Basal inotropic state in rats with renal hypertension: influence of coronary flow and perfusion pressure

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SUMMARY Cardiac performance in renal hypertensive animals has been variously reported as normal, increased, or decreased. Because of the many factors that can alter cardiac function in vivo ventricular contractility was investigated in isolated, paced, isovolumic heart preparations (modified Langendorff). Twenty one hypertensive rats (2 kidney, 1 clip Goldblatt) and 21 matched sham operated controls, were studied. Mean(SD) blood pressure and left ventricular weight were significantly higher in the study rats (228(5) vs 125(2) mmHg and 4.16(0.12) vs 2.35(0.04) mg·kg⁻¹ respectively). In the first experiment performed at 50 mmHg perfusion pressure left ventricular +dP/dt and myocardial flow rate were lower in 12 study rats (mean(SD) 1782(79) vs 2270(105) mmHg·s⁻¹, 5.8(0.4) vs 14.0(1.0) ml·g⁻¹ LV weight·min⁻¹, respectively) than in the controls. In a second experiment performed at 80 mmHg perfusion pressure (nine study hearts and seven controls) the increased myocardial flow rate resulted in a higher left ventricular +dP/dt in both groups, but the study hearts still had a lower mean(SD) myocardial flow rate than the controls (12.5(0.9) vs 19.8(2.2) ml·g⁻¹ LV weight·min⁻¹; the difference in left ventricular +dP/dt became non-significant (2646(186) vs 2951(136) mmHg·s⁻¹). Similarly, at equal myocardial flow rates (study hearts at 80 mmHg perfusion pressure and controls at 50 mmHg perfusion pressure) ventricular performance was similar in the two groups (mean(SD) left ventricular +dP/dt 2270(105) in controls vs 2646(186) mmHg·s⁻¹ in study hearts). In addition, beta blockade by propranolol (10⁻⁷ mol·litre⁻¹) did not affect the results obtained in non-blocked preparations as regards either left ventricular +dP/dt or myocardial flow rate.

These results indicate that coronary perfusion pressure significantly influences ventricular contractility and that left ventricular performance in hypertensive left ventricular hypertrophy depends on a balance between left ventricular mass, perfusion pressure, and myocardial flow rate.

Despite intensive investigations, there is still considerable disagreement on whether hypertensive left ventricular hypertrophy is associated with normal, improved, or depressed cardiac performance. Some of the discrepancies could be related to the type of hypertension investigated (spontaneous or renal hypertension), to the methods used (papillary muscle studies or in vivo experiments), or to the aspect of function investigated (response to pressure, volume overload, or inotropic stimuli). Nevertheless, discrepancies still persist among studies of well defined renal hypertensive models, and there are many factors responsible for the divergence in conclusions. Among these, one factor has received relatively little attention until recently. The role of aortic pressure has mostly been analysed in terms of the load it imposes on the left ventricle; its effect on coronary perfusion and flow and hence on myocardial performance has usually been referred to only indirectly.

In order to study this aspect and avoid interference by the many factors that can influence cardiac function in vivo, this study was performed in isolated, paced, isovolumically contracting hearts from renovascular hypertensive rats and precisely matched sham operated controls. This model of hypertension (1 clip 2 kidney Goldblatt) was chosen because of the greater precision it allows in setting its controls compared with the spontaneously hypertensive rat model. An initial set of experiments was performed at the perfusion pressure (50 mm Hg) usually used in these...
preparations, and the same experiments were then repeated at a higher pressure (80 mm Hg). Since myocardial flow rates vary with changes in perfusion pressure it was then possible to compare cardiac performance in the hypertensive rats with that in their normotensive controls, first at equal perfusion pressures and then at equal myocardial flow rates. Moreover, since cardiac contractility is reportedly influenced by myocardial catecholamine concentrations, contractility was also assessed in similar groups of normal and hypertrophied hearts during beta adrenoceptor blockade with propranolol ($10^{-7}$ mol·litre$^{-1}$) in order to determine if this variable contributed significantly to the differences in inotropy seen in the first set of experiments.

**Material and methods**

Male Sprague-Dawley rats (Hilltop Lab, Scottsdale, AR) of equal body weight and age were obtained from the same source. After three to four days’ rest in the laboratory, when they were 6 weeks of age, half of them underwent left renal artery clipping leaving the right kidney untouched, as previously described, and half underwent sham surgery at the same time under the same conditions. Blood pressure was monitored weekly in all by the tail cuff method, and all were accommodated in a climate controlled room, fed a regular diet of Purina Rodent Lab Chow 5001 with unlimited intake of water, and handled in the same way by the same person throughout the study. Only those rats whose blood pressure rose to $\geq 160$ mmHg within two weeks of surgery were considered to be hypertensive and were included in the study group; all sham operated rats remained normotensive.

Eight weeks after clipping (at 14 weeks of age) the rats were killed. The Langendorff preparation used in our laboratory has been described in detail previously. In summary, under pentobarbital anaesthesia (30 mg·kg$^{-1}$ intraperitoneally) and adequate ventilation the heart was rapidly dissected out and placed in a cold (0°C) Krebs-Henseleit bicarbonate buffer solution saturated with oxygen; it was then securely attached via its aortic stump to the grooved tip cannula of the Langendorff apparatus. Retrograde perfusion was begun immediately through the aorta with the same oxygenated (95% O$_2$ and 5% CO$_2$) Krebs-Henseleit bicarbonate buffer solution maintained at 37°C and pH 7.4; the solution consists of (in mmol·litre$^{-1}$): NaCl 117, KCl 4.7, CaCl$_2$ 2.5 plus 0.5 to balance EDTA, KH$_2$PO$_4$ 1.2, NaHCO$_3$ 25, Na$_2$ EDTA 0.5, and dextrose 8.5. Immediately after the start of perfusion, the base of the pulmonary artery was incised to allow efficient drainage of the right ventricle from the coronary sinus and Thebesian vessel flow. The left ventricular cavity was drained via a Pe-50 intramedic polyethylene tube inserted in the left ventricle through the left atrium and pulled out at the apex of the heart. The distal end of the tube was bevelled to reduce possible injury to the myocardium; the intraventricular end was slightly dilated by heating to prevent outward slippage of the tube. This step was followed by insertion of a balloon transducer tipped catheter in the left ventricle via the mitral valve. The atrioventricular node was then crushed and the isolated heart paced at a constant rate of 260 beats·min$^{-1}$, which was maintained throughout the experiment, using an S-5 stimulator (Grass Instrument Co, Quincy, MA); duration of the stimulus was 5 ms at 2.3 V.

The balloon inserted in the left ventricle was prepared in a 37°C water bath from a thin rubber material (Akwell Industries Inc, Dothan, Alabama) and fastened around the tips of a Millar transducer tip catheter (MIKRO-TIP), 5F, Millar Instruments, Houston, TX) and a PE-50 tube attached together by a silk thread placed 1 cm above the tip of the Millar catheter. The catheter was used to record intraventricular pressures, and the PE-50 tubing served to fill the balloon with water and adjust its volume. The frequency response of the transducer system with the balloon attached was 200 Hz.

At the start of the experiment the deflated balloon was inserted into the left ventricle via the left atrium; its volume was then adjusted so that the end diastolic left ventricular pressure was set at 0 mmHg; the volume of the balloon was then left unchanged throughout the experiment. From 25 to 30 minutes were allowed for equilibration. During this time, the left ventricular systolic pressure (LVP), its first derivative (LV +dP/dt), and the left ventricular end diastolic pressure were continuously monitored and recorded at a paper speed of 0.05 mm·s$^{-1}$ (Brush recorder, Gould Inc, Cleveland, Ohio). At the end of this equilibration period, the paper speed was increased to 50 and 200 mm·s$^{-1}$ to obtain control readings.

Myocardial flow was measured by collecting the perfusate for two consecutive periods of 5 min each. Myocardial flow rate was calculated in ml·min$^{-1}$ and ml·g$^{-1}$ left ventricular weight·min$^{-1}$; the ventricular weight was obtained at the end of the experiment using a Mettler PC 440. The accuracy of myocardial flow rate, in our preparation, had been previously determined both by simultaneous recording of flow rate through the aortic stump by a T shape electromagnetic flowmeter and by infusion of radioactive microspheres into the aorta; the microspheres recovered from the perfusate did not exceed 3.5%, indicating that most of the coronary flow actually perfused the myocardial capillaries with minimum leakage compatible with the results expected from the microsphere technique.
EXPERIMENTAL DESIGN

Experiment (1) — The first set of experiments included 14 normal hearts from sham operated rats and 12 hypertrophied hearts from 2 kidney 1 clip hypertensive rats. A perfusion pressure of 50 mmHg was used. Experiment (2) — The second set of experiments was performed in seven controls and nine hypertrophied hearts at a perfusion pressure of 80 mmHg. Experiment (3) — The effect of beta adrenergic blockade on baseline cardiac performance was investigated in a separate group of 34 hearts (eight hypertensive and eight sham operated at perfusion pressure 50 mmHg, and nine hypertensive and nine sham operated at perfusion pressure 80 mmHg). In order to maintain the same duration of the experiment as in the non-propranolol group, propranolol (10-7 mol·litre-1) was added to the perfusate at the beginning of the experiment. After an equilibration period of 30 minutes, the left ventricular developed pressure, +dP/dt, and end diastolic pressure were recorded at a paper speed of 200 mm·s-1. Myocardial flow was collected over two consecutive periods of 5 min each. This dose of propranolol was chosen because previous experience had shown that it blocked effectively the response to isoproterenol (10-8 mol·litre-1) in our preparation.

STATISTICAL ANALYSIS
The unpaired Student’s t test was used to test statistical significance of differences between groups; least squares regression analysis was used to determine correlation coefficients; statistical significance was set at p<0.05.

Results
Compared with sham operated controls, renal hypertensive rats had a lower mean(SD) body weight (332(17) vs 471(12) g, p<0.001), higher (mean(SD) blood pressure (230(8) vs 126(3) mmHg, p<0.001), and heavier mean(SD) left ventricular weight, both in absolute value and in relation to body weight (1342(40) vs 1085(24) g and 4.16(0.16) vs 2.33(0.07) mg·g-1 body weight respectively, p<0.001 for both).

In experiment I performed at a perfusion pressure of 50 mmHg, the hypertrophied hearts from renal hypertensive rats had a significantly lower left ventricular +dP/dt and left ventricular developed pressure than controls (mean(SD) 1782(79) mmHg·s-1 vs 2270(105) and 58(2) vs 73(4) mmHg respectively, p<0.01 for both) (table). Myocardial flow rate was also significantly reduced in the hypertrophied hearts (5.8(0.4) vs 14.0(1.0) ml·g-1·LV·wt·min-1, p<0.001).

Increasing the perfusion pressure to 80 mmHg (experiment 2) led to a significant increase in both myocardial flow rate and left ventricular +dP/dt in both normal and hypertensive hearts; the magnitude of this increase was not different between the two groups (table). At these new increased perfusion pressures and myocardial flow rate, the left ventricular +dP/dt of the study hearts became near normal (2646(186) vs 2951(136) mmHg·s-1), and the difference between the study hearts and controls was not statistically significant.

In order to study the influence of these differences in myocardial flow rate on left ventricular +dP/dt, we compared the findings in control hearts perfused at 50 mmHg with those in study hearts perfused at 80 mmHg. The higher perfusion pressure study hearts was associated with a myocardial flow rate equal to that of the control hearts perfused at the lower pressure (12.5(0.9) vs 14.0(1.0) ml·g-1·LV·wt·min-1, NS); left ventricular dP/dt under these conditions was not significantly different between the two groups (2646(186) vs 2270(105) mmHg·s-1, NS).

A significant correlation was found between left ventricular +dP/dt and myocardial flow rate in all the rats studied (21 study hearts and 21 controls), as well as within each group (study hearts: r = 0.781, p<0.001; controls: r = 0.535, p<0.05).

EFFECT OF PROPRANOLOL (Table)
The rats used in this experiment had the same general

TABLE Influence of perfusion pressure on myocardial flow rate and contractile indices of isolated rat hearts of renovascular hypertensive rats (RHR) and sham operated controls. Values are mean (SEM)

<table>
<thead>
<tr>
<th>Perfusion pressure (mm Hg)</th>
<th>Without propranolol</th>
<th>With propranolol</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>LV +dP/dt</td>
</tr>
<tr>
<td>50</td>
<td>14 sham</td>
<td>2270(105)</td>
</tr>
<tr>
<td>50</td>
<td>12 RHR</td>
<td>1782(79)**</td>
</tr>
<tr>
<td>80</td>
<td>7 sham</td>
<td>2951(136)†</td>
</tr>
<tr>
<td>80</td>
<td>9 RHR</td>
<td>2646(186)†</td>
</tr>
</tbody>
</table>

**p<0.01 from corresponding sham; <0.05 from corresponding sham; †p<0.01 from sham 50; †p<0.01 from RHR 50.
LVP=left ventricular developed pressure; MFR=myocardial flow rate.
characteristics as those in the first experiment; the study rats had a lower body weight (338(22) vs 448(9) g, p<0.001), higher blood pressure (224(6) vs 124(3) mmHg, p<0.001), and heavier left ventricular weight (4.17(0.18) vs 2.38(0.05) mg·g⁻¹ body weight, p<0.001) than normal. These data were similar to those of the non-propranolol experiments described above. No significant difference in left ventricular +dP/dt was found between beta blocked hearts (propranolol 10⁻⁷ mol·litre⁻¹) and those not infused with propranolol; this applied to the two perfusion pressures used (50 and 80 mmHg) and the three variables studied — left ventricular +dP/dt, left ventricular developed pressure, and myocardial flow rate — as well as the correlation between left ventricular +dP/dt and myocardial flow rate (figure).

Discussion

The results of these experiments indicate that different conclusions can be reached regarding the inotropic state of hypertensive left ventricular hypertrophy depending on the perfusion pressure used. The effect of altering aortic pressure on myocardial performance in our study was not due to changes in afterload since the left ventricle contracted isovolumetrically but was related to its influence on myocardial flow rate and coronary perfusion pressure. Previous studies by Hallback and colleagues and others had pointed out that the hypertrophied hearts of spontaneously hypertensive rats performed better than control hearts under conditions of increased load. Nevertheless the preparation that they used could not differentiate between the many consequences of increased aortic pressure by determining whether left ventricular performance was improved because of the higher afterload or better myocardial perfusion or both. Although the first mechanism is undoubtedly operative in in vivo situations, our results indicate that coronary perfusion does play an important role in that respect.

This conclusion is in agreement with the suggestions advanced by others and with the results of Alfaro and colleagues regarding the influence of myocardial perfusion on left ventricular performance in hypertensive hypertension. The significant correlation obtained between left ventricular dP/dt and myocardial flow rate further supports that suggestion. Coronary flow reserve has been shown to be reduced to a variable extent in left ventricular hypertrophy as a result of pressure overload; in hypertension this encroachment on coronary reserve can be further accentuated by the medial hypertrophy and vascular disease associated with the hypertensive disease.

It is therefore plausible that different degrees of impairment in cardiac performance could occur depending both on the load imposed on the heart and on the ability of the coronary circulation to respond to myocardial demands. Wendt and colleagues commented that the wide standard deviation encountered in their studies of rats with sustained hypertension could be related to the natural inhomogeneity of vascular structural lesions.

This conclusion regarding the relation between myocardial perfusion and performance is not meant to explain by itself all the differences in inotropic state between hypertensive and normal hearts. Differences in actomyosin composition, in energy transport systems, in neurohumoral factors, and in myocardial collagen can all play major roles in that respect. Our study points out that under in vitro controlled conditions, with unchanged and constant preload and afterload, left ventricular performance was considerably influenced by coronary perfusion pressure and flow. That influence was pronounced enough that opposite conclusions could be reached in the same model of hypertensive hypertrophy — namely, that hypertrophied hearts had a depressed contractility (first set of experiments) or were not different from controls (second set of experiments).
The results obtained when propranolol was added to the perfusate indicate that myocardial catecholamines did not play a major role either in the differences between normal and hypertrophied hearts or in the effect of alterations in coronary perfusion. Some investigators had suggested that the stores of catecholamines in the myocardial cells and in the remnants of nerve ending could influence basal resting contractility, but this is not confirmed by others. Nevertheless since Tarazi and colleagues had shown that myocardial catecholamine concentration tended to be reduced in the hypertrophied heart of renal hypertensive rats, the study was repeated under cover of propranolol. Addition of the beta blocker (propranolol 10^-7 mol·litre^-1) to the perfusing solution did not produce any pronounced alteration in basal left ventricular +dP/dt in either renal hypertensive or sham operated rats; the changes in ventricular contractility indices with variations in perfusion pressure gave essentially the same conclusions in beta blockade as in control hearts (figure), indicating that the differences in performance of the isolated hearts between real hypertensive rats and controls could not be ascribed to their myocardial catecholamine stores. Nor could these differences be ascribed to discrepancies in heart rate since all studies were performed at the same rate.

There are two aspects of the influence of coronary perfusion on ventricular performance in our results; one is the improvement in performance with improvement in myocardial perfusion in renal hypertensive rats and the second is related to both the normal and hypertensive groups and what was described as the Gregg phenomenon. The greater dependence of myocardial performance on coronary perfusion in hypertensive hearts reflects in part the combined effect of reduction in coronary reserve, coronary vascular changes, alterations in capillary density, and greater heterogeneity in oxygenation of the hypertrophied myocardium. In addition, however, it has been repeatedly shown in both normal and hypertrophied ventricles that increases in coronary perfusion pressure or flow rate or both lead to enhanced cardiac contractility. Increased perfusion pressure and distention of the coronary vessels appear to be more important in that respect than changes in flow rate per se, a conclusion supported indirectly by our results. This enhancement of cardiac contractility has been ascribed to an erectile effect due to the increased coronary pressure stretching the myocardium and resulting in enhanced myocardial contraction (Frank-Starling mechanism). This effect can even be shown at the biochemical level; Kira and colleagues, have reported that myocardial protein synthesis in non-working isolated hearts is enhanced by increased aortic (coronary) perfusion pressure, suggesting that increased distension of the coronary vessels is effectively sensed as increased tension, resulting in greater contractility and a stimulus to protein synthesis.

In conclusion, coronary perfusion pressure was shown to be an important determinant of myocardial performance both in normotensive and hypertensive hearts. Judgments regarding cardiac performance in hypertension should therefore account for possible differences in coronary dynamics.

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References


