Vasoactive Intestinal Peptide Receptor in Failing Human Ventricular Myocardium Exhibits Increased Affinity and Decreased Density

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We investigated vasoactive intestinal peptide (VIP)-receptor pharmacology in failing and nonfailing human ventricular myocardium by examining [125I]VIP binding in membrane fractions of left ventricle and inotropic effects of VIP in isolated right ventricular trabeculae mounted in tissue baths. [125I]VIP binding demonstrated upwardly concave, curvilinear Scatchard plots consistent with two classes of binding sites. Only the high-affinity (dissociation constant \( K_d \) 400–800 pM) site could be regulated by guanine nucleotides. Compared with nonfailing heart, membranes derived from failing heart exhibited a twofold reduction in the \( K_d \) of the high-affinity VIP binding site, whereas the receptor density (\( B_{max} \)) was decreased by 62%. In concordance with this decreased receptor density and increased affinity, the maximal contractile response of right ventricular trabeculae from failing right ventricles was decreased by 61%, and the dose-response curve to VIP was left-shifted approximately threefold. We conclude that the VIP receptor in failing human ventricular myocardium exhibits novel regulatory behavior consisting of increased receptor affinity and decreased receptor density.

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Vasoactive intestinal peptide (VIP) is a 28-amino acid-peptide neurotransmitter widely distributed in neural tissue of the mammalian central nervous system and other organs.1,2 VIP is present in cardiac tissues of several mammalian species.3–6 The majority of immunoreactive material is found in atria; much smaller amounts are present in ventricular myocardium.4 VIP mediates physiological effects through VIP-specific membrane receptors that are coupled to adenylate cyclase in myocardial membranes.5

Failing human ventricular myocardium is characterized by selective \( \beta_1 \)-receptor down-regulation8 and partial uncoupling of \( \beta_2 \)-receptors from adenylate cyclase.9 Failing ventricular myocardium is also subsensitive to \( \beta 
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 agonist stimulation of adenylate cyclase and muscle contraction.8,9 In contrast, we have reported that the failing human heart is supersensitive to VIP stimulation of adenylate cyclase.10

The purpose of the current investigation was to determine the mechanism for this VIP-mediated supersensitivity response by directly measuring VIP receptors and VIP-mediated muscle contraction responses in preparations derived from nonfailing and failing human ventricular myocardium.

Materials and Methods

Cardiac Tissue Procurement

Human cardiac tissue was obtained from the Utah and Stanford cardiac transplant programs. Ventricles of explanted hearts removed from heart transplant recipients were used as sources of failing heart; hearts from prospective donors not used for transplant and the left ventricles of heart-lung transplant recipients were used as nonfailing controls. Myocardial membranes were prepared from 13 severely failing (average cardiac index, 2.1±0.2 l/min/m²; mean pulmonary wedge pressure, 19.8±2.7 mm Hg; mean right atrial pressure, 6.5±1.6 mm Hg; all values are mean±SEM) left ventricles explanted from patients with end-stage biventricular failure secondary to idiopathic dilated cardiomyopathy of whom none had received \( \beta 
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 receptor agonist infusions before transplantation and from six nonfailing hearts from organ donors with normal cardiac function by history and/or by echocardiogram. The
hearts from the donors were not used for transplantation because of late-developing recipient exclusions, because of recipient blood type or size mismatch, or in two cases, because of age exclusion. In all cases, repeated attempts were made to place these hearts for organ donation through the United Network for Organ Sharing. Three additional non-failing left ventricles were from heart-lung recipients with primary pulmonary hypertension and normal left ventricular function by echocardiogram.

Medications taken by all subjects with biventricular failure included diuretics and vasodilators, and two were also taking digoxin. Subjects with primary pulmonary hypertension were all taking diuretics, with two taking vasodilators and one taking digoxin. Organ donors were taking no cardiovascular medications before brain death. No subject received a β-receptor active agent (agonist or antagonist) within 48 hours of tissue procurement.

For tissue bath studies, 28 additional hearts were used, including 20 hearts with severe biventricular failure (average cardiac index, 2.0±0.1 l/min/m²; mean pulmonary wedge pressure, 24.6±1.3 mm Hg; mean right atrial pressure, 6.9±0.9 mm Hg; all values are mean±SEM) procured from the Utah Cardiac Transplant Program. Fourteen of these hearts had idiopathic dilated cardiomyopathy, and six hearts had ischemic cardiomyopathy. Non-failing trabeculae were obtained from eight right ventricles with normal function by ejection fraction and echocardiographic criteria.

Myocardial Membrane Preparations

Crude myocardial membrane preparations suitable for VIP- and β-receptor–binding studies were made as previously described. Briefly, 5-g aliquots of left ventricular free wall were minced in 10 volumes of a solution containing ice-cold 10 mM Tris and 1 mM EGTA (pH 8.0) and then homogenized with a polytron (three bursts for 5 seconds each). An equal volume of ice-cold 1 M KCl was added and stirred for 15 minutes. The preparation was centrifuged at 50,000 × g for 15 minutes; then the final pellet was resuspended in a solution of 75 mM Tris and 10 mM MgCl₂ (pH 7.5). After two more centrifugations and a resuspension, the final pellet was resuspended in a solution of 250 mM sucrose, 50 mM Tris buffer, and 1 mM EGTA (pH 7.5) to give a final protein concentration of 5–8 mg/ml. This preparation was frozen at −80°C until used.

Crude particulate preparations suitable for measuring adenylate cyclase activity were made from a 2-g aliquot of left ventricular free wall, as previously described.(

Receptor Binding Assays

VIP receptor binding assays were performed with [¹²⁵I]VIP radiolabeled to 2,000 Ci/mmol. Initial experiments were directed at decreasing nonspecific binding and indicated that increasing concentration of bovine serum albumin (BSA) decreased nonspecific binding of [¹²⁵I]VIP to polypropylene incubation tubes from 20–30% without BSA to 2–3% in the presence of 3% BSA. Presoaking Whatman GF/C glass fiber filters (Whatman, Clifton, New Jersey) in 0.5% polyethyleneimine combined with 3% BSA in the incubation buffer reduced nonspecific filter binding to less than 1% of added radioligand. Nonspecific binding under these conditions was linear for added [¹²⁵I]VIP. Saturation binding experiments with all reagents present except myocardial preparations (no membrane "buffer blank" tubes) demonstrated that no specific binding was present. Additional preliminary experiments demonstrated that hypotonic buffer (10–20 mM) and a pH of 7.40 resulted in optimal specific binding. Sodium, calcium, and magnesium had no effects on binding other than to lower nonspecific binding by a small degree. Because increasing magnesium concentration decreased nonspecific binding and because magnesium promotes agonist binding in some systems, 5 mM MgCl₂ was used in the assay buffer. Dissociation kinetics revealed reversible specific binding, with a T½ of approximately 30 minutes.

Saturation binding assays were performed with 20 increasing concentrations of [¹²⁵I]VIP (Tyro-¹²⁵I)-VIP (5 pM–50 nM), with total and nonspecific binding tubes in triplicate. Specific binding approached 80% at low radioligand concentrations and decreased to 30–40% at higher radioligand concentrations.

In order to vary radioligand concentrations over five orders of magnitude, unlabeled VIP was added to [¹²⁵I]VIP at higher concentration ranges (50 pM–50 nM). The first four concentrations (5–25 pM) were obtained with only [¹²⁵I]VIP; the next 10 concentrations (50 pM–5 nM) were obtained with a 30-fold dilution of [¹²⁵I]VIP with unlabeled VIP; the final six concentrations (5–50 nM) were obtained with a further 10-fold dilution with unlabeled VIP. Left ventricular myocardial membranes were suspended in a final volume of 900 μl buffer solution containing 10 mM Tris, 5 mM MgCl₂, and 3% BSA, pH 7.40, to give a final protein concentration of 50–75 μg/ml and were incubated for 100 minutes at 22°C in the presence or absence of 3 μM unlabeled VIP. Bound ligand was trapped by vacuum filtration through Whatman GF/C 2.4-cm glass fiber filters presoaked in 0.5% polyethyleneimine. The filters were immediately washed with 20 ml of 10 mM Tris and 5 mM MgCl₂ and then were dried and counted on a gamma counter (model 4200, Micromedic, Horsham, Pennsylvania) with a counting efficiency of 65%.

For competition binding experiments, the final assay conditions were the same as those for saturation binding assays, except that a fixed concentration of radioligand (1 nM) was used to yield occupancy of approximately the same number of high- and low-affinity binding sites.

β-receptor saturation binding assays were performed, as previously described, with [¹²³I]iodocyanopindolol (ICYP).
Adenylate Cyclase

Adenylate cyclase assays were performed by a radioenzymatic technique with previously described methods.\(^1\)

Tissue Bath Studies

Isolated right ventricular trabeculae were used to measure contractile response of human ventricular myocardium to VIP, isoproterenol, and calcium with previously described methods.\(^1\) Trabeculae from 20 failing and eight nonfailing right ventricles were dissected into uniform strips of 1-2 mm × 6.0-7.0 mm and mounted in a plastic multichamber tissue bath maintained at 37° C. The isolated tissues were bathed in physiological salt solution (Tyrode’s buffer (mM): NaCl 118, KCl 4, MgSO\(_4\) 1.2, NaHCO\(_3\) 25, NaH\(_2\)PO\(_4\) 1.2, dextrose 5, and CaCl\(_2\) 2) bubbled with 95% O\(_2\)-5% CO\(_2\) to give a pH of 7.4. The isolated trabeculae were placed at a resting tension that yielded maximum contractile response, usually of approximately 1 g tension. Trabeculae were field-stimulated at 5-10% above threshold voltage at 1 Hz with a 5-msec pulse wave delivered with platinum electrodes. Trabeculae were attached to stationary force transducers, and isometric developed tension was recorded with a calibrated strip chart recorder. Agonists were added in cumulative doses.

For studies with VIP, BSA was added to each bath to give a final concentration of 0.1%. BSA alone did not alter the tension responses. In three preliminary experiments, a further increase in inotropic effect was not found with concentrations of VIP greater than 1 \(\mu\)M, and because of the substantial expense of using VIP in concentrations greater than 1 \(\mu\)M, this concentration was considered maximal response. VIP was added to baths at 8-minute intervals; isoproterenol was added at 2-minute intervals. At the completion of other pharmacological studies, the inotropic response to 10 mM calcium chloride was measured.

Creatine Kinase

Creatine kinase activity was measured in the supernatants of adenylate cyclase preparations, as previously described.\(^1\)

Materials

Synthetic VIP (>99% peptide purity by high-performance liquid chromatography) was purchased from Bachem, Torrance, California. \([^{125}\text{I}]\text{VIP}\) and \([^{125}\text{I}]\text{ICYP}\) were from Amersham, Arlington Heights, Illinois. Polyethyleneimine, non-hydrolyzable guanine nucleotide GppNHp, \((-)\text{isoproterenol}, \text{and BSA (radioimmunoassay grade)}\) were purchased from Sigma Chemical, St. Louis, Missouri.

Data and Statistical Analyses

Receptor binding data. VIP receptor maximum binding estimates (\(B_{\text{max}}\) and \(B_{\text{max}}\)) and dissociation constants (\(K_{\text{d1}}\) and \(K_{\text{d2}}\)) were determined by nonlinear least-squares computer modeling for the best fit of the specific binding curve (total binding less nonspecific binding), with the dependent variable bound counts per minute and the independent variable counts per minute of free \([^{125}\text{I}]\text{VIP}\). Two separate computer programs were used, BDATA (EMF Software, Philadelphia, Pennsylvania) and LIGAND (Peter Munson, National Institutes of Health, Bethesda, Maryland). The weighting function used in both programs was 1/F\(^2\), where F is the free radioligand concentration. Both programs use an \(F\) test to determine the number of binding sites present. In all cases but one, a two-site fit was superior (\(p<0.05\)) to a one-site model. Receptor \(B_{\text{max}}\) was expressed in femtomoles (fmol) \([^{125}\text{I}]\text{VIP}\) bound/mg protein, as measured by the Peterson modification of the Lowry method.\(^1\) \([^{125}\text{I}]\text{VIP}\) competition binding data were analyzed with BDATA, a part of the BDATA package, for the best fit of a two-site model. \(\beta\)-Receptor \(B_{\text{max}}\) and \(K_{\text{d}}\) were determined by nonlinear least-squares computer modeling for the best fit of the specific binding curve as previously described.\(^6\)

Receptor density and \(K_{\text{d}}\) were compared in the two groups by nonpaired Student’s \(t\) test. A \(p<0.05\) in the two-tailed distribution was considered statistically significant.

Tissue bath data. Data were expressed as milligrams tension developed above basal tension. For experiments in which full VIP (13 experiments) and isoproterenol (12 experiments) dose-response curves were performed, the tension responses of all trabeculae that received VIP or isoproterenol were averaged together. Dose-response curves were modeled to a four-parameter logistic equation of DeLean et al\(^{16}\) to find the best fit of a sigmoidal curve, including minimum, maximum, \(ED_{50}\), and slope. For VIP dose-response curves, the maximum developed tension (value at 1 \(\mu\)M) was assigned to 10 \(\mu\)M and 100 \(\mu\)M concentrations in order to enhance curve fitting because the expense of synthetic VIP prohibited routine use of higher concentrations than 1 \(\mu\)M and preliminary experiments demonstrated no additional inotropic effect beyond this concentration.

Individual VIP and isoproterenol dose-response curve \(ED_{50}\), slopes, and maxima were compared in failing and nonfailing groups by a nonpaired Student’s \(t\) test. Additionally, entire dose-response curves were compared in the failing and nonfailing groups by an analysis of covariance, as previously described.\(^9\)

Results

Characterization of VIP Receptor Binding

The time course and effects of temperature on VIP binding are shown in Figure 1. In this initial experiment, the 22° C isotherm was only extended to 80 minutes, but in four additional experiments that were extended to 120 minutes and one experiment that was extended to 240 minutes, specific
TIME AND TEMPERATURE EFFECTS ON \[^{125}\text{I}]\text{VIP}\) BINDING

Graphs of time courses of \[^{125}\text{I}]\text{vasoactive intestinal peptide (VIP)} binding at five temperatures. Specific binding is plotted vs incubation time in minutes. Note that specific binding is maximal at 22° C, with evidence of decreasing specific binding at 30° C and 37° C. Time-temperature isotherms were repeated twice more for 15° C and 30° C with similar results. Because in the initial experiment shown here, the 22° C time course was only extended to 80 minutes, four additional 22° C isotherms were performed; three were extended to 120 minutes, and one was extended to 240 minutes. Specific binding was maximal by 80 minutes, with no decrease in specific binding even after 240 minutes. Thus, 100 minutes and 22° C were selected as standard assay conditions. CPM, counts per minute.

\[^{125}\text{I}]\text{VIP Receptor Binding in Preparations Derived From Nonfailing and Failing Ventricles}

A representative VIP saturation binding curve is shown in Figure 2. Because of the range of VIP concentrations necessary to occupy both the high- and low-affinity binding sites, the data are divided into two different ranges. The entire data set is presented as a Scatchard plot in Figure 3; it can be seen that the plot is curvilinear and upwardly concave, consistent with two classes of binding sites.

Saturation binding assays were performed on myocardial membrane preparations from nine failing and nine nonfailing left ventricles (LVs). Each experiment was designed such that preparations from both failing and nonfailing ventricles were measured in the same assay. As can be seen in Table 1, BDATA and LIGAND data analyses yield similar results, with the high-affinity VIP \(K_d\) value being approximately 49% lower in the failing group. Likewise, the high-affinity \(B_{\text{max}}\) in failing LVs was approximately 40% (36.8% and 38.7%, respectively) of the value in nonfailing LVs. For the low-affinity site, no differences between failing and nonfailing LVs were found for either the \(K_d\) or the \(B_{\text{max}}\) values.

During the characterization of the VIP receptor binding assay, we also performed competition curves between \[^{125}\text{I}]\text{VIP}\) and unlabeled VIP to assess the reliability of the \(K_d\) measured by saturation binding assays. A representative experiment is presented in Figure 4; this is the same preparation of failing LV used for the saturation binding curves shown in Figure 3. The \(K_d\) of 165 pM versus 226 pM and 27.9 nM versus 26.6 nM at the high- and low-affinity binding sites, respectively, represent reasonable agreement of \(K_d\) values for these two types of experiments.

Guanine nucleotides may modulate receptor affinity for agonists, and for adenylate cyclase-coupled receptors, such modulation suggests the presence of a "physiological" binding site, that is, a receptor coupled to a signal transduction pathway. To determine if the two VIP binding sites are modulated by guanine nucleotides, we examined the effects of increasing concentrations of the non-hydrolyzable guanine nucleotide GppNHp on \[^{125}\text{I}]\text{VIP binding}. With low (<1 nM) VIP concentrations, GppNHp (10 nM-100 \(\mu\text{M}\)) demonstrated linear inhibition of binding from 0.1 to 100 \(\mu\text{M}\) GppNHp, with higher concentrations of GppNHp resulting in greater than 80% inhibition of specific binding at 200 pM \[^{125}\text{I}]\text{VIP}. Additionally, three saturation binding experiments were conducted in the presence and absence of 0.1 mM GppNHp. In all three experiments, the high-affinity VIP binding site shifted to a lower affinity in the presence of GppNHp without change in receptor density, whereas binding reached plateau by 100 minutes and specific binding remained stable to 240 minutes. In three experiments, increasing the concentration (35-200 \(\mu\text{g/ml}\)) of myocardial membrane protein gave linear increases in specific binding.
**FIGURE 2.** Graphs of saturation binding isotherm. Total (△) nonspecific (■), and specific (●) binding points are shown for a representative 20-point saturation binding experiment performed with myocardial membranes derived from a failing human left ventricle. In the top panel, points 1–10 are shown, and points 8–20 are shown in the bottom panel. [125I]vasoactive intestinal peptide concentrations on the x-axis are given in picomolar units (pM) in both panels.

**FIGURE 3.** A representative BDATA Scatchard plot of [125I]vasoactive intestinal peptide (VIP) binding is shown, bound/free versus bound, in counts per minute. The curve was modeled for a two-site fit, with the high-affinity binding site $K_d$ of 228 pM and $B_{max}$ of 114 fmol/mg protein and the low-affinity binding site $K_d$ of 26.6 nM and $B_{max}$ of 12,100 fmol/mg protein. The two-site fit was statistically superior compared with a one-site fit, $p<0.001$. In this experiment, the myocardial membrane preparation was derived from failing human left ventricular myocardium.
TABLE 1. Summary of Vasoactive Intestinal Peptide Saturation Curve Binding Data Derived From BDATA and LIGAND Nonlinear Regression Fitting Programs

<table>
<thead>
<tr>
<th></th>
<th>Nonfailing LV (n=9)</th>
<th>Failing LV (n=9)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>BDATA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d$ (pM)</td>
<td>846±147.7</td>
<td>418±112.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$B_{\text{max}}$ (fmol/mg)</td>
<td>389±76.2</td>
<td>143±29.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td>94.1±33.0</td>
<td>59.2±19.5</td>
<td>NS</td>
</tr>
<tr>
<td>$B_{\text{max}}$ (fmol/mg)</td>
<td>23,380±9,704</td>
<td>20,600±6,636</td>
<td>NS</td>
</tr>
<tr>
<td><strong>LIGAND</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d$ (pM)</td>
<td>902±163.8</td>
<td>457±113.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$B_{\text{max}}$ (fmol/mg)</td>
<td>411±85.6</td>
<td>159±33.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td>112±41.5</td>
<td>69.3±23.7</td>
<td>NS</td>
</tr>
<tr>
<td>$B_{\text{max}}$ (fmol/mg)</td>
<td>25,800±10,830</td>
<td>22,300±7,065</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV, left ventricle; $K_d$, $K_d$, $K_d$, and $K_d$, dissociation constants; $B_{\text{max}}$, $B_{\text{max}}$, $B_{\text{max}}$, receptor density; NS, not significant.

the low-affinity VIP binding site demonstrated no change in receptor density or affinity. A representative experiment with myocardial membranes prepared from a failing LV is shown in Figure 5. In this experiment, the high-affinity VIP binding site $K_d$ in the presence of 0.1 mM GppNHp shifted from 144 pM to 1.03 nM without a change in $B_{\text{max}}$ (156 fmol/mg vs. 133 fmol/mg), whereas the low-affinity VIP binding site demonstrated no change in $K_d$ or $B_{\text{max}}$ (35.0 vs. 35.8 nM, 4.8x10^4 vs. 4.9x10^4 fmol/mg). For the three experiments, the mean±SEM values for the high-affinity $K_d$ shift were from 380±236 pM in the absence of GppNHp to 2,120±1,500 pM in the presence of GppNHp. Under both conditions, a two-site fit was superior to a one-site fit in all cases ($p<0.001$ by F test, as described above).

**$\beta$-Receptor Binding Data in Nonfailing and Failing Ventricles**

$\beta$-adrenergic receptor maximum binding values were reduced in the failing group (47.4±5.7 fmol/mg protein vs. 73.4±5.8 fmol/mg protein, $p<0.001$, failing vs. nonfailing) without change in ICYP $K_d$ (10.3±1.4 vs. 7.0±0.8 pM, failing vs. nonfailing, $p=NS$). These values were similar to previous reports from this laboratory.8,11

**Myocardial Membrane Markers**

Adenylate cyclase activity in the presence of 10 mM sodium fluoride was not different between failing (n=7) and nonfailing (n=9) preparations (respective values of 37.06±3.14 vs. 41.38±4.83 pmol cyclic AMP/min/mg, $p=NS$). Soluble creatine kinase activity likewise was not different between failing and nonfailing heart (respective values of 961±127 vs. 1,153±117 IU/g, $p=NS$).

**Tissue Bath Data**

VIP muscle bath data were compiled in two data sets because 13 initial experiments were performed with either one or three concentrations of VIP, and the subsequent 15 experiments consisted of nine-point VIP dose-response curves. The first data set includes the responses of trabeculae from all 28 hearts exposed to 10 nM, 100 nM, and 1 μM VIP concentrations. These data are summarized in Table

**FIGURE 4.** Graph of a [125]vasoactive intestinal peptide (VIP) competition binding curve is shown for the best two-site fit, as determined by CDATA. The myocardial membrane preparation used for this experiment was from the same failing left ventricle used for the saturation binding curves shown in Figure 3. The curve was modeled for a high-affinity binding site $K_d$ of 165 pM and for a low-affinity binding site $K_d$ of 27.9 nM, in agreement with the $K_d$s determined by BDATA and LIGAND for the saturation binding experiment above.
FIGURE 5. Graphs of the nonhydrolyzable guanine nucleotide GppNHz effects on vasoactive intestinal peptide (125I)VIP binding. The upper panel shows a LIGAND displacement plot of a saturation binding isotherm without GppNHz; the middle panel shows a saturation binding isotherm performed side-by-side in the same experiment in the presence of 0.1 mM GppNHz. The lower panel shows the two LIGAND plots superimposed on one another. The initial parts of the binding isotherms result primarily from the high-affinity VIP binding site, and this portion of the binding curve shifted to a lower affinity in the presence of GppNHz. In contrast, the terminal portions of the binding curves that result primarily from the low-affinity binding sites are virtually superimposed on one another and show that GppNHz had little effect at the low-affinity VIP binding site.
Table 2. Inotropic Responses of Human Right Ventricular Trabeculae to Vasoactive Intestinal Peptide and Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>Nonfailing RV (n=8)</th>
<th>Failing RV (n=20)</th>
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<tbody>
<tr>
<td></td>
<td>Number of trabeculae</td>
<td>Tension (mg)</td>
</tr>
<tr>
<td>VIP 10 nM</td>
<td>27</td>
<td>101±21.0</td>
</tr>
<tr>
<td>VIP 100 nM</td>
<td>27</td>
<td>443±107.2</td>
</tr>
<tr>
<td>VIP 1 μM</td>
<td>27</td>
<td>810±129.6</td>
</tr>
<tr>
<td>Iso max</td>
<td>24</td>
<td>2,070±331.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>61</td>
<td>1,590±122.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. RV, right ventricle; VIP, vasoactive intestinal peptide; Iso max, isoproterenol maximal tension responses; Calcium, maximal inotropic effect of 10 mM calcium chloride.

2, along with maximum contractile responses for isoproterenol and calcium. The VIP data are also shown graphically in Figure 6, where data inspection reveals two general findings. First, in failing heart, contractile response at the highest concentration of VIP is markedly decreased (810±129.6 vs. 313±44.1 mg, p<0.001), whereas contractile response at the lowest concentration of VIP demonstrates a twofold increase (179±24.6 vs. 101±21.0 mg, p<0.02). Second, the apparent slopes of these “three-point dose-response curves” are very different with the responses from failing heart demonstrating a lower slope. In an attempt to obtain further quantitation of these apparent differences, we performed additional experiments evaluating a complete range of VIP concentrations between 10⁻¹⁰ and 10⁻⁶ M VIP.

The results of these nine-point VIP dose-response curves compared with their corresponding isoproterenol dose-response curves are summarized in Table 3 and in Figures 7 and 8. In failing ventricle, the mean ED₅₀ of the individual VIP dose-response curves was shifted leftward by 2.9-fold (4.04±1.28 vs. 11.70±2.17×10⁻⁸ M, p<0.02). Although maximum responses from full VIP dose-response curves (987 vs. 350 mg, Table 3) were in good agreement with the responses at 1 μM VIP presented in Table 2, in the smaller data set of fewer dose-response curves, this difference was not statistically significant (p=0.08).

Isoproterenol maximal tension responses, as given in Table 2 and Figure 8, were likewise decreased in failing versus nonfailing trabeculae (985±103.2 vs. 2,070±331.5 mg, p<0.005). In contrast, maximum inotropic effect of 10 mM calcium chloride (in 61 trabeculae from eight nonfailing and 120 trabeculae from 16 failing right ventricles) gave no difference (1,590±122.4 vs. 1,286±103.2 mg, p=NS).

In contrast to left-shifted (supersensitive) ED₅₀s derived for VIP augmented muscle contraction data in failing heart, the average isoproterenol dose-response curve ED₅₀ from these same hearts was right-shifted by 2.4-fold (ED₅₀×10⁻₈ M isoproterenol: 2.91±0.556 vs. 1.23±0.169, p<0.02, failing vs. nonfailing), as shown in Table 3. The maximal isoproterenol tension was reduced in the failing hearts (1,018±225.5 vs. 2,173±266.7 mg, p<0.01), also in agreement with the larger data set given in Table 2.

To better appreciate the quantitative differences in ED₅₀s, individual VIP and isoproterenol dose-response curves were also normalized to their own maximal responses and then averaged together to give “percent maximal” dose-response curves, as shown in Figures 7 and 8. The VIP dose-response curves from failing heart are left-shifted by approximately twofold in agreement with the average of individual ED₅₀s, as presented in Table 3. Likewise, average and percent maximal isoproterenol dose-response curves from failing heart are right-shifted by approximately 1.5–2-fold, both in close agree-
ment with the average of individual dose-response curve data given in Table 3.

Discussion

In the current investigation, the pharmacology of VIP in failing and nonfailing human ventricular myocardium was examined by receptor binding and muscle contraction studies. \[^{[2]}\] VIP receptor binding revealed biphasic, upwardly concave, curvilinear Scatchard plots that could be resolved into high- ($K_a$, 400–800 nM) and low-affinity ($K_a$, 50–100 nM) binding sites, consistent with what has previously been described for the VIP receptor in other tissues.\[^{18,19}\]

The high-affinity binding site could be regulated by guanine nucleotides, which is consistent with what would be expected for a G-protein-coupled "physiological" agonist binding site.\[^{17}\] These observations are in agreement with previous findings of the significance of high- and low-affinity VIP binding sites in other tissues.\[^{18,19}\] In contrast, agonist binding to the low-affinity site was not affected by guanine nucleotides. This occurrence suggests that this site may not be biologically active.

\[^{[2]}\]VIP binding in membrane preparations derived from failing human left ventricle demonstrated a twofold decrease in $K_d$ (a twofold increase in affinity) compared with preparations derived from nonfailing heart. Additionally, a 62% decrease in VIP maximum binding or receptor density was present in failing heart. The decreased VIP receptor density observed in failing heart follows the precedent of decreased $\beta_1$-receptor density,\[^{8}\] whereas the observation of decreased VIP receptor $K_d$ or increased receptor affinity is a novel finding for adenylyl cyclase-coupled receptors in failing human heart.

The dual observations of decreased density and increased affinity were surprising because these two alterations may give functional effects that oppose one another. That is, a decrease in receptor density may lead to a subsensitive response, whereas an increase in receptor affinity may lead to a supersensitive response. Furthermore, these observations were initially of some concern because the presence of the high-capacity (96% of the total), low-affinity VIP binding site creates the potential for erroneous resolution of the high-affinity portion of biphasic binding curves,\[^{21}\] especially since the $K_d$ and $B_{max}$ both decreased in preparations derived from failing heart. To provide assurance that these alterations were not simply a system artifact, the binding curves were analyzed by two different nonlinear-regression curve-fitting computer programs that use different methods for determining the best fit, and the data from each type of analysis were in close agreement. Moreover, it was possible to observe and quantitate an effect of guanine nucleotides on the $K_d$ of the high-affinity, low-capacity VIP binding site. This observation provided further support for the contention that the heart failure-associated changes in VIP receptor affinity was truly biologic. However, the best support for the contention that the radioligand-measured differences in VIP receptor affinity and density were biologic phenomena present in failing heart was the directional concordance of the muscle bath data.

The VIP-mediated muscle contraction responses were in close agreement with the binding data: maximal contractile force was decreased by 62% in failing heart compared with a 61% decrease in VIP receptor density. Also, VIP dose-response curve position was left-shifted in failing heart by approximately twofold to threefold, in agreement with the twofold increase in VIP receptor affinity measured directly at \[^{[2]}\]VIP binding. Thus, we found that both VIP receptor alterations identified at \[^{[2]}\]VIP receptor binding, decreased receptor density and increased affinity, were apparently linked to functional effects.

In contrast to VIP muscle contraction data, isoproterenol dose-response curves in failing heart demonstrated a right-shifted $ED_{50}$ as well as a decreased maximal response, the latter in agreement with earlier reports.\[^{9,11}\] These observations suggest that some degree of affinity change of

<table>
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<th>Table 3. Summary of Vasoactive Intestinal Peptide and Isoproterenol Dose-Response Curves From Failing and Nonfailing Human Right Ventricular Trabeculae Isolated in Tissue Bath</th>
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<tr>
<td>Nonfailing RVs</td>
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<tr>
<td>$ED_{50}\times 10^{-8} \text{ M [VIP]}$</td>
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<tr>
<td>Slope</td>
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<td>Maximum tension (mg)</td>
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<tr>
<td>Nonfailing RVs</td>
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<tr>
<td>$ED_{50}\times 10^{-8} \text{ M [ISO]}$</td>
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<td>Maximum tension (mg)</td>
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Values are mean±SEM. RV, right ventricle; VIP, vasoactive intestinal peptide; ISO, isoproterenol.
In the upper panel, the average vasoactive intestinal peptide (VIP) augmented muscle contraction dose-response curves are shown from seven failing (○) and six nonfailing (●) right ventricles. Again, as in Figure 6, the response in failing heart is markedly decreased at higher concentrations of VIP, but at lower concentrations, failing heart gave increased contractile response. Each curve was normalized to its own maximal response, and the average percent maximal dose-response curves from failing and nonfailing are shown in the lower panel. The degree of left shift is readily apparent. The results of the four-parameter fits for the average curves: minimum 38.58 vs. 33.17 mg; maximum 360 vs. 867 mg; ED$_{50}$×10$^{-9}$ M [VIP] 5.21 vs. 9.18; and slope -0.614 vs. -1.297; failing vs. nonfailing, respectively. The results of the four-parameter fits for percent maximal contraction curves: minimum 13.9% vs. 4.5%; maximum 99.5% vs. 99.5%; ED$_{50}$×10$^{-9}$ M [VIP] 4.06 vs. 12.47; slope -0.692 vs. -1.303; failing vs. nonfailing, respectively.

β-adrenergic receptors (either β$_1$ or β$_2$) had occurred in failing myocardium, which was combined with the reduction in β-receptor density. In that regard, we have reported that β$_1$-receptors down-regulate whereas β$_2$-receptors partially uncouple in the failing human heart, and it may be that one or both also demonstrate an agonist affinity shift to a lower, but still coupled, affinity state. At any rate, the right shift in the isoproterenol dose-response curves in failing preparations is in marked contrast to the left shift in VIP dose-response curves.

In an attempt to evaluate the VIP and β-adrenergic receptor pathways beyond the receptor, 10 mM calcium chloride was routinely applied to all trabeculae at the end of muscle bath studies, and maximal tension was measured. The tension response to maximal concentrations of calcium, which presumably acts independently of receptor function, was not different in right ventricular trabeculae removed from failing and nonfailing heart in this or in previous investigations by us or by others. Additionally, muscle contraction responses to for-
skolin in isolated tissue preparations have not been different in failing and nonfailing heart.\textsuperscript{21} Taken together, these findings suggest that the differences observed between failing and nonfailing heart for the VIP and \(\beta\)-adrenergic pathways are caused by changes at the receptor level.

Although the decrease in VIP and \(\beta\)-receptor density in failing human heart could have been an artifact of diseased or damaged tissue, flouride-stimulated adenyate cyclase as a membrane marker and creatine kinase as a marker of viable myocardium were not different in tissue removed from failing ventricles compared with nonfailing controls. Moreover, previous studies in membranes extracted from failing human ventricular myocardium have shown that a variety of membrane markers are unchanged compared with nonfailing controls.\textsuperscript{8} Thus, the changes in VIP and \(\beta\)-receptor density present in membranes derived from failing myocardium are likely to be pharmacologically specific abnormalities rather than being secondary to "nonspecific" membrane dysfunction. Although medications such as digitalis, diuretics, and vasodilators taken by patients before cardiac transplantation could have had an effect on VIP receptors, no direct interaction of any of these agents with the VIP receptor is known.

In this series of investigations, right ventricular trabeculae were used to assess inotropic responses because they are more suited to quantitative pharmacological study than are left ventricular preparations. Although biochemical and binding data were measured in left ventricular preparations, right and left ventricular function was similar within respective classes of cardiac function. Moreover, we have previously shown that in left and right human ventricle, \(\beta\)-receptor behavior is identical, provided that the functional status is similar.\textsuperscript{8,11} Thus, it is likely that the muscle-contraction behavior of right ventricular trabeculae was representative of both right and left ventricular tissue.

We observed functional ED\(_{50}\)'s of VIP-augmented muscle contraction in the 10+ nM range, whereas the agonist ED\(_{50}\)s measured directly by \(^{[\text{125I}]\text{VIP}}\) binding were in the 0.4–0.8 nM range. Similarly, the agonist \(K_d\) measured for \(\beta\)-receptors, is in the 1–2 nM range,\textsuperscript{22} yet the agonist ED\(_{50}\) for muscle contraction is 10–30 nM. Differences such as these between agonist-binding-determined \(K_d\)s, and physiological-response ED\(_{50}\)s are not unusual when working with isolated tissue preparations. Multiple factors may explain these differences, including a nonlinear receptor-occupancy response relation\textsuperscript{23} or a barrier to drug diffusion into trabeculae in whole-tissue preparations. We also observed that without the addition of BSA to decrease the nonspecific binding to the walls of the tissue baths, the contractile responses to VIP were markedly diminished or absent. As with many peptides, VIP will bind nonspecifically to glass and plastics, and the large surface areas of our 80-ml plastic tissue baths may bind a substantial amount of VIP despite the measures taken to minimize this problem.

This study has not addressed possible mechanisms accounting for VIP receptor regulatory changes in failing heart. Possible explanations include changes in tissue VIP levels derived locally from cardiac neural tissue or changes in circulating VIP. With regard to the latter, changes in circulating cardiac\textsuperscript{24} and noncardiac\textsuperscript{25} peptide hormones do occur in heart failure and are related to compensatory processes accompanying this clinical syndrome.

Alternatively, the change in the VIP receptor to a higher-affinity state could have been a primary compensatory change. If this were true, this change may lead to enhanced cardiac cell binding of VIP, which would initially provide inotropic support to the failing heart but which might ultimately be followed by receptor down-regulation. We have recently shown in a model system that both isoproterenol and VIP caused homologous desensitization of myocardial responses.\textsuperscript{26} That is, exposure to VIP caused rapid desensitization of myocardial contractile response with no evidence of a supersensitivity response (a left shift in dose-response curve).\textsuperscript{26} Heterologous desensitization of VIP receptors by \(\beta\)-receptor agonists was not observed in this study.\textsuperscript{26} On the other hand, altered VIP receptor density and affinity have recently been described in lung tissue after perturbations of certain membrane phospholipids.\textsuperscript{19} It is also possible that the VIP receptor density and affinity changes are not causally related but that they may be independent events related temporally in the progression of myocardial failure. Prospective studies in human subjects with heart failure should help to resolve these issues.

It is not clear at this time if the VIP receptor alterations identified in this report have any relation to the pathogenesis or clinical course of human heart failure. However, of \(G_\text{i}\)-coupled receptors, only \(\beta\)-adrenergic (markedly decreased \(\beta_1\) density\textsuperscript{8} and mild uncoupling of \(\beta_2\) receptors\textsuperscript{9}) and VIP receptors (decreased density, increased affinity) have demonstrated alterations in failing human ventricular myocardium. In contrast, multiple receptors\textsuperscript{27–29} or receptor pathways\textsuperscript{12,30} in failing human ventricular myocardium are apparently not altered, including the \(H_2\) histamine receptor pathway,\textsuperscript{12,27} the \(\alpha_1\)-receptor,\textsuperscript{28} the \(A_1\) adenosine receptor pathway,\textsuperscript{30} and the calcium channel-dihydropyridine receptor.\textsuperscript{29} The lack of alteration of these multiple receptors in failing human heart suggests that generalized or nonspecific panreceptor processes probably do not occur. Furthermore, the observed change in the \(\beta\)-receptor appears to be a relatively specific pharmacological response in failing human heart,\textsuperscript{31} and by inference the VIP receptor alterations may also be pharmacologically specific responses. Such specificity may increase the likelihood that these receptor regulatory events may be causally related to human heart failure. However, the final determination of the significance of the VIP and \(\beta\)-receptor

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alterations observed in the failing human heart remains to be elucidated.

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References


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