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Research Article

Pressure overload left ventricular (LV) hypertrophy was produced by banding the ascending aorta of puppies and allowing them to grow to adulthood. LV free wall weight per body weight increased by 87% from a normal value of 3.23 +/- 0.19 g/kg. Hemodynamic studies of conscious dogs with LV hypertrophy and of normal, conscious dogs without LV hypertrophy showed similar base-line values for mean arterial pressure, heart rate, and LV end-diastolic pressure and diameter. LV systolic pressure was significantly greater, P less than 0.01, and LV stroke shortening was significantly lss, P less than 0.01, in the LV hypertrophy group. In both normal and LV hypertrophy groups, increasing bolus doses of norepinephrine or isoproterenol produced equivalent changes in LV dP/dt. beta-adrenergic receptor binding studies with [3H]-dihydroalprenolol ([3H]DHA) indicated that the density of binding sites was significantly elevated, P less than 0.01, in the hypertrophied LV plasma membranes (111 +/- 8.8, n = 8), as compared with normal LV (61 +/- 5.6 fmol/mg protein, n = 11). The receptor affinity decreased, i.e., disassociation constant (KD) increased, selectively in the LV of the hypertrophy group; the KD in the normal LV was 6.8 +/- 0.7 nM compared with 10.7 +/- 1.8 nM in the hypertrophied LV. These effects were observed only in the LV of the LV hypertrophy group and not in […]

Effects of Pressure Overload, Left Ventricular Hypertrophy on β-Adrenergic Receptors, and Responsiveness to Catecholamines

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Abstract. Pressure overload left ventricular (LV) hypertrophy was produced by banding the ascending aorta of puppies and allowing them to grow to adulthood. LV free wall weight per body weight increased by 87% from a normal value of 3.23±0.19 g/kg. Hemodynamic studies of conscious dogs with LV hypertrophy and of normal, conscious dogs without LV hypertrophy showed similar base-line values for mean arterial pressure, heart rate, and LV end-diastolic pressure and diameter. LV systolic pressure was significantly greater, $P < 0.01$, and LV stroke shortening was significantly less, $P < 0.01$, in the LV hypertrophy group. In both normal and LV hypertrophy groups, increasing bolus doses of norepinephrine or isoproterenol produced equivalent changes in LV dP/dt.

β-adrenergic receptor binding studies with [^{3}H]-dihydroalprenolol ([^{3}H]DHA) indicated that the density of binding sites was significantly elevated, $P < 0.01$, in the hypertrophied LV plasma membranes (111±8.8, $n = 8$), as compared with normal LV (61±5.6 fmol/mg protein, $n = 11$). The receptor affinity decreased, i.e., disassociation constant (K_D) increased, selectively in the LV of the hypertrophy group; the K_D in the normal LV was 6.8±0.7 nM compared with 10.7±1.8 nM in the hypertrophied LV. These effects were observed only in the LV of the LV hypertrophy group and not in the right ventricles from the same dogs. The plasma membrane marker, 5' -nucleotidase activity, was slightly lower per milligram protein in the LV hypertrophy group, indicating that the differences in β-adrenergic receptor binding and affinity were not due to an increase in plasma membrane protein in the LV hypertrophy group. The EC_{50} for isoproterenol-stimulated adenylate cyclase activity was similar in both the right and left ventricles and in the two groups. However, maximal-stimulated adenylate cyclase was lower in the hypertrophied left ventricle. Plasma catecholamines were similar in the normal and hypertrophied groups, but myocardial norepinephrine was depressed in the dogs with LV hypertrophy (163±48 pg/mg) compared with normal dogs (835±166 pg/mg).

Thus, severe, but compensated LV hypertrophy, induced by aortic banding in puppies, is characterized by essentially normal hemodynamics in adult dogs studied at rest and in response to catecholamines in the conscious state. At the cellular level, reduced affinity and increased β-adrenergic receptor number characterized the LV hypertrophy group, while the EC_{50} for isoproterenol-stimulated adenylate cyclase activity was normal. By these mechanisms, adequate responsiveness to catecholamines is retained in conscious dogs with severe LV hypertrophy.

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Introduction

Hypertrophy is a major mechanism by which the left ventricle compensates to a chronic pressure overload. The sympathetic

nervous system is another major compensatory mechanism, and is recruited in response to stress. It is conceivable that β-adrenergic mechanisms of the heart are responsible in part for the preservation of mechanical function in the presence of hypertrophy, and alterations in autonomic control may be involved in the process of decompensation associated with the development of cardiac failure.

The goal of the present investigation was to characterize β-adrenergic regulation of the severely hypertrophied left ventricle, which is induced by chronic, severe pressure overload. These studies were designed to examine inotropic responsiveness to catecholamines in the intact, conscious animal with left ventricular (LV)[1] hypertrophy, and then to investigate the extent to which the biochemical determinations could explain the physiological responses. The biochemical analyses initially were directed towards investigation of β-adrenergic receptor number and affinity, and progressed to the study of coupling of β-adrenergic receptors to adenylate cyclase. Finally, catecholamines were measured in the hypertrophied left ventricle and in the plasma of these dogs, and compared with values obtained in the normal animals.

Methods

Preparation of model. Mongrel puppies of either sex at 7–10 wk of age were anesthetized with halothane (1 vol per 100 ml), ventilated with a respirator, and a right thoracotomy was performed through the fifth intercostal space. The ascending aorta above the coronary arteries was isolated and dissected free of surrounding tissue. In the puppies designated as sham controls, the chests were closed at this time. In the group designated for LV hypertrophy, a Teflon cuff (6–8 mm in diameter) was placed around the aorta and the chest was closed. Both groups of puppies were then allowed to recover and grow for 10–16 mo to adulthood before experimentation. The cuff produced a fixed lesion. 60% of the puppies survived the initial 10–16-mo period after aortic banding.

Instrumentation of animals. At operation, six adult dogs with LV hypertrophy, three sham operated dogs, and three normal, mongrel dogs were sedated with propriopromazine HCl (Tranvet; Diamond Laboratories, Inc., Des Moines, IO) and anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Using sterile technique and through an incision in the left fifth intercostal space, Tygon (Norton Co., Plastics and Synthetic Div. Akron, OH) catheters were implanted in the descending thoracic aorta and left atrium, piezoelectric transducers were implanted on opposing anterior and posterior endocardial surfaces of the left ventricle, and a solid state pressure gauge (P 22, Konigsberg Instruments, Inc., Pasadena, CA) was inserted into the left ventricle via an apical stab wound. The incision then was closed in layers, the pneumothorax reduced, and the animals were allowed to recover.

Arterial and left atrial pressures were measured using the implanted catheters and Statham P23Db strain gauge manometers (Statham Instruments, Inc., Oxnard, CA). LV pressure was measured with the solid state miniature pressure gauge and calibrated in vitro against a mercury manometer and in vivo against the arterial and left atrial pressure measurements. An improved ultrasonic transit-time dimension gauge (1, 2) was used to measure LV diameter. The instrument generates a voltage linearly proportional to the transit time of acoustic impulses traveling at the sonic velocity of 1.5×10^6 mm/s between the 3 MHz piezoelectric crystals, thus giving a record of instantaneous LV diameter. The frequency response of the dimension gauge is flat to 60 Hz. At a constant room temperature, the thermal drift of the instrument is minimal, i.e., <0.01 mm in 6 h. Any drift in the measurement system was eliminated during the experiment by periodic calibrations. This involved substituting pulses of known duration from a crystal-controlled pulse generator with a stability of 0.001%. The position of all transducers was confirmed at autopsy.

The cardiac responses of conscious animals to exogenously administered norepinephrine and isoproterenol were examined in six dogs with LV hypertrophy and in six normal dogs, of which three were sham operated controls. Norepinephrine was administered in bolus doses of 0.05, 0.1, 0.5, 1.0, and 1.5 μg/kg, while isoproterenol was administered in bolus doses of 0.01, 0.05, 0.10, and 0.50 μg/kg. Bolus injections were administered in one 12-h period. Care was taken to allow full recovery of LV function before the next dose was administered. Reproducibility was assured by administering the same doses on a different day at least 3–7 d later.

The data were recorded on a multichannel tape recorder (Hewlett Packard Co., Palo Alto, CA) and played back on a direct writing oscillograph (Gould-Brush). A cardio-tachometer (Beckman No. 9857B Beckman Instruments, Inc., Fullerton, CA) triggered by the pressure pulse provided instantaneous and continuous records of heart rate. Continuous records of LV dP/dt were derived from LV pressure signals using Philbrick operational amplifiers (Teledyne Philbrick, Dedham, MA), operated as differentiators, with a frequency response of 700 Hz. A triangular wave signal was substituted for the pressure signal to calibrate the differentiators directly.

Biochemical studies. These studies were conducted at least 2 d and not >1 wk after the bolus doses of isoproterenol and norepinephrine. These studies were also carried out in five normal dogs and in two dogs with LV hypertrophy that were neither instrumented nor used for the experiments with sympathomimetic amines. After the dogs were anesthetized with 30 mg/kg sodium pentobarbital, the hearts were immediately excised and placed into iced Krebs-Ringer's solution. All subsequent procedures were carried out at 4°C. Approximately 1 mm of epicardium and endocardium were removed with a scissors and discarded. LV myocardium was minced coarsely in buffer (sucrose 0.25 M, $MgCl_2$ 1 mM, and $KHCO_3$ 1 mM), and homogenized with a PT-10ST Polytron (Brinkmann Instruments, Inc., Westbury, NY) tissue disruptor. The homogenate was filtered through one layer of Japanese silk screen, size 12, and centrifuged in a Sorvall RC-2 (DuPont Instruments, DuPont Co., Wilmington, DE) at 1,000 *g* for 15 min. The supernatant was respun at 18,000 *g* for 15 min and the pellet was resuspended in buffer (Tris 0.1 M, $MgCl_2$ 5 mM, and EDTA 1 mM), using a Teflon pestle in a Potter-Elvehjem homogenizer. The homogenization and 28,000 *g* spin were repeated twice. The pellet was resuspended to a protein concentration of 3 mg/ml and stored at −70°C until assayed. At the time of assay, the membranes were again washed in the Tris buffer and centrifuged.

All studies were performed in triplicate in the presence of Tris 0.1 M, $MgCl_2$ 5 mM, and EDTA 1 mM, pH 7.2. For the determination of β-adrenergic receptor binding saturation, 100 μl of the cardiac membrane preparation (2–3 mg protein per milliliter) was incubated at 37°C for 30 min with increasing concentrations (1.0–50 nM) of [^{3}H](−)dihydroalprenolol ([^{3}H]DHA) (New England Nuclear, Boston, MA), with or without unlabeled *d,l*-propranolol (10 μM), in a final reaction volume

1. *Abbreviations used in this paper:* [^{3}H]DHA, dihydroalprenolol; LV, left ventricular; RV, right ventricular.

of 150 μl. A concentration of 10 μM propranolol was required to displace the ligand at the higher concentration of [^{3}H]DHA. After incubation, the 150 μl of reaction mixture was rapidly filtered under vacuum onto Whatman GF/C glass fiber filters (Whatman Laboratory Products, Inc., Clifton, N. J.). The filters were quickly washed (<10 s) three times with 4 ml Tris-$MgCl_2$-EDTA buffer, at 4°C. The filters were counted for 10 min in 10 ml of Hydrofluor (New England Nuclear) in a Packard Tricarb 300 scintillation counter (Hewlett Packard Co.) with a counting efficiency of 50%. Isoproterenol competition curves were performed in triplicate with 100 μl of the membrane preparation (2–3 mg protein per milliliter), 25 μl of [^{3}H]DHA 10 nM, and 25 μl of isoproterenol (10^{-8}–10^{-3} M). The incubation, filtering, and counting were performed as above for the Scatchard analysis.

For the adenylate cyclase assay, 50 μl of solution containing 1 mM ATP (2–3 × 10^6 cpm of [^{32}P]ATP), 20 mM creatine phosphate, 1 U creatine phosphokinase, 1 mM cyclic AMP (2,000–3,000 cpm of ^{3}H-cyclic AMP), 25 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA, and the test substance, i.e., 10 mM isoproterenol, 1.0 mM GTP, and cardiac membranes containing 200–300 μg of protein, were incubated for 10 min in a shaking water bath at 37°C (3). 100 μl of a stopping solution (20 mM ATP, 10 mM cyclic AMP, and 2% sodium dodecyl sulfate) was added to each tube to terminate the reaction, and the tube was heated on a dry bath at 100°C for 3 min. 0.9 ml of water was added and the mixture (1.0 ml) was passed over a column of packed Dowex AG50 W-X4, 200–400 mesh, hydrogen form. The column was eluted with 2 ml of water, and then stacked on top of a 6-mm diameter column containing 1.2 g of alumina (Brockman Grade I) so that the eluate of one column ran onto the other. The Dowex column was eluted with 3 ml of water, and the eluent flowed onto the alumina column. The alumina column was then eluted with 3 ml of 50 mM imidazole, pH 7.4, directly into counting vials containing Hydrofluor (New England Nuclear) (10 ml), and was counted in a Packard TriCarb 300 scintillation counter (Hewlett Packard Co.). Recovery of added cyclic AMP was 40–80%. Zero time controls approached background. Maximal adenylate cyclase activity was assessed by measuring cyclic AMP production in the presence of either 10 mM NaF, 0.1 mM GppNHp.

5′ Nucleotidase (5′ribonucleotide phosphohydrolase) was assayed by the enzyme kinetic method of Arkesteijn (4). The rate of NAD formation from coupled reactions involving AMP and 2-oxoglutarate is measured by the decrease in absorbance at 340 μm, which is directly proportional to 5′ -nucleotidase activity.

Plasma epinephrine, norepinephrine, and tissue norepinephrine levels were determined according to the method of DaPrada and Zurcher (5).

The protein concentrations for each membrane assay were determined by the Lowry method (6). Analysis of saturation binding assays was performed according to the method of Scatchard (7). The data were then analyzed with the iterative curve-fitting program "Ligand" of Munson and Rodbard (8).

Statistical analysis. Data were expressed as mean value±SEM. Data were stored in a digital computer (PDP-11/34) and statistical evaluation was performed by a one-way analysis of variance for linear contrasts and a multiple-way analysis of variance to determine significance between groups. Significance was determined using Scheffe's test (9).

Results

In the LV hypertrophy group, LV free wall weight per body weight (6.03±0.36 g/kg) was almost twice that observed in the normal control group (3.23±0.19 g/kg). Right ventricular (RV) free wall weight per body weight was only slightly elevated (Table I). Since there were no differences in morphology, hemodynamic response, and adrenergic receptor number in the sham-operated controls and the normal adult animals, the data were pooled from these two groups and are referred to as the normal control group.

Table I. Morphology

	Normal (n = 11)	LV Hypertrophy (n = 8)	Confidence level
Body weight (*kg*) (mean±SEM)	25±3	20±1	NS
LV free wall weight (*g*)	81±9	123±9	$P < 0.05$
LV free wall weight/ body weight (*g/kg*)	3.23±0.19	6.03±0.36	$P < 0.01$
LV free wall and septum weight (*g*)	110±11	166±12	$P < 0.05$
LV free wall and septum weight/body weight (*g/kg*)	4.41±0.19	8.15±0.53	$P < 0.01$
RV free wall weight (*g*)	39±4	43±3	NS
RV free wall weight/ body weight (*g/kg*)	1.56±0.07	2.10±0.12	$P < 0.05$

NS, not significant.

Physiological studies

Base-line values. Except for LV systolic pressure and LV stroke shortening, base-line values for mean arterial pressure, heart rate, and LV end-diastolic pressure and diameter were similar in the two groups (Tables II and III). In the group of dogs with LV hypertrophy, LV systolic pressure (250 mmHg) was twice ($P < 0.01$) that in the normal control group (125 mmHg), while LV stroke shortening (5.0 mm) was less, $P < 0.01$, in LV hypertrophy than in the normal animals (8.9 mm).

Responses to norepinephrine. Typical responses to the same dose of norepinephrine, 1.0 μg/kg, are shown in a normal dog (Fig. 1) and in a dog with severe LV hypertrophy (Fig. 2). Dose-response curves for normal dogs and dogs with LV hypertrophy are shown in Table II and illustrated in Fig. 3. In both groups of dogs, increasing doses of norepinephrine (Table II) or isoproterenol (Table III) produced equivalent changes in LV dP/dt and arterial pressure. The increases in LV systolic pressure were greater in dogs with LV hypertrophy, but base-line values were higher in that group. The only other significant differences were that LV end-systolic diameter fell less at some doses, which was most likely due to the higher LV systolic pressure. With isoproterenol, LV end-diastolic diameter tended to fall more in the normal dogs than in the dogs with LV hypertrophy.

Myocardial β-adrenergic receptor binding studies

Saturation plots of [^{3}H]DHA binding demonstrated a significant increase in β-adrenergic receptor concentration in the membranes prepared from the hypertrophied LV. Specific [^{3}H]DHA

Table II. Effects of Norepinephrine, 0.05–1.50 μg/kg

	Changes±SEM from base line at the following doses (μg/kg)					
	Base line±SEM	0.05	0.10	0.50	1.00	1.50
LV Systolic pressure (*mmHg*)						
Normal	126±3.8	7.7±2.4	21.4±6.1	70.0±7.0	96.2±12.7	122±18
LVH	249±24.9*	26.5±8.0†	55.0±11.4†	156±35†	168±37	184±30
LV End-diastolic pressure (*mmHg*)						
Normal	8.8±1.1	0.6±0.4	0.1±0.6	0.2±0.9	1.2±0.6	2.1±1.7
LVH	10.5±0.9	0.7±0.3	1.7±0.2	2.3±0.3	2.0±1.2	3.7±1.4
LV dP/dt (*mmHg/s*)						
Normal	3,721±172	805±308	1,354±404	4,930±516	6,650±196	7,182±633
LVH	3,831±438	644±218	1,482±192	5,736±628	6,766±664	7,617±473
LV End-diastolic diameter (*mm*)						
Normal	35.0±2.5	−0.3±0.3	0.3±0.4	0.1±0.1	0.5±0.5	−0.2±0.2
LVH	30.0±2.2	0.5±0.3	0.5±0.1	0.4±0.2	0.3±0.1	−0.2±0.5
LV End-systolic diameter (*mm*)						
Normal	25.9±3.0	−0.7±0.3	−1.1±0.3	−1.6±0.4	−1.3±0.4	−1.7±0.5
LVH	25.0±2.1	−0.3±0.1	−0.4±0.1†	−0.8±0.2	−0.6±0.1	−0.4±0.1†
LV Stroke shortening (*mm*)						
Normal	9.2±1.2	0.3±0.3	1.4±0.5	1.7±0.5	1.8±0.8	1.5±0.5
LVH	4.9±0.9*	0.8±0.2	0.8±0.2	1.1±0.2	1.0±0.1	0.2±0.5
Mean arterial pressure (*mmHg*)						
Normal	98.3±3.4	13.7±4.2	22.4±4.3	54.0±8.5	73.0±11.3	87.3±8.2
LVH	89.0±4.6	9.2±3.5	21.3±6.4	46.7±3.7	61.0±4.9	71.8±10.1
Heart rate (*beats/min*)						
Normal	92.5±3.3	−13.3±3.3	−23.8±5.4	−20.3±2.5	−19.8±2.8	−16.2±4.9
LVH	94.2±5.0	−12.0±4.8	−14.0±5.7	−23.0±3.3	−20.8±4.3	−20.4±8.9

LVH group (n = 6) significantly different from control group (n = 6).
* $P < 0.01$.
‡ $P < 0.05$.

binding to the myocardial membrane preparation was saturable, yielding a single component, linear Scatchard relationship (7). Typical Scatchard analyses of [^{3}H]DHA binding to normal LV myocardial membranes and LV hypertrophied membrane preparations are shown in Fig. 4. In these experiments, the affinity for [^{3}H]DHA was decreased, $P < 0.05$, in the LV of the hypertrophy dog (disassociation constant [K_D] = 10.7±1.8 nM), as compared with the normal LV preparation (K_D = 6.8±0.7 nM). The density of binding sites, as determined from the Scatchard analysis, was significantly higher ($P < 0.01$) in the LV hypertrophy preparation (n = 8) as compared with the normal membranes (n = 11) (111±8.8 vs. 61±5.6 fmol/mg protein) (Fig. 5). The density of binding sites and K_D in the left ventricle of the dogs with LV hypertrophy was significantly greater ($P < 0.01$) than the RV receptor number (79.4±8.4 fmol/mg protein) and K_D (5.4±0.7 nM) for the same dogs. It is also important to note that the β-adrenergic receptor number (63.2±7.0 fmol/mg protein) and K_D (5.4±0.7 nM) in the right ventricles in the normal group were similar to those in the right ventricles of the LV hypertrophy group.

To be sure that the increase in β-adrenergic receptor density in the LV hypertrophy membranes did not reflect a difference

Table III. Effects of isoproterenol, 0.01–0.50 μg/kg

	Changes±SEM from base line at the following doses (μg/kg)				
	Base line±SEM	0.01	0.05	0.10	0.50
LV Systolic pressure (*mmHg*)					
Normal	123±6.7	−1.7±1.7	0.2±3.3	5.2±2.3	29.1±10.5
LVH	250±26*	4.8±4.8	44.2±22.9	80.7±25.8†	173±46†
LV End-diastolic pressure (*mmHg*)					
Normal	8.8±1.2	−2.0±1.3	−3.3±1.0	−4.2±1.3	−4.8±1.8
LVH	9.9±1.3	0.1±0.1	−2.0±0.6	−2.7±0.4	−4.3±0.7
LV dP/dt (*mmHg/s*)					
Normal	3,565±167	346±82	1,325±289	2,681±371	5,883±386
LVH	3,819±395	410±104	944±296	2,921±347	7,687±742
LV End-diastolic diameter (*mm*)					
Normal	34.9±2.1	−0.5±0.2	−1.9±0.5	−3.0±0.9	−3.5±1.0
LVH	29.7±2.2	−0.2±0.2	−0.3±0.2†	−1.4±0.4	−1.4±0.6†
LV End-systolic diameter (*mm*)					
Normal	26.6±2.3	−0.8±0.4	−2.2±0.6	−3.1±0.6	−4.6±1.3
LVH	24.7±2.2	−0.9±0.3	−1.3±0.4	−1.4±0.4†	−1.8±0.7†
LV Stroke shortening (*mm*)					
Normal	8.3±0.9	0.3±0.2	0.3±0.2	0.1±0.2	1.1±0.5
LVH	5.0±1.0*	0.7±0.3	1.0±0.5	0.1±0.2	−0.5±0.6
Mean Arterial pressure (*mmHg*)					
Normal	96.5±4.1	−6.4±2.6	−20.0±5.7	−22.0±3.3	−35.2±2.1
LVH	85.0±5.3	−10.3±2.5	−19.7±3.8	−23.3±3.5	−32.0±5.5
Heart rate (*beats/min*)					
Normal	93.3±1.2	13.7±1.9	50.2±13.2	74.2±18.8	99.7±16.8
LVH	97.3±6.6	28.5±11.4	41.3±10.0	61.2±13.0	104±9

LVH group (n = 6) significantly different from control group (n = 6).
* $P < 0.01$.
† $P < 0.05$.

in protein content induced by the preparation of the membranes, they were assayed for their content of another membrane-associated protein uninvolved in receptor ligand binding. The membrane-associated activity of 5′ nucleotidase was higher in normal (64±6.6 mIU/mg protein) (n = 8) than in LV hypertrophy preparations (35±7.1 mIU/mg protein) (n = 5).

In order to determine if there was an alteration in receptor affinity for agonists in the hypertrophied LV, isoproterenol competition curves were performed with and without 0.1 mM GppNHp. No differences in the IC_{50} were seen in the left ventricles of control vs. hypertrophied dogs or when compared with the right ventricle. The IC_{50} for the left ventricles of control animals with isoproterenol and 0.1 mM GppNHp was 1.0±0.2 μM, as compared with 0.8±0.3 μM (n = 4) without GppNHp. In the left ventricles from hypertrophied animals the IC_{50} with isoproterenol and 0.1 mM GppNHp was 1.2 μM, and without GppNHp the IC_{50} was 0.6 μM (n = 2). At 100 μM isoproterenol the specific binding was 60%.

β-adrenergic receptor-mediated isoproterenol stimulation of adenylate cyclase was examined in the membrane preparations from both normal and LV hypertrophied hearts. As shown in Fig. 6, there were no significant differences in the dose-response curves for isoproterenol-stimulated adenylate cyclase activity in either normal and LV hypertrophied hearts, or in the left or right ventricles from the dogs with LV hypertrophy. The EC_{50} in each case was 6 μM. However, examination of maximal-stimulated adenylate cyclase activity did demonstrate differences between normal and hypertrophied left ventricles. For example, NaF stimulation resulted in adenylate cyclase activity of 5,726±1,508 pmol/min per mg protein in normals, (n = 6) compared with 2,014±491 pmol/min per mg protein in LV hypertrophy (n = 6). With GppNHp 1 mM, the maximal ad-

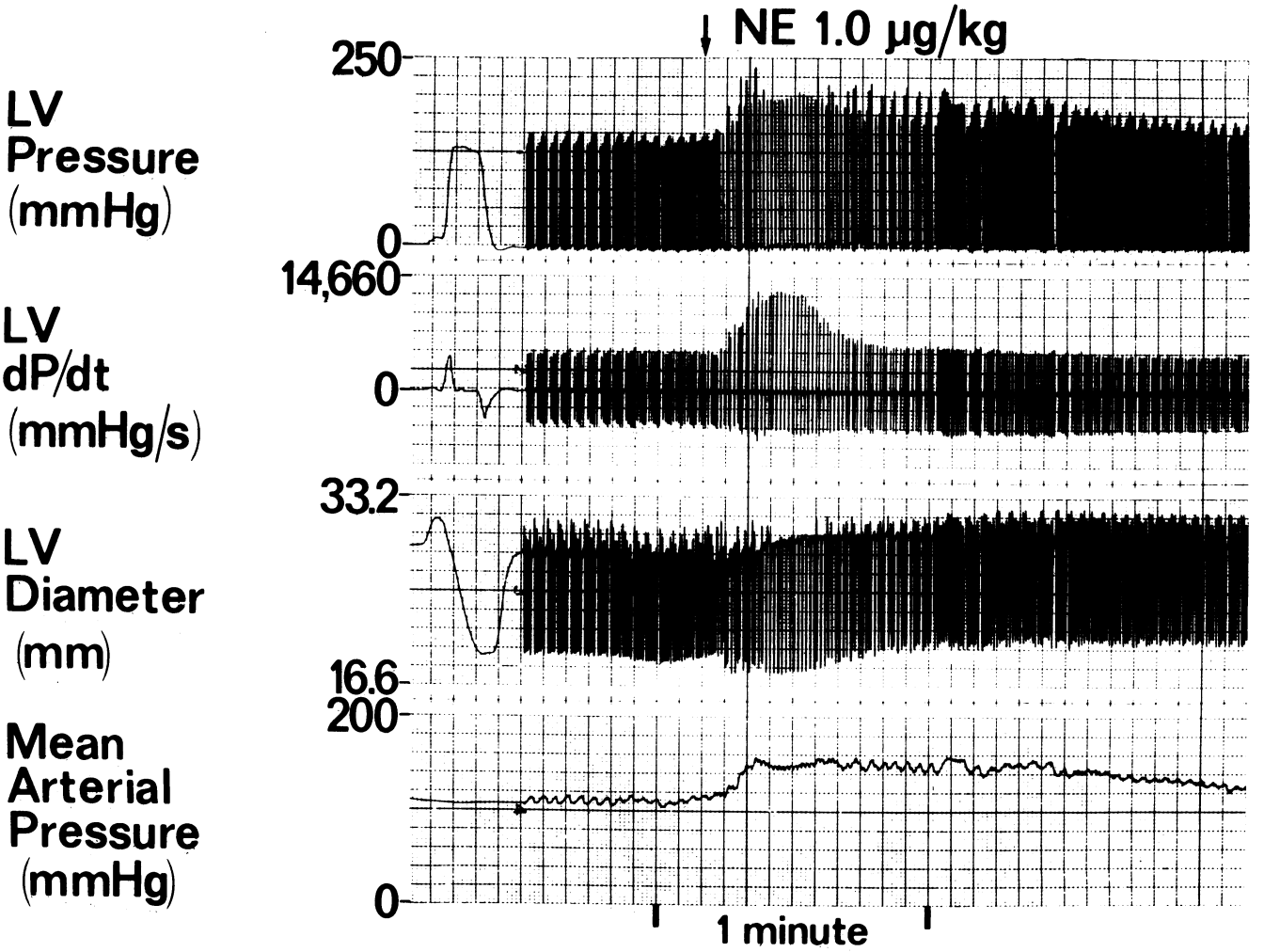

Figure 1. The effects of a bolus of norepinephrine (1.0 µg/kg) are shown on phasic measurements of LV pressure, LV dP/dt, LV diameter, and mean arterial pressure in a normal dog.

enylate cyclase stimulation for the normal left ventricle was 1,628±345 pmol/min per mg protein as compared with 599±238 pmol/min/per mg protein for hypertrophied left ventricles, $P < 0.05$.

Myocardial and plasma catecholamines

LV norepinephrine was higher, $P < 0.01$, in the normal control group (835±166 pg/mg) than in the group with LV hypertrophy (163±48 pg/mg). Plasma norepinephrine and epinephrine were similar in dogs with LV hypertrophy (223±26 and 81±13 pg/ml) as compared with normal controls (251±23, and 122±17 pg/ml).

Discussion

Base-line hemodynamics were remarkably similar in the normal dogs and dogs with LV hypertrophy. Only LV systolic pressure was significantly elevated, and LV stroke shortening was reduced in the dogs with hypertrophy. The dogs with LV hypertrophy appeared fully compensated in terms of normal daily activities and with respect to hemodynamics, particularly in the normal values for LV end-diastolic pressure and LV end-diastolic diameter and LV dP/dt. Specifically, the ventricles, while hypertrophied, were not dilated and did not exhibit any reduction in LV peak dP/dt. Moreover, when the ventricles were stressed with norepinephrine in increasing bolus doses, the responses of the normal and LV hypertrophy hearts, particularly with respect to LV dP/dt, were almost superimposable (Fig. 3). In fact, the hypertrophied hearts were able to generate even greater pressure, in some instances up to 600 mmHg (Fig. 2), without failing. This finding of similar inotropic response in normal and pressure overload LV hypertrophy differs from studies in anesthetized dogs with pressure overload LV hypertrophy, which showed a depressed length-contractile force curve in response to isopro-

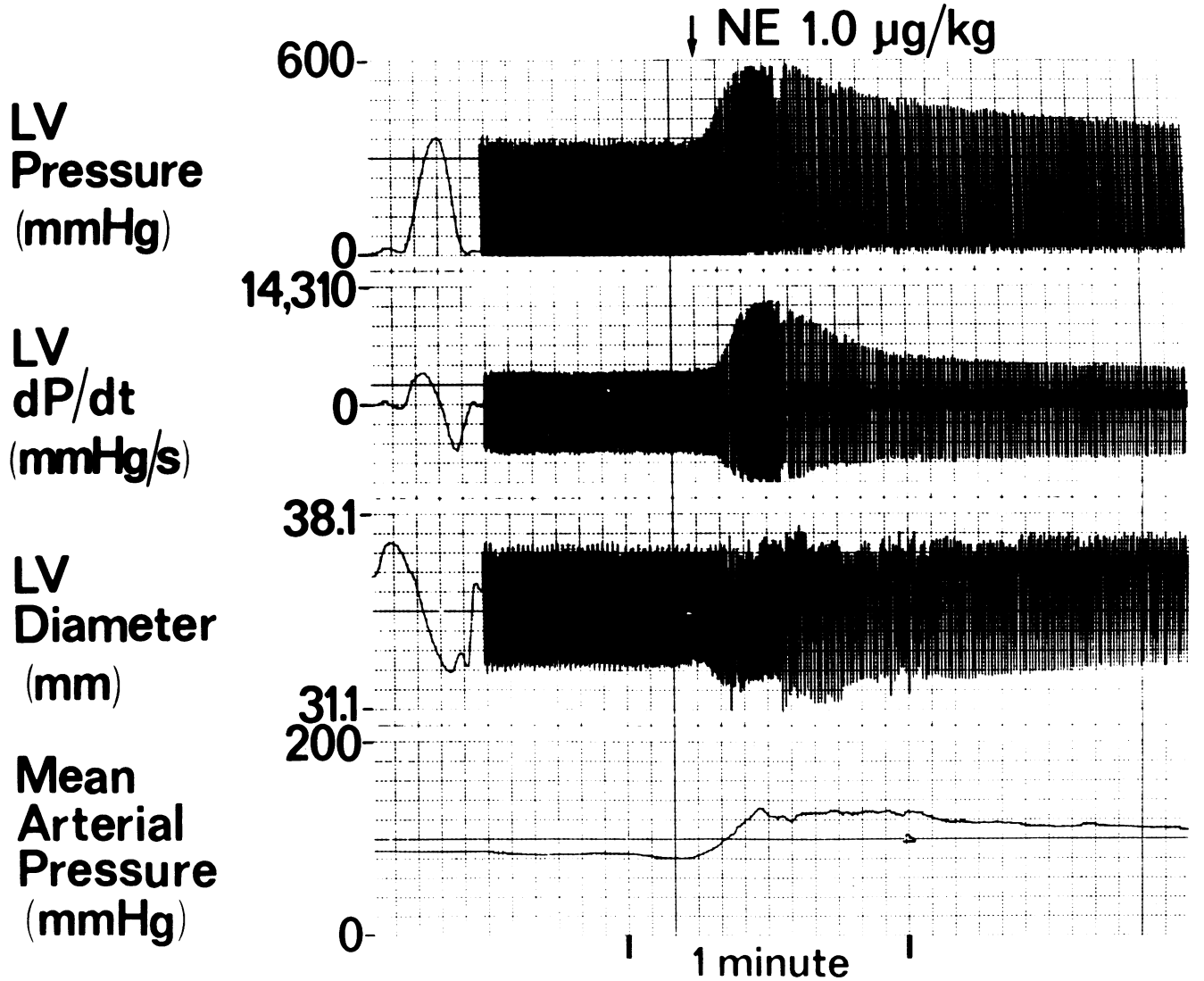

Figure 2. The effects of a bolus of norepinephrine (1.0 µg/kg) are shown on phasic measurements of LV pressure, LV dP/dt, LV diameter, and mean arterial pressure in the dog with pressure overload LV hypertrophy. The response of LV dP/dt was nearly identical to that observed in the normal animal (Fig. 1). In this animal, this dose of norepinephrine increased LV systolic pressure to nearly 600 mmHg without inducing LV decompensation.

terenol (10). Also, in rats with renovascular hypertension and LV hypertrophy, the inotropic response to isoproterenol, as measured by LV dP/dt, was depressed from those observed in normal hearts (11). It is possible that the depressed inotropic response in previous studies (10, 11) is secondary to the use of general anesthesia or the open chest preparation.

Despite the maintenance of normal physiologic responsiveness to catecholamines in the hypertrophied myocardium, direct characterization of β-adrenergic receptor number and affinity revealed definite differences. An unexpected finding in this study was the decreased β-adrenergic receptor affinity that was consistently observed in the membranes from the hypertrophied left ventricle. One explanation may be that a different form of the receptor is expressed during the development of LV hypertrophy. Such receptor microheterogeneity might explain the altered affinity and may be compared with the shift in isozyme patterns seen in other proteins in cardiac hypertrophy, which include: (*a*) the shift towards the V_3 isozyme of the myosin heavy chain in the rat with aortic banding (12), (*b*) the marked increase in MB-creatine kinase in the dog myocardium with pressure overload hypertrophy (13), and (*c*) the increase in the anerobic M-type isozyme of LDH in the heart with pressure overload hypertrophy (14–16). These proteins appear to change towards a more developmentally immature type of isozyme.

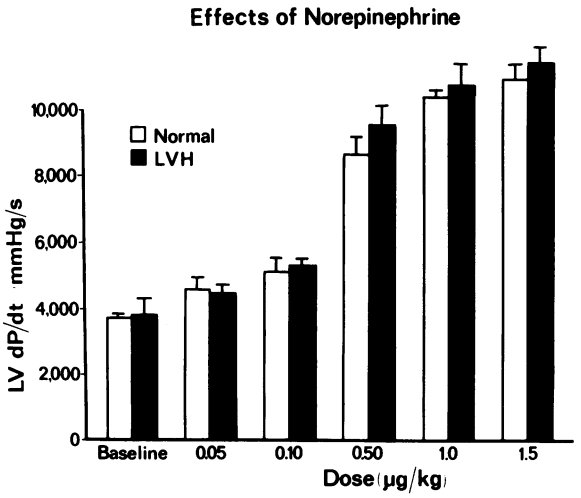

Figure 3. The peak LV dP/dt levels achieved by increasing doses of norepinephrine are compared in normal (n = 6) and LV hypertrophied dogs (n = 6) on LV dP/dt (mmHg/s). There were no differences in the responses of peak LV dP/dt to norepinephrine in the two groups.

The future development of methods that permit both purification (17, 18) and photoaffinity labeling of the hormone-binding subunit (17–20) will permit this hypothesis to be examined at the molecular level.

The decrease in β-adrenergic receptor affinity in the presence of chronic pressure overload LV hypertrophy may be offset by the increase in receptor number, which results in the normal inotropic response of the LV hypertrophied ventricle to catecholamines. These changes in receptor number and affinity were unique to the left ventricles from the dogs with LV hypertrophy, since they were not observed in the normal dogs and were not observed in the right ventricles from the dogs with LV hypertrophy.

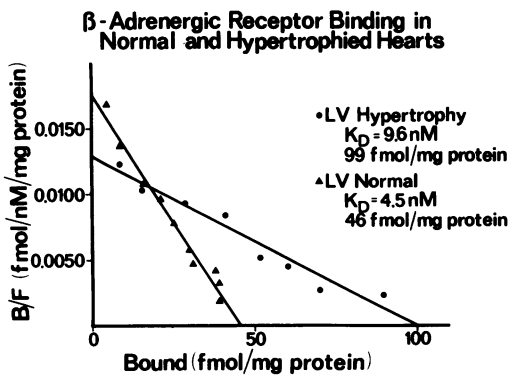

Figure 4. Scatchard analyses of β-adrenergic receptor binding are compared in the normal (triangles) and hypertrophied (circles) LV plasma membranes. The LV hypertrophied heart shows a lower affinity and more receptors per milligram protein than the normal LV.

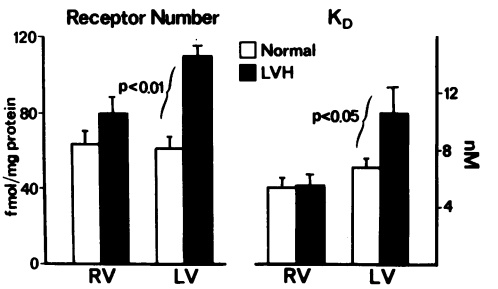

Figure 5. β-adrenergic receptor concentration and affinity constants (K_D) are compared in the right and left ventricles of normal dogs (n = 11) and dogs with LV hypertrophy (n = 8). Note that the marked increases in both receptor number and K_D are only observed in the LV of dogs with LV hypertrophy.

Our finding of a significant increase ($P < 0.01$) in receptor density in the hypertrophied left ventricle as compared with the normal left ventricle differs from the majority of prior work conducted in this field. Most prior studies were conducted in rodents with either renovascular hypertension (21–24) or in SHR rat models (25, 26). In these studies, β-adrenergic receptor number was either normal (24, 26) or depressed (21–24), with normal affinity. These differences could be due to different species or to the model. Favoring the latter is the finding of Limas (27), with aortic banded rats, showing increased β-adrenergic receptor number. In contrast, a study by Cervoni et al. (28) demonstrated no changes in β-adrenergic receptor number or in affinity in rats with aortic banding. In addition, Kumano et al. found an increase in β-adrenergic receptor density and no change in K_D in the renal hypertensive rat, and found a decreased receptor density in the SHR rat with no change in affinity (29). Another consideration is the presence or absence of congestive failure. Karliner et al. (30) found increased β-adrenergic receptor number and no change in affinity in guinea pigs with experimental congestive heart failure, whereas Bristow et al. (31) found significantly reduced β-adrenergic receptor number and no change in K_D in failing human hearts. Other important differences in experimental design include the severity of the hypertrophy in the dogs in the present study where LV weight per body weight rose by 87% compared with results in prior studies in rodents (21, 23, 28), where LV per body weight increased 20–50%. In

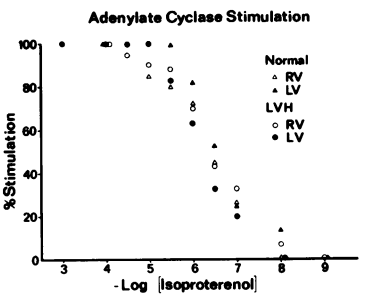

Figure 6. This graph of adenylate cyclase stimulation with isoproterenol compares the RV and LV of normal (n = 6) and hypertrophied hearts (n = 6) and shows no significant differences.

these animals, the receptor number decreased without any change in affinity. Thus, the severity of hypertrophy is another factor to be considered.

The increased number of receptors found in the membrane preparations in the dogs with LV hypertrophy could not be attributed to a difference in the membrane preparations. Another membrane marker, 5′ nucleotidase, was found to be significantly depressed in the membrane preparation from the animals with LV hypertrophy. A second sarcolemmal marker, maximal-stimulatable adenylate cyclase was also lower in the hypertrophied LV, whether it was determined in the presence of NaF or GppNHp, at maximally effective concentrations. One can only speculate on the reasons for the decreased maximal adenylate cyclase activity in these preparations. In view of the observed decrease in two sarcolemmal markers, however, 5′ nucleotidase and adenylate cyclase, it is possible that the membrane preparation from the hypertrophied LV was contaminated with non-plasma membrane proteins, as might occur if fibrosis or increased collagen deposition had occurred during the development of LV hypertrophy. These data imply that the observed increase in β-receptor density in the hypertrophied myocardium, the major goal and finding of the present investigation, may have been an underestimation.

Other problems due to the method of raising the puppies to adulthood or due to the operation at an early age were considered. To rule out that these possibilities accounted for the differences, sham-operated litter mates, indistinguishable in terms of β-adrenergic receptor number or affinity from the other normal, mongrel adult control animals, were studied. However, it is still conceivable that a pressure-overload stimulus applied at a young age results in a different response in terms of β-adrenergic receptor response than when the stimulus is applied to the adult.

While plasma levels of both epinephrine and norepinephrine were similar in the two groups, it is also conceivable that the increased β-adrenergic receptor number represents "upregulation" secondary to the depleted catecholamine stores of the hypertrophied left ventricle. This phenomenon of reduced tissue norepinephrine levels has been a consistent feature of cardiac failure (32).

In the normal dogs and dogs with LV hypertrophy, although the maximally stimulatable adenylate cyclase differed, the dose-response curves for adenylate cyclase stimulation by isoproterenol were similar. A small decrease in the EC_{50} for adenylate cyclase stimulation secondary to an increase in receptor number would have been difficult to detect, particularly in the setting of the decreased affinity in this preparation. It is postulated that the increased β-adrenergic receptor number may be an important control mechanism compensating for the reduced adrenergic innervation (decreased tissue norepinephrine levels) and the decreased affinity of the β-adrenergic receptor in the hypertrophied myocardium. It should also be apparent that the hemodynamic and cellular profiles observed with hypertrophy may differ depending upon (*a*) species, e.g., rat vs. large mammal and man; (*b*) inducing stimulus, pressure vs. volume overload, mechanical pressure overload vs. systemic hypertension; (*c*) whether the stimulus is applied to the immature or adult animal; (*d*) severity of the stimulus; (*e*) the duration of the stimulus; (*f*) the chamber involved; and (*g*) the presence or absence of cardiac failure.

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