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Hemodynamic Factors and Vascular Density as Potential Determinants of Blood Flow in Hypertrophied Rat Heart (39685)

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When a chronic pressure overload is imposed upon the heart, additional myocardial tissue accumulates as a compensatory response. The additional heart mass creates increased tissue oxygen demands which, under normal circumstances, would be met primarily by increasing coronary blood flow (1). However, recent studies have shown that total coronary blood flow (2) and flow per unit mass of tissue (3) are reduced below normal levels in hypertrophied hearts.

The apparent failure to meet the additional oxygen demands of the hypertrophied heart may be related to hemodynamic determinants of coronary blood flow (4) which accompany the pressure overload. Alternatively, coronary vascularity may fail to increase in proportion to the increase in myocardial mass. Since blood flow to the endocardial portion of the left ventricle is considered to be marginally adequate under normal conditions (5, 6), it seemed likely that this portion of the ventricle would be particularly susceptible to pressure overload stress.

Therefore, hemodynamic factors related to coronary blood flow regulation were measured in control and aortic constricted rats. Alkaline phosphatase specific activity was utilized as a quantitative chemical index of vascular density in the epicardial (EPI) and endocardial (ENDO) portions of control and hypertrophied left ventricles. In this way, the relative potential contribution of hemodynamic factors and/or coronary vascularity to blood flow regulation in the hypertrophied heart could be evaluated.

Materials and Methods. Animals and surgical procedures. Male Sprague–Dawley rats weighing 250–275 g were used in these experiments. Pressure-induced left ventricular (LV) hypertrophy was created by constricting the abdominal aorta above the renal vessels (7). Control animals received sham operations. Aortic constricted and control animals were studied 3 weeks following surgery at which time stable LV hypertrophy would be expected in aortic constricted animals (8).

Hemodynamic measurements. Animals were anesthetized with sodium pentobarbital (approx 50 mg/kg, ip), weighed and placed on positive pressure ventilation with room air via a tracheostomy. LV pressure, heart rate, mean arterial pressure, and the maximum rate of LV pressure development (max dP/dt) were measured as previously described (9). The max dP/dt was utilized as an index of myocardial contractility. After hemodynamic measurements were completed, the heart was excised and placed into a beaker of ice-cold 10 m*M* Tris–saline buffer, pH 7.4.

Tissue preparation. The excised heart was cleared of blood by perfusing 7 ml Tris–saline buffer retrograde through the aorta. Atria, great vessels and the right ventricle were then carefully removed. The remaining LV plus interventricular septum was separated into EPI and ENDO portions by dissecting the ventricle at the midwall. These tissue samples were weighed separately and the combined values were taken as LV weight.

Chemical analyses. In the rat heart, alkaline phosphatase has been histochemically localized primarily in coronary vessels and capillary endothelial cells (10, 11). Therefore, the chemical determination of alkaline phosphatase specific activity in heart tissue was utilized as a quantitative estimation of vascular density. EPI and ENDO portions of the LV from one group of animals were homogenized in Tris–saline buffer using a ground-glass homogenizer. Following centrifugation at 27,000*g* for 20 min, the supernatant was removed and alkaline phosphatase specific activity was determined (12). Supernatant protein was measured by the biuret reaction. Enzyme activity was deter-

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mined under linear conditions with respect to incubation time and enzyme concentration.

In another group of animals, a 10% (w/v) homogenate of each LV portion (EPI and ENDO) was prepared using ice-cold distilled water. Aliquots were analyzed for protein, RNA, DNA, and hydroxyproline as previously described (13).

Statistical methods. Within groups, EPI and ENDO values were compared using Student's paired *t* analysis. Comparisons between control and aortic constricted animals were made by unpaired *t* analysis. A *P* value of 0.05 or less was considered statistically significant.

Results. At the time of sacrifice, the combined control animals used in this study weighed 384 ± 9 g (mean ± SE). Since the body weights of aortic constricted animals were similar to those of control animals (374 ± 8 g), the efficacy of aortic constriction in producing hypertrophy could be evaluated without resorting to body weight normalizing procedures. LV weight was 792 ± 22 mg in control animals and was significantly increased to 981 ± 40 mg in aortic constricted animals.

The hemodynamic measurements obtained from control and aortic constricted animals are shown in Table I. Abdominal aortic constriction produced a substantial LV pressure overload, as evidenced by a 40 mm Hg elevation in peak LV pressure. In addition, significant increases in heart rate, mean arterial pressure and max *dP/dt* accompanied the LV pressure overload.

The alkaline phosphatase results are shown in Table II. In control animals, the specific activity of the ENDO portion of the LV was significantly higher than that of the EPI portion and the ratio of ENDO-EPI specific activities was approximately 1.2. In the LV of aortic constricted animals, there was an increase in EPI alkaline phosphatase specific activity which was accompanied by a decreased enzyme activity in the ENDO portion. These alterations did not achieve statistical significance when the LV portions were compared on a group basis. However, the ENDO and EPI enzyme activities were no longer significantly different in aortic constricted animals and the normal ENDO-EPI vascular density relationship was eliminated. This alteration resulted in a significant reduction in the ENDO-EPI specific activity ratio.

TABLE I. HEMODYNAMIC MEASUREMENTS FROM CONTROL AND AORTIC CONSTRICTED ANIMALS[a]

	Control (9)	Aortic constricted (15)
LVP[b], mmHg	120 ± 4	162 ± 5[d]
Heart Rate, bpm	398 ± 8	438 ± 9[d]
MAP[c], mmHg	104 ± 4	118 ± 4[d]
Max. dP/dt, mm Hg/sec	6550 ± 350	8420 ± 340[d]

[a] Values are mean ± SE with number of animals in parentheses.
[b] Peak left ventricular pressure.
[c] Mean arterial pressure.
[d] $P < 0.05$ *vs* control.

TABLE II. ALKALINE PHOSPHATASE SPECIFIC ACTIVITY IN EPICARDIAL AND ENDOCARDIAL PORTIONS OF LEFT VENTRICLE FROM CONTROL AND AORTIC CONSTRICTED ANIMALS[a]

	Control (7)	Aortic constricted (8)
Epicardium	6.38 ± 0.48	7.03 ± 0.32
Endocardium	7.40 ± 0.38[c]	6.85 ± 0.48
Endo/Epi[b]	1.21 ± 0.07	0.97 ± 0.04[d]

[a] Enzyme activity is expressed as nmole *p*-nitrophenol released/mg protein/min under conditions described in Methods. Values are mean ± SE with number of animals in parentheses.
[b] Ratio of endocardial-epicardial specific activities.
[c] $P < 0.05$ vs epicardium by paired *t* analysis.
[d] $P < 0.05$ vs control.

The observed alterations in enzyme activity can not be accounted for by differences in separating LV tissue. In control animals, 59 ± 1% and 41 ± 1% of the total LV was designated EPI and ENDO, respectively. Identical values were obtained in aortic constricted animals.

In contrast to vascular density, there was no indication of marked transmural differences in tissue levels of protein, nucleic acids and connective tissue in control animals (Table III). Normal tissue levels and normal transmural relationships were maintained in aortic constricted animals (Table III).

Discussion. The increased LV mass resulting from pressure overload would elevate myocardial oxygen demands. Moreover, from the hemodynamic measurements

TABLE III. PROTEIN, NUCLEIC ACIDS AND CONNECTIVE TISSUE LEVELS IN EPICARDIAL AND ENDOCARDIAL PORTIONS OF LEFT VENTRICLE FROM CONTROL AND AORTIC CONSTRICTED ANIMALS.[a]

	Control (11)			Aortic constricted (8)		
	Epi[b]	Endo[c]	Endo/Epi[d]	Epi	Endo	Endo/Epi
Protein	185 ± 7	176 ± 7	0.95 ± .02	176 ± 10	161 ± 5	0.92 ± .03
RNA	3.72 ± .11	3.53 ± .14	0.95 ± .02	3.67 ± .09	3.53 ± .09	0.96 ± .02
DNA	2.76 ± .10	2.43 ± .10	0.88 ± .03	2.90 ± .23	2.68 ± .29	0.92 ± .06
OH-P[e]	0.89 ± .03	0.81 ± .04	0.92 ± .04	0.92 ± .05	0.92 ± .04	1.00 ± .07

[a] All tissue level values are mg/g wet weight with mean ± SE shown. Number of animals given in parentheses.
[b] Epicardium.
[c] Endocardium.
[d] Ratio of endocardial-epicardial tissue levels.
[e] Hydroxyproline.

obtained from aortic constricted rats in the present study, it is clear that elevations in LV pressure, heart rate, max. *dP/dt* and afterload also dictate increased myocardial oxygen consumption (4). The heart normally extracts all but approximately 5 vol% of available oxygen from arterial coronary blood (1, 14). Since little additional oxygen can be obtained by increasing oxygen extraction, coronary blood flow would be expected to increase in order to supply the additional oxygen required by the hypertrophied LV. However, recent studies have shown that total blood flow to the hypertrophied LV (2) as well as blood flow/g tissue (3) is reduced below normal levels.

A combination of multiple hemodynamic factors influences net coronary blood flow (1). The elevated mean arterial pressure observed in aortic constricted rats would increase coronary perfusion pressure and tend to evoke a similar directional change in blood flow. In contrast, the elevated LV pressure created by aortic constriction would increase vascular compression and reduce blood flow. The observed elevation in max. *dP/dt* would also contribute to reduced blood flow by a similar mechanism. In the normal LV, the majority of blood flow occurs during diastole when extravascular compressive forces are minimal (14). The tachycardia which accompanied pressure overload in rats would restrict the coronary inflow period by impinging upon the diastolic phase of the contractile cycle. Thus, in summing the hemodynamic factors which influence coronary blood flow, a reduction in overall coronary flow with LV pressure overload would not be completely unexpected. Furthermore the ENDO portion of the LV is considered to be marginally perfused under normal circumstances (5, 6) and would be profoundly affected by even a minor reduction in coronary blood flow. Sympathomimetic agents have been shown to divert blood flow away from the ENDO portion of the LV (15). Taken together, the hemodynamic measurements obtained in the present study suggest that the cardiovascular system of the aortic constricted animals was operating under conditions of enhanced sympathetic stimulation. Increased sympathetic activity in aortic constricted animals would further reduce the already limited oxygen available to the ENDO portion of the hypertrophied LV.

Previous work by Myers and Honig (16) has shown that the ENDO portion of the normal LV possesses a greater vascular density than the EPI portion. In the present studies, a similar relationship was found in the ENDO-EPI alkaline phosphatase specific activities of control rat LV. Therefore, alkaline phosphatase specific activity would appear to be a valid chemical marker for myocardial vascular density. Concentric LV hypertrophy is created by pressure overload conditions. If concomitant vascular growth does not accompany ENDO tissue accumulation, then a decreased ENDO vascular density would result. Morphological studies (17, 18) and an evaluation of vascular capacity by a radioisotopic method (19) have shown a decreased vascular density in the LV of hypertrophied hearts. In the present studies it would appear that accumulated ENDO tissue outstrips its vascularity in the LV which has enlarged in response to a

pressure overload stimulus. In contrast, protein, nucleic acids and connective tissue increased in proportion to the increase in LV mass. The resulting loss of a preferential ENDO vascular density gradient could impose further flow limitations upon the normally, marginally perfused portion of the ventricle.

Since blood flow was not measured in the present studies, no conclusions can be drawn regarding the actual level of blood flow in the hypertrophied LV. Nevertheless, alterations in both hemodynamic factors and coronary vascular density were identified as being potentially capable of reducing blood flow to the hypertrophied LV, particularly to the ENDO portion.

Summary. LV pressure overload was created in adult male rats by abdominal aortic constriction. Three weeks following surgery, LV weight was increased by approximately 25% in aortic constricted animals. Aortic constriction produced a substantial pressure overload, as indicated by a 40 mmHg increase in peak LV pressure. Significant increases in heart rate and max *dP/dt* accompanied the pressure overload. The above hemodynamic factors would tend to reduce blood flow to the LV by (a) increasing vascular compression and (b) restricting the coronary inflow period. Alkaline phosphatase specific activity was utilized as an index of vascular density in the EPI and ENDO portions of control and hypertrophied LV. In control LV, the specific activity of the ENDO portion was significantly greater than the EPI portion, indicating a greater ENDO vascular density. The preferential ENDO vascular density gradient was eliminated in the LV which had enlarged in response to a pressure overload stimulus. Both hemodynamic factors and alterations in vascular density were identified as being potentially capable of reducing blood flow to the hypertrophied LV.

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