Beta-Receptors and Contractile Reserve in Left Ventricular Hypertrophy

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SUMMARY The inotropic responsiveness to adrenergic stimulation is diminished in hypertension associated with left ventricular hypertrophy (LVH). This was shown in hypertrophied hearts from renal hypertensive rats (RHR) (two-kidney, one clip hypertension, Goldblatt) 6 weeks after renal artery clipping when compared to age-matched normotensive sham-operated controls (NR). The isoproterenol-stimulated inotropic responses (Δ peak dP/dt) of isolated hearts perfused at constant pressure were significantly lower in RHR than in NR (p < 0.001 by analysis of variance and covariance, including repeated measures). This reduction in ventricular responsiveness of isolated hearts from RHR did not extend to other inotropic agents such as calcium ions and the cardiotonic cardiac glycoside scillaren. Assay of beta-adrenergic receptors by binding to (−)[3H] dihydroalprenolol showed that left ventricular beta-receptor numbers (fmol per mg membrane protein) were significantly reduced in RHR compared to NR (28.2 ± 1.1 vs 36 ± 2.6, p < 0.01) with no significant change in affinity (Kd, nM) (1.9 ± 0.27 vs 2.26 ± 0.34, NS). The results of this study suggest that LVH in renovascular hypertension is associated with impairment in inotropic responsiveness to beta-receptor stimulation parallel with and, in part, related to, a reduction in ventricular beta-receptor concentrations. Such blunting of inotropic responsiveness to beta-adrenergic stimulation may be one of the mechanisms in the progression from LVH to heart failure in hypertensive disease.

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KEY WORDS • renovascular hypertension • rats • (−)[3H] dihydroalprenolol • Langendorff preparation • isoproterenol • LV inotropic responsiveness • calcium ions • scillaren

The contractile reserve of the heart is determined by its inotropic responsiveness to adrenergic stimulation and has been shown to be diminished in hypertrophied hearts of spontaneously hypertensive rats (SHR).1-3 Saragoca and Tarazi4 have also reported a reduction in cardiac responses to isoproterenol from in vivo experiments in renovascular hypertensive rats (RHR) (two-kidney, one clip hypertension, Goldblatt), and this reduction was correlated with the degree of left ventricular hypertrophy (LVH).4 The reduced inotropic responsiveness might be due to many factors, either intrinsic or extrinsic to the heart. Further, previous studies in hypertensive rats did not define whether the reduced responsiveness extended to other positive inotropic stimuli or involved alterations in beta-receptor mechanisms which were described in other models.5-8

We have therefore studied responses to isoproterenol in the same model of hypertension (two-kidney, one clip hypertension, RHR), utilizing a Langendorff preparation in which the physical and chemical milieu of isolated hearts were strictly controlled. In parallel experiments, we also investigated the responses of hearts isolated from RHR to other inotropic agents, such as calcium ions and scillaren; further, we have measured ventricular beta-receptors in another group of hearts from the same model.

Methods

Animals

Renal hypertension (two-kidney, one clip Goldblatt hypertension) was induced in male Sprague-Dawley rats, 150-174 g (Hilltop Laboratories, Scottsdale, New Jersey). The left renal artery was constricted in some by a silver clip (10.2 mm internal width) under ether anesthesia, while other rats underwent sham surgery (NR). The animals were allowed to recover; body weight and blood pressure (tail-cuff technique) were recorded twice weekly, and only those rats whose blood pressure rose within 2 weeks to 160 mm Hg or more were accepted for study, provided they remained healthy with stable body weight (RHR).

Six weeks after surgery, two groups of rats (sham = 7, RHR = 7) were used for beta-receptor assay, and, in another parallel group, cardiac pharmacological studies were obtained in a Langendorff preparation.
Beta-Receptor Assay

Hearts freshly excised under ether narcosis were carefully cleaned from blood, fat, and fibrous tissue; the atria and right ventricular wall were excised, and the left ventricle (LV wall and septum) blotted dry and rapidly weighed in a precision balance. The LV was homogenized with Polytron in hypotonic buffer, washed with 3 volumes of 1 M KCl, filtered, and pelleted. Preparation of the membranes and their binding were carried out according to the technique of Baker and Potter9 using six different concentrations (0.6 - 15 nM) of (-) [3H] dihydroalprenolol (New England Nuclear, 90 Ci/mmol). Specific binding was defined as the difference between total binding in the absence of propranolol and the nonspecific binding found in presence of 5 × 10^-6 M dl propranolol. Protein concentration was determined by the method of Lowry et al.10 using bovine serum albumin as a standard. The maximum number of receptor sites (Bmax) and dissociation constants (Kd) were calculated by Scatchard analysis;11 Bmax was expressed as specific membrane activity (fmol per mg membrane protein), as density (pmol per g wet ventricular weight) and as total ventricular receptors (pmol per ventricle).

Isolated Heart Perfusion

Cardiac responses to inotropic agents were determined in vitro in a Langendorff preparation with retrograde perfusion under constant pressure (=55 mm Hg) without recirculation. This approach was used to exclude the effects of anesthesia, respiratory movements, cardiovascular reflexes, and neural or humoral factors that might influence in vivo studies, and to minimize variabilities of preloading and/or afterloadings during the study of inotropic responses of the heart.

Procedure

Rats were given an i.p. injection of heparin (1000 units) followed 1 hour later by anesthesia with pentobarbital (30 mg/kg); the trachea was rapidly cannulated with a PE-240 tube (Clay Adams, New Jersey), and ventilation with atmospheric air was begun under positive pressure at a rate of 60 strokes per minute (Harvard Rodent Respirator). The heart was rapidly removed, Keeping a 0.5 cm long aortic stump, and immediately placed in ice-cold Krebs-Henseleit bicarbonate buffer, where it rapidly stopped beating. The aorta was slipped over a grooved perfusion cannula and secured by a silk ligature along the groove; retrograde perfusion was begun from a reservoir 75 cm above the heart. The perfusion medium consisted of a modified Krebs-Henseleit bicarbonate buffer (KHB), pH 7.4, equilibrated with O2:CO2 (95:5) at 32°C, and containing (mM): NaCl-118; KCl-4.7; CaCl2-0.8 plus 0.5 to balance EDTA; MgSO4-1.2; KH2PO4-1.2; and NaHCO3-25. Na2EDTA (0.5 mM) was included to chelate the trace quantities of heavy metals present in reagents. Dextrose (11 mM) was added as substrate and hydroxyethyl starch (HES, American Critical Care) was added in a concentration of 3% to adjust the oncotic pressure of the medium. Adjustment of oncotic pressure was necessary to prevent cardiac tissue edema; this was controlled by ensuring that the heart weight remained unchanged after perfusion.

Recording of Cardiac Responses

The transducer needle assembly consisted of a small needle (21 G) directly connected to a larger blunt needle which, through a steel stopcock, was connected to a pressure transducer (Micron MP-15) that was firmly attached to the arm of a triaxial micromanipulator (Brinkman, Inc.). The fluid-filled needle transducer system had a natural resonant frequency of 144 Hz and a dampening coefficient of 0.63. The needle was advanced through a window in the glass cardiac chamber to puncture the left ventricle approximately 0.5 cm above the apex. The needle was positioned to obtain the best undampened LV pressure curve tracing; the first derivative of LV pressure, dP/dt, was recorded electronically by a differentiator module with a frequency response of 1-100 Hz ( = 3 dB). The LV end-diastolic pressure recorded in our experiments averaged 0 ± 1 mm Hg under the conditions described above, both in normotensive and hypertensive hearts. Previous experience in this laboratory showed no significant difference in the passive pressure volume curve between normotensive and hypertensive hearts in the same model of hypertension,4 and the same observation was reported by other investigators in the hypertrophied hearts of spontaneously hypertensive rats.13 Thus, it can be reasonably assumed that the response to various inotropic agents was derived from the same preload conditions in normotensive and hypertensive preparations.

Once adequate undampened LV pressure recordings were obtained, the heart was left to stabilize for 45 minutes. A baseline recording was then obtained at a paper speed of 200 mm/sec to determine the baseline (preinfusion) heart rate and peak dP/dt. The inotropic agent was infused through a catheter (PE-50) opening just above the aortic cannula, at sequential rates using a Harvard infusion pump; each dose level was continued for 5 minutes, at the end of which recordings were obtained at 200 mm/sec paper speed. The changes in peak dP/dt from baseline level were used as an index of inotropic responses as described by Braunwald et al.13

Protocol of Infusions

Isoproterenol

Isoproterenol (Isuprel (dl isoproterenol) Breon Laboratories) was infused sequentially (0.34 to 6.8 × 10^-5 mg/min), to obtain final concentrations of 1.0 × 10^-9 to 3 × 10^-8 M. This was carried out in two sets of experiments.

Experiment 1. On hearts from six RHR and six NR, KHB was used with CaCl2 reduced to 0.8 mM and the temperature adjusted at 32°C.

Experiment 2. On hearts from nine RHR and nine NR, KHB was used with its regular CaCl2 concentration (2.5 mM) and the temperature adjusted at 37°C.
Calcium Chloride

Increasing concentrations of calcium chloride (0.065 to 1.3 μg/min) were added sequentially to the perfusing KHB fluid, beginning from a concentration of 0.8 mM to end with a final concentration of 2.7 mM. The temperature was adjusted to 32°C, and the study was performed on the hearts from six RHR and six NR.

Scillaren

Scillaren (Sandoz), a cardiotonic glycoside obtained from Scilla leaves, was chosen because of the rat heart’s notorious resistance to digitalis. Because of its prolonged action, we could not achieve a dose response curve. The response to scillaren was therefore measured from a dose level determined from preliminary experiments to stop short of signs of toxicity in this preparation, namely, an infusion rate of 10.3 μg/min, giving a final concentration of 1.65 × 10⁻⁶ M. Scillaren was infused for 15 minutes; the perfusing KHB fluid was low in CaCl₂ concentration (0.8 mM) and its temperature adjusted to 32°C, in order to demonstrate clearly any inotropic effect of the glycoside and to minimize the chances of toxicity of the preparation. This study was performed on eight RHR and eight NR.

Statistical Analysis

Unpaired t tests were used to test differences between the different groups; the paired t test was used to test the effect of scillaren from within the same group. For analysis of dose responses to graded doses of isoproterenol or calcium in RHR vs NR, the increments in response at each dose level were evaluated by analysis of variance and covariance, including repeated measures (Public Program, BMDP-2V, UCLA (1977) available on PROPHET).

Results

Clipping of the renal artery induced hypertension associated with LVH. The systolic blood pressure (mm Hg) was significantly higher in RHR than in NR (202 ± 8 vs 124 ± 3, p < 0.001) and ventricular weights of RHR were significantly increased than those of NR controls whether expressed in absolute value (g) (1.14 ± 0.06 vs 0.87 ± 0.01, p < 0.001) or normalized for body weight (mg/g) (3.36 ± 0.18 vs 2.1 ± 0.08, p < 0.001).

Ventricular Beta-Adrenergic Receptors

As shown in table 1, the number of beta-receptors, whether expressed as per mg membrane protein (specific membrane activity) or per g wet ventricular weight (receptor density), was significantly reduced in RHR (n = 7) compared to age-matched controls (n = 7) (p < 0.01 and p < 0.005, respectively). The total ventricular receptor number and the Kₐ were not significantly altered, (p > 0.05 for both).

### Table 1. Left Ventricular Beta-Adrenergic Receptors in Renal Hypertensive (Two-Kidney, One Clip) Rats (RHR)

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 7)</th>
<th>RHR (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane yield (mg protein/g ventricle)</td>
<td>71.4 ± 4</td>
<td>69.8 ± 3.3</td>
</tr>
<tr>
<td>Specific membrane activity (fmol/mg protein)</td>
<td>36 ± 2.6</td>
<td>28.2 ± 1.1*</td>
</tr>
<tr>
<td>Receptor density (fmol/g ventricle)</td>
<td>2.47 ± 0.08</td>
<td>1.93 ± 0.1†</td>
</tr>
<tr>
<td>Total receptors (pmol/left ventricle)</td>
<td>2.14 ± 0.07</td>
<td>1.97 ± 0.20</td>
</tr>
<tr>
<td>Dissociation constant (Kₐ) (nM)</td>
<td>2.26 ± 0.34</td>
<td>1.91 ± 0.27</td>
</tr>
</tbody>
</table>

Values are means ± one standard error.
* p < 0.01. † p < 0.005.

EC₅₀ = concentration of isoproterenol at 50% of the maximal response.
Baseline Indices of the Perfused Heart's Activity

Neither the baseline heart rate nor peak dP/dt were significantly different in RHR from control rats (tables 2 and 3, figs. 1-3).

Chronotropic Responses to Isoproterenol Graded Infusion

Isoproterenol graded infusion increased heart rate significantly in all groups whether hypertensive or normotensive. There was no difference between RHR and NR in their chronotropic responsiveness to isoproterenol, whether the increments in heart rate were analyzed by analysis of variance and covariance including repeated measures or by comparison of the maximum responses and of EC₅₀ obtained by fitting the sigmoid function of the dose response curve (tables 2 and 3).

Inotropic Responsiveness to Isoproterenol

The inotropic responses to isoproterenol were significantly lower in RHR than in NR whether isoproterenol was infused at 37°C and at 2.5 mM [Ca]₀ or at 32°C and at 0.8 mM [Ca]₀ (p < 0.001; p < 0.05, respectively) (fig. 1). The maximum responses obtained by fitting the sigmoid function of the dose response curves were significantly higher in NR than in RHR under both conditions (p < 0.001 and p < 0.05 respectively), but differences in EC₅₀ were not significantly different (tables 2 and 3).

Inotropic Responsiveness to CaCl₂ and Scillaren

Increasing the extracellular concentration of calcium ions from 0.8 to 2.7 mM showed no difference in response between RHR and NR (p = 0.45), as seen in figure 2. Scillaren increased ventricular dP/dt significantly in both NR and RHR (p < 0.01 for both); this inotropic response was equal in both groups (p = 0.6) as seen in figure 3.

Discussion

Our results indicate that LVH in renal hypertensive rats (two-kidney, one clip) is associated with a decreased concentration of ventricular beta-adrenergic receptor as well as with a diminished inotropic response to beta-agonists but not to other inotropic agents.

Inotropic Responses

Besides confirming the earlier data obtained both in SHR and RHR, our results have further documented that the defect in responsiveness to isoproterenol is intrinsic to the heart and appears restricted to the beta-adrenergic system. The latter result is in contrast with findings reported following aortic banding in dogs. In that study, Newman and Webb found that the inotropic impairment associated with hypertrophy was not restricted to beta-agonists but extended to calcium and glucagon; the responses to cardiac glycosides however, remained normal as in our study (fig 3). The discrepancy between these results and ours might be due to fundamental differences in pathophysiology of the two types of hypertrophy or possibly to species differences.
Since responsiveness to calcium ions was normal in our model, the process of LVH in renovascular hypertension does not appear to affect the transsarcolemmal calcium fluxes, the sensitivity of myofilament to calcium ions, or the contractile machinery. In contrast, hypercalcium fluxes, the sensitivity of myofilament to calcium ions, and the contractile machinery of LVH in this model does not interfere with the contractile machinery or the sodium and calcium fluxes. Since responsiveness to calcium ions was normal in the hypertrophied hearts of SHR, we tried to minimize these factors by reducing CaCl₂ concentration and the temperature of the infusing fluid. The same differences in response to isoproterenol were found to be associated with altered myosin-ATPase activity. The normal responsiveness to scillaren in RHR may also indicate that LVH did not affect the sodium and potassium ATPase activity and/or the sodium and calcium fluxes. Since the contractile effect of cardiac glycosides is ultimately dependent on calcium ions, these data, together with those of the calcium studies (fig. 2) suggest that LVH in this model does not interfere with the contractile machinery or sensitivity of the contractile elements to calcium ions; the defect seems rather to be restricted to the beta-adrenergic system.

Since differences in inotropic responsiveness to isoproterenol could be related to metabolic imbalance between actual O₂ supply and increased O₂ demands in LVH, we tried to minimize these factors by reducing the inotropic status and basal heart rate by diminishing CaCl₂ concentration and the temperature of the infusing fluid. The same differences in response to isoproterenol persisted between NR and RHR, whether the preparation was set at temperature 32°C and at 0.8 mM [Ca], (fig. 1, table 2) or at a temperature of 37°C and at 2.5 mM [Ca], (fig. 1, table 3). Thus, the reduction of the hypertrophied hearts' inotropic responsiveness to isoproterenol was not apparently dependent on the level of metabolic and contractile state and may probably not be related to a deficit in energy production or improper oxygenation in LVH.

Beta-Receptors

Ventricular beta-adrenergic receptor densities were reduced in RHR with no change in the apparent affinity; these results are in agreement with most reports regarding beta-receptors in the hypertrophied hearts of other hypertension models, such as in one-kidney, one clip renal hypertension and DOCA-salt hypertension. This is not common to all models, however, since reports of beta-receptor changes in hypertrophied hearts of SHR are variable and as are the reports of receptor changes following constriction of the abdominal aorta.

In that context, even more important than a difference in receptor numbers between RHR and NR, would be the question of whether changes in receptor numbers could account for or contribute to the changes in ventricular inotropic responsiveness in RHR. According to Cryer, whether receptors are rate-limiting or not (spare receptors), changes in their number would lead to a parallel change in responses in the submaximal range of stimulation, which is probably the usual physiologic range of activity. In a current study, we have found that ventricular beta-receptor density correlated significantly with the inotropic responsiveness of the same hearts to beta-agonist stimulation (r = 0.54, p < 0.001). This correlation, as well as the results of this study, suggest that a reduction in receptor numbers contributes at least partially to the lowered responsiveness in RHR; however, they do not exclude that other post-receptor defect(s) in the adrenergic system could also contribute to that phenomenon.

Chronotropic Responses

The increase in heart rate by isoproterenol was equal in RHR and NR. Although inotropic and chronotropic responses are mediated by beta-receptors, this dissociation suggests that atrial or sinus receptors can be regulated differently from ventricular receptors. Based on the data available, it is not possible to speculate on differences in numbers or Kᵣ between atrial and ventricular beta-receptors in RHR. However, pharmacological and binding data have suggested the presence of two subtypes of beta-receptors in the heart, one dominantly for inotropy, and the other mediating both inotropy and chronotropy.

Conclusions

The ability of the heart to augment its performance in response to adrenergic stimulation constitutes an important contractile reserve mechanism. However, we found that inotropic responsiveness to beta agonist stimulation was compromised as left ventricular hypertrophy developed in renal hypertension in parallel with a decrease in density of ventricular beta-receptors. Although preservation of chronotropic responsiveness may spare another mechanism of cardiac reserve (heart rate), it is possible that the heart may come to depend more on Frank-Starling forces than on adrenergic support. Whether this contributes to the progression to cardiac dilatation and failure merits further investigation.
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