Effects of Chronic Dobutamine Administration on Hearts of Normal and Hypertensive Rats

Peter Buttrick, Ashwani Malhotra, Stephen Factor, David Geenen, and James Scheuer

We have previously shown that physical conditioning in the rat improves cardiac mechanics and biochemistry and normalizes the cardiac contractile protein abnormalities associated with renovascular hypertension. Since chronic adrenergic stimulation with dobutamine simulates some aspects of physical conditioning, this study was undertaken to investigate the effects of chronic dobutamine administration on normal and hypertensive rat hearts. Four groups of female animals were studied: controls, dobutamine-treated (2 mg/kg twice daily), renovascular hypertensives, and dobutamine-treated hypertensives. Animals were killed after 8–10 weeks and cardiac histology, myosin biochemistry, and mechanics in an isolated heart perfusion apparatus were studied. Dobutamine, unlike hypertension, was not associated with histological evidence of myocardial damage but did increase cardiac mass by 10% and calcium-activated myosin ATPase activity by 13%. Hypertension was associated with a 24% increase in mass, a 24% decrease in ATPase activity, and a shift in the myosin isoenzyme pattern from V1 to V3. The combined stimuli caused additive hypertrophy (44%) and normalized myosin biochemistry and isomyosin distribution. Dobutamine treatment was not associated with significant improvements in pump or muscle function in control or hypertensive hearts. Thus chronic dobutamine treatment, like physical conditioning, induces a physiological cardiac hypertrophy in rats that is associated with improved myosin enzymology and normalization of the contractile protein abnormalities associated with hypertension. Unlike physical conditioning, however, these biochemical alterations do not result in improved contractile function as measured in an isolated buffer-perfused heart apparatus. (Circulation Research 1988;63:173-181)

Varying degrees of cardiac hypertrophy can be induced in humans and experimental animals by chronic exercise conditioning. This physiological increase in cardiac mass is associated in rats and other rodents with an increase in the V1 (aa) myosin isoenzyme and its associated ATPase activities. In addition, exercise conditioning in the rat, either by swimming and to a lesser degree by running, is associated with improved cardiac contractile performance and reverses the cardiac dysfunction and biochemical alterations seen as a result of the hypertrophic response to renovascular hypertension and to other forms of systolic overload.

Intermittent catecholamine stimulation necessarily accompanies exercise programs, and there have been several investigations in recent years to determine if catecholamine administration alone can simulate the cardiovascular adaptations observed with chronic conditioning. Low-dose isoproterenol infusions over a 4–6-week period induce mild to moderate cardiac hypertrophy in rats that is associated with normal metabolic and functional capacity. Chronic intermittent adrenergic stimulation with dobutamine, on the other hand, induces a variety of cardiovascular adaptations that are similar to those seen in response to exercise conditioning, including the maintenance of exercise capacity during a period of deconditioning in normal human volunteers, improvement in exercise duration in patients with congestive heart failure, and a resting bradycardia in dogs that is associated with a diminished catecholamine response to submaximal exercise. The link between chronic dobutamine treatment and physiological cardiac adaptations has not been clearly elucidated.

The present study was performed to characterize the histological, physiological, and biochemical features of chronic intermittent dobutamine therapy in
cise conditioning have been well-defined. In addition, since exercise conditioning in this animal model has been shown to exaggerate the cardiac hypertrophy but neutralize the biochemical and mechanical adaptations associated with renovascular hypertension, the effect of chronic intermittent dobutamine therapy on hypertensive rat hearts was also investigated.

Materials and Methods

Animal Models

Female Wistar rats (Charles River, Wilmington, Massachusetts) 10–12 weeks old and weighing 175–200 g were used in all experiments. Animals were provided food and water ad libitum. Initial studies were done to determine the dose of dobutamine that induced hemodynamic changes analogous to those seen in our standard exercise protocols. For these preliminary studies, normotensive animals were anesthetized with ketamine (87 mg/kg) and xylazine (13 mg/kg), the left carotid artery cannulated and a 3F ultraminiature pressure transducer (Millar Instruments, Houston, Texas) advanced retrograde into the left ventricle. The frequency of this catheter system is flat to 2,000 Hz. Acute aortic insufficiently was probably not created because end-diastolic pressure did not change during the observation period. Heart rate, peak left ventricular pressure, and positive dP/dt were monitored for 40 minutes or until stable and then after subcutaneous injection of the drug. Dobutamine [a racemic mixture of (+) and (−) isomers provided by Eli Lilly Co., Indianapolis, Indiana], 2 mg/kg dissolved in normal saline, was administered in two daily subcutaneous injections 4–6 hours apart 5 days/week for 9–11 weeks. The study duration and twice-daily dosing schedule was used to simulate timed interventions used in our previous exercise studies.11 Animals displayed no adverse side effects during or after injection.

Animals were made hypertensive by placing silver clips (0.18–0.25 mm i.d.) around the left renal artery as described previously.4,12 Control animals were matched litter mates. Blood pressure was monitored by the tail-cuff method under light ether anesthesia 3 weeks after surgery (time 0) and animals with cuffs pressures greater than 145 mm Hg were considered to be hypertensive. Blood pressure was checked at the midpoint and the conclusion of the protocol 18–24 hours after the previous dobutamine injection, and only those animals who were consistently hypertensive were included in the final data analysis. One half of the control (C) and hypertensive (H) animals were randomly assigned to receive chronic intermittent dobutamine treatment (D, DH). There was no difference in the initial body weights among the four groups or in blood pressure between C and D or between H and DH.

Heart Perfusions

Full descriptions of the isolated working heart apparatus have been published previously.13,14 Hearts were removed from ether-anesthetized rats who had not received dobutamine for 18–24 hours and immersed immediately in iced saline. The period of cardioplegia prior to attachment to the perfusion apparatus was 2–3 minutes. The perfusate was a modified Krebs-Henseleit buffer at 37°C gassed with a 95% O2–5% CO2 mixture and contained 15 mM glucose, 0.01 units/ml regular insulin (Lilly), and 2.0 mM calcium with 0.5 mM EDTA yielding 1.5 mM free calcium. Left ventricular pressure was measured through a 2.5 cm polyethylene (PE-60) catheter inserted through the apex of the heart and attached to a Statham P23d strain gauge pressure transducer (Gould, Cleveland, Ohio). A second catheter (PE-20) was placed through the left ventricular apex for dye injection. Aortic pressure was measured from a sidearm on the aortic cannula 7–8 mm above the aortic valve. Instantaneous aortic flow was measured using a cannulating 2.5 mm i.d. flow probe (Statham-Gould, frequency response set to 50 Hz) inserted in the aortic outflow tubing. Coronary flow was measured directly as right heart outflow, and cardiac output was measured as aortic flow plus coronary flow. Dye concentrations in the aorta were measured by a densitometer system placed in the aortic cannula. All hearts were paced from the right atrium at a rate of 350 beats/minute. Oxygen tension was measured in the perfusate from the left atrial reservoir and from a pulmonary arterial catheter. Whenever possible, hearts from all experimental and control groups were perfused on the same day.

Cardiac function was assessed at two levels of preload at each of two levels of afterload. Afterload was varied by changing the height of the aortic outflow chamber, which determined the aortic diastolic pressure. In the present study, the aortic outflow chamber was set at 80 or 140 cm above the heart, corresponding to aortic diastolic pressures of 59 and 103 mm Hg. Preload at each level of aortic pressure was varied by changing the height of the left atrial reservoir to produce varying levels of left atrial pressure. The moderate preload corresponded to an end-diastolic pressure of 6–7 mm Hg while the high preload corresponded to an end-diastolic pressure of 10–14 mm Hg. It is important to note that pump function at the high left atrial pressure represents a near maximal Frank-Starling response of any heart at a given aortic pressure and at the paced heart rate of 350 beats/minute.

In all studies after a 10–15-minute period of retrograde perfusion, during which the catheters for left ventricular pressure and dye injections were positioned, antegrade perfusion was begun by unclamping the left atrial catheter. Measurements at each atrial pressure were made at an aortic pressure of 59 and 103 mm Hg. The experiment was completed with a repeat measurement at the high left atrial pressure and the low aortic pressure to ensure that deterioration of the preparation had not occurred. If the results for cardiac output from the
repeat measurements differed by more than 10%, the data for the experiment was discarded. At each loading condition records were made of dynamics, multiple dye-dilution curves were recorded, and coronary flow and cardiac output were measured over a 1 minute interval.

End-diastolic volumes were estimated from the dye-dilution curves. A full description of this method and the validation for measuring ventricular volumes have been published previously. Ejection fraction (EFx) was calculated from the equation EFx = V1 - V3, where K = Cn/Cd and C is the dye concentration at beat n. End-diastolic volume was calculated by dividing the directly measured stroke volume (cardiac output divided by the heart rate) by the ejection fraction. Wall stress (σ) was calculated from the equation \( \sigma = \frac{P \cdot r}{2h} \) where \( P \) is instantaneous pressure, \( r \) is ventricular radius, and \( h \) is wall thickness. Measurements of ventricular dimensions assume a spherical model with uniform wall thickness. We have previously demonstrated the utility of this model system. Flow and pressure analog data were digitized on an IBM 9000 laboratory computer, and the measurements of cardiac dynamics were determined as previously described. At the end of each experiment, the atria and great vessels were dissected free and the right ventricular free wall was removed. The left ventricle (including the septum) was weighed to determine wet weight. The remaining portion of the left ventricle was stored at -80°C in 50% glycerol buffer containing 50 mM KCl and 10 mM KHPO₄ (pH 7.0) prior to preparation of the myosin extracts. Myosin was extracted and purified by previously described techniques and was shown by sodium dodecyl sulfate gel electrophoresis to be free of actin, troponin, and tropomyosin and without evidence of proteolytic degradation. Calcium myosin ATPase activities were assayed at 30°C in 0.3 M KCl, 50 mM Tris-Cl (pH 7.6), 10 mM CaCl₂, 5 mM ATP, and 5 mM sodium azide. The reaction was initiated by the addition of substrate and terminated after 10 minutes with 1.0 ml cold 10% trichloracetic acid. Results are expressed as micromoles inorganic phosphate per mg per minute.

Myosin isoenzymes were analyzed by electrophoresis of purified myosin on polyacrylamide gels using nondissociating conditions at 2°C as reported by d’Albis et al16 in the Pharmacia Apparatus (GE 2/4, Piscataway, New Jersey). Approximately 2–3 μg of myosin was layered on each gel and run at a constant voltage gradient 14 V/cm for 20–22 hours. The gels were then stained with Coomassie Blue, destained, and densitometric scans recorded at 605 nm on an E-C apparatus densitometer. The semi-quantitative estimate of each isoenzyme was calculated from the height of each peak by a Hewlett-Packard Integrator (model 3390 A, Palo Alto, California) attached to the densitometer. The relative contribution of V₂ was distributed equally between the V₁ and V₃ isoenzymes.

**Histological Analysis**

A representative section of the left ventricle that included portions of the septum and the free wall was placed in 3.7% phosphate-buffered formaldehyde immediately after heart perfusion was completed. Specimens were then imbedded in paraffin and slices stained with hematoxylin and eosin for routine histological analysis and with Masson’s trichrome stain for identification of connective tissue. Slides were reviewed by an experienced pathologist (S.F.) who was blinded as to treatment group. Three or four representative levels per heart, including the septum and the left ventricular free wall, were reviewed. The whole section was scanned at low magnification and then abnormalities were confirmed under higher magnification. The morphological changes in the tissue were subjectively graded according to the following scale: 0 = normal; 1+ = mild; 2+ = moderate; and 3+ = severe changes. Interstitial fibrosis was defined as the presence of connective tissue between viable myocytes extending away from perivascular spaces; replacement fibrosis was defined as the presence of connective tissue in areas in which cells were either undergoing or had undergone active necrosis; and vascular sclerosis was defined as connective tissue deposition and thickening of muscular arterial walls in which luminal narrowing was observed and around which there was perivascular fibrosis. Cellular hypertrophy was estimated from a qualitative assessment of cell diameter in association with an increase in hyperchromic nuclei.

**Cardiac Contractile Proteins**

The remaining portion of the left ventricle was collected from the pulmonary artery outflow, which in this model represents coronary effluent, using a commercially available kit (Behring Diagnostics, La Jolla, California).

Lactate content was determined in the buffer collected from the pulmonary artery outflow, which was stored at -80°C in 50% glycerol buffer containing 50 mM KCl and 10 mM KHPO₄ (pH 7.0) prior to preparation of the myosin extracts. Myosin was stored at -80°C in 50% glycerol before extraction and purification. The data for the experiment was discarded. At each loading condition records were made of dynamics, multiple dye-dilution curves were recorded, and coronary flow and cardiac output were measured over a 1 minute interval.

**Thyroid Hormone Determinations**

Triiodothyronine and thyroxine determinations were done on plasma obtained from animals at the time of killing using commercially available RIA kits (Pantex Corp, Santa Monica, California). The accuracy of the tracers in rat plasma was established by adding known amounts of the hormone to plasma obtained from thyroidectomized animals.

**Statistical Analysis**

Results were submitted to a two-factor analysis of variance using presence of drug or hypertension as the two factors. The mean square error within groups was then used in a Newman-Keuls multiple comparison test to evaluate differences between
Results

The acute hemodynamic effects of 2 mg/kg subcutaneous dobutamine injection are shown in Figure 1. In this study, animals were under general anesthesia with ketamine and xylazine, and therefore, the initial hemodynamics do not reflect a resting state. Abrupt increases in both heart rate and positive dP/dt were observed within minutes of injection, the former increasing by approximately 50% and the latter by greater than 100%. Maximum dP/dt returned to near baseline values approximately 50% and the latter by greater than 100%. Hearts from any two groups. \(^{17}\) Significance is reported at the 0.05 level.

animals. Although differences in blood pressure between hypertensive and normotensive groups were highly significant \((p<0.001)\), there were no significant differences within normotensive or hypertensive groups. Blood pressures did not change significantly in any group throughout the duration of the study.

Body weights were similar among all four groups. Compared with C, the dry heart weight was 10% increased in D, 24% increased in H, and 44% increased in DH. Hearts from DH were significantly more hypertrophied than hearts from H. Similar relations were seen for dry left ventricular weights.

Histological results are summarized in Table 2. Both septal and left ventricular free wall sites were examined. There was no increase in interstitial fibrosis, replacement fibrosis, or vascular sclerosis in D versus C. H and DH had increased fibrosis and vascular sclerosis relative to both D and C but were not different from each other. Qualitative myocellular hypertrophy was seen in D, H, and DH. Representative photomicrographs demonstrating these findings are shown in Figure 2.

Table 3 summarizes results from the isolated perfused heart studies. Data at moderate and high preload and 103 mm Hg aortic diastolic pressure are shown. Left ventricular end-diastolic pressure was slightly higher in DH than in C and D at high preload. End-diastolic volume was the same in all four groups at both preloads. Normalized end-

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<tr>
<th>C</th>
<th>D</th>
<th>H</th>
<th>DH</th>
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<tbody>
<tr>
<td>BP (mm Hg)</td>
<td>99±3</td>
<td>98±4†</td>
<td>165±5*</td>
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<td>BW (g)</td>
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<td>196±6*</td>
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<td>DLVW (mg)</td>
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<td>184±5*</td>
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<td>Wet/dry (mg/g)</td>
<td>0.22</td>
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C; control; D, dobutamine-treated; H, hypertensive; DH, dobutamine-treated hypertensive; BP, tail-cuff blood pressure at the end of the study; BW, body weight; DHW, dry heart weight; and DLVW, dry left ventricular weight.

\(^{*}p<0.05\) vs. C; \(^{†}p<0.05\) H vs. D or DH; \(^{††}p<0.05\) D vs. DH.

Data are mean±SEM of 10–12 studies.

Table 1. Blood Pressure and Body and Heart Weights For Control, Dobutamine-Treated, Hypertensive, and Dobutamine-Treated Hypertensive Rats

Table 2. Histology of Control, Dobutamine-Treated, Hypertensive, and Dobutamine-Treated Hypertensive Rats

Table 3. Comparison of Histological Findings

Morphological changes were graded on a scale of 0–3 where 0, no abnormality; 1+, mild; 2+, moderate; and 3+, severe changes. See "Materials and Methods" for details. C, control; D, dobutamine-treated; H, hypertensive; DH, dobutamine-treated hypertensive.

\(^{*}p<0.05\) vs. C; \(^{†}p<0.05\) H vs. D or DH; \(^{††}p<0.05\) D vs. DH.

Data are mean±SEM of nine hearts.
**Discussion**

Several previous reports have suggested that chronic adrenergic stimulation with dobutamine simulates some features of physical conditioning. Liang et al. documented a decreased resting heart rate and a decreased heart rate at submaximal exercise in dogs who had received the drug for 5 weeks. Both Leier et al. and Liang et al. demonstrated sustained beneficial effects of dobutamine treatment in patients with congestive heart failure who received the drug over 72 hours including a reduction in New York Heart Association class, an increase in exercise duration and a slight increase in ejection fraction weeks after the drug was discontinued. These authors have suggested that these effects may be mediated both by direct myocardial effects as well as by beneficial adjustments in skeletal muscle metabolism, which are analogous to those induced by chronic exercise conditioning. This later conclusion has been emphasized by Sullivan et al. who have shown that some deconditioning effects of prolonged bedrest can be prevented in normal subjects by chronic dobutamine administration and that the peripheral muscle adaptations that occur, increased oxygen extraction across a submaximally exercised muscle, and diminished lactate...
tion as evidenced by increased fractional shortening and $V_{ch}$ at equivalent wall stress when compared with control hearts.\textsuperscript{5,11} This result was not seen in the hearts of dobutamine-treated animals that did not differ from controls in these derived measurements of left ventricular function at high preload and afterload when end-diastolic wall stress was equivalent. Similarly, the drug did not alter cardiac performance in hypertensive hearts, although the hypertensive hearts in this study did not show depressed mechanical performance that we have observed in previous studies\textsuperscript{5,12} perhaps because of the relatively modest (24\%) increase in heart mass observed, although depressed left ventricular performance is not uniformly seen following systolic overload in rats and larger animals.\textsuperscript{26,27} Of note, however, both C and H hearts failed to show an increase in systolic wall stress as preload was increased, suggesting that these hearts were functioning at a maximal Frank-Starling relation. In contrast, both D and DH hearts increased systolic wall stress with the change in load, and it is therefore possible that further increases in preload might have unmasked real augmentation in cardiac performance in these two groups.

The contractile protein data showing enhanced ATPase activity in both dobutamine-treated groups would support this presumption. Alternately, and more likely, drug treatment with dobutamine may not be as potent a stimulus or may not precisely mimic the cardiac response to physical conditioning. Other biochemical features of the myocardium, such as the regulation of sarcoplasmic reticular and sarcosomal calcium movement are altered with physical conditioning.\textsuperscript{2,26-30} It is likely that the adaptation to physical conditioning involves an interplay among several regulatory systems and that intermittent dobutamine administration stimulates protein synthesis and influences myosin biochemistry but does not affect these other as yet unstudied control systems.

Dobutamine treatment did not appreciably alter coronary flow in normotensive animals nor did it improve the depressed coronary flow seen in association with hypertension. The depressed myocardial oxygen consumption seen in both hypertensive groups is probably due to the diminished wall stresses noted with lower energy demands and not to any coronary flow limitation since the effluent lactate values were all within the normal range for this workload.\textsuperscript{19,20} Thus, the dobutamine-associated hypertrophy in both normotensive and hypertensive hearts did not incur any mechanical or metabolic cost and may in fact have resulted in improved cardiac performance under extreme loading conditions.

There is considerable evidence from other in vivo studies that chronic catecholamine administration, specifically with norepinephrine and isoproterenol, induces cardiac hypertrophy via hemodynamic independent mechanisms.\textsuperscript{31} The hypertrophy induced by norepinephrine is not blocked by a nonselective $\beta$-blocker,\textsuperscript{32} whereas that induced by isoproterenol is prevented by propranolol.\textsuperscript{33} These results are consistent with the hypothesis that catecholamine-induced hypertrophy is mediated in vivo by postsynaptic $\alpha$-adrenergic stimulation. Simpson,\textsuperscript{34,35} investigating terminally differentiated myocytes in a serum-free culture system, has confirmed this, demonstrating that $\alpha$- and not $\beta$-adrenergic stimulation increases cell size and protein synthesis. Thus, it is not surprising that adrenergic stimulation with dobutamine, which certainly leads to presynaptic $\beta$- and postsynaptic $\alpha$- and $\beta$-stimulation, results in myocardial hypertrophy.

Dobutamine-induced hypertrophy, however, contrasts with other types of catecholamine stimulation in that it induces an increase in myosin ATPase activity and a preferential expression of the $V_{1}$ myosin isoform. Baldwin et al\textsuperscript{7} investigated actomyosin and myofibrillar ATPase activities in isoproterenol-induced cardiac hypertrophy and found no change from control values after four weeks of treatment with 200–400 $\mu$g/kg/day. Cuffman et al\textsuperscript{36} in vivo and White and Simpson\textsuperscript{37} in vitro have shown that norepinephrine stimulation increases $V_{1}$ myosin expression. It is difficult to compare these studies with the present work because the manner of administration and the hemodynamics may not have been similar and also because these catecholamines, even at relatively low doses, have been shown to structurally damage cardiac muscle.\textsuperscript{6,23,31} Nonetheless, the results suggest that dobutamine and other catecholamines exert different influences on isomyosin expression.

One possible explanation of this might lie in the fact that isoproterenol treatment down-regulates $\beta$-receptor number,\textsuperscript{38,39} whereas chronic intermittent dobutamine treatment has not been shown to influence receptor number or physiology,\textsuperscript{24} although the design of this study differed slightly from our own. It is of interest, in this context, to note that two other stimuli that clearly influence cardiac myosin expression, physical conditioning and thyroid hormone excess, may also in part be mediated by $\beta$-adrenergic stimulation. Conditioning, associated with intermittent catecholamine production, does not change $\beta$-receptor number or affinity,\textsuperscript{40} and thyroid hormone stimulation increases, among other proteins, $\beta$-adrenergic receptor number.\textsuperscript{41} This theoretical construct could be tested in a cell culture system or in vivo using specific adrenergic blocking agents in concert with dobutamine.

In conclusion, chronic intermittent dobutamine treatment results in a unique cardiac response that has features in common with those induced by other catecholamines and by physical conditioning. The increase in mass is common to all stimuli, but dobutamine, unlike other catecholamines, does not appear to structurally damage the muscle or to down-regulate beta adrenergic receptor number in the physiological doses used. The preferential expression of the $V_{1}$, high ATPase isomyosin, and the correction of the myosin biochemical abnormalities...
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