Relationship of Cardiac Hemodynamic and Biochemical Adaptations to Mortality during Long-Term Aortic Constriction (43314)

DAVID L. CRANDALL,[1] BRIAN M. GOLDSTEIN, GREGORY D. FERRARO, AND PETER CERVONI
Cardiovascular Research, Medical Research Division, American Cyanamid Company, Pearl River, New York 10965

Abstract. **To determine the biochemical and hemodynamic responses to aortic ligation, and to assess the survival rate after the induction of hypertension, 90 normotensive rats were subjected to surgical constriction of the abdominal aorta. Mortality, left ventricular hemodynamics, myocardial biochemical assays, and plasma renin assays were determined 1 week, 1 month, 3 months, or 1 year later. Mortality was greatest between 1 week and 3 months after aortic ligation, during which plasma renin activity was significantly elevated. The rate of left ventricular pressure rise, contractile index, and myocardial α-adrenoceptor number were increased at 1 month, but were comparatively depressed at 3 months after the operation, suggesting that the heart was in failure at this time. At 1 year after ligation, hemodynamic and biochemical parameters continued toward normalization. Our data suggest that, in this rodent model, cardiac pump failure occurs through a combination of time-dependent, pressure-induced mechanical adaptations and myocardial biochemical changes that involve both the renin-angiotensin and sympathetic nervous systems. The observed relationship between mortality, myocardial hemodynamics, and biochemical parameters may be used for additional basic research investigations concerning the early periods of cardiac failure.** [P.S.E.B.M. 1991, Vol 198]

Animal models investigating hypertension-induced cardiac hypertrophy have often been used to determine the events contributing to heart failure (1–4). Because the presentation of the clinical situation follows a progression of events occurring over a considerable period of time, some investigators have used animal models with defined myocardial aging effects together with superimposed hypertension as a correlate to the human condition (5–7). One purpose of experimental studies linking duration of hypertension to left ventricular adaptative responses is to identify the point at which cardiac muscle can no longer respond to the pressure-induced stress, thereby signaling the beginning of a series of events that will result in cardiac pump failure. Incisive data concerning the time course of the disease in animal models might, therefore, allow intervention before irreversible failure, leading to new insight concerning the mechanisms involved.

[1]To whom correspondence and requests for reprints should be addressed at 200-4603 Lederle Labs, Pearl River, NY 10965.

Received February 20, 1991. [P.S.E.B.M. 1991, Vol 98]
Accepted June 11, 1991.

0037-9727/91/1982-0747$3.00/0

Most recently, data from such studies have been predominantly obtained by analyzing the hemodynamics following pressure-induced hypertrophy in a rodent strain known to exhibit heart failure during aging (4–6). In these and other rodent studies, the experimental design involved induction of hypertension in normotensive, aged animals or analysis of hemodynamic and cardiac morphometric adaptations at only the initial and final time points of the study. While of considerable value, the possibility exists that additional groups at more time points would be helpful for considering the course of pressure-induced failure in rodents. In addition, the effects of the surgical intervention upon the renin-angiotensin system were not assessed, even though a correlation between elevated plasma renin and cardiac disease is well-documented (7).

The present investigation describes the hemodynamic and cardiac muscle adaptations to elevated systemic pressure in young and aged rats, and at several intervals between the induction of the hypertension and analysis in the oldest group 1 year later. Interpretation of these studies has relevance toward understanding both the duration of hypertension required to initiate experimental heart failure and the separate effect of

aging in the etiology of congestive heart disease observed clinically.

Materials and Methods

One hundred and eighteen male Sprague-Dawley rats were used initially in this study, 90 of which were subjected to aortic ligation and 28 of which were used as sham-operated controls. Rats (Charles River Breeding Laboratories, Wilmington, MA) were housed individually at 25°C with a controlled 12:12-hr. light:dark cycle and with free access to food and water. At 60 days of age, hypertension was induced by ligating the abdominal aorta between the two renal arteries. The surgical procedure involved ether anesthesia, a retroperitoneal incision in the left flank, and retraction of the kidney (8, 9). The aorta was subsequently isolated to the origin of the renal arteries, and a 000 silk ligature was placed around the aorta between the renal arteries and tied as tightly as possible, thereby causing severe constriction of the aorta and restriction of blood flow to the left kidney and the organs below the ligature. Surgery was performed on approximately six rats per day over a 4-week period and, in addition, some rats were used for right carotid and left femoral artery pressure measurements during the actual ligation in order to estimate changes in blood flow proximal and distal to the ligature.

Rats were returned to their cages and monitored on a weekly basis for survival rate and body weight changes. At appropriate time intervals following surgery, rats were randomly selected for hemodynamic monitoring. They were anesthetized with ether, placed on a heating pad maintained at 37°C (Aquamatic K-Pad; Hamilton Industries, Cincinnati, OH) and intubated, and the left femoral vein was cannulated. A 3-F Millar Micro-Tip pressure transducer (Millar Instruments, Houston, TX) was introduced into the right carotid artery. Following these surgical procedures under ether anesthesia, sodium pentobarbital (30mg/kg) was delivered via the femoral vein cannula in preparation for hemodynamic monitoring. Systolic, diastolic, and mean pressure, as well as heart rate, were monitored for 5 min, followed by passage of the transducer into the left ventricle.

Hemodynamics were subsequently recorded from the left ventricle for 5 min, the transducer was returned to the carotid artery, and another 5-min recording period elapsed. All pressure recordings were visualized on a Grass model 7 physiological recorder, which was interfaced to a computer accessing data at a rate of 200/sec (MINC 11/23; Digital Equipment, Maynard, MA). Data were updated at 1-min intervals, and the average of the values obtained from the 5-min recording period was considered representative of the hemodynamics of each animal. In addition to standard hemodynamic indices, data were expressed as contractile index, which was equivalent to dP/dt per unit of left ventricular mass.

Rats were killed by decapitation, cavity blood was collected, and hearts were quickly excised, rinsed free of blood in cold saline, blotted of excess moisture, and weighed to the nearest tenth of a gram. The atria and right ventricle were dissected and the separated left ventricle was weighed. An incision was then made along the vertical plane of the left ventricular free wall, and muscular thickness was determined along the line of the incision with three separate measurements using a microprocessor-controlled, digital readout, stainless steel caliper with a designated accuracy of 0.02 mm. The average of the three measurements was considered as a representative value of chamber-free wall thickness. The heart remained moistened with cold saline throughout this procedure, which was completed within 90 sec.

Following morphometric measurements, hearts were frozen with tongs precooled in liquid nitrogen and stored at −65°C. Within 6 weeks, ventricular tissue was thawed on ice and prepared for analysis of cardiac α-adrenoceptor density and affinity. Membranes were prepared from cardiac tissue, as described previously (10). Isolated cardiac membrane protein (500 μg) was then assayed for α-adrenoceptor binding by the method of Karliner *et al.* (11). Briefly, membrane protein was incubated in triplicate at five different concentrations of [^{3}H]prazosin ranging from 0.3 to 5 nM, with or without 10 μM phentolamine. Following a 20-min incubation period, specifically bound prazosin was separated with a Millipore sampling manifold, and α-adrenoceptor concentration (B_{max}) and affinity (K_d) were assessed by standard Scatchard analysis.

For renin analysis, plasma was collected on the day of the experiment by draining a sample from the left femoral vein directly into a tube containing EDTA, and plasma renin activity was determined on fresh samples using a commercially available radioimmunoassay (Baxter Healthcare Corp., Cambridge, MA). Serum prepared from cavity blood was subjected to chemical analysis using an automated system with appropriate controls.

Comparisons between and within groups were made using a two-way analysis of variance, with the factors being time after coarctation and its associated hemodynamic or morphologic ramifications. The comparisons between group means were made with a Student's t test by using the pooled error term from the analysis of variance. Values were tabulated as the mean ± SE and were considered significantly different at $p < 0.05$.

Results

Figure 1 illustrates survival data in rats with aortic ligation. Mortality was greatest between 1 week and 3

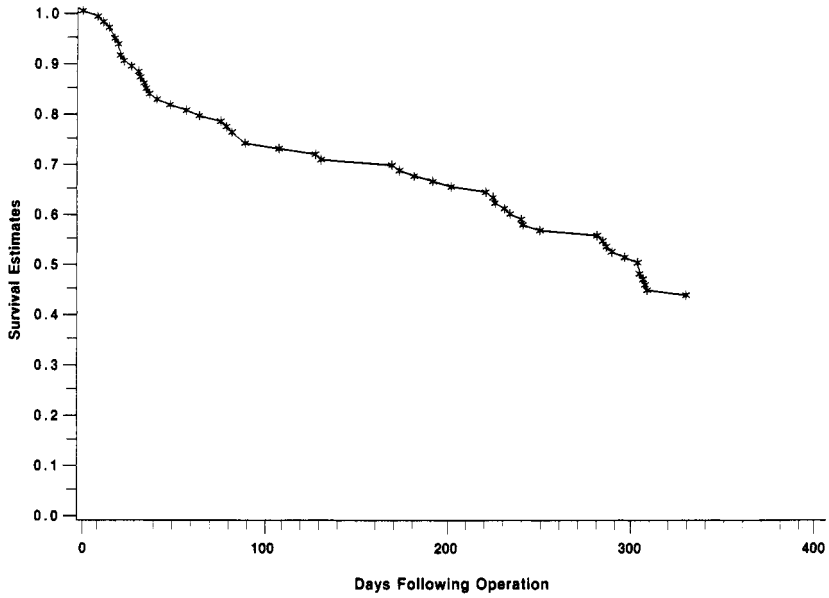

Figure 1. Cumulative percentage of survival of rats in which the aorta was ligated between the renal arteries at Day 0. Each x represents the death of an individual animal, and the curve can be used as a reference for future survival estimates.

Table I. Heart, Ventricular Weights, and Ratios to Body Weight after Various Stages of Aortic Ligation[a]

Parameter	Treatment	Duration of ligation			
		1 Week	1 Month	3 Months	12 Months
Body wet wt. (g)	Sham	356 ± 10	399 ± 16	524 ± 17	665 ± 16
	Ligated	247 ± 7	305 ± 23	505 ± 7	649 ± 19
	P	<0.001	<0.01	NS	NS
Heart wet wt. (mg)	Sham	982 ± 29	1120 ± 62	1209 ± 42	1567 ± 55
	Ligated	1013 ± 29	1132 ± 53	1463 ± 53	1676 ± 82
	P	NS	NS	<0.01	NS
Heart wet wt./body wet wt. (mg/g)	Sham	2.77 ± 0.06	2.80 ± 0.07	2.33 ± 0.14	2.36 ± 0.09
	Ligated	4.12 ± 0.16	3.83 ± 0.34	2.90 ± 0.11	2.59 ± 0.14
	P	<0.001	<0.05	<0.01	NS
Left ventricular wet wt. (mg)	Sham	692 ± 22	815 ± 44	960 ± 55	1108 ± 68
	Ligated	738 ± 19	841 ± 49	1084 ± 47	1241 ± 68
	P	NS	NS	NS	<0.08
Right ventricular wet wt. (mg)	Sham	176 ± 8.7	193 ± 13.2	241 ± 6.9	301 ± 15.9
	Ligated	168 ± 12.2	181 ± 10.2	236 ± 6.6	271 ± 10.3
	P	NS	NS	NS	NS
n	Sham	7	6	6	8
	Ligated	10	6	11	8

[a] Results are expressed as mean ± SE, with *n* indicating sample size. NS = not significantly different between groups.

months after surgery, continuing at a lower rate for the remainder of the study. In this study, 90 rats underwent aortic ligation, 52 of which died at some time during the 1-year monitoring period (58% mortality). There were no deaths among the 28 sham-operated rats. Specific mortality data, with percentage of contribution to total mortality in parentheses, were as follows: one rat died within the first week (1.1%), 11 rats died between the second week and the first month (12.2%), 12 rats between the second and third months (13.3%), seven rats between the fourth and sixth months (7.8%), 13 rats between the seventh and ninth months (14.4%), and eight rats from the beginning of the tenth month through the completion of the 1-year study (8.9%).

The initial effects of aortic ligation on body and heart weight are shown in Table I. While of an initially equal body weight, aortic constriction was associated with a rapid decrease in body weight, as 1 week after surgery the ligated group weighed about 100 g less then the sham-operated group. Differences in body weight remained at 1 month after the operation, but they were not apparent at 3 months after the surgery.

Absolute heart weight was significantly greater in aortic-constricted rats at 3 months after the surgery, and heart weight to body weight ratios indicated the

Table II. Left Ventricular Morphologic and Hemodynamic Adaptations to Different Periods of Aortic Ligation[a]

Parameter	Treatment	Duration of ligation			
		1 Week	1 Month	3 Months	12 Months
Left ventricular thickness (mm)	Sham	3.54 ± 0.20	3.62 ± 0.18	3.81 ± 0.21	4.13 ± 0.14
	Ligated	3.86 ± 0.15	3.73 ± 0.20	4.30 ± 0.10	4.34 ± 0.18
	P	NS	NS	<0.05	NS
Peak systolic pressure (mmHg)	Sham	157 ± 8.4	143 ± 4.0	142 ± 7.6	133 ± 10.0
	Ligated	200 ± 7.5	218 ± 19.9	172 ± 9.3	153 ± 9.6
	P	<0.01	<0.01	<0.05	NS
Rate of pressure rise (mmHg/s)	Sham	6044 ± 234	5891 ± 140	5399 ± 167	5556 ± 262
	Ligated	7465 ± 334	7724 ± 575	6095 ± 265	6220 ± 316
	P	<0.01	<0.05	NS	NS
Contractile index (mmHg/s/mg)	Sham	8.83 ± 0.55	7.34 ± 0.47	5.73 ± 0.41	5.05 ± 0.35
	Ligated	10.16 ± 0.51	9.16 ± 0.34	5.66 ± 0.20	5.12 ± 0.39
	P	NS	<0.01	NS	NS

[a] Values are mean ± SE. For sample size, see footnote to Table I. The contractile index is equivalent to the rate of pressure rise, except that is corrected for left ventricular mass.

existence of cardiac hypertrophy from 1 week through 3 months of ligation. Left ventricular weight was increased at 1 year ($P = 0.08$), and left ventricular thickness was significantly increased in hearts from rats 3 months after the ligation (Table II). Left and right kidney weights were also obtained in order to verify the effect of the surgery on blood flow distal to the ligation. Without exception, kidneys were of equivalent weight in the sham-operated groups, while the left kidneys were severely atrophied in the ligated rats. Mean right kidney weight was 2.72 ± 0.21 g, while left kidney weight was 0.52 ± 0.14 g ($P < 0.001$), when pooling weights from all ligated groups. Postmortem evidence of left renal atrophy was a prerequisite for including these animals in the ligated group data analysis.

Aortic ligation resulted in a rapid onset of severe systemic arterial hypertension. In acute experiments used for validating the degree of aortic stenosis, immediately following tightening of the ligature, right carotid mean arterial pressure slowly increased approximately 50 mm Hg, while left femoral arterial pressure was rapidly reduced to a stable mean pressure of approximately 25 mm Hg, a decrease of over 80 mm Hg in less than 15 sec. In chronic experiments, mean arterial blood pressure averaged 137 mm Hg for the sham-operated group, with a range of 142 ± 5 mm Hg 1 week after the surgery to 130 ± 7 mm Hg 1 year later. Aortic ligation resulted in the following mean arterial blood pressure recordings: 1 week exhibited 170 ± 7 mm Hg, 1 month resulted in a reading of 190 ± 11 mm Hg, 3 months yielded 171 ± 10 mm Hg, and at 1 year after ligation, mean arterial blood pressure was 147 ± 9 mm Hg. With the exception of the pressures recorded 1 year after surgery, aortic ligation resulted in a statistically significant elevation in systemic pressure ($P < 0.01$). Interestingly, the heart rates did not differ between groups at any point in the study, with mean heart rates ranging from 400 to 430 beats per minute.

Left ventricular hemodynamic adaptations to different periods of aortic constriction are shown in Table II. The most dramatic changes in hemodynamics occurred at 1 month after ligation, at which time peak systolic pressure, rate of pressure rise, and contractile index were all significantly elevated, compared with the appropriate sham-operated control group.

Figure 2 represents changes in plasma renin activity (PRA) associated with the surgery. PRA was significantly elevated at 1 week and remained elevated 1 month after ligation. However, PRA was normalized to values equivalent to those observed in the sham-operated group at 3 months after surgery. To ensure that the PRA values were not affected by the surgical procedure, blood was collected and processed from nine normotensive rats of ages equivalent to those of the 3-month sham-operated and ligated groups. The PRA in these normotensive rats was 8.90 ± 1.19 ng/ml/hr, which was not significantly different from any of the sham-operated group values between 1 week and 3 months after ligation. Binding of radiolabeled prazosin to cardiac membrane preparations also indicated that

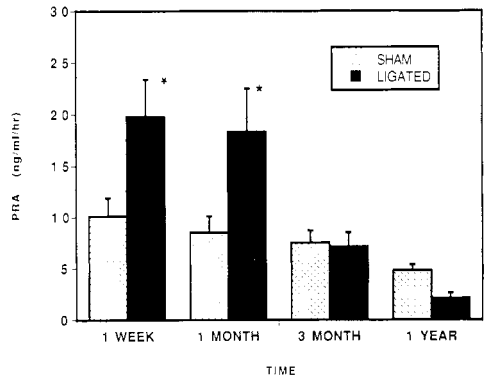

Figure 2. Plasma renin activity in sham-operated and aortic-ligated rats. *Significantly different from sham-control at $p < 0.05$.

1 month of aortic ligation was associated with an elevation of α-adrenoceptor number (Table III). No differences in receptor affinity were observed between groups.

Blood chemistry is shown in Table IV. Basal urinary nitrogen was greater in the ligated rats at all time points of analysis after the surgery, but these elevations were quantitatively minor. In general, aortic ligation did not result in dramatic abnormal alterations in serum chemistry.

Discussion

Investigations concerning the time course of adaptations to aortic constriction by rodent hearts have often used a strain of laboratory rat selected on the basis of a well-defined life span (4–6). The experimental design of these aforementioned studies has employed ligation at different ages for relatively short periods of time, whereas our study used ligation at the same, young age for a very long period of time. While both experimental designs can provide data concerning the effect of age on the ability of the heart to respond to pressure-induced hypertrophy, our experiments are unique in that they allow determination of the period of greatest mortality during a 1-year study. By investigating mortality rate, cardiac muscle adaptations, biochemical changes, and hemodynamic responses, our data indicated that cardiac pump failure was most likely to occur between 1 and 3 months after aortic constriction.

Insight into the mechanistic basis of the high mortality during the initial phase of the study is provided through both hemodynamic and biochemical data. Analysis of left ventricular hemodynamics indicated that the aortic constriction produced a ventricular hypertrophy that was associated with a depressed peak rate of pressure rise and contractile index between 1 and 3 months, suggesting that the heart was incapable of continued response to the elevated afterload during this time. Biochemical data revealed increased levels of plasma renin activity during this same period, which is an established result of aortic constriction, especially

Table III. Effect of Duration of Aortic Ligation on Left Ventricular α_1-Adrenoceptor Binding Properties[a]

Duration of ligation	Treatment	n	B_{max}	K_d
1 Week	Sham	9	50.8 ± 4.26	0.504 ± 0.186
	Ligated	8	42.2 ± 4.48	0.481 ± 0.031
1 Month	Sham	7	59.6 ± 4.61	0.800 ± 0.150
	Ligated	7	70.3 ± 7.89[b]	0.544 ± 0.111
3 Months	Sham	4	51.9 ± 7.91	0.817 ± 0.038
	Ligated	8	52.3 ± 4.59	0.647 ± 0.096
12 Months	Sham	7	47.7 ± 5.96	0.766 ± 0.091
	Ligated	7	58.6 ± 8.57	0.883 ± 0.054

[a] Values are mean ± SE. B_{max} is expressed in fmole/mg protein. K_d is expressed in nanomolars.
[b] Significantly different from pooled sham value at $P < 0.05$.

Table IV. Serum Chemistry of Sham-Operated and Aortic-Ligated Rats[a]

Parameter	Treatment	Duration of ligation			
		1 Week	1 Month	3 Months	1 Year
Blood urea nitrogen (mg/dl)	Sham	18 ± 0.88	18 ± 1.2	19 ± 0.69	15 ± 1.0
	Ligated	27 ± 2.4	26 ± 3.0	22 ± 1.01	31 ± 3.2
	P	<0.05	<0.05	<0.05	<0.001
Sodium (mmol/liter)	Sham	145 ± 1.1	143 ± 1.1	144 ± 1.1	144 ± 1.1
	Ligated	140 ± 1.6	144 ± 1.5	142 ± 1.1	146 ± 2.1
	P	<0.05	NS	NS	NS
Potassium (mmol/liter)	Sham	5.0 ± 0.38	5.7 ± 0.56	5.3 ± 0.48	5.5 ± 0.33
	Ligated	5.8 ± 0.59	5.7 ± 0.32	5.9 ± 0.35	5.4 ± 0.28
	P	NS	NS	NS	NS
Chloride (mmol/liter)	Sham	102 ± 1.2	112 ± 1.2	112 ± 1.4	111 ± 0.7
	Ligated	100 ± 1.5	114 ± 0.63	114 ± 1.5	111 ± 1.9
	P	NS	NS	NS	NS
Glucose (mg/dl)	Sham	148 ± 5.6	161 ± 18.2	157 ± 9.3	166 ± 8.4
	Ligated	181 ± 8.7	176 ± 40.6	147 ± 3.9	146 ± 5.5
	P	<0.05	NS	NS	NS
Creatinine (mg/dl)	Sham	0.24 ± 0.03	0.33 ± 0.07	0.27 ± 0.04	0.38 ± 0.05
	Ligated	0.41 ± 0.03	0.47 ± 0.09	0.38 ± 0.02	0.75 ± 0.08
	P	<0.01	NS	<0.05	<0.01
Calcium (mg/dl)	Sham	9.4 ± 0.22	9.3 ± 0.33	9.4 ± 0.17	9.5 ± 0.21
	Ligated	8.6 ± 0.82	10.9 ± 1.07	9.0 ± 0.38	10.4 ± 0.20
	P	NS	NS	NS	<0.01
Cholesterol (mg/dl)	Sham	36 ± 3.1	53 ± 5.6	57 ± 3.1	81 ± 10.0
	Ligated	52 ± 3.5	53 ± 5.03	66 ± 4.1	157 ± 16.1
	P	<0.01	NS	NS	<0.01

[a] Values are mean ± SE. For the number of animals in each group, see the footnote to Table I. NS, not significant ($P > 0.05$).

during the early postoperative stages (12, 13). The serum chemistry profile indicated that while basal urinary nitrogen and creatinine were significantly elevated in most ligated rats, in agreement with other studies, these values were not indicative of severe renal dysfunction (6). The most striking effect on blood chemistry, therefore, was elevated plasma renin activity, which has direct detrimental effects on cardiac muscle cells *in vitro* (14); *in vivo,* the angiotensin II component of the axis has been shown to facilitate norepinephrine release (15).

Our data support the concept of involvement of the sympathetic nervous system, since the myocardial α-adrenoceptor number increased during the period of greatest mortality. Similar increases in the α-adrenoceptor number have, in fact, been observed by other investigators during periods of *in vivo* ischemia (16, 17), and are suggested to be a component of clinically observed congestive heart failure (18). In separate studies, others have shown that cardiac β-adrenoceptor binding sites can either increase or decrease as a result of specific experimental interventions (19, 20). While our binding data suggest that α-adrenoceptors may be specifically regulated in this model, a complete interpretation of receptor physiology requires additional analysis of both receptor subtypes in the same animal, as well as agonist and antagonist binding data. In the present model, therefore, both hemodynamic and biochemical data indicate that components of the renin-angiotensin system and sympathetic nervous system are most affected during the 1-year period following aortic ligation at time points between 1 and 3 months after the insult, suggesting that interactions between these systems may be contributing to the significant mortality.

Heart failure is a frequently diagnosed disease, yet the mechanisms contributing to irreversible cardiac failure remain poorly understood (21). Animal models are necessarily required for investigating the disease, and the large number of studies using hypertension as a stimulus for the induction of heart failure have provided an important data base for future experimentation. While, albeit rarely, long-term studies have been used to assess the course of heart failure in rodents subjected to myocardial infarction (22, 23), our study is the first to follow mortality, hemodynamics, and PRA for 1 year after the induction of renovascular hypertension. In fact, many authors using this model do not routinely monitor mortality (2, 13, 24), while in reports concerning other methods of surgical intervention producing heart failure, long-term studies are difficult to perform because of the prohibitively high mortality rate approaching 75% of total (25). Our mortality data indicated that the possibility of impaired cardiac contractility and death was greatest through the first 3 months after aortic ligation, and that mechanistically, successful pharmacologic intervention at this time could include modulators of the renin-angiotensin system. Future studies investigating this question, as well as the successful adaptations occurring in those animals surviving past 3 months, could provide important data concerning the pathophysiologic processes involved.

1. Laird JF, Spadone JC. Regional circulations in experimental coarctation of the aorta in conscious dogs. J Hypertension **3**:281–291, 1985.
2. Stanek KA, Coleman TG, Murphy WR. Overall hemodynamic pattern in coarctation of the abdominal aorta in conscious rats. Hypertension **9**:611–618, 1987.
3. Spann JF, Covell JW, Eckberg DL, Sonnenblick EH, Ross J, Braunwald E. Contractile performance of the hypertrophied and chronically failing cat ventricle. Am J Physiol **223**:1150–1157, 1972.
4. Isoyama S, Wei J, Izumo S, Fort P, Schoen F, Grossman W. Effect of age on the development of cardiac hypertrophy produced by aortic constriction in the rat. Circ Res **61**:337–345, 1987.
5. Capasso JM, Palackal T, Olivetti G, Anversa P. Severe myocardial dysfunction induced by ventricular remodeling in aging rat hearts. Am J Physiol **259**:H1086–H1096, 1990.
6. Capasso JM, Malhotra A, Scheuer J, Sonnenblick EH. Myocardial biochemical, contractile, and electrical performance after imposition of hypertension in young and old rats. Circ Res **58**:445–460, 1986.
7. Brunner HR, Laragh JH, Baer L, Newton MA, Goodwin FT, Krakoff LR, Bard RH, Buhler FR. Essential hypertension: Renin and aldosterone, heart attack and stroke. N Engl J Med **286**:441–449, 1971.
8. Rojo-Ortega J, Genest J. A method for production of experimental hypertension in rats. Can J Physiol Pharmacol **46**:883–885, 1968.
9. Crandall DL, Goldstein BM, Lizzo FH, Lozito RJ, Cervoni P. Development of an animal model for investigating the disparate myocardial effects of obesity and hypertension. J Appl Physiol **64**:1094–1097, 1988.
10. Crandall DL, Lai FM, Huggins FJ, Tanikella TK, Cervoni P. Effect of caloric restriction on cardiac reactivity and beta-adrenoceptor concentration. Am J Physiol **244**:H444–448, 1983.
11. Karliner JS, Barnes P, Hamilton CA, Dollery CT. Alpha$_1$-adrenergic receptors in guinea pig myocardium: Identification by binding of a new radioligand, ^{3}[H]-prazosin. Biochem Biophys Res Commun **90**:142–149, 1979.
12. Lai FM, Tanikella T., Thibault L., Chan PS, Cervoni P. Effects of different stages of aortic coarctation hypertension on aortic contraction and relaxation in rats. J Pharmacol Exp Therap **214**:388–394, 1980.
13. Salgado HC, Krieger EM. Mechancial and renin-angiotensin system components in acute aortic coarctation hypertension. Hypertension **8**(suppl):I133–I136, 1986.
14. Tan L, Jalil J, Janicki J, Weber KT, Clark WA. Cardiotoxic effects of angiotensin II [Abstract]. J Am Coll Cardiol **13**:2A, 1989.
15. Zimmerman JB, Roberston D, Jackson EK. Angiotensin II-noradrenergic interactions in renovascular hypertensive rats. J Clin Invest **80**:443–457, 1987.
16. Corr PB, Shayman JA, Kramer JB, Kipnis RJ. Increased alpha-adrenergic receptors in ischemic cat myocardium. J Clin Invest **67**:1232–1236, 1981.
17. Heathers GP, Yamada KA, Kanter EM, Corr PB. Long-chanin acylcarnitines mediate the hypoxia-induced increase in alpha$_1$-

adrenergic receptors on adult canine myocytes. Circ Res **61**:735–746, 1987.
18. Leier CV, Binkley PF, Cody RJ. Alpha-adrenergic component of the sympathetic nervous system in congestive heart failure. Circulation **82**(suppl):I68–I76, 1990.
19. Mukherjee A, Bush LR, McCoy, KE, Duke RJ, Hagler H, Buja LM, Willerson JT. Relationship between β-adrenergic receptor numbers and physiological responses during experimental canine myocardial ischemia. Circ Res **50**:735–741, 1982.
20. Kenakin TP, Ferris RM. Effects of *in vivo* β-adrenoceptor down-regulation on cardiac responses to prenalterol and pirbuterol. J Cardiovasc Pharmacol **5**:90–97, 1983.
21. Packer M. Vasodilator and inotropic drugs for the treatment of chronic heart failure: Distinguishing hype from hope. J Am Coll Cardiol **12**:1299–1317, 1988.
22. Sweet CS, Ludden CT, Stabilito II, Emmert SE, Heyse JF. Beneficial effects of milrinone and enalapril on long-term survival of rats with healed myocardial infarction. Eur J Pharmacol **147**:29–37, 1988.
23. Sweet CS, Emmert S, Stabilito II, Riberio LG. Increased survival of rats with congestive heart failure treated with enalapril. J Cardiovasc Pharmacol **10**:636–642, 1987.
24. Overbeck HW, Magargal WW. Aortic hypertrophy and "water-logging" in the development of coarctation hypertension. Hypertension **14**:316–321, 1989.
25. Flaim SF, Minteer WJ, Nellis SH, Clark DP. Chronic arteriovenous shunt: Evaluation of a model for heart failure in rat. Am J Physiol **5**:H698–H704, 1979.