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Research Article

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Noncoordinate Regulation of Alpha-1 Adrenoreceptor Coupling and Reexpression of Alpha Skeletal Actin in Myocardial Infarction-induced Left Ventricular Failure in Rats

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Abstract

To determine the effects of myocardial infarction-induced left ventricular failure on the regulation of surface alpha-1 adrenoreceptors and signal transduction, large infarcts were produced in rats and the animals killed seven days later. After the documentation of impaired left ventricular pump performance, radioligand binding studies of the alpha-1 adrenoreceptor, norepinephrine-stimulated phosphoinositol turnover, and ADP ribosylation of 41 kD substrate by pertussis toxin were examined in the hypertrophying unaffected myocardium. Moreover, the expression of sarcomeric actin isoforms was analyzed by Northern blots and hybridization with specific oligonucleotide probes. Alpha-1 adrenoreceptor density was found not to be altered in membranes obtained from the spared left ventricular tissue, whereas phosphoinositol turnover was increased 3.1-fold in the viable myocytes of infarcted hearts. Furthermore, pertussis toxin substrate was augmented 2.5-fold in membranes prepared from the surviving left ventricular myocardium. Finally, an upregulation of the skeletal actin isoform was detected in the tissue of the failing left ventricle. In conclusion, the possibility is raised that in the presence of severe myocardial dysfunction and ongoing reactive hypertrophy, effector pathways linked to the alpha-1 adrenoreceptor may stimulate the myocyte hypertrophic response which would tend to normalize cardiac hemodynamics. The reexpression of alpha skeletal actin may be a molecular indicator of the persistance of an overload on the myocardium. (*J. Clin. Invest.* 1990. 86:1451–1458.) Key words: regulatory protein • phosphoinositol turnover • ventricular failure • hypertrophy

Introduction

Cardiac hypertrophy develops after myocardial infarction and the magnitude of this compensatory response correlates with infarct size (1, 2). Infarcts comprising 40% or more of the left ventricle lead to severe myocardial dysfunction that may result in acute congestive heart failure or sudden cardiac death (1, 3, 4). This phenomenon has been observed in man and animal models, although the latter are able to sustain more severe destruction in cardiac mass (1, 3, 5). In both cases, however, the signal that induces cellular growth is the critical factor for short and long term survival. Myocyte hypertrophy after infarction is an early event (1, 3, 6) that progresses with time (2) in an attempt to restore ventricular mass and improve function (1, 3, 6). Moreover, this growth adaptation involves augmentations in myocyte length which exceed those in cell diameter (1–3), implying that the altered hemodynamic state after infarction affects the preload more than the afterload (3). This prevailing volume overload on the surviving myocardium of the injured left ventricle contrasts with the growth pattern of the right ventricular myocardium which is primarily exposed to an elevated afterload with increases in myocyte diameter alone (1, 7). Importantly, a similar mechanism of myocardial response to infarction has been documented to be operative in the human heart (8).

Recent investigations have indicated that the mechanism of myocyte growth in the adult rat heart exposed to pressure overload is associated with reexpression of alpha skeletal actin (9, 10). The signal that pressure overload transduces to the myocyte nucleus initiating transcription of the gene for alpha skeletal actin is unknown. However, stimulation of surface alpha-1 adrenoreceptors in neonatal rat cardiac myocytes in culture induces reexpression of alpha skeletal actin and myocyte hypertrophy (11). On the other hand, norepinephrine-stimulated adult cardiac cells have failed to demonstrate an effect of alpha receptors on cellular hypertrophy (12). Although potential differences may exist in the response of neonatal and adult ventricular myocytes to alpha-1 receptor stimulation (9–12), the hypothesis was advanced that activation of the sympathetic nervous system in left ventricular failure associated with myocardial infarction may induce transcription of alpha skeletal actin in the surviving myocytes. Moreover, because of the role of the alpha-1 adrenoreceptor in myocyte hypertrophy and gene expression (11, 13), the regulation of surface alpha-1 adrenoreceptors and signal transduction was examined in adult cardiac myocytes one week postinfarction. This time interval was chosen to allow for some consolidation of infarct size as well as to expose the surviving myocardium to the hyperadrenergic state present in left ventricular failure.

Methods

Coronary occlusion

Experiments were carried out in 3-mo-old male Sprague-Dawley rats weighing ~ 250 g (Charles River Breeding Laboratories, North Wilmington, MA). Ligation of the left coronary artery near its origin was performed in 110 animals in order to produce infarcts of the left ventricle associated with left ventricular failure (1, 3). Briefly, under ether anesthesia the thorax was opened and the heart exteriorized by applying a light pressure upon the thorax. The left coronary artery was then ligated, the chest closed, and the animals allowed to recover. 44

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rats died shortly after the operation mostly because of pulmonary edema. The remaining 66 animals were killed a week later. 34 sham-operated rats were used as controls.

Functional measurements

Just before killing, animals were anesthetized with chloral hydrate (300 mg/kg body wt, i.p.), and the external right carotid artery was exposed and cannulated with a micro-tip pressure transducer catheter (PR 249; Millar Instruments, Houston, TX) connected to an electrostatic chart recorder (Gould ES 2000; Gould Inc., Cleveland, OH). After monitoring arterial blood pressure in all animals, in a subgroup of 20 animals, the catheter was advanced into the left ventricle for the evaluation of left ventricular pressures and *dP/dt*. This part of the experimental protocol was restricted to only some animals because of the high mortality associated with very large infarcts during this procedure. Thus, measurements were made of systolic, diastolic, and mean arterial blood pressure, and in a subset of animals, made of ventricular pressures and *dP/dt* in the close chest preparation. These events were monitored and inscribed on recording paper for subsequent analysis. Ventricular pressure and *dP/dt* were measured in 10 sham-operated rats.

The hemodynamic measurements were employed not only for the assessment of global ventricular performance 1 wk after the induction of coronary artery occlusion, but also for an indirect evaluation of infarct size. Previous work conducted in our laboratory has shown that characteristic features of pump function impairment with a fall in systemic arterial blood pressure develops in this animal model when the destruction in myocardial mass involves nearly 50% of the myocyte population of the left ventricle inclusive of the septum (1, 3). This correlation has been shown to be present acutely (1, 3) and chronically (14, 15) after myocardial infarction. On this basis, 51 rats exhibiting indices of left ventricular failure and extensive myocardial infarction at death were included in the study. The remaining 15 animals did not fulfill these criteria and were excluded from subsequent determinations.

Alpha-1 adrenoreceptors

Membrane preparation. After the measurement of physiologic parameters, 15 rats from the infarcted group and 10 rats from the sham-operated group were killed by decapitation. The hearts were rapidly excised, weighed, and placed in ice-cold saline. The aorta and great vessels were discarded, and the left and right ventricles were separated. The ventricles were subsequently coarsely minced and homogenized in 4:1 weight volume of 0.25 M sucrose 30 mM histidine with a Polytron (setting 8 for 10 s × 2; Brinkmann Instruments Co., Westbury, NY). 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 a final protein concentration of 1 to 2 mg/ml. Protein concentration was determined by the method of Lowry using bovine serum albumin 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]±BE 2254 (sp act 2,200 Ci/mmol; New England Nuclear, Boston, MA), the iodinated derivative of the potent alpha-1 adreno-receptor antagonist I-2-[-(4-hydroxyphenyl) ethylaminomethyl] tetralone, was employed to label alpha-1 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 to 120 μg of membrane protein and 100 μl of agonists and antagonists in assay buffer. This mixture was incubated for 30 min and binding was terminated by rapid vacuum filtration over glass fiber filters (Gelman Sciences, Inc., Ann Arbor, MI). The filters were washed with 20 ml of assay buffer and bound radioactivity was determined by an automatic gamma counter (Micromedic Systems, Horsham, PA) at a counting efficiency of 75%. Specific binding was defined as the portion of total counts displaced by 1 mM l-epinephrine. At ligand concentrations equivalent to the K_D, specific binding averaged 85%. All values in figures and tables refer to specific binding.

Regulatory protein

Preparation of myocardial membranes. Membranes from 11 infarcted and 8 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 weight volume of 24 mM NaH_2CO_3 30 mM histidine with a Brinkmann Polytron (setting 8 for 10 seconds × 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 (TED), 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.

Pertussis toxin [^{32}P]NAD labeling. Radiolabeling of membranes was performed in the presence and absence of pertussis toxin. Membranes were diluted 1:5 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; 0.14 mM GTP; 3.46 mM $MgCl_2$; 1.4 mM EDTA; 13.85 mM DTT; 1.4 mM [^{32}P]NAD (46 Ci/mmol); 692.3) μM NADP; pertussis toxin 400 μg/ml. Reactions were initiated by adding 37 μg of membranes to 26 μl of reaction mixture, vortexed, and incubated for 1 h at 30°C. Reactions were terminated by adding 20 μl of 2× sampling buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, bromophenol blue, 125 mM Tris) and boiling samples for 5 min. The samples were applied to a 17% sodium dodecyl sulfate polyacrylamide gel according to the method of Laemmli (16). 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, 0.20% coomasie blue, and then destained with 10% methanol and 10% glacial acetic acid. The gels were dried and subjected to autoradiography using Kodak XAR5 film at −70°C. The incorporation of radioactive label was quantitated directly from the gel (17–19) by a computer assisted radioanalytic imaging system (AMBIS Radioanalytic Imaging System, San Diego, CA). The AMBIS system images and quantitates with a degree of sensitivity equal to the combined use of autoradiography and scintillation counting (20). 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.

Norepinephrine stimulated phosphoinositol turnover

Myocyte preparation. Ventricular myocytes were isolated by a modification of the method described by Brown (21). Under anesthesia and following the measurements of physiologic parameters, animals were injected with heparin (1,500 U/kg, i.p.), and after 20 min, hearts were removed and placed in a large culture dish containing media (0.1% bovine serum albumin and 1 mM $CaCl_2$). The aorta was isolated and severed proximal to the brachiocephalic trunk. A 14-gauge catheter was placed near the origin of the coronary arteries and media injected in order to flush the blood from the coronary circulation. The heart was then attached by the aorta to a perfusion apparatus for collagenase digestion (1 mg/ml) over a period of 50 min during which the myocardium was continuously gassed (95% O_2, 5% CO_2) and maintained at 32°C. After digestion, the heart was minced and placed in a siliconized trypsinizing flask containing 50 ml of collagenase for 10 min. The suspension was subsequently filtered through a nylon mesh cloth into a plastic receiving vessel. Cells were then centrifuged at 50 *g* for 2 min and the pellet resuspended in the same media and allowed to settle. Viability was determined by exclusion of trypan blue and found to be nearly 90%. The average yield per heart was 7–9 million of cells in sham-operated controls and 3–4 million of cells in infarcted rats.

Phosphoinositol assay. Ventricular myocytes were isolated from 12 infarcted and 8 sham-operated rats at 3 mo of age. Myocytes were suspended in 20 ml of basal Eagle's medium containing 1 mM $CaCl_2$, 0.1% bovine serum albumin, and 5 μCi/ml [^{3}H]myoinositol (14–20 Ci/mmol). Myocytes were incubated for 1 h at 35°C (shaking and gassed with 95% O_2, 5% CO_2). Myocytes were then centrifuged at 50 *g* for 2 min, washed, and resuspended in medium without labeled inositol. Experiments were initiated by the addition of labeled myocytes (4 × 10^6 cells/assay) into incubation vessels containing norepinephrine (10^{-4} M), diluent (ascorbate 100 μM), and 10 mM LiCl. 60 min later, the myocytes were collected centrifugally (Beckman Microfuge B; Beckman Instruments, Inc., Fullerton CA), medium removed, and trichloroacetic acid was added. The cells were subsequently sonicated and centrifuged at 3,000 *g* and the supernatant extracted with water saturated ether. The aqueous phase was transferred to an anion exchange column to separate [^{3}H]inositol phosphate by formate gradients. Samples were counted in a beta counter and the net stimulation calculated by subtracting background counts from total cpm.

Detection of alpha skeletal actin mRNA transcripts in the infarcted left ventricle

Northern blot. After the measurements of physiologic parameters, hearts from 15 infarcted and 8 sham-operated rats were excised, weighed, and placed in ice-cold saline. The left ventricles were separated and total cellular RNA extracted by the hot phenol method (11). Briefly, tissues were homogenized in 4 M guanidinium isothiocyanate and extracted with phenol, $CHCl_3$ at 65°C. Nucleic acids were collected by centrifugation after ethanol precipitation. RNA (10 μg) was size fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters (22). Oligonucleotide probes for alpha skeletal and cardiac actins (Research Genetics, Huntsville, AL) were labeled with gamma [^{32}P]ATP. The probes were homologous to the 3′ untranslated regions of alpha skeletal and cardiac actins mRNA's. The sequence of the probe for alpha skeletal actin was as follows: 5′-GCAACCATAGCACGATGGTC-3′ (23). The sequence of the probe for alpha cardiac actin was 5′-TGCACGTGTGTAAACAAACT-3′ (23). Hybridization was carried out at 42°C for 16 h in 6× standard saline citrate (SSC) (0.9 M NaCl, 0.09M Na Citrate, pH 7.0), 0.1% SDS, 5× Denhardt's (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), and 100 μg/ml single stranded, sheared, herring sperm DNA. Filters were washed in 6× SSC, 0.1% SDS at 42°C. Filters were exposed to Kodak XAR-5 film at −80°C with an intensifying screen and the hybridization signal quantitated by an AMBIS Radioanalytic Imaging System.

Statistical analysis

Values are reported as means±SE. Comparisons between values were performed using a two-tailed, unpaired Student's *t* test. *P* values < 0.05 were considered to be significant. Because measurements presented could not all be obtained in every animal and at times samples were pooled, *n* values for each determination are listed in the text or the legend of each figure.

Results

Heart weight and physiologic parameters. Acute myocardial infarction in rats 7 d after ligation of the left coronary artery near its origin showed an increase in heart weight and in the ratio of heart weight-to-body weight (Table I). Systolic, diastolic, and mean arterial pressures were all decreased in infarcted animals. Moreover, left ventricular end diastolic pressure increased whereas left ventricular peak systolic pressure was decreased after infarction. A substantial decrease in peak positive and negative *dP/dt* was also evident in the infarcted hearts indicating that a significant impairment of global ventricular function was present (Table I).

Table I. Effects of Myocardial Infarction on Cardiac Function of 3-mo-old Rats

	Sham-operated	Infarcted	% Difference
Body weight, *g*	298±4 (*n* = 34)	266±3.5 (*n* = 51)	−11
Heart weight, *mg*	892±11 (*n* = 34)	1078±19 (*n* = 51)	21*
Heart weight/body weight, *mg/g*	2.99±0.03 (*n* = 34)	4.05±0.08 (*n* = 51)	35*
Diastolic arterial pressure, *mmHg*	78±2.2 (*n* = 34)	51±1.4 (*n* = 51)	−35*
Systolic arterial pressure, *mmHg*	99±1.7 (*n* = 34)	74±1.4 (*n* = 51)	−25*
Mean arterial pressure, *mmHg*	86±1.9 (*n* = 34)	59±1.3 (*n* = 51)	−31*
Left ventricular end diastolic pressure, *mmHg*	9.4±1.04 (*n* = 10)	24.4±1.03 (*n* = 20)	160*
Left ventricular systolic pressure, *mmHg*	103±1.0 (*n* = 10)	78.5±3.6 (*n* = 20)	−24*
+ d*P*/d*t*, *mmHg/s*	9578±395 (*n* = 10)	5021±394 (*n* = 20)	−48*
−d*P*/d*t*, *mmHg/s*	8153±334 (*n* = 10)	4564±295 (*n* = 20)	−44*

Results are presented as mean±SE. * A difference that is statistically significant; $P < 0.05$.

[^{125}I]±BE 2254 binding to myocardial membranes. In a previous study, the specificity of the radioligand for the myocardial alpha-1 adrenoreceptor was established by performing saturation isotherms and competition curves with alpha-1 adrenoreceptor agonists and antagonists (24). Binding of [^{125}I]±BE 2254 to the alpha-1 adrenoreceptor was saturable and of high affinity. Competition curves with agonists demonstrated the expected rank order of potency for the alpha-1 adrenoreceptor: epinephrine > clonidine > phenylephrine > norepinephrine. Antagonists competed for binding sites in the following order: BE 2254 > phentolamine > corynanthine > yohimbine (24).

Fig. 1 shows a representative saturation isotherm and Scatchard analysis performed on left ventricular membranes obtained from infarcted hearts. Binding was saturable and Scatchard transformation indicated a homogeneous set of binding sites with high affinity.

Table II lists the density of alpha-1 adrenoreceptors (B_{max}) and the affinity of the receptor for the radioligand (K_D) in the left and right ventricles of sham-operated and infarcted rats 7 d after surgery. Alpha-1 adrenoreceptor density remained essentially constant in both ventricles after myocardial infarction. However, a 54% increase in K_D was measured in the infarcted left ventricle and this difference was found to be statistically significant ($P < 0.005$). In contrast, a 33% decrease in K_D was detected in the right ventricular myocardium of experimental rats.

Pertussin toxin [^{32}P]NAD ribosylation. Pertussis toxin catalyzes the transfer of an ADP ribose group from NAD to the

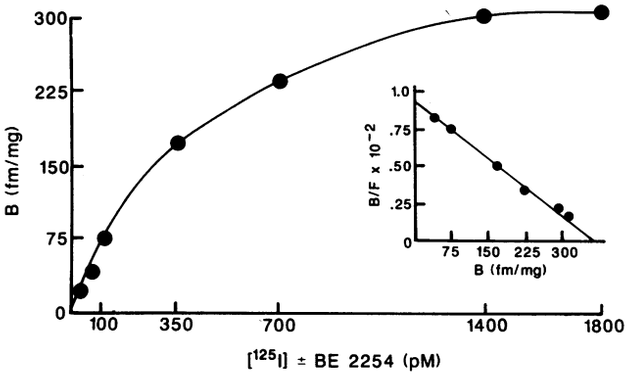

Figure 1. Saturation isotherm and Scatchard analysis of [^{125}I]±BE 2254 binding to membranes prepared from the left ventricle of infarcted hearts. Membranes were incubated with increasing concentrations of [^{125}I]±BE 2254, and specific binding determined. (*Inset*) Scatchard transformation of saturation isotherm data.

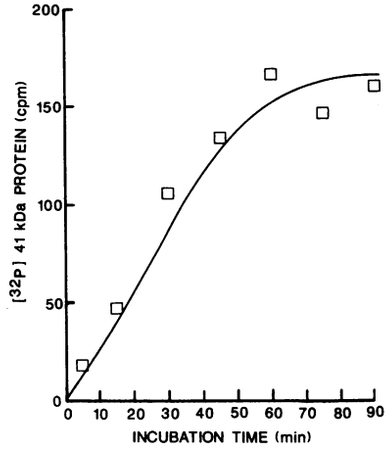

Figure 2. Incorporation of pertussis toxin specific counts into membranes prepared from control hearts as a function of time. Incorporation of [^{32}P]NAD was maximal at 60 min. Gels were counted by AMBIS radioanalytic scanner.

alpha subunit of guanine regulatory proteins Go and Gi (25). By employing [^{32}P]NAD as substrate, the labeled components of the membrane can be resolved on SDS polyacrylamide gels and identified by autoradiography. ADP ribosylation has been shown to inhibit agonist stimulated phosphoinositol hydrolysis in some systems (26), implying that a pertussis sensitive G protein links alpha-1 adrenoreceptor occupancy by agonists with phospholipase C. Therefore, pertussis toxin was employed to identify and quantitate the relative amounts of pertussis sensitive G protein in membranes from infarcted and control ventricles.

To establish that the ADP ribosylation catalyzed by pertussis toxin was complete 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 90 min (Fig. 2). The results obtained indicated 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. 3).

By employing this approach, labeled membranes from the left ventricles of infarcted and sham-operated rats showed bands at 41 kD molecular weight that correspond to the regulatory protein coupled with the alpha-1 adrenoreceptor (26). Gels were quantitated with an AMBIS radioanalytic scanner. Results demonstrated that G protein content was 2.5-fold greater in the surviving myocardium of the infarcted left ventricle than that present in the corresponding control ventricle (Fig. 4). Moreover, no increase in G protein amount was measured in the right ventricle of infarcted rats with respect to sham-operated controls.

Table II. Effects of Myocardial Infarction on Alpha-1 Adrenoreceptor Density and Affinity of 3-mo-old Rats

	Sham-operated	Infarcted
Left ventricle		
B_{max}, *fM/mg*	363±15	395±12
	(*n* = 3)	(*n* = 5)
K_D, *pM*	254±20	392±12*
	(*n* = 3)	(*n* = 5)
Right ventricle		
B_{max}, *fM/mg*	517±12	480±5
	(*n* = 3)	(*n* = 3)
K_D, *pM*	501±26	336±6*
	(*n* = 3)	(*n* = 3)

Results are presented as mean±SE. * A value that is statistically significantly different; $P < 0.05$.

Norepinephrine stimulated phosphoinositol turnover. In preliminary studies, the specificity of norepinephrine in stimulating phosphoinositol turnover via the alpha-1 adrenoreceptors was established. This was accomplished by incubating isolated ventricular myocytes, labeled with [^{3}H]myoinositol, in the presence of norepinephrine and adrenergic agonists and antagonists. Fig. 5 illustrates that phosphoinositol turnover was almost completely abolished by the alpha-1 antagonist BE 2254, while it was not blunted by the beta adrenoreceptor antagonist propranolol. Moreover, the beta adrenoreceptor agonist, isoproterenol, failed to stimulate hydrolysis of phosphoinositol.

To determine whether norepinephrine stimulates phosphoinositol turnover in a dose-dependent fashion, isolated ventricular myocytes labeled with [^{3}H]myoinositol were incubated with various concentrations of norepinephrine (Fig. 6). The rate of phosphoinositol turnover increased with increasing doses of norepinephrine and was found to be maximal at 100 μM.

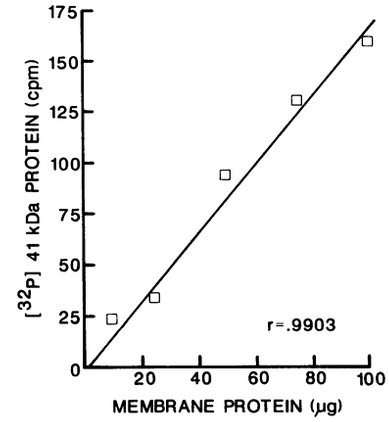

Figure 3. Incorporation of pertussis toxin specific counts into membranes prepared from control hearts as a function of increasing amounts of membrane protein. Radiolabeling of membranes increased linearly with increasing amounts of membrane protein.

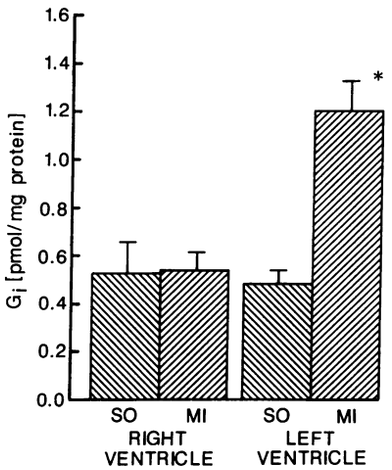

Figure 4. Quantitation of the inhibitory guanine nucleotide binding protein (G_i) in left and right ventricles of infarcted and sham-operated rats. *A value that is statistically and significantly different; $P < 0.001$; $n = 4$ in all determinations.

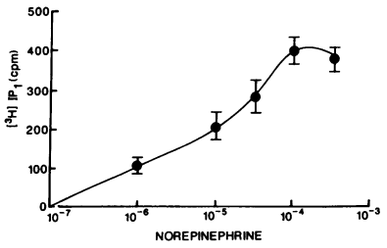

Figure 6. Dose-dependent stimulation of [^{3}H]IP_1 formation by norepinephrine. Myocytes labeled with [^{3}H]-myoinositol were incubated at various concentrations of norepinephrine for 10 min. $n = 2$ or 3 separate determinations at each point.

Fig. 7 illustrates phosphoinositol turnover in isolated ventricular myocytes labeled with [^{3}H]myoinositol and stimulated with 100 μM norepinephrine. Cells were obtained from infarcted, sham-operated and nonoperated rats. The latter group was included as an internal control. Net stimulation of phosphoinositol turnover by norepinephrine was found to be 3.1-fold greater in myocytes from infarcted hearts than in myocytes from sham-operated ventricles. This difference was highly statistically significant ($P < 0.005$). Moreover, comparable levels of phosphoinositol turnover were seen in sham-operated and nonoperated controls indicating that net stimulation in myocytes from infarcted hearts was independent from the surgical intervention and reflected an enhanced coupling of agonist-mediated phosphoinositol turnover.

Alpha skeletal and cardiac actins. The expression of sarcomeric actin isoforms was examined in the left ventricular myocardium of infarcted and sham-operated rats. This was performed by analyzing Northern blots hybridized with oligonucleotide probes for alpha skeletal and cardiac actin mRNA's. The specificity of the probe for fetal sarcomeric actin isoform was demonstrated by detection of alpha skeletal actin in RNA isolated from hearts of fetal- and up to 10-h-old neonatal rats (Fig. 8, lane *1*). In contrast, mRNA transcripts for alpha skeletal actin were barely detectable in RNA obtained from adult rat hearts (lane *2*). The oligonucleotide probe also cross hybridized with the 18S ribosomal RNA as illustrated in lanes *1* and *2*. RNA isolated from the surviving myocardium of the infarcted left ventricle contained mRNA transcripts for alpha skeletal actin (lane *3*). On the other hand, mRNA transcripts for alpha skeletal actin were barely detectable in RNA from the left ventricle of sham-operated control rats (lane *4*). No differences in the expression of alpha cardiac actin were seen between infarcted and noninfarcted ventricles (data not shown).

Discussion

The results of this study demonstrate that occlusion of the left coronary artery near its origin was associated 7 d after surgery with severe myocardial dysfunction and an increase in the heart weight to body weight ratio indicative of cardiac hypertrophy. The growth response of the myocardium was accompanied by a marked augmentation in the content of pertussis toxin substrate in cardiac membranes, which was coupled with enhanced norepinephrine-stimulated phosphoinositol turnover. In contrast, alpha-1 adrenoreceptor density remained essentially constant in both ventricles and alpha-1 adrenoreceptor affinity for the radioligand decreased in the infarcted left ventricle. Finally, in the setting of an increased pertussis toxin substrate and elevated level of phosphoinositol turnover, an upregulation of the alpha skeletal actin was found in the surviving myocardium of the injured ventricle.

Myocardial infarction and ventricular performance. The current results demonstrate that left ventricular failure was present following infarcts comprising most of the free wall of the left ventricle, 7 d after coronary occlusion. Left ventricular end diastolic pressure was elevated, whereas systolic arterial and ventricular pressures and peak positive and negative *dP/dt* were significantly depressed. These observations are consistent with previous findings in which alterations in ventricular function have been reported in the same animal model at 2 d (3), 3 d (1), and at the completion of the healing process, ~ 1 mo after surgery (14–16). In all cases, the impairment in car-

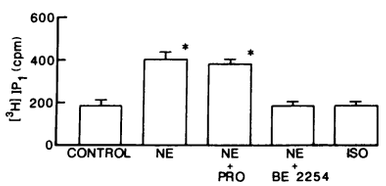

Figure 5. Receptor specificity of norepinephrine stimulated phosphoinositol turnover. [^{3}H]myoinositol-labeled myocytes were incubated for 40 min with 100 μm norepinephrine and alpha-1 antagonist (BE 2254 10^{-6}), beta antagonist (propranolol 10^{-6}), and beta antagonist (isoproterenol 10^{-4}). *A value that is statistically and significantly different; $P < 0.05$; $n = 3$ or 4 separate determinations in each case.

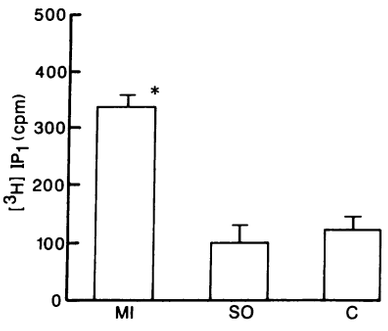

Figure 7. Norepinephrine stimulated [^{3}H]IP_1 formation in myocytes isolated from infarcted, sham-operated, and nonoperated control rat hearts. Rats were labeled with [^{3}H]myoinositol and incubated with 100 μm norepinephrine. *A value that is statistically and significantly different; $P < 0.05$; $n = 4$ in all determinations.

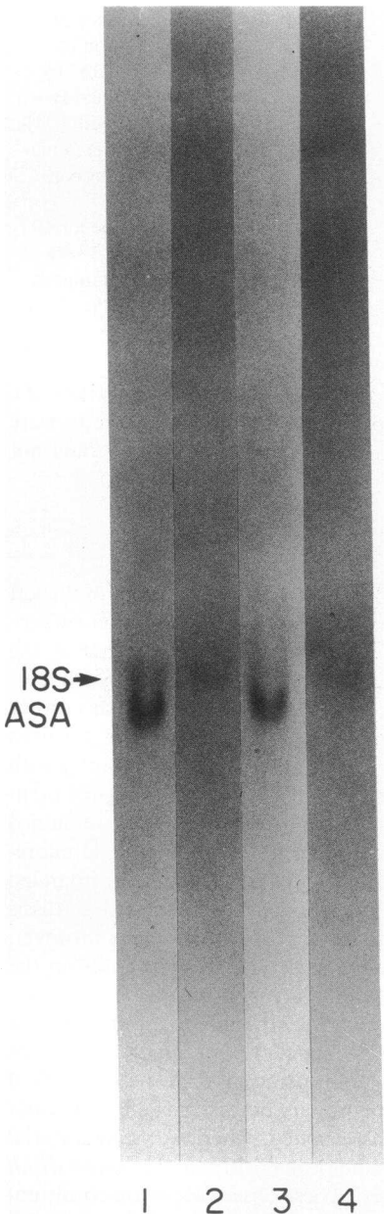

Figure 8. Total RNA was isolated from the left ventricles of fetal/neonatal, infarcted, and sham-operated rats as described in Methods. RNA (10 μg) was size fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters. The filters were hybridized with an oligonucleotide ^{32}P-labeled probe, homologous to the 3′ untranslated region of alpha skeletal actin mRNA. Autoradiography was performed by exposing the filter to Kodak XAR-5 film with intensifying screens. (Lane *1*) Hybridization signal of RNA extracted from fetal/neonatal rat hearts. Fetal hearts expressed alpha skeletal mRNA while this isoform was barely detectable in normal adult rat hearts (lane *2*). (Lane *3*) Alpha skeletal actin mRNA transcripts were reexpressed in the left ventricle of infarcted hearts. (Lane *4*) Left ventricle of sham-operated rats. Hybridization signal for alpha skeletal actin mRNA was barely detected. Arrow denotes the location of the 18S ribosomal RNA band.

diac hemodynamics was detected only when the infarcted region involved nearly 50% of the ventricle (1, 3, 14, 15). Moreover, infarcts of this size are associated with an increased right ventricular systolic pressure (14), and the right ventricle has to sustain a greater work load in order to maintain the pressure gradient across the pulmonary vascular bed (1, 7, 14). Although quantitative estimation of the magnitude of infarction was not performed in this investigation, the abnormalities in pump performance provide indirect supportive evidence that coronary occlusion produced an ~ 50% loss of viable tissue (1, 3). Consequently, as previously demonstrated in similar studies (1–3, 6, 14, 15), a significant increase in the loading state of the spared myocardium of the left and right ventricles had to occur stimulating a growth hypertrophic reaction in the attempt to compensate for the destruction in mass and impaired cardiac function.

Myocardial infarction and cardiac hypertrophy. Results in this study indicate that left ventricular failure induced by myocardial infarction was characterized by an increase in heart weight and in the ratio of heart weight to body weight indicative of cardiac hypertrophy. Although the changes in myocyte cell volume after infarction would have provided a direct measurement of the hypertrophic reaction of the muscle compartment of the myocardium (1–3), a close correlation has previously been shown between the enlargement of myocytes and the extent of myocardial infarction (2). Hypertrophy of left and right ventricular myocytes occurs early (1, 3), progresses with time (2), and is dependent on infarct size (2). The response of left ventricular myocytes, however, is significantly greater than that of right ventricular myocytes (1, 7, 27). For example, at 3 d, enlargement of myocytes of the right and left ventricles was 21 and 28%, respectively (1). 1 mo after coronary occlusion, right and left myocyte hypertrophy was 32 and 81% (7, 27). On this basis, it can be inferred that cellular growth mechanisms were operative 7 d after coronary artery occlusion.

Alpha-1 adrenoreceptor. The density of surface alpha-1 adrenoreceptors in the surviving myocardium of the left ventricle was not altered following myocardial infarction and subsequent ventricular failure. The observation that alpha-1 adrenoreceptor density was essentially unchanged in the failing infarcted heart is in contrast with previous reports in which pathological states of the myocardium have been found to be associated with an increased number of alpha-1 adrenergic receptors in both human (28) and animal models (29). In particular, myocardial ischemia is characterized by a two-fold increase in alpha-1 adrenoreceptor number (29) which contributes to the occurrence of malignant ventricular arrhythmias (30). Although a reduction in the oxygenation potential of the surviving myocardium early after infarction has been claimed at the level of the capillary microvasculature (3, 6), measurements of coronary blood flow distribution have failed to demonstrate abnormalities in coronary perfusion consistent with myocardial ischemia (31). Moreover, the alterations in alpha-1 adrenergic receptor number in congestive dilated cardiomyopathy in humans (28) occur over a period of many years of decompensated overload, whereas in the current experiments in rats, alpha-1 adrenoreceptor density was determined 1 wk after coronary occlusion. Multiple differences between the present animal model and the failing cardiomyopathic heart in patients may also explain the decrease in K_D found in the infarcted left ventricle and the absence of such a change in the human heart (28). However, in this study, the right ventricle showed an increase in K_D, indicating a heterogenous response of the two ventricles after infarction. The reduction in alpha-1 adrenoreceptor affinity suggests that a fundamental change in the recognition or ligand binding unit of the receptor may have occurred in the surviving myocardium of the infarcted left ventricle. A similar possibility has been suggested for the beta adrenoreceptor response following pressure overload and left ventricular hypertrophy in which receptor affinity was significantly reduced (32). Moreover, the decrease in alpha-1 adrenoreceptor affinity in the failing heart may participate in the reduction of the inotropic and chronotropic state of the myocardium and, in turn, diminish oxygen consumption in an already compromised ventricle.

Regulatory protein and phosphoinositol turnover. The current results demonstrate an increase in the 41-kD membrane component labeled by pertussis toxin, in the left ventricle of infarcted hearts. Moreover, norepinephrine-stimulated phos-

phoinositol turnover increased threefold in ventricular myocytes isolated from infarcted hearts. An important finding in this study was that alpha-1 adrenoreceptor density and phosphoinositol turnover were not coordinately regulated. The parallel adaptations of regulatory protein and phosphoinositol turnover, which were concurrently enhanced after infarction, would appear to support the concept that a guanine nucleotide-binding protein may be involved in this signal transduction pathway of the alpha-1 adrenoreceptor. Although the identity of the G protein coupled to the phospholipase C has not been demonstrated with certainty, a pertussis sensitive regulatory protein has been linked to the hydrolysis of phosphoinositol in a number of systems (26). Furthermore, the increased pertussis toxin substrate in membranes from infarcted left ventricles, suggests that alpha-1 adrenoreceptor effector responses can be maintained through a postreceptor mechanism in a decompensated failing heart (33). In contrast, the beta adrenoreceptor adenylate cyclase system has been found to be attenuated in the presence of myocardial dysfunction associated with long term pressure overload hypertrophy. This defect in beta adrenoreceptor signal transduction was characterized by an impairment in the efficiency of the coupling protein G_s (34). On the other hand, compensated, physiologic hypertrophy induced by dynamic exercise has been shown to be accompanied by an increase in the level of G_s in spite of no changes in left ventricular beta adrenoreceptor density (35). The combination of these findings tends to suggest that perturbations in regulatory proteins may reflect the nature and characteristics of the hemodynamic overload. Moreover, the activation of postreceptor mechanisms in the presence of pump failure and ongoing reactive hypertrophy, raises the possibility that abnormal mechanical loading and enhanced signal transduction by the alpha-1 adrenoreceptors are integral components of the myocyte hypertrophic response after infarction. Alternatively, the increased G_i found in this study may account for impaired contractile function and disordered calcium cycling in the infarcted ventricle in view of the potential inhibitory effect of this regulatory protein on adenyl cyclase (36). This possibility has been suggested to be operative in the failing human heart (36, 37).

It should be recognized that the demonstration in vitro of enhanced phosphoinositol turnover in the presence of norepinephrine does not necessarily imply that a similar response is operative in vivo since pharmacological doses of norepinephrine have been employed in this assay. Furthermore, levels of IP_3 and diacylglycerol, intracellular messengers of this effector pathway, were not determined. By inference, however, the threefold augmentation in IP_1 after infarction suggests that these second messengers may have accumulated as well. In a related line of investigation, enhanced IP_3 synthesis was observed acutely after hypoxia in myocytes stimulated by norepinephrine (38). Contrary to our findings, the increase in alpha-1 adrenoreceptor signal transduction during early ischemia was found to be coupled with a twofold elevation in alpha-1 adrenoreceptor density (29).

Reexpression of alpha skeletal actin. The upregulation of the fetal sarcomeric actin isoform in the infarcted left ventricle suggests a potential link between severe myocardial dysfunction and enhanced signal transduction via the alpha-1 adrenoreceptors. It is noteworthy that in an in vitro system, the alpha-1 adrenoreceptor has been shown to be a molecular mediator of transcriptional changes involved in the altered expression of sarcomeric actins (39). Moreover, protein kinase C, which is activated through the alpha-1 adrenoreceptors, has also been implicated in this response (40). Previous studies in various models of myocardial hypertrophy have demonstrated that the reexpression of the fetal sarcomeric actin isoform in vivo is dependent upon acute pressure overload and is self limited (9, 10). In contrast, in thyroxine-induced ventricular hypertrophy no effect on the expression of mRNA encoding the fetal sarcomeric actin isoform has been described (23). Cardiac hypertrophy following myocardial infarction is a response to an abnormal workload on the surviving myocytes in which the augmentation in diastolic cell stress markedly exceeds the rise in systolic cell stress (3). Thus, in both pressure and volume overload hypertrophy, the reexpression of alpha skeletal actin appears to be associated with the persistence of the overload on the myocardium before normalization of wall and cell stress may occur.

In conclusion, the possibility is raised that effector pathways linked to the alpha-1 adrenoreceptor may be involved in the reactive hypertrophic response associated with myocardial infarction-induced ventricular failure. Moreover, the elevated loading state on the myocardium may be responsible for the reexpression of alpha skeletal actin in the surviving tissue which may also be mediated by activation of alpha-1 postreceptor mechanisms.

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