN3, and 3 mM Tris-ATP (pH 7.4). Na⁺,K⁺-ATPase was evaluated as the difference between the activity in the presence and in the presence of 1 mM ouabain. Ca²⁺-ATPase activity was measured in a medium containing 40 μg/ml of vesicle protein, 3 mM MgCl2, 50 mM DL-histidine, 100 mM KCl, 0.1 mM CaCl2, 5 mM NaN3, and 3 mM Tris-ATP (pH 7.4). Ca²⁺-ATPase was evaluated as the difference between the activity in the absence and in the presence of 2 mM Tris-EGTA. ATPase assays were also performed after a previous incubation (20 minutes at 20° C) with alamethicin (alamethicin/protein ratio 0.4:1, wt/wt) to unmask enzyme activity inside intact vesicles.11–13 The ATPase activities were determined by measurement of the inorganic phosphorus released from ATP during an incubation at 37° C for 20 minutes. The reaction was stopped with trichloroacetic acid at the final concentration of 5%; the inorganic phosphorus was measured by the Fiske-SubbaRow assay in the supernatant after centrifugation at 3,500g for 15 minutes to remove the precipitate.

**Binding Assay**

[3H]Dihydroalprenolol binding. Crude homogenate (100 μg) or sucrose density membranes (20–50 μg) were incubated in polypropylene tubes containing 0.05–6 nM [3H]DHA at 37° C for 30 minutes. Nonspecific binding was determined by addition of 1 μM (-)-propranolol.14,15 Crude homogenate was also preincubated with alamethicin (alamethicin/protein ratio 0.4:1, wt/wt) to evaluate the effect of this pore-forming drug on β-adrenergic receptor binding.

[3H]Prazosin binding. Crude homogenate (100–200 μg) or sucrose density membranes (20–60 μg) were incubated in polypropylene tubes at 37° C for 60 minutes in the presence of 0.05–5 nM [3H]prazosin. The final volume was 200 μl. Nonspecific binding was determined by addition of 1 mM (-)-norepinephrine.2 Crude homogenate membranes were also preincubated with alamethicin (alamethicin/protein ratio 0.4:1, wt/wt) to evaluate if unmasking procedures could permit detection of α₁-adrenoceptors in this fraction.
In displacement experiments [3H]prazosin was used at the concentration of 0.8–1 nM, and norepinephrine was protected from oxidation by addition of superoxide dismutase and catalase.\(^6\) After incubation, the reaction was stopped by addition of 3 ml ice-cold buffer (50 mM Tris-HCl and 10 mM MgCl\(_2\) [pH 7.5]), and the samples were immediately vacuum filtered on Whatman GF/C filters (Whatman Int Ltd, Maidstone, England), which were then quickly rinsed twice with 10 ml of the same buffer.

The effect of 0.1 mM GTP on [3H]prazosin saturation curves in plasma membranes from both normal and diseased myocardium was evaluated. The GTP effect on plasma membranes from normal myocardium was also evaluated after preincubation for 30 minutes at 37\(^\circ\)C in the presence of agonist [1 mM (−)-norepinephrine] and successive extensive washing in an attempt to eliminate endogenous GDP. Filters were dried overnight and placed in scintillation vials containing 10 ml Filter-Count (Packard Instrument, Downers Grove, Illinois). Radioactivity was determined in a liquid scintillation spectrometer (1500 Tri-Carb, Packard Instrument) at the efficiency of 49.5%.

**Protein Determination**

Protein concentration was measured by Bradford assay.\(^7\)

**Binding Data**

Scatchard plots\(^8\) of concentration-dependent [3H]prazosin and [3H]DHA binding were linear and were analyzed by simple regression analysis. Displacement curves of [3H]prazosin with (−)-norepinephrine in the presence and the absence of 0.1 mM GTP were analyzed with a computer-assisted modeling technique.\(^9\) The goodness of fit for one-site or two-site binding models of displacement curves was evaluated according to Akaike.\(^20\)

**Statistical Analysis**

Enzymatic and binding data are expressed as mean±SD. Student’s t test was used for comparison of enzymatic activities and binding between normal and diseased hearts, with a statistical significance of \(p<0.05\) in a two-tailed distribution.

**Results**

**Subcellular Distribution of Na\(^+\),K\(^+-\)ATPase, Ca\(^2+-\)ATPase, and \(\beta\)-Adrenergic Receptors**

**Normal hearts.** In membrane vesicles from normal human left ventricle (Figure 1a), four subfractions were isolated at the interface between the gradients. Na\(^+\),K\(^+-\)ATPase, a sarcolemmal marker, was enriched in the light-density fractions A and B by about sixfold and fivefold, respectively, relative to the corresponding activity of the crude homogenate. Similar enrichment was observed in [3H]DHA binding (about sixfold in fraction A and fivefold in fraction B) (Figure 1a). The Scatchard analysis of [3H]DHA binding to membrane vesicles in fraction A from a normal heart is shown in Figure 2; these data are representative of those obtained from three normal hearts (mean values are reported in Table 1).

Conversely, the activity of the sarcoplasmic reticulum marker Ca\(^2+\)-ATPase was lowest in fractions A and B. In the denser fractions C and D, Ca\(^2+\)-ATPase activity was higher, whereas Na\(^+\),K\(^+-\)ATPase activity was lower (Figure 1a). Fraction D exhibited minimal Na\(^+\),K\(^+-\)ATPase activity, representing 4% of the activity in fraction A. The [3H]DHA binding was very low in fraction D (Figure 1a), indicating negligible contamination of this subfraction with sarcolemma.

**Diseased hearts.** In membrane vesicles from patients with dilated cardiomyopathy (Figure 1b), the distribution of Na\(^+\),K\(^+-\)ATPase and \(\beta\)-adrenergic receptors was similar to that observed in normal hearts. Figure 2 shows the Scatchard analysis of [3H]DHA binding to membrane vesicles in fraction A from a patient with dilated cardiomyopathy; these data are representative of those obtained from nine diseased hearts (mean values are reported in Table 1). The number of \(\beta\)-adrenergic receptors was lower in membrane vesicles from diseased hearts (\(p<0.01\)). No difference was observed in Na\(^+\),K\(^+-\)ATPase and Ca\(^2+\)-ATPase between normal and diseased hearts (Table 1).

**Alamethicin treatment.** Preincubation with alamethicin increased Na\(^+\),K\(^+-\)ATPase activity in fraction A in both normal and diseased hearts (\(p<0.05\) in both cases). The increase in Ca\(^2+\)-ATPase in fraction D after unmasking procedures was not statistically significant. No difference was observed between normal and diseased hearts in alamethicin-unmasked Na\(^+\),K\(^+-\)ATPase and Ca\(^2+\)-ATPase (Table 1). Alamethicin treatment did not affect the density (\(B_{max}\)) or the affinity (\(K_D\)) of \(\beta\)-adrenergic receptors in crude homogenate from either the normal hearts (\(B_{max}\): 189±10 fmol/mg, \(K_D\): 0.98±0.09 nM without alamethicin; \(B_{max}\): 175±13 fmol/mg, \(K_D\): 1.01±0.09 nM with alamethicin) (n=3) or the diseased hearts (\(B_{max}\): 81±13 fmol/mg, \(K_D\): 0.70±0.08 nM without alamethicin; \(B_{max}\): 73±10 fmol/mg, \(K_D\): 0.81±0.07 nM with alamethicin) (n=9).

**[3H]Prazosin Binding Studies**

Membrane fractions enriched in sarcolemma were used as source to characterize cardiac \(\alpha\)-adrenergic receptors since specific binding for [3H]prazosin was undetectable in the crude homogenate even after unmasking procedures with alamethicin.

**Normal hearts.** [3H]Prazosin binding to the membrane vesicles (fraction A) obtained from normal hearts was saturable when nonspecific binding was assessed with 1 mM (−)-norepinephrine. A Scatchard plot of the saturation curve, representative of the data obtained from three normal subjects, is shown in Figure 3 (mean values are reported in Table 1).
Binding of \(^{3}H\)prazosin to sarcolemma-enriched membrane vesicles from normal hearts was stereospecific. As shown in Figure 4, (−)-norepinephrine was about 100 times more potent than (+)-norepinephrine in displacing \(^{3}H\)prazosin from the membranes. The (−)-norepinephrine displacement curve was better explained by a one-site binding model when evaluated with Akaike test\(^{20}\) (p<0.01).

The data in Figure 3 are representative of those obtained from three normal hearts; the mean values are reported in Table 2.

The effects of GTP on the density of the receptors are reported in Table 1 and Figure 3. The addition of 0.1 mM GTP failed to modify the density of α\(_{1}\)-adrenergic receptors or their \(K_{D}\) (Table 1).

The addition of 0.1 mM GTP in the (−)-norepinephrine displacement experiments was not followed by a right shift. The data in Figure 3 are representative of those obtained in three normal hearts; mean values are reported in Table 2. After incubation at 37° C in the presence of agonist and
successive washings in an attempt to eliminate endogenous GDP, we were unable to detect any right shift with GTP in normal hearts.

Diseased hearts. [3H]Prazosin binding to the membrane vesicles (fraction A) obtained from patients with dilated cardiomyopathy was saturable [nonspecific binding in presence of 1 mM (-)-norepinephrine]. A Scatchard plot of the saturation curve representative of the data obtained from nine diseased hearts is shown in Figure 2; mean values are reported in Table 1. α1-Adrenergic receptor density was significantly higher in patients (Bmax, 58±5 fmol/mg protein) than in normal hearts (Bmax, 30±5 fmol/mg protein) (p<0.01). The KD of α1-adrenergic receptors from diseased hearts (KD, 0.90±0.08 nM) was similar to that observed in normal hearts (KD, 0.90±0.03 nM) (Table 1).

Binding of [3H]prazosin to sarcolemma-enriched membrane vesicles from diseased hearts was stereospecific. As shown in Figure 5, (-)-norepinephrine was about 100 times more potent than (+)-norepinephrine in displacing [3H]prazosin from the

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**TABLE 1. Characteristics of Adrenergic Receptors, Na⁺,K⁺-ATPase, and Ca²⁺-ATPase in Membrane Vesicles of Normal and Diseased Hearts**

<table>
<thead>
<tr>
<th></th>
<th>α-receptors</th>
<th></th>
<th>β-receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without GTP</td>
<td>With GTP</td>
<td>Without GTP</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Bmax (fmol/mg)</td>
<td>KD (nM)</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>30±5</td>
<td>0.90±0.03</td>
</tr>
<tr>
<td>Diseased</td>
<td>9</td>
<td>58±5*</td>
<td>0.90±0.08</td>
</tr>
</tbody>
</table>

n, number of hearts; Bmax, maximum density of binding sites; KD, dissociation constant.

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**FIGURE 3.** Scatchard analysis of saturation binding curve of [3H]prazosin to sarcolemma-enriched membrane fractions from normal heart (○) and from heart of patient with idiopathic dilated cardiomyopathy (●). Data represent nine experiments on diseased hearts and three experiments on normal hearts. Bmax, maximum density of binding sites; KD, dissociation constant.

**FIGURE 4.** Competition curve for [3H]prazosin binding sites by (-)-norepinephrine (○) and (+)-norepinephrine (●) in sarcolemma-enriched membrane fractions from normal heart. Data represent three experiments on normal hearts. [3H]prazosin displacement by (-)-norepinephrine in normal heart after addition of 0.1 mM GTP (●) was also evaluated. Curve in absence of GTP (○) is better explained by one-site binding model (Ki, 9.5 μM). Addition of GTP does not cause right shift (Ki, 10 μM). Data represent three experiments on normal hearts. NE, norepinephrine; Ki, equilibrium dissociation constant.
membranes. The (−)-norepinephrine displacement curve was best explained by a two-site binding model (p<0.01) and disclosed both high- and low-affinity binding sites (Table 1). Data in Figure 5 are representative of displacement experiments with (+)-norepinephrine on three hearts and with (−)-norepinephrine on nine hearts; mean values are reported in Table 2. The addition of 0.1 mM GTP did not modify the density or the affinity of α₁-adrenergic receptors for [3H]prazosin in vesicles from diseased hearts (Table 1). The addition of 0.1 mM GTP in the (−)-norepinephrine displacement experiments was followed by a significant rightward shift; the displacement curve in the presence of GTP was best explained by a one-site binding model (p<0.01). The data in Figure 5 are representative of those obtained from nine diseased hearts; mean values are reported in Table 2.

Discussion

This study identified and characterized α₁-adrenergic receptors in sarcolemma-enriched membrane vesicles. The separation of sarcolemma from sarcoplasmic reticulum was obtained by identifying biochemical activities unique to each membrane fraction. The activities analyzed were Na⁺,K⁺-ATPase and Ca²⁺-ATPase. The first is a membrane-embedded transport protein that establishes and maintains gradients for Na⁺ and K⁺ ions across cell membranes by coupling the translocation of these ions to the enzymatic hydrolysis of ATP. The second is a sarcoplasmic reticulum enzyme that hydrolyzes ATP in the presence of micromolar concentration of Ca²⁺ to yield ADP and inorganic phosphate. β-Adrenergic receptors were identified in Na⁺,K⁺-ATPase-enriched fractions. The density of β-adrenergic receptors in the lightest fractions was considerably higher than that observed in the crude homogenate, confirming that a substantial enrichment of sarcolemma in these fractions was obtained. The density of β-adrenergic receptors was considerably lower in membrane vesicles from diseased hearts than in membrane vesicles from normal hearts, confirming the data by Bristow et al. whereas Vatner et al. reported an increased density of β-adrenergic receptors with loss of high affinity. The discrepancy between data obtained in myocardium from patients with idiopathic congestive cardiomyopathy in this study and others and in dogs with pressure-overload left ventricular failure may be related to species difference and/or different pathogenetic mechanisms of heart failure.

While Bristow et al. reported in a preliminary form the detection of a small density of α₁-adrenergic receptors on crude homogenate, others have reported a marginal density of α₁-adrenergic receptors (less than 10 fmol/mg tissue) that precludes biochemical analysis. The failure to identify α₁-adrenergic receptors in our crude homogenate

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**TABLE 2. Affinity and Percentage of α₁-Adrenergic Receptors in High- and Low-Affinity States Plasma Membrane Fractions of Normal and Diseased Hearts**

<table>
<thead>
<tr>
<th></th>
<th>Without GTP</th>
<th>With GTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>α₁-H (%)</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>28±4</td>
</tr>
<tr>
<td>Diseased</td>
<td>9</td>
<td>98±4</td>
</tr>
</tbody>
</table>

n, number of hearts; Kᵢ, equilibrium dissociation constant. Measurements taken of high (α₁-H) and low (α₁-L) affinity states in sarcolemma-enriched plasma membrane fractions from normal and idiopathic dilated cardiomyopathic hearts; mean±SD of n hearts.

**FIGURE 5. Competition curve for [3H]prazosin binding sites by (−)-norepinephrine (○) and (+)-norepinephrine (●) in sarcolemma-enriched membrane fraction from patient with idiopathic dilated cardiomyopathy. Data represent nine experiments with (−)-norepinephrine and three experiments with (+)-norepinephrine on diseased hearts. [3H]prazosin displacement by (−)-norepinephrine in diseased heart after addition of 0.1 mM GTP (○) is also evaluated. Curve (○) is better explained by a two-site binding model, one with high affinity (α₁-H) (Kᵢ=0.1 µM, 29% of total number of receptors) and the other with lower affinity (α₁-L) (Kᵢ=22 µM, 71% of total number of receptors). Addition of GTP causes an evident right shift; curve (○) is explained by a one-site binding model (Kᵢ, 10 µM). Data represent nine experiments on diseased hearts. NE, norepinephrine; Kᵢ, equilibrium dissociation constant.**
preparation even after alamethicin treatment may be due to an extremely unfavorable noise-to-signal ratio.

The characteristics of membrane vesicles were also investigated after alamethicin treatment to evaluate possible variations of Na⁺, K⁺-ATPase activity and radioreceptor binding in plasma membrane vesicles treated to increase their permeability. The increased activity of Na⁺, K⁺-ATPase after alamethicin treatment suggests the possible formation of "inside-out" vesicles. However, alamethicin treatment did not consistently affect the binding of β-adrenergic antagonists to membrane vesicles. It is tempting to speculate that since [³H]DHA is a lipophilic drug, it is able to cross plasma membranes and to equally label adrenergic receptors in both right-side-out and inside-out vesicles.

The density of α₁-adrenergic receptors was higher in sarcolemma-enriched membranes from patients with idiopathic dilated cardiomyopathy, whereas the affinity of [³H]prazosin for the receptors was similar in normal subjects and in patients. Sarcolemma from patients with idiopathic dilated cardiomyopathy thus displays a decreased density of β-adrenergic receptors and an increased density of α₁-adrenergic receptors. The preparation of sarcolemma-enriched membrane vesicles was suitable for the characterization of the coupling of these receptors to the GTP binding protein. As is known, the interaction of the receptors with the GTP binding protein may be demonstrated by the presence of two affinity states of the receptor and the loss of high-affinity state in the presence of GTP. Our results suggested that the α₁-adrenergic receptors of normal heart do not fulfill these two requirements. In fact, the agonist displacement curve with (-)-norepinephrine was best explained by a one-site binding model; the addition of GTP did not induce a rightward shift, suggesting that α₁-adrenergic receptors in normal human sarcolemma are not linked to a GTP binding protein.

By contrast, in membrane vesicles from patients, the [³H]prazosin displacement with (-)-norepinephrine clearly delineated two subpopulations of α₁-adrenergic receptors. After addition of GTP a significant rightward shift of the competition curve was obtained in these membrane vesicles with disappearance of the high-affinity subpopulation. This suggested that α₁-adrenergic receptors in idiopathic dilated cardiomyopathy are functionally coupled to a guanine-nucleotide binding protein.

In conclusion, α₁-adrenergic receptors were identified in sarcolemma-enriched subcellular fractions from both normal and idiopathic dilated cardiomyopathic hearts. These receptors were coupled to GTP binding protein in pathological hearts but not in normal hearts.

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