Chronic nonocclusive coronary artery constriction in rats. Beta-adrenoceptor signal transduction and ventricular failure.

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Research Article

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Chronic Nonocclusive Coronary Artery Constriction in Rats

β-Adrenoreceptor Signal Transduction and Ventricular Failure

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Abstract

To determine the effects of chronic coronary artery constriction on the relationship between cardiac function and regulation of β-adrenoreceptor signal transduction, the left main coronary artery was narrowed in rats and the animals were killed 5 mo later. An average reduction in coronary luminal diameter of 44% was obtained and this change resulted in an increase in left ventricular end-diastolic pressure and a decrease in positive and negative *dP/dt*. Significant increases in left and right ventricular weights indicative of global cardiac hypertrophy were observed. Radioligand binding studies of β-adrenoreceptors, agonist-stimulated adenylate cyclase activity, and ADP ribosylation of 45-kD substrate by cholera toxin were all depressed in the failing left ventricle. In contrast, in the hypertrophic nonfailing right ventricle, β-adrenoreceptor density was preserved and receptor antagonist affinity was increased. In spite of these findings at the receptor level, agonist stimulated cyclic AMP generation was reduced in the right ventricular myocardium. The quantity of the 45-kD substrate was also decreased. In conclusion, longterm nonocclusive coronary artery stenosis of moderate degree has profound detrimental effects on the contractile performance of the heart in association with marked attenuation of adrenergic support mechanisms. (*J. Clin. Invest.* 1991. 88:1940–1946.) Key words: ventricular dysfunction • cardiac hypertrophy • receptor signaling • regulatory protein • cyclic AMP

Introduction

In coronary artery disease, focal narrowing of the main coronary arteries by atherosclerosis is the most frequent pathologic process responsible for the reduction in coronary blood flow to the myocardium. Although the severity of the atherosclerotic involvement of the coronary circulation seems to correlate with the impairment of blood supply to the tissue (1), in a large number of patients there is no correlation between the clinical manifestations of ischemia and the angiographic evaluation of the magnitude of constriction of the epicardial arteries (2). This discrepancy is further emphasized by anatomical findings which indicate that in congestive ischemic heart disease (3, 4) only relatively small amounts of myocardium are lost and replaced by fibrotic tissue, providing no basis for the ultimate events of irreversible congestive heart failure and death (3, 4). Consistent with these observations, acute (5) and subacute (6) nonocclusive coronary artery stenoses in rats have been found to be associated with severe alterations in cardiac function, and sites of reparative fibrosis and myocytolytic necrosis primarily affecting the subendocardium and midmyocardium of the left ventricular wall (5, 6). However, the relatively moderate extent of myocyte necrosis and replacement scarring cannot fully account for the depression in ventricular hemodynamics, because similar alterations in cardiac function are commonly seen after total occlusion of the coronary artery and infarcts affecting nearly 50% of the wall acutely (7) and chronically (8) in rats. Although ischemia may be implemented as the prevailing mechanism of myocardial dysfunction (9), coronary narrowing, involving a reduction in luminal diameter up to 62%, has no effect on resting coronary blood flow (5, 6) to support the hypothesis of a decreased oxygen availability to the tissue and ventricular failure. In view of these contrasting observations, the possibility may be advanced that nonocclusive coronary constriction may result in a depressed force-generating capacity of the myocardium mediated by abnormalities in the adrenergic mechanisms supporting myocardial contractility. Because the inotropic state of heart muscle is mostly controlled by the β-adrenoreceptor adenylate cyclase complex (10), a decrease in β-adrenoreceptor density and/or attenuation in the activation of the regulatory protein coupling these receptors with adenylate cyclase may be implicated in the depression of mechanical pump performance after coronary artery narrowing. The chronic effects of coronary constriction have been investigated here because of their relevance to the human disease.

Methods

Experiments were carried out in male Sprague-Dawley rats at 3 mo of age weighing ~ 250–275 g (Charles River Breeding Laboratories, North Wilmington, MA). Coronary artery narrowing was performed in 27 animals. Eight animals in this group died within 2–3 wk after coronary constriction mostly because of pulmonary edema. 22 sham-operated rats served as controls. All animals were killed 5 mo after surgery.

Coronary artery narrowing. Under ether anesthesia, thoracotomy via the third left intercostal space was performed, the atrial appendage elevated, the left coronary artery located, and a suture positioned around the vessel 1–2 mm from its origin. Subsequently, a probe 275 μm in diameter was held in contact with the wall of the exposed coronary artery. The entire vessel and the probe were ligated and the probe quickly removed (5, 6). The chest was closed and the animals allowed to recover. Sham-operated control rats were treated similarly except that the ligature around the coronary artery was not tied.

Functional measurements. Just before being killed, animals were anesthetized with chloral hydrate (300 mg/kg body weight, ip), and the external right carotid artery was exposed and cannulated with a micro-

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tip pressure transducer catheter (model PR 249; Millar Instruments, Houston, TX) connected to an electrostatic chart recorder (model ES 2000; Gould Inc., Cleveland, OH). After monitoring arterial blood pressure the catheter was advanced into the left ventricle for the evaluation of left ventricular pressures and dP/dt. Thus, measurements were made of arterial and ventricular pressures, and dP/dt in the closed chest preparation. These events were monitored and inscribed on recording paper for subsequent analysis.

Measurement of the degree of coronary artery constriction. At death (see below), in each heart, the initial 2–3-mm segment of the left coronary artery was dissected free to localize the level of coronary constriction. The vessel was then cut transversely to expose the lumen of the coronary artery at the region of the ligature. The luminal diameter of the proximal portion of the vessel adjacent to the narrowed site and at the constricted region were measured with a dissecting microscope having an incorporated ocular reticle. The degree of constriction was evaluated by comparing the vessel diameter above the stenosis with the diameter at the level of the stenosis (5, 6).

β-Adrenoreceptors

Membrane preparation. After the measurement of physiologic parameters, 15 rats from the coronary-constricted (CC)[1] group and 15 rats from the sham-operated group were killed by decapitation. The hearts were rapidly excised and placed in ice-cold saline. The aorta and great vessels were discarded, and the left and right ventricles were separated and weighed. The ventricles were minced and homogenized in 4:1 wt/vol of 0.25 M sucrose/30 mM histidine with a Brinkman Polytron (Westbury, CT) (setting: 8; 10 s × 2). The crude homogenate of each ventricle was then centrifuged twice at 14,000 *g* for 20 min, and the supernatant centrifuged at 45,000 *g* for 30 min. Pellets were resuspended in 0.25 M sucrose and 30 mM histidine to final protein concentration of 1–2 mg/ml. Protein concentration was determined by the method of Lowry using BSA standards. All preparatory procedures were performed at 4°C. Membranes were immediately stored at −70°C until the radioligand binding assay was performed.

Radioligand binding assay. The radioligand ^{125}I±CYP (sp act 2,200 Ci/mmol; New England Nuclear, Boston, MA), the iodinated derivative of cyanopindolol, was employed to label β-adrenoreceptors in myocardial membranes. Assays were performed in 75 mM Tris, 25 mM $MgCl_2$, pH 7.4 at 37°C. Total incubation volume was 1 ml, consisting of 80–120 μg of membrane protein, and 100 μl of agonist and antagonists in assay buffer. This mixture was incubated for 30 min and binding was terminated by rapid vacuum filtration over Gelman AE glass fiber filters (Ann Arbor, MI). The filters were washed with 20 ml of assay buffer and bound radioactivity was determined by Micromedic Automatic Gamma Counter (Micromedic Systems, Inc., Horsham, PA) at a counting efficiency of 75%. Specific binding was defined as the portion of total counts displaced by 1 mM *l*-isoproterenol. At ligand concentrations equivalent to the K_d, specific binding averaged 85%. All values in the figures and tables refer to specific binding.

Adenyl cyclase assay. Adenyl cyclase activity was measured in rat myocardial membrane by the method of Salomon (11). Assay conditions were based on those that gave maximal isoproterenol stimulation. The substrate was 0.1 mm ATP trace labeled with 1.0 μCi of α-[^{32}P]-ATP, with 10 mM $MgCl_2$ in excess. Incubation volume was 70 μl and contained 25 μg of membrane protein, 10 mM creatine phosphate, 12.5 mM cyclic AMP, 5 mM KCl, 9 mM theophylline, and 10^{-5} M GTP. The assay buffer was 0.05 M Tris, pH 7.5 and incubations were performed at 37°C for 15 min. Assay blanks yielded < 10% of basal activity in all cases. Recovery of ^{3}H-cyclic AMP ranged from 65 to 80%.

Regulatory protein

Preparation of myocardial membranes. Membranes from four coronary-constricted and four sham-operated rats were prepared following the determination of physiologic parameters. Hearts were rapidly excised and weights determined. The tissues were homogenized in 4:1 wt/vol of 24 mM NaH_2CO_3/30 mM histidine with a Brinkman Polytron (setting 8; 10 s × 2). The homogenates were centrifuged at 12,000 *g* for 20 min and the pellets were saved. Subsequently, the pellets were homogenized in 25 mM sucrose/30 mM histidine, centrifuged at 12,000 *g* for 20 min and the supernatants saved. Supernatants were then centrifuged at 37,000 *g* for 90 min, pellets resuspended in 20 mM Tris, pH 8, 1 mM EDTA, and 1 mM DTT, and membranes extracted with 2% cholate. The suspensions were kept on ice for 60 min and finally centrifuged at 20,000 *g* for 20 min. The supernatants were stored at −70°C until used. Protein concentration was determined by the method of Lowry.

Cholera toxin [^{32}P]NAD labeling. Radiolabeling of membranes was performed in the presence and absence of cholera toxin. Membranes were diluted 1:10 with TED/lubrol (0.05%) before labeling. The final reaction mixture contained 138.5 mM Tris HCl, pH 8.0, 13.85 mM thymidine, 1.4 mM ATP, 1.4 mM GTP, 3.46 mM $MgCl_2$, 1.4 mM EDTA, 13.85 mM DTT, 2.8 mM [^{32}P]NAD (30 Ci/mM), 692.3 μM NADP, cholera toxin 2 mg/ml. Reactions were initiated by adding 100 μg of membranes to 26 μl of reaction mixture; vortex and incubate for 1 h at 37°C. Reactions were terminated by adding 20 μl of 2 × sampling buffer (4% SDS, 20% glycerol, 10% 2-mercapethanol, bromophenol blue, 125 mM Tris) and boiling samples for 5 min. The samples were applied to a 12% SDS polyacrylamide gel according to the method of Laemmli (12). Molecular weight markers were also applied to the gel and electrophoresis was terminated when the dye front left the bottom of the gel. The gels were stained with 50% methanol, 10% glacial acetic acid, and 0.20% coomassie blue, and then disdained with 10% methanol and 10% glacial acetic acid. The gels were dried and subjected to autoradiography using Kodak XAR5 film (Eastman Kodak Co., Rochester, NY) at −70°C. The incorporation of radioactive label was quantitated directly from the gel (13) by a computer-assisted radioanalytic imaging system (AMBIS Systems, Inc., San Diego, CA). The molar amount of incorporated label was calculated from the total counts contained in the labeled bands and the specific activity of the [^{32}P]NAD.

Alkaline phosphatase activity. A spectrophotometric assay (14, 15) was employed to measure alkaline phosphatase activity in myocardial membranes from coronary-constricted and control hearts. The determinations were performed on aliquots of pooled membrane vesicles used in receptor and adenylate cyclase assays. Values are expressed as units of alkaline phosphatase activity per microgram of membrane protein.

Statistical analysis. Values are reported as means±SE. Comparisons between values in controls and coronary-constricted rats were performed using a two-tailed, unpaired Student's *t* test. Comparisons between the left and right ventricles within each group were done utilizing a paired Student's *t* test. P values < 0.05 were considered to be significant. Because at times samples were pooled, n values for each determination are listed in the text or the legend of each figure.

Results

Coronary constriction and ventricular hemodynamics. The experimental procedures used resulted in a 44±2.4% reduction in the luminal diameter of the left coronary artery near its origin. This change in linear dimension corresponded to a 61% decrease in mean cross-sectional area of the coronary lumen. Sham operation had no effect on coronary artery luminal diameter in control rats. 5 mo after coronary artery narrowing, systolic, diastolic, and mean arterial blood pressures were decreased by 7, 6, and 4%, respectively. Heart rate was increased by 4%. However, all these differences were found not to be statistically significant (data not shown). In contrast, left ventricular minimal diastolic pressure increased by 6.9-fold, from 1.02±0.32 to 6.99±0.44 mm Hg ($P < 0.001$). Left ventricular end diastolic pressure augmented 2.7-fold, from 10±1.58 to

1. *Abbreviations used in this paper:* CC, coronary-constricted.

27±1.40 mm Hg (Fig. 1 A). Whereas peak systolic ventricular pressure did not change in coronary-constricted rats (Fig. 1 B), peak positive *dP/dt* was reduced by 29%. Moreover, negative *dP/dt* was also decreased by 15%. However, there were no signs of liver congestion and ascites to indicate global cardiac failure.

In summary, impairment of left ventricular function was present 5 mo after moderate degrees of coronary artery narrowing.

Gross cardiac characteristics. Fig. 2 illustrates the changes in the weight of the heart and its major subdivisions 5 mo after coronary artery constriction. Overall heart weight was found to be increased by 16% in experimental animals (control: 1,204±15 mg; CC: 1,391±49) as a result of a 12% augmentation in left ventricular weight (control: 935±17 mg; CC: 1,045±31 mg) and a 29% expansion in the weight of the right ventricular free wall (control: 269±2.3 mg; CC: 346±18 mg). These changes were all statistically significant.

Because body weight did not differ in control (499±3 g) and experimental (487±9 g) animals, the ratios of heart weight/, left ventricular weight/, and right ventricular weight/body weight were increased in coronary-constricted rats. In particular, the augmentations in the left and right ventricular weight/body weight ratios were 15 and 31%, respectively (data not shown).

In summary, chronic coronary artery constriction was associated with biventricular hypertrophy. However, the increase in right ventricular mass exceeded the increase in left ventricular mass.

^{125}I±CYP binding to myocardial membranes. In a previous study, the specificity of the radioligand for the myocardial β-adrenoreceptor was established by performing saturation isotherms and competition curves with β-adrenoreceptor agonist and antagonists (16). Binding of ^{125}I±CYP to the β-adrenoreceptor was saturable and of high affinity. Competition curves with agonist and antagonists demonstrated the expected rank order of potency for the β-adrenoreceptor: *dl*-propranolol > *l*-isoproterenol > *l*-epinephrine > *l*-norepinephrine > *dl*-isoproterenol (16).

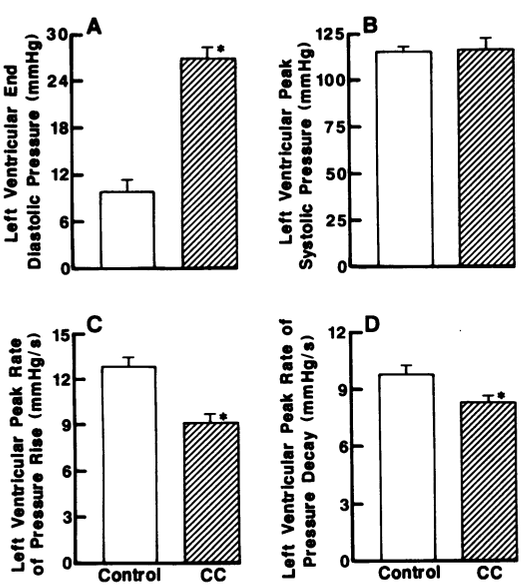

Figure 1. Effects of chronic coronary artery constriction (CC) on left ventricular function. Results are presented as mean±SEM (controls: n = 10; CC: n = 19). *Indicates a change that is statistically significant, $P < 0.05$.

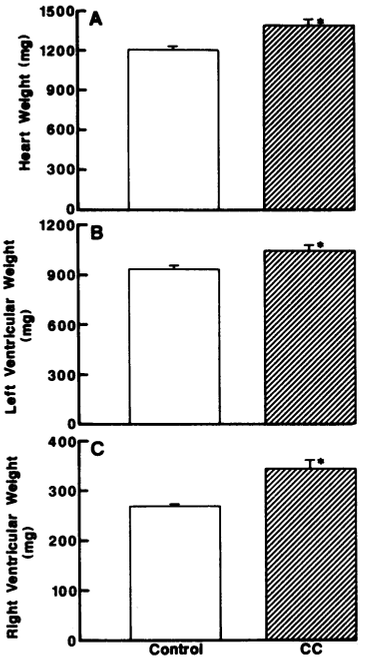

Figure 2. Effects of chronic coronary artery constriction (CC) on the weights of the heart and its major subdivisions. Results are presented as mean±SEM, (controls: n = 22; CC: n = 19). *Indicates a change that is statistically significant, $P < 0.05$.

Table I lists the density of β-adrenoreceptors (B_{max}) and the affinity of the receptor for the radioligand (K_d) in the myocardium of control and experimental animals. A 28% reduction ($P < 0.005$) in the density of β-adrenoreceptors was found in the left ventricle of coronary-constricted rats, whereas receptor density in the right ventricle remained essentially constant. In the left ventricle, K_d was not affected by the experimental procedure, but a 58% decrease ($P < 0.05$) in this parameter was measured in the right ventricle. When left and right ventricular data were compared in each of the two groups of animals, it was noted that receptor density was 70% higher ($P < 0.03$) in the right myocardium than in the left myocardium of control rats. A similar pattern but of larger magnitude was present in coronary constricted animals, because B_{max} was 111% greater (P

Table I. Effects of Coronary Constriction on β-Adrenoreceptor Density and Affinity

	Sham-operated	Coronary-constricted
	fM/mg	*fM/mg*
Left ventricle		
B_{max}	130±6.5	94±3.6*
K_d, pM	49±3.6	57±2.1
	(n = 3)	(n = 4)
Right ventricle		
B_{max}	221±26	198±37
K_d, pM	370±66	154±37*
	(n = 3)	(n = 3)

Results are presented as mean±SE. *Indicates a value that is significantly different, $P < 0.05$. B_{max}, density of β-adrenoreceptors; K_d, affinity of β-adrenoreceptors for the radioligand ^{125}I±CYP.

< 0.01) in the right ventricle. Moreover, K_d values were 7.6-fold ($P < 0.01$) and 2.7-fold ($P < 0.03$) higher in the right than in the left ventricle of control and experimental rats, respectively.

In summary, chronic coronary artery constriction led to a down regulation of β-adrenoreceptor density in the left ventricular myocardium. Moreover, receptor affinity for the radioligand was increased in the right myocardium. Coronary narrowing reduced the differences in the binding properties of the β-adrenoreceptors normally present between the two ventricles.

Cholera toxin [^{32}P]NAD ribosylation. Cholera toxin catalyzes the transfer of an ADP ribose group from NAD to the α subunit of the stimulatory guanine regulatory protein Gs (17). By employing [^{32}P]NAD as substrate, the labeled components of the membrane can be resolved on SDS polyacrylamide gels and identified by autoradiography. Covalent modification of the α subunit of the stimulatory guanine nucleotide binding protein Gs by cholera toxin inhibits intrinsic GTPase activity, enhancing activation of the catalytic moiety of adenyl cyclase (17). Therefore, cholera toxin was employed to identify and quantitate the relative amounts of Gs in membranes from hearts of control and experimental rats.

To establish that maximal covalent modification of Gs was present at 60 min, the incorporation of [^{32}P]NAD substrate into myocardial membranes from control and experimental hearts was analyzed at different time intervals up to 120 min (Fig. 3). The results obtained demonstrated that radiolabeling of membranes was time dependent and maximal at 60 min. Moreover, incorporation of the radioisotope was found to increase linearly with increasing amounts of membrane protein (Fig. 4).

By employing this approach, quantification of Gs was performed from myocardial membranes of control and experimental rats (Fig. 5). As shown in the insert of Fig. 5, the 45-K_d band was labeled in the presence of cholera toxin, but when cholera toxin was omitted no incorporation [^{32}P]NAD was detected. Coronary constriction resulted in a 39% ($P < 0.05$) and a 54% ($P < 0.02$) reduction in Gs in the left and right ventricular myocardium, respectively. Whereas similar amounts of Gs were present in the left and right ventricles of coronary artery–narrowed animals, a 75% greater content ($P < 0.04$) was detected in the right myocardium with respect to the left in control rats.

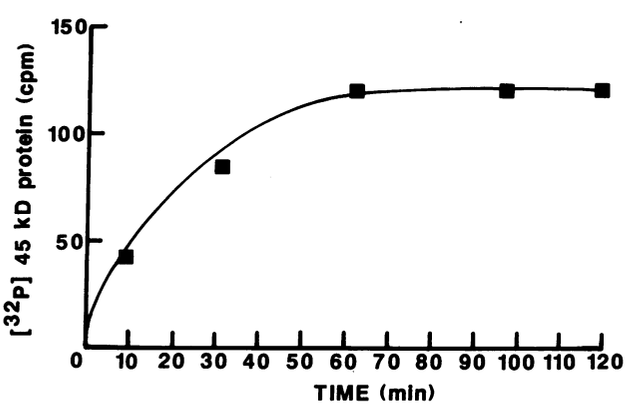

Figure 3. Incorporation of cholera toxin–specific counts into membranes prepared from control hearts as a function of time. Incorporation of [^{32}P]NAD was maximal at 60 min. Gels were counted by AMBIS radioanalytic scanner.

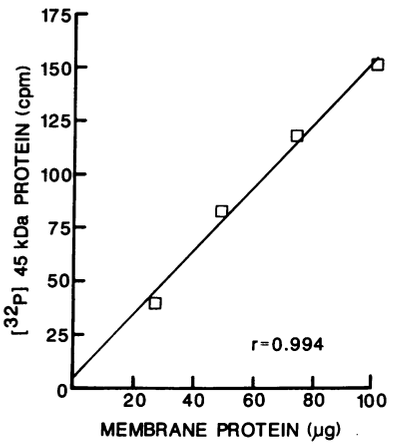

Figure 4. Incorporation of cholera toxin–specific counts into membranes prepared from control hearts as a function of increasing amounts of membrane protein. Radiolabeling of membranes increased linearly with increasing amounts of membrane protein.

In summary, chronic coronary artery constriction resulted in a biventricular reduction in the content of the stimulatory guanine nucleotide binding protein. Coronary narrowing abolished the difference in relative amounts of this regulatory protein normally present between the left and right ventricle.

Adenylate cyclase activity. Adenylate cyclase activity was assessed in myocardial membranes by *l*-isoproterenol stimulated ^{32}P-cyclic AMP generation. In a preliminary study (16), *l*-isoproterenol was found to stimulate adenylate cyclase activity in a dose-dependent fashion with maximal stimulation at 100 μm. Therefore, this dose was employed to compare the activity of this enzyme in the myocardium of sham-operated and coronary-constricted rats.

Fig. 6 illustrates that maximal stimulation of adenylate cyclase in myocardial membranes decreased significantly 5 mo after coronary artery narrowing. In comparison with controls, 84% ($P < 0.01$) and 45% ($P < 0.03$) reductions in the generation of cyclic AMP were found in the left and right myocardium of experimental rats, respectively. Moreover, agonist-stimulated adenylate cyclase activity was 45% greater ($P < 0.01$) in the right ventricle than in the left ventricle of sham-operated animals. Coronary constriction enhanced this difference between the ventricles, resulting in a fivefold greater ability of the right myocardium to generate cyclic AMP in response to *l*-isoproterenol stimulation.

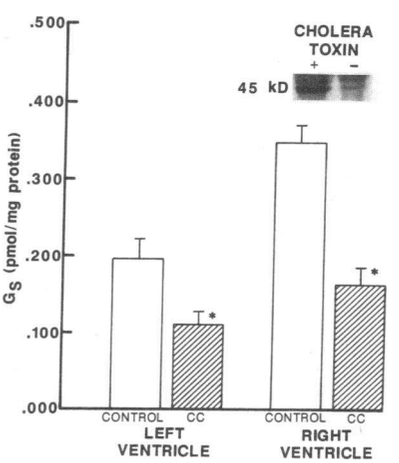

Figure 5. Quantitation of the stimulatory quanine nucleotide binding protein (Gs) in left and right ventricles of coronary-constricted and sham-operated rats. *Indicates a value that is statistically significantly different, $P < 0.05$; $n = 5$ in all determinations. (*Inset*) Radiolabeling of myocardial membranes incubated in the presence (+) and in the absence (−) of cholera toxin.

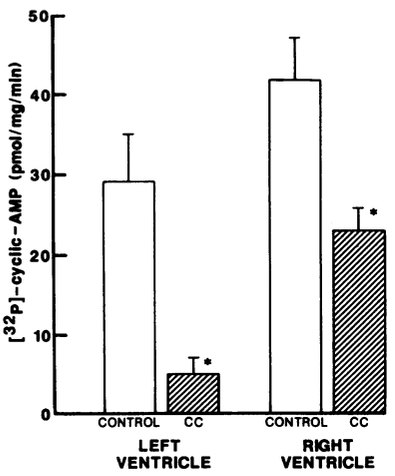

Figure 6. Hormone sensitive adenylate cyclase in the left and right ventricles of coronary-constricted and sham-operated rats. *l*-Isoproterenol, 100 μM, was employed to stimulate adenylate cyclase activity. *Indicates a change that is statistically significant, $P < 0.05$; $n = 4$ in all determinations.

In summary, chronic coronary artery constriction was associated with a marked attenuation of adenylate cyclase activity in the left and right ventricles. The experimental procedure enhanced the difference between the ventricles in agonist stimulated cyclic AMP generation.

Alkaline phosphatase activity. To ensure equivalency of membrane preparations employed in this investigation, the activity of an alternate membrane marker was determined. The activity of alkaline phosphatase was found not to differ in myocardial membranes obtained from ventricles of control and experimental animals: control (n = 3): 447±16 U/μg membrane protein; experimental (n = 4) 449±44 U/μg membrane protein.

Discussion

Coronary artery constriction and cardiac function. Data in the present study demonstrate that cardiac function was markedly depressed 5 mo after moderate degrees of coronary artery constriction. This observation raises the important question of whether magnitudes of coronary stenosis considered to represent clinically insignificant lesions may have to be reevaluated in terms of their potential impact on ventricular dynamics. Moreover, these findings are consistent with previous reports in which abnormalities in ventricular pump function have been found as early as 45 min (5), and at 3 and 5 d (6) following coronary artery stenosis. In all cases, ventricular performance was impaired, indicating that coronary artery narrowing may lead to sudden alterations of physiological parameters that persist during the progression of the disease state and long thereafter. The depression in the inotropic ability of the myocardium found in this model cannot be attributed only to global or regional myocardial ischemia because resting coronary blood flow is not affected under these conditions (5, 6). Decreases in basal flow of 10–20% are required to impair regional endocardial function (18), and nearly complete interruption of resting coronary blood flow must be present before active shortening of the myocardium is completely abolished (18). Several factors other than coronary perfusion may have participated in the initiation and persistence of diastolic dysfunction and depression in the force generating ability of the myocardium. Changes in myocardial pH and the degree of bonding between actin and myosin (19–21), abnormalities in calcium sequestration by the sarcoplasmic reticulum (19), and increased myocardial stiffness due to myocyte loss and collagen accumulation (6, 21) may all be involved in the impairment of the mechanical properties of the ventricle (22–24).

Coronary artery constriction and cardiac hypertrophy. The current findings indicate that cardiac hypertrophy was present after coronary artery narrowing. However, this tissue response failed to normalize ventricular performance chronically. Although the temporal sequence of events that took place over the 5-mo period of observation was not investigated here, the possibility may be advanced that pathological hypertrophy developed in association with long-term coronary artery constriction. Moreover, the 5-mo interval may represent the phase of transition from decompensated ventricular hypertrophy and myocardial dysfunction to overt failure and death in this animal model.

The observation that the growth adaptation of the right ventricle was greater than the hypertrophic reaction of the left ventricle was unexpected and surprising. Although this difference cannot be explained at present, coronary narrowing leads to myocyte loss in the left myocardium (5, 6) and this phenomenon results in an underestimation of the degree of hypertrophy in the unaffected myocytes (25–30), when evaluated on the basis of tissue weight or volume measurements alone. Because the changes in myocyte volume were not determined here, it cannot be excluded that myocyte cellular hypertrophy in the injured left ventricle exceeded that in the right ventricle in spite of a greater increase in right ventricular mass. Recent observations in the aging heart (30), and following acute and healed myocardial infarction (7, 25) favor this possibility.

The functional determinants of cardiac hypertrophy after coronary artery narrowing may be found in the characteristics of ventricular hemodynamics investigated here and in previous studies of this animal model (5, 6). Impairment of left ventricular pump performance and chamber dilatation develop immediately after the constriction (5), resulting in an increase in diastolic and systolic wall stress that may persist chronically, providing a mechanical stimulus for myocyte growth. Moreover, myocytolytic necrosis generates a greater work load on the remaining myocytes, which may increase with time, leading to an additional load-dependent mechanism of myocyte hypertrophy in the left ventricle. The bases of right ventricular hypertrophy are presently unknown. One possibility is that right ventricular systolic pressure is increased to maintain the pressure gradient across the pulmonary bed in left ventricular failure, or the right ventricle is acting in concert with the injured left ventricle to sustain systemic arterial pressure. Should this be the case, however, the left ventricle would be exposed to a combination of pressure and volume overload stress (5), whereas the right ventricle would be subjected to pressure overload stress only.

Coronary artery constriction and β-adrenoreceptors. Data in the current study document, for the first time, that distinct regulatory modification of surface β-adrenoreceptors occur in the left and the right myocardium in association with biventricular hypertrophy induced by chronic coronary artery constriction. The downregulation of these receptors in the left ventricle coupled with their preservation in the right ventricle may reflect the different anatomical and hemodynamic conditions discussed above: decompensated pressure and volume overload eccentric hypertrophy of the left ventricle and compensated pressure overload concentric hypertrophy of the right

ventricle. The combination of these effects would result in an attenuation of β-adrenoreceptor–mediated support of contractility in the compromised left ventricle. In contrast, the lack of β-receptor downregulation in the overloaded right ventricle indicates a difference in the modulation of β-adrenoreceptors between the two ventricles. This may result in a more effective transmission of adrenergic signals to the right ventricular myocardium at the receptor level. Moreover, these changes in β-adrenoreceptor density may further characterize the properties of pathologic left ventricular hypertrophy and physiologic right ventricular hypertrophy. Such a contention is consistent with previous studies in which the number of β-adrenoreceptors has been found to be decreased in the failing human heart (31, 32). However, ventricular hypertrophy in its compensated stage (33, 34) and at the onset of myocardial dysfunction (35) typically shows increases in β-adrenoreceptor density.

The increase in β-adrenoreceptor antagonist affinity in the hypertrophied right ventricle suggests a fundamental modification in the ligand recognition unit of the receptor. Furthermore, a persistent decrease in β-adrenoreceptor antagonist affinity has been reported in a canine model of pressure overload left ventricular hypertrophy and failure (34, 35). At the molecular level, alterations in the transcription of the gene for β-adrenoreceptors may result in the expression of a modified primary structure of the receptor protein (35), with a corresponding increase or decrease in receptor antagonist affinity, dependent on the nature of the substitution or deletion.

Differences in β-adrenoreceptor density and affinity between the left and right myocardium of control hearts were found to be present. The lower number of receptors in the left ventricle with respect to the right may reflect variation in the intensity of postsynaptic adrenergic activity in the two sides of the heart coupled with the hemodynamic characteristics of the respective chambers. The left ventricle is faced with a high systemic vascular resistance and consequently operates as a pressure pump. On the other hand, the right ventricle is exposed to the low vascular resistance of the pulmonary circulation and acts as a volume pump. Similarly, such distinct loading conditions between the ventricles may influence turnover rate and synthesis of receptor protein, and account for the K_d expressed in the left and right myocardium.

Coronary artery constriction and regulatory protein. The present results indicate that left ventricular dysfunction and β-adrenoreceptor downregulation in the presence of coronary stenosis were associated with a decrease in the turnover of the stimulatory protein that links agonist occupancy of the β-adrenoreceptors with the catalytic unit of adenylate cyclase. In contrast, a dissociation occurred between β-adrenoreceptor density and Gs in the overloaded hypertrophied right ventricle: receptor density remained constant while the quantity of radiolabeled substrate decreased.

The observations here are at variance with previous studies in which left ventricular dysfunction and failure (36) exhibited an upregulation of β-adrenoreceptor density, and a decrease in the quantity of Gs. On the other hand, dynamic exercise has been shown to induce an increase in this regulatory protein with no change in the number of β-adrenoreceptors in the left ventricle (37). Moreover, the greater mechanical stimulus on the right ventricle during exercise (38) resulted in a down regulation of these receptors and in an increased amount of regulatory protein (37). All these findings point to the possibility that the altered adrenergic responsiveness of the stressed myocardium can be modulated through postreceptor mechanisms. The greater quantity of regulatory protein and β-adrenoreceptors found in the right myocardium under normal conditions supports the notion that the right side of the heart may have an enhanced capacity for the transduction of adrenergic signals to cardiac myocytes.

Coronary artery constriction and adenylate cyclase activity. The current data indicate that hormone-sensitive adenylate cyclase activity was decreased in the myocardium of coronary artery–narrowed rats. However, the reduced activity of this membrane-bound enzyme was almost twofold greater in the left than in the right ventricle. Thus, cyclic AMP generation was affected more in the failing left ventricle than in the hypertrophic nonfailing right ventricle. This is consistent with the kinetics of β-adrenoreceptors and regulatory protein interaction that can be inferred from the present results. Whereas both β-adrenoreceptor density and the quantity of stimulatory guanine nucleotide binding protein were severely depressed in the functionally impaired left ventricle, receptor density was preserved in the right ventricle, maintaining a more favorable stoichiometric relationship between receptors and regulatory protein. On the other hand, the alterations in the β-adrenoreceptor adenylate cyclase complex found in the hypertrophic right ventricle suggest that impaired transmission of adrenergic signals may occur before ventricular failure supervenes. Furthermore, under normal loading conditions, the molecular components that mediate agonist-stimulated cyclic AMP generation are present in greater quantities in the right than in the left ventricle, strengthening the contention of an enhanced interaction potential between adrenergic influences and effector responses in the right side of the heart. These observations in coronary artery–narrowed animals are in agreement with previous work in experimental models of pressure overload–induced myocardial dysfunction (35, 36) and in the failing human heart (39–41).

In conclusion, the depression in left ventricular myocardial performance in long-term coronary artery constriction may be mediated by impairment of the transmission of adrenergic signals to myocytes with attenuation of their force-generating ability and pump failure. Although extrapolation of results from animals to humans requires considerable caution, the current findings indicate that moderate degrees of coronary artery narrowing may have a significant impact on the molecular events controlling muscle contractile behavior and global cardiac function in the clinical setting.

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References

1. Knoebel, S. B., W. C. Elliot, P. L. McHenry, and E. Ross. 1971. Myocardial blood flow in coronary artery disease: correlation with severity of disease and treadmill exercise response. *Am. J. Cardiol.* 27:51–58.

2. Welch, C. C., W. L. Proudfit, and W. C. Sheldon. 1975. Coronary arteriographic findings in 1000 women under age 50. *Am. J. Cardiol.* 35:211–215.

3. Schuster, E. H., and B. A. Bulkley. 1980. Ischemic cardiomyopathy: a clinicopathologic study of fourteen patients. *Am. Heart J.* 100:506–512.

4. Pantely, G. A., and J. D. Bristow. 1984. Ischemic cardiomyopathy. *Prog. Cardiovasc. Dis.* 27:95–114.

5. Capasso, J. M., M. W. Jeanty, T. Palackal, G. Olivetti, and P. Anversa. 1989. Ventricular remodeling induced by acute nonocclusive constriction of coronary artery in rats. *Am. J. Physiol.* 257:H1983–H1993.

6. Capasso, J. M., P. Li, and P. Anversa. 1991. Non-ischemic origin of myocardial damage induced by short-term nonocclusive constriction of the coronary artery in rats. *Am. J. Physiol.* 260:H651–H661.

7. Anversa, P., A. V. Loud, V. Levicky, and G. Guideri. 1985. Left ventricular failure induced by myocardial infarction. I. Myocyte hypertrophy. *Am. J. Physiol.* 248:H876–H882.

8. Pfeffer, M. A., J. M. Pfeffer, M. C. Fishbein, P. J. Fletcher, J. Spadaro, R. A. Kloner, and E. Braunwald. 1979. Myocardial infarct size and ventricular function in rats. *Circ. Res.* 44:503–512.

9. Masari, A., and S. Chierchia. 1982. Coronary artery spasm: demonstration, definition, diagnosis and consequences. *Prog. Cardiovasc. Dis.* 25:169–192.

10. Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. 1984. β-Adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiol. Rev.* 64:661–743.

11. Salomon, Y., C. Londos, and M. Rodbell. 1974. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* 58:541–547.

12. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

13. Meggs, L. G., J. Tillotson, H. Huang, E. H. Sonnenblick, J. M. Capasso, and P. Anversa. 1990. Noncoordinate regulation of alpha-1 adrenoreceptor coupling and reexpression of α skeletal actin in myocardial infarction-induced left ventricular failure in rats. *J. Clin. Invest.* 86:1451–1458.

14. Bowers, H., Jr., H. U. Bergmeyer, and D. W. Moss. 1975. International Federation on Clinical Chemistry. Provisional recommendations on IFCC methods for the measurement of catalytic concentration of enzymes: expert panel on enzymes as accepted by the committee on standards. *Clin. Chim. Acta.* 61:F11–F24.

15. Frajola, W. J., R. D. Williams, and R. A. Austad. 1965. The kinetic spectrophotometric assay for serum alkaline phosphatase. *Am. J. Clin. Pathol.* 43:261–264.

16. Meggs, L. G., J. Ben-Ari, D. Gammon, M. Choudhury, and A. I. Goodman. 1990. Adaptive myocardial hypertrophy in the renal ablation model. *Am. J. Hypertens.* 3:33–38.

17. Stryer, L., and H. R. Bourne. 1986. G proteins: a family signal transducers. *Annu. Rev. Cell Biol.* 2:391–419.

18. Vatner, S. F. 1980. Correlation between acute reduction in myocardial blood flow and function in conscious dogs. *Circ. Res.* 47:201–207.

19. Katz, A., and M. Tada. 1972. The "stone heart": a challenge to the biochemist. *Am. J. Cardiol.* 29:578–580.

20. Hearse, D. J., P. B. Garlick, and S. M. Humphrey. 1977. Ischemic contracture of myocardium. Mechanisms and prevention. *Am. J. Cardiol.* 39:986–993.

21. Wexler, L. F., E. O. Weinberg, J. S. Ingwall, and C. S. Apstein. 1986. Acute alterations in diastolic left ventricular chamber distensibility: mechanistic differences between hypoxemia and ischemia in isolated perfused rabbit and rat hearts. *Circ. Res.* 59:515–528.

22. Serizawa, T., W. M. Vogel, C. S. Apstein, and W. Grossman. 1981. Comparison of acute alterations in left ventricular relaxation and diastolic chamber stiffness induced by hypoxia and ischemia. *J. Clin. Invest.* 68:91–102.

23. Momomura, S. I., A. B. Bradley, and W. Grossman. 1984. Left ventricular pressure-segment length relations and end-diastolic distensibility in dogs with coronary stenoses. *Circ. Res.* 55:203–214.

24. Paulus, W. J., W. Grossman, T. Serizawa, P. D. Bourdillon, A. Pasipoularides, and I. Mirsky. 1985. Different effects of two types of ischemia on regional left ventricular systolic and diastolic function. *Am. J. Physiol.* 248:H719–H728.

25. Anversa, P., C. Beghi, Y. Kikkawa, and G. Olivetti. 1986. Myocardial infarction in rats: infarct size, myocyte hypertrophy and capillary growth. *Circ. Res.* 58:26–37.

26. Anversa, P., T. Palackal, E. H. Sonnenblick, G. Olivetti, L. G. Meggs, and J. M. Capasso. 1990. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ. Res.* 67:871–885.

27. Anversa, P., T. Palackal, E. H. Sonnenblick, G. Olivetti, and J. M. Capasso. 1990. Hypertensive cardiomyopathy. Myocyte nuclei hyperplasia in the mammalian rat heart. *J. Clin. Invest.* 85:994–997.

28. Capasso, J. M., T. Palackal, G. Olivetti, and P. Anversa. 1990. Left ventricular failure induced by long-term hypertension in rats. *Circ. Res.* 66:1400–1412.

29. Olivetti, G., M. Melissari, J. M. Capasso, and P. Anversa. 1991. Cardiomyopathy of the aging human heart: myocyte loss and reactive cellular hypertrophy. *Circ. Res.* 68:1560–1568.

30. Anversa, P., B. Hiler, R. Ricci, G. Guideri, and G. Olivetti. 1986. Myocyte cell loss and myocyte hypertrophy in the aging rat heart. *J. Am. Coll. Cardiol.* 8:1441–1448.

31. Bristow, M. R., R. Ginsburg, W. Minobe, R. S. Cubicciotti, W. S. Sageman, K. Lurie, M. E. Billingham, D. C. Harrison, and E. B. Stinson. 1982. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med.* 307:205–211.

32. Bristow, M. R., R. Ginsburg, V. Umans, M. Fowler, W. Minobe, R. Rasmussen, P. Zera, R. Menlove, P. Shah, S. Jamieson, and E. B. Stinson. 1986. β_1- and β_2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1-receptor down-regulation in heart failure. *Circ. Res.* 59:297–309.

33. Limas, C. J. 1979. Increased numbers of β-adrenergic-receptors in the hypertrophied myocardium. *Biochim. Biophys. Acta.* 588:174–178.

34. Vatner, D. E., C. J. Homcy, S. P. Sit, W. T. Manders, and S. F. Vatner. 1984. Effects of pressure overload, left ventricular hypertrophy on β-adrenergic receptors and responsiveness to catecholamines. *J. Clin. Invest.* 73:1473–1482.

35. Vatner, D. E., S. F. Vatner, A. M. Fujii, and C. J. Homcy. 1985. Loss of high affinity cardiac β-adrenergic receptors in dogs with heart failure. *J. Clin. Invest.* 76:2259–2264.

36. Longabaugh, J. P., D. E. Vatner, S. F. Vatner, and C. J. Homcy. 1988. Decreased stimulatory guanosine binding protein in dogs with pressure-overload left ventricular failure. *J. Clin. Invest.* 81:420–424.

37. Hammond, H. K., L. A. Ransnas, and P. A. Insel. 1988. Noncoordinate regulation of cardiac Gs protein and β-adrenergic receptors by a physiological stimulus, chronic dynamic exercise. *J. Clin. Invest.* 82:2168–2171.

38. Anversa, P., V. Levicky, C. Beghi, S. L. McDonald, and Y. Kikkawa. 1983. Morphometry of exercise-induced right ventricular hypertrophy in the rat. *Circ. Res.* 52:57–64.

39. Feldman, M. D., L. Copelas, J. K. Gwathmey, P. Phillips, S. E. Warren, F. J. Schoen, W. Grossman, and J. P. Morgan. 1987. Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. *Circulation.* 5:331–339.

40. Gwathmey, J. K., L. Copelas, R. MacKinnon, F. J. Schoen, M. D. Feldman, W. Grossman, and J. P. Morgan. 1987. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circ. Res.* 61:70–76.

41. Bristow, M. R., R. E. Hershberger, J. D. Port, W. Minobe, and R. Rasmussen. 1989. β_1- and β_2-adrenergic receptor-mediated adenylate cyclase stimulation in nonfailing and failing human ventricular myocardium. *Mol. Pharmacol.* 35:295–303.