Overexpression of α_{1B}-adrenergic receptor induces left ventricular dysfunction in the absence of hypertrophy

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Departments of [1]Pharmacology and Cell Biophysics, [2]Molecular and Cellular Physiology, [3]Internal Medicine, and [4]Pathology, University of Cincinnati, Cincinnati, Ohio 45267; and [5]Department of Medicine, University of Montreal, and Montreal Heart Institute, Montreal, Quebec, Canada H1T 1C8

Grupp, Ingrid L., John N. Lorenz, Richard A. Walsh, Gregory P. Boivin, and Hansjörg Rindt. Overexpression of α_{1B}-adrenergic receptor induces left ventricular dysfunction in the absence of hypertrophy. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H1338–H1350, 1998.—The stimulation of cardiac α_1-adrenergic receptors (AR) modulates the heart's inotropic response and plays a role in the induction of cardiomyocyte hypertrophy. We have analyzed transgenic mouse lines overexpressing a wild-type α_{1B}-AR specifically in the heart. Basal level systolic and diastolic left ventricular (LV) contractile function was depressed both in the anesthetized closed-chest mouse and the perfused working-heart preparation. Intrinsic LV function was further characterized under controlled preload and afterload conditions using the perfusion model. Contractile parameters were restored by chronic treatment with the α-AR antagonist prazosin. In ventricular function curves, the load-dependent force increases (length-tension effects) remained intact, although the transgenic curve was shifted to lower levels. The basal level contractile deficits were paralleled by a decrease in calcium transients in isolated LV cardiomyocytes. LV function comparable to controls was restored by isoproterenol stimulation. The physiological changes occurred in the absence of cardiomyocyte hypertrophy. This transgenic model will be useful for studying the potential role of α_1-AR in cardiac contractility and hypertrophy.

heart; myocardial contractility; muscle; transgenic mouse

THE α_1-ADRENERGIC RECEPTORS (AR) mediate many effects of the sympathetic nervous system. They belong to the superfamily of G protein-coupled receptors and are found in the membranes of a number of cell types, including vascular smooth muscle cells and cardiomyocytes. Pharmacological studies have functionally identified at least two subtypes, α_{1A}-AR and α_{1B}-AR, and more recently, three distinct receptor cDNA, α_{1B}-AR, α_{1C}-AR, and α_{1D}-AR, have been isolated and characterized (43). The structure and function of α_1-AR subtypes and their coupling to specific G proteins have been reviewed recently (15, 38).

The activation of α_1-AR has been ascribed several roles in cardiac homeostasis, including the induction of myocyte hypertrophy. In cultured neonatal rat cardiomyocytes, agonist treatment results in an increase in cell size and in the activation of the β-myosin heavy chain (MHC) gene (22). Similar results were obtained by stimulation or forced expression of protein kinase C (PKC), indicating the role of PKC in α_1-AR-mediated hypertrophy. The transgenic overexpression of the α_1-AR G protein partner G_q was recently shown to induce cardiac hypertrophy and cardiac contractile failure (10). In addition to effects on myocyte growth, stimulation of α_1-AR protects the myocardium from ischemia-reperfusion injury (reviewed in Ref. 9) and has been implicated in the generation of ischemia-induced cardiac arrhythmias (reviewed in Ref. 47).

α_1-AR may also play a role in mediating inotropic stimuli in the heart. Inotropic and chronotropic responses to sympathetic activity are affected primarily by β-AR. In the failing human heart, however, β_1-AR density may be reduced, and β_1-AR gene expression is downregulated while α_1-AR generally remain unchanged (4–6). Increases in α_1-AR densities in the myopathic heart and after chronic β-AR blockade have also been reported (34a, 50). This leads to an increase of the α_1-AR-to-β-AR ratio, and it has been suggested that α_1-AR may therefore assume a greater functional role in the failing heart by acting as a secondary inotropic system.

In several mammalian species studied, stimulation of α_1-AR elicits a positive inotropic effect. Preparations of rat and rabbit ventricular muscle, as well as isolated cardiomyocytes, respond with increased force of contraction to stimulation by agonist (reviewed in Ref. 14). Similarly, in the conscious dog, the rate of left ventricular (LV) pressure development is increased after α_1-AR stimulation, although the effect is not as pronounced as in the rat (40). In human atrial and ventricular muscle preparations, α_1-AR-mediated increases in force of contraction have been reported (7, 39). Intracoronary perfusion of the α_1-AR agonist phenylephrine also leads to increased LV pressure development in humans (26). The mechanisms by which α_1-AR exert their positive inotropic effect include the Ca^{2+} sensitization of myofilaments and the prolongation of action potential repolarization.

In contrast to these findings, α_1-AR are associated with a depression of contractility in a number of experimental situations. In isolated rabbit papillary muscle, the agonist-mediated positive inotropy is reversed by higher frequencies of stimulation (13). Stimulation of α_1-AR has also been shown to diminish β-AR-driven velocity of shortening and Ca^{2+} entry, suggesting an integration of adrenergic inputs by the cardiomyocyte (11). Furthermore, a decrease in maximum shortening velocity after α_1-AR stimulation has been demonstrated in skinned rat ventricular myocytes (44). These data as well as studies on the regulation of inotropic force, cAMP accumulation, and inositol phosphate production (3, 17, 36, 41, 51) suggest that the inputs of the

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β-AR and α_1-AR systems are integrated within the cardiomyocyte.

The present transgenic model of cardiac-specific α_{1B}-AR overexpression has been previously analyzed by Akhter et al. (1). An increase in β-AR kinase activity and the reduction of cAMP levels in transgenic membrane preparations supported the notion that β-AR signaling is modulated by α_1-AR. We were interested in the physiological consequences of cardiac α_{1B}-AR overexpression and have analyzed this model using both the anesthetized closed-chest mouse and the isolated work-performing perfusion preparation. We show here that the overexpression of α_{1B}-AR led to a dramatic basal level reduction in contractility that was correlated with a decrease of systolic Ca^{2+} transients. Stimulation of β-AR restored most contractile parameters. However, perfused transgenic hearts were more sensitive to work load, indicating an impaired LV function. The functional changes occurred in the absence of cardiac hypertrophy.

MATERIALS AND METHODS

RNA isolation and blots. The generation and initial characterization of the transgenic mice has been described previously (1). Animals were euthanized by CO_2 inhalation, and hearts were excised. Atria and vessels were dissected, and the ventricles were homogenized (Polytron, Brinkmann) directly in Trizol reagent (Boehringer Mannheim). Total cellular RNA was isolated according to the manufacturer's instruction with two modifications. The homogenate was passed through a 25-gauge needle to shear DNA, and a precipitation step with 0.1 vol of 3.2 M Tris·HCl (pH 8.2) and 2 vol ethanol was added. The RNA was resuspended in water, and the concentration was determined by measuring the optical density at 260 nm. RNA dot blots were performed by applying twofold serial dilutions, starting with 7.5 μg, to a nitrocellulose membrane. The blots were then hybridized to a probe specific for ventricular regulatory myosin light chain-2 (MLC-2v). Hybridizations were performed in 6× saline sodium citrate (SSC) (1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate), 0.5% SDS, 5× Denhardt's solution (1× Denhardt's solution is 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% BSA, fraction V), and 100 μg/ml denatured sonicated salmon sperm DNA at 60°C for 16 h. Filters were washed three times in 0.2× SSC and 0.5% SDS at 60°C and exposed to Kodak X-Omat film. Northern blots were performed by separating 10 μg of total cellular RNA on a 0.7% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred to a nitrocellulose membrane by capillary blotting. Hybridizations were carried out as above with oligonucleotide probes specific to the 3′-untranslated regions of the α- and β-MHC RNA. Filters were washed three times in 0.5× SSC and 0.5% SDS and exposed as above.

Protein isolation and SDS gels. Protein was isolated from ventricular tissue by homogenization in buffer containing 600 mM KCl, 25 mM $Na_4P_2O_7$, 50 mM Tris·HCl, pH 7.0, and 1 mM dithiothreitol. After insoluble material was pelleted, the protein concentration of the supernatant was determined using Bradford reagent (Bio-Rad), and 10 μg of total protein were separated on a 10% SDS/polyacrylamide gel containing 30% glycerin. After electrophoresis in a Bio-Rad minigel apparatus for 3 h at 120 V, MHC protein was visualized by Coomassie brilliant blue staining (Bio-Rad).

Transgenic animals. The transgene DNA construct and the generation of the transgenic founder animals have been described previously (1). Throughout the study, adult age-matched animals (genetic background C57BL6xSJL) of comparable weight (transgenics, 27.7 ± 4.5 g; controls, 29.3 ± 4.2 g) and of either sex were used. The age range of the transgenic group was 19.1 ± 1.3 wk; that of the littermate control group was 19.0 ± 0.8 wk. The sex distribution for individual sets of experiments is indicated in Tables 1 and 2 and Figs. 1–8 where appropriate.

Work-performing perfused heart preparation. The anterogradely perfused preparation was carried out essentially as described (16). The animals were anesthetized with 30 μg/g body wt pentobarbital sodium. In a first step, a 20-gauge cannula was inserted into the ascending aortic stump and, for stabilization of the heart, retrograde (Langendorff) perfusion was temporarily performed with oxygenated (95% O_2-5% CO_2) Krebs-Henseleit buffer at 37.6°C. A flanged polyethylene catheter (PE-50) was inserted through a pulmonary vein, guided through the mitral valve into the left ventricle, and exited through the apex. It was then connected to a larger more compliant catheter and to a COBE (CDXIII) pressure transducer (COBE Cardiovascular, Arvada, CO) to record intraventricular pressures. In the second step, a cannula was inserted in one of the pulmonary veins (tying off the other), and retrograde perfusion was switched to the anterograde mode. Venous return (preload) and aortic pressure (afterload) were regulated with a custom-made micrometer. Optimal basal level preload and afterload conditions (5 ml/min cardiac output and 50 mmHg aortic pressure) had been determined previously (16), and the hearts were allowed to stabilize at this basal work load of 250 $ml \cdot min^{-1} \cdot mmHg$ before obtaining ventricular function curves (Frank-Starling curves). Heart rate and pressures were continuously monitored, and the first derivative of left ventricular pressure (LVP), peak LV $+dP/dt$ and $-dP/dt$, as well as time to peak pressure (TPP)/mmHg and half-time of relaxation ($RT_{1/2}$)/mmHg were calculated using a custom-designed computer program. Venous return and aortic flow were measured with a dual-channel flowmeter (Transonic Systems, Ithaca, NY). Isoproterenol was infused cumulatively to the venous return in increasing concentrations from 0.8 to 800 nM.

Anesthetized closed-chest preparation. Animal surgery and the experimental protocol have been described in detail (31). Briefly, animals selected according to the criteria described above were anesthetized with intraperitoneal injections of 50 μg/g body wt ketamine and 100 μg/g body wt thiobutabarbital (Inactin). Body temperature was held constant on a thermally controlled surgical table and monitored via a rectal probe. Tracheotomy was performed with a short length of PE-90 tubing. The right femoral artery was cannulated, and the catheter connected to a low-compliance COBE CDXIII fixed-dome pressure transducer (COBE Cardiovascular) to measure arterial blood pressure. The right femoral vein was cannulated for infusion of drugs via a CMA/100 microinjection pump. For the measurement of myocardial function, the right carotid artery was cannulated with a 2-Fr Millar MIKRO-TIP transducer (SPR-407, Millar Instruments, Houston, TX). The tip of the transducer was advanced through the ascending aorta and into the left ventricle under constant monitoring of the blood pressure wave. The transducer was then anchored in place with 7–0 sutures. All wounds were closed with cyanoacrylate to minimize evaporative fluid loss, and the animals were allowed to stabilize for 45 min. Pressure signals were then digitized, recorded at 1,000 samples/s, and analyzed with a MacLab 4/s data acquisition system.

Myocyte isolation and Ca^{2+} transients. The method for the isolation of adult calcium-tolerant ventricular cardiomyocytes has been described in detail previously (12). In short,

animals were anesthetized, and the excised hearts were placed in oxygenated, nominally Ca^{2+}-free solution P containing 132 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose, and 10 mM HEPES, pH 7.2. The aorta was cannulated with a 23-gauge cannula and flushed gently, and the heart was mounted on the perfusion apparatus. Perfusion was carried out as follows: 8 min with the Ca^{2+}-free solution P; 7 min with Joklik's MEM (GIBCO) supplemented with 75 U/ml each of type I and type II collagenase (Worthington), 0.1% BSA, and 2% calf serum; and finally 6 min of washout with low Ca^{2+} Joklik's MEM (Joklik's supplemented with 0.025 mM $CaCl_2$). LV tissue was then dissected and incubated for 5 min at 37°C in low Ca^{2+} Joklik's MEM, and released cells were filtered through 200-μm nylon mesh. Remaining pieces were reincubated and refiltered. The filtrates were combined, and the cells were collected by gravity sedimentation for 30 min. The pelleted cells were then resuspended twice in low Ca^{2+} Joklik's MEM with increasing Ca^{2+} concentration (0.5 and 1.4 mM). The yields of intact rod-shaped myocytes were routinely ~80% for control and ~60% for transgenic hearts. To record intracellular free Ca^{2+} transients, cells were loaded with the fluorescent Ca^{2+} chelator fura 2-AM for 30 min at 37°C in low Ca^{2+} Joklik's MEM. Cells were washed once and resuspended in solution P supplemented with 1.8 mM $CaCl_2$. Measurement of field-stimulated cardiomyocytes was performed as described previously (12, 21). Intracellular free Ca^{2+} was monitored and reported as the ratio of 340/380 nm fluorescence of fura 2 at 500-nm emission wavelength using a photo scan dual-beam spectrofluorophotometer (Photon Tech) coupled to an Olympus IMT-2 ultraviolet fluorescent microscope with ultraviolet transparent optics.

Fixation of hearts and electron microscopy. Specimens for electron microscopy were prepared according to standard procedures. Briefly, mice were anesthetized, and their hearts were exposed. Cardioplegic solution (25 mM KCl, 5% dextrose in PBS) was perfused (column height 65 cm) to relax the muscle. The heart was then fixed by perfusion with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, and postfixed for 24 h. Blocks of ~1 mm^2 from the LV wall at midsection were embedded, oriented, and sectioned. Thin sections were viewed using a Zeiss 912 transmission electron microscope. Sarcomere length comparisons were made from photographs of these sections.

Statistics. Results are expressed as means ± SE. Unpaired Student's *t*-tests were performed for pairwise comparisons, and a level of $P < 0.05$ was considered significant. ANOVA with Fisher's least-squares difference post hoc analysis was performed to determine differences between multiple groups.

RESULTS

We were interested in the role of α_1-AR in cardiac homeostasis and have analyzed transgenic mouse lines overexpressing the α_{1B}-AR in cardiomyocytes (1). Transgene expression is driven by the α-MHC promoter that is specific for cardiomyocytes (45), directing high levels of expression to the adult mouse heart. The offspring of two independent transgenic mouse lines were analyzed. The levels of α_1-AR overexpression (Tg47, 26-fold, and Tg43, 43-fold) have been determined previously (1).

Reduced basal level contractility of α_{1B}-AR overexpressors in vivo. The functional consequences of α_{1B}-AR overexpression were determined in the anesthetized closed-chest mouse model. It has been shown previously that differences in contractile performance in mice with altered adrenergic signaling status can be measured reliably in this system (10, 49). The comparison of basal level contractile parameters showed a reduction in LV performance in the transgenics (Table 1). Mean arterial pressure (MAP) and LVP tended to be reduced, but the differences were not statistically significant. The rate of pressure development, peak LV +dP/d*t* (the first derivative of LVP over time) was reduced by 36%. In addition, +dP/d*t* at 40 mmHg developed pressure (a parameter which attempts to correct for variations in afterload) was also decreased, as was the maximum value of +dP/d*t* divided by developed pressure at peak +dP/d*t* (a parameter which attempts to correct for variations in preload) (Table 1). During relaxation, peak LV −dP/d*t* was reduced by 36% in transgenic hearts. Basal level heart rate was comparable between the two groups. These data indicate a dysfunction of the left ventricle in α_{1B}-AR overexpressing mice resulting in a reduced basal level contractile performance.

Intrinsic LV performance is reduced. In the anesthetized closed-chest animal, sympathetic innervation might influence the performance of the α_{1B}-AR overexpressing hearts to a different degree than controls. In addition, the application of drugs can have peripheral effects that modulate cardiac function. The trend to lower MAP observed in the closed-chest preparation may also affect afterload. We therefore measured intrinsic LV contractile function in the isolated perfused working-heart preparation. This protocol also allows the strict control of preload and afterload in the absence of external inputs and therefore experimentally induced changes in work. To establish contractile function at basal level, hearts were perfused under conditions of constant preload (venous return, 5 ml/min) and afterload (aortic pressure, 50 mmHg). These basal level parameters have been established in previous experiments (16). Representative polygraph tracings of a transgenic (Tg47) and a control heart are shown in Fig. 1. The contractile function of the transgenic heart was severely compromised. Under identical preload

Table 1. *Cardiac performance in the anesthetized closed-chest animal*

	Control	Transgenic	*P* Value
HR, beats/min	357 ± 9	349 ± 19	0.71
MAP, mmHg	74.4 ± 5.1	64 ± 5.5	0.20
LVP, mmHg	101.6 ± 7.4	87.8 ± 7.3	0.22
LVEDP, mmHg	1.8 ± 2.4	2.3 ± 1.8	0.88
Peak +dP/d*t*, mmHg/s	8,126 ± 627	5,207 ± 552	0.008
Peak −dP/d*t*, mmHg/s	8,822 ± 624	5,676 ± 390	0.003
+dP/dt_{40}, mmHg/s	7,210 ± 382	5,280 ± 303	0.01
+dP/d*t*/DP, s^{-1}	153 ± 5.1	124 ± 6.5	0.004

Values are means ± SE; each group contained 3 female and 2 male animals. Contractile parameters were measured in anesthetized mice with a pressure transducer placed in left ventricle. Basal level left ventricular pressure (LVP) was recorded continuously, and its derivatives were calculated. Right femoral artery was cannulated to measure mean arterial pressure (MAP). HR, heart rate; LVEDP, left ventricular end-diastolic pressure; dP/dt_{40}, rate of pressure development at 40 mmHg developed pressure; +dP/d*t*/DP, dP/d*t* divided by developed pressure.

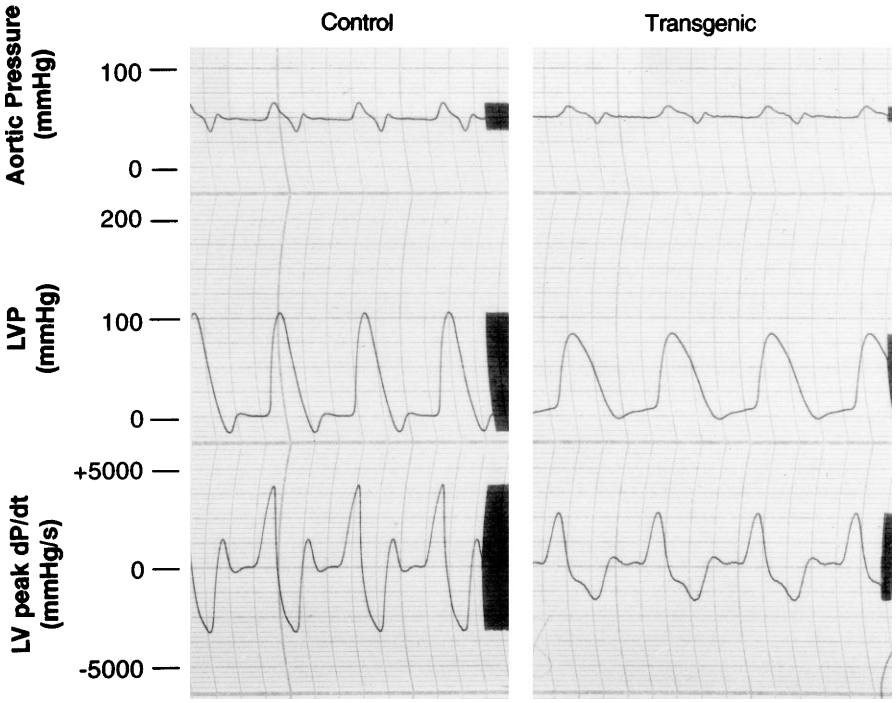

Fig. 1. Polygraph tracing of work-performing perfused hearts. Contractile parameters were determined in isolated, anterogradely perfused hearts. Representative traces obtained from a control (*left*) and transgenic (*right*) animal are shown. LVP, left intraventricular pressure; dP/d*t*, first derivative of LVP over time; LV, left ventricular.

and afterload, LVP was drastically reduced in the transgenic hearts. Analysis of the myocardial contractile parameters (Table 2) showed that LVP was reduced by ~20 mmHg, whereas both diastolic and end-diastolic pressure were significantly increased. Left atrial pressure was also significantly increased. In the transgenics, peak LV +dP/d*t* was reduced by 32% of control, and TPP was prolonged by 48%. Peak LV −dP/d*t* was decreased by 42% of control. Similarly, $RT_{1/2}$ was prolonged by 66%. These data indicate that both systolic and diastolic LV functions are compromised in the transgenic hearts. These measurements are similar in direction and magnitude to the results obtained in the anesthetized closed-chest model. Hearts isolated from line Tg43 displayed a comparable reduction of LV contractile function in the perfusion preparation (data not shown), indicating that the observed phenotype is not due to integration site effects of the transgene. Further experiments were therefore carried out with animals from line Tg47.

Table 2. *Cardiac performance in the working-heart preparation at controlled pre- and afterload conditions*

	Control	Transgenic	*P* Value
Minute work, ml·min^{-1}·mmHg	249 ± 7.1	251.1 ± .01	0.79
HR, beats/min	317 ± 24	315 ± 15	0.93
LVP, mmHg	104.9 ± 1.5	83.3 ± 1.0	<0.0001
LVDP, mmHg	−5.9 ± 0.9	0.9 ± 0.5	<0.0001
LVEDP, mmHg	8.9 ± 0.7	13.8 ± 0.5	<0.0001
LAP, mmHg	7.9 ± 0.9	12.4 ± 1.2	0.004
Peak +dP/d*t*, mmHg/s	3,780 ± 66	2,600 ± 61	<0.0001
Peak −dP/d*t*, mmHg/s	3,239 ± 101	1,906 ± 81	0.0005
TPP, ms/mmHg	0.431 ± 0.007	0.640 ± 0.012	<0.0001
$RT_{½}$, ms/mmHg	0.479 ± 0.014	0.797 ± 0.026	0.0004
Coronary flow, ml/min	2.67 ± 0.64	3.16 ± 1.34	0.11

Values are means ± SE; control group contained 4 female and 5 male animals, and transgenic group contained 4 female and 4 male animals. Definitions are as in Table 1. Hearts were excised and cannulated through a pulmonary vein and aorta for a work-performing preparation. LVP was measured continuously by a fluid-filled catheter connected to a pressure transducer. Derivatives of LVP, as well as time to peak pressure (TPP) and half-time of relaxation ($RT_{½}$), were calculated. Coronary flow was determined by collecting perfusate on a digital electronic balance. All parameters were determined at identical preload and afterload conditions, which is reflected in comparable values for minute work.

Positive inotropic response to α_1-AR stimulation in the mouse heart. Varying effects on inotropy by α_1-AR agonists have been described. We therefore wanted to determine the response of mouse hearts to phenylephrine, an α_1-AR agonist. Hearts from control animals were perfused anterogradely with increasing doses of phenylephrine. As shown in Fig. 2, a trend toward increased peak +dP/d*t* and −dP/d*t* was observed. Similarly, TPP and $RT_{1/2}$ were shortened. A positive chronotropic response (130% of baseline) was also observed. This experiment demonstrates that in the perfused mouse heart α_1-AR stimulation results in positive inotropic and chronotropic responses.

Increased inotropy by the β-AR agonist isoproterenol. Agonist-mediated stimulation of β-AR has a positive inotropic and chronotropic effect. To test if α_{1B}-AR overexpression modulates the heart's response to β-AR stimulation, work-performing perfused hearts were challenged with increasing doses of the β-AR agonist isoproterenol. No adverse effects were elicited by the process of infusion itself (see suboptimal doses in Fig. 3). In controls, the expected dose-dependent positive inotropic and chronotropic responses were observed. The increase in heart rate was accompanied by an increase in +dP/d*t* and −dP/d*t* (Fig. 3, *B* and *C*) and a

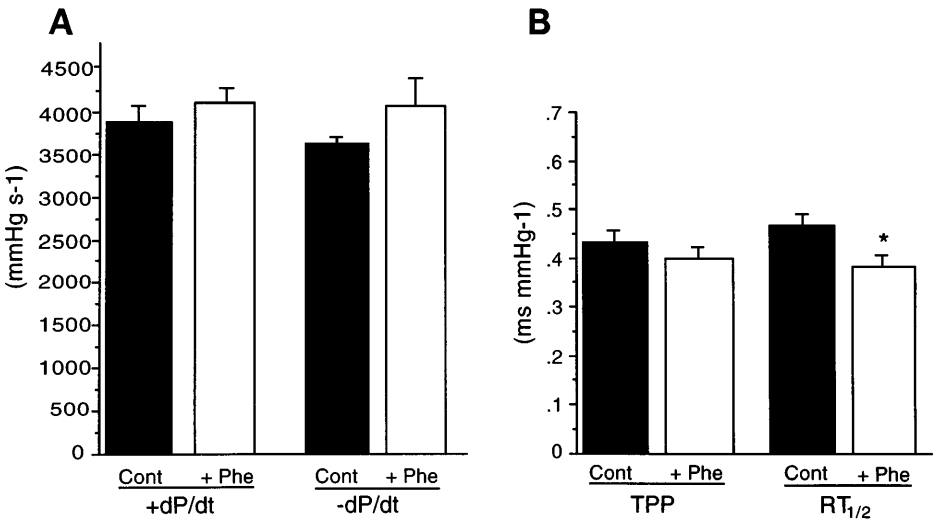

Fig. 2. Positive inotropic effect of α_1-adrenergic receptor (AR) stimulation. Hearts from nontransgenic mice (n = 5, 3 females, 2 males) were perfused anterogradely, and contractile parameters were determined at baseline (Cont) and at 4×10^{-6} M phenylephrine (+Phe). TPP, time to peak pressure; $RT_{1/2}$, half-time of relaxation. *$P < 0.05$, Cont vs. +Phe.

shortening of TPP and $RT_{1/2}$ (Fig. 3, *D* and *E*). Transgenic hearts also displayed positive inotropic and chronotropic responses. Baseline heart rate was similar to controls and increased in a dose-dependent manner comparable to controls (Fig. 3*A*). Baseline +dP/d*t* and −dP/d*t* were lower in the absence of agonist (0 in Fig. 3, *B* and *C*; see also Table 2) but increased in a dose-dependent manner. Interestingly, at higher doses of isoproterenol, transgenic +dP/d*t* and −dP/d*t* were restored to control levels, indicating that the depression of basal contractility is overcome by β-AR stimulation. When reported on a percent basis, the relative agonist-dependent increase over basal level was actually higher in the transgenic hearts (+dP/d*t*, 139 ± 34 vs. 194 ± 28%; −dP/d*t*, 152 ± 33 vs. 239 ± 41%; control vs. transgenic, $P < 0.05$). Similarly, baseline TPP and $RT_{1/2}$ were longer in the absence of agonist but shortened with increasing isoproterenol doses, and finally reached control values at higher doses (Fig. 3, *D* and *E*). The ED_{50} values for both groups remained close to 6×10^{-9} M. These results indicate that the maximal β-AR-stimulated inotropic response is not attenuated by α_{1B}-AR overexpression.

Response to changes in work load. To further investigate the decrease in the performance of the transgenic hearts, afterload challenges were carried out by increasing aortic pressure while keeping venous return constant. Parameters were recorded in the work-performing perfusion protocol. In control hearts, changes in peak LV +dP/d*t* were directionally related to minute work (Fig. 4, *A* and *B*; baseline is 250 ml·min^{-1}·mmHg). Peak LV −dP/d*t* was also proportionally changed in response to altered work. Despite a lower baseline contractility, transgenic hearts responded in a similar fashion. The relationships between work and peak LV dP/d*t* were parallel, but the *y*-intercepts were significantly lower in transgenic hearts (Fig. 4, *A* and *B*). The activation of the sliding filaments remained intact (similar slopes), but only moderate work loads were tolerated. Interestingly, transgenic TPP and $RT_{1/2}$ were shortened to a greater extent at moderately high work loads, which becomes apparent from the convergence of the regression lines in Fig. 4, *C* and *D*.

Control and transgenic hearts differed at highly elevated work loads. To determine work load tolerance of the perfused hearts, minute work was increased until maximal peak LV +dP/d*t* was reached. Further increases of work failed to elicit higher values or even reduced dP/d*t*, indicating that the maximal response point had been reached or exceeded. In control animals, the minute work generating maximal +dP/d*t* was 500 ± 10.5 ml·min^{-1}·mmHg (n = 5). Transgenic hearts could sustain minute work of maximally 363 ± 26.5 ml·min^{-1}·mmHg (n = 6; control vs. trangenic, $P < 0.01$, Student's *t*-test). These findings indicate that the transgenic hearts are limited in their capacity to perform against high work loads.

Lack of hypertrophy in α_{1B}-AR transgenic hearts. The potential induction of cardiac hypertrophy in the transgenic animals could have profound effects on the contractile performance of the hearts. Because myocyte hypertrophy has been linked to α_1-AR stimulation both in cell culture and in vivo, we measured several hypertrophy indicators in the transgenic animals.

Cardiac hypertrophy is characterized by the growth of cardiomyocytes, leading to an increase in heart size and weight. In our model, the heart weight-to-body weight ratio was not increased in the transgenic group (control 7.1 ± 0.216 vs. transgenic 6.7 ± 0.36, P = NS, n = 10 for each group), indicating that myocyte hypertrophy does not occur as result of α_{1B}-AR overexpression.

On the molecular level, cardiomyocyte hypertrophy is characterized by a modulation of myosin gene expression. We therefore wanted to rule out the possibility that changes in the myosin complement are responsible for the altered contractile function of the transgenic hearts. In the mouse, α-MHC is the prominent adult ventricular isoform. Reduction of the circulating level of thyroid hormone, or the experimental induction of acute pressure overload, induces the reappearance of the fetal β-MHC (19). Similarly, the β-MHC gene is

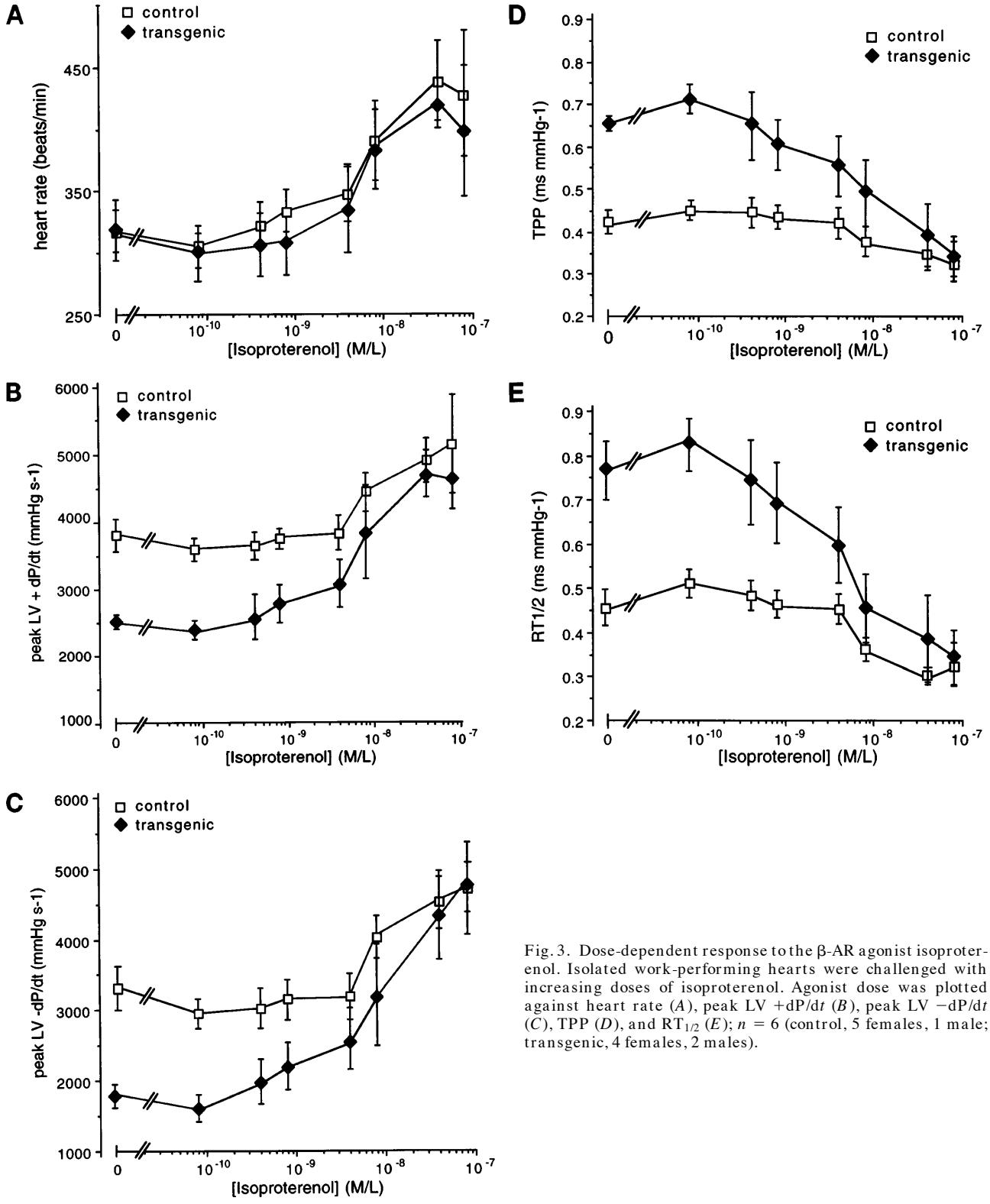

Fig. 3. Dose-dependent response to the β-AR agonist isoproterenol. Isolated work-performing hearts were challenged with increasing doses of isoproterenol. Agonist dose was plotted against heart rate (*A*), peak LV +dP/d*t* (*B*), peak LV −dP/d*t* (*C*), TPP (*D*), and $RT_{1/2}$ (*E*); $n = 6$ (control, 5 females, 1 male; transgenic, 4 females, 2 males).

positively regulated by α_1-AR agonists in cultured neonatal cardiomyocytes (22). Because β-MHC has an intrinsically lower ATPase activity, the isoform switch results in functional changes. We therefore examined the MHC content of transgenic hearts both on the mRNA and protein level. Total RNA was fractionated by agarose gel electrophoresis and transferred to nitrocellulose by Northern blotting. The mRNA for the two cardiac isoforms were detected using oligonucleotides specific for the repective 3′-untranslated regions (Fig. 5*A*). Both transgenic and control ventricles contained exclusively the adult-stage α-MHC mRNA. No

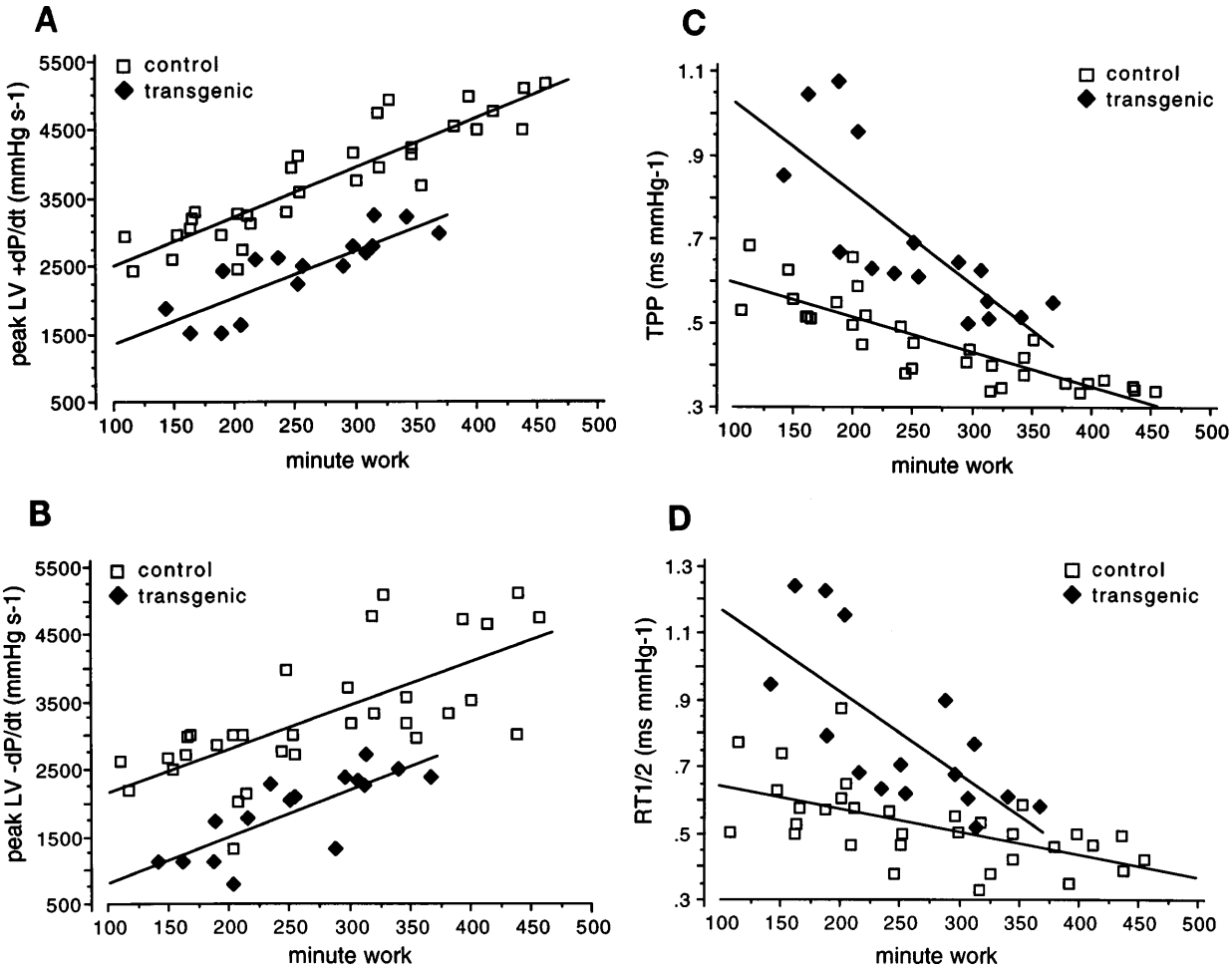

Fig. 4. Ventricular function curves during increased and decreased loading of heart. Hearts were stressed by experimentally changing aortic pressure (afterload). Venous return (preload) was held constant. Minute work was plotted against rates and times of contraction and relaxation. *A*: peak LV +dP/d*t*: control, *y*-intercept = 1,765; slope = 7.31; $r = 0.90$; transgenic, *y*-intercept = 680; slope = 7.0; $r = 0.84$. *B*: peak LV −dP/d*t*: control, *y*-intercept = 1,511; slope = 6.30; $r = 0.69$; transgenic, *y*-intercept = 80; slope = 7.0; $r = 0.78$. *C*: TPP: control, *y*-intercept = 0.68; slope = 0.0008; $r = 0.84$; transgenic, *y*-intercept = 1.24; slope = 0.0022; $r = 0.78$. *D*: $RT_{1/2}$: control, *y*-intercept = 0.71; slope = 0.0007; $r = 0.59$; transgenic, *y*-intercept = 1.42; slope = 0.0025; $r = 0.71$.

β-MHC mRNA was detected. Furthermore, we analyzed the MHC protein content in transgenic ventricles. Protein extracts were subjected to SDS-PAGE in the presence of glycerin, and MHC protein was visualized by Coomassie brilliant blue staining. A difference in the electrophoretic mobility allows the discrimination between the α-MHC and the β-MHC protein isoforms. No β-MHC protein was detected in transgenic ventricles (Fig. 5*B*), corroborating the results of the mRNA analysis. Therefore, the observed reduction in cardiac contractility is not caused by a switch in cardiac MHC isoforms.

MLC-2v is upregulated in response to hypertrophic signals in in vitro and in vivo experimental systems (19, 48). It is unclear whether a common mechanism exists for the differential regulation of most or all genes involved in hypertrophy. Recent evidence suggests that MLC-2v expression is governed by a distinct mechanism during development (32). This raises the possibility that the MLC-2v gene may be upregulated in the α_{1B}-AR transgenics even though the β-MHC gene is not (see Fig. 5, *A* and *B*). We therefore compared the steady-state level of MLC-2v mRNA from ventricles of transgenic and control animals. Total RNA was isolated and subjected to RNA dot-blot analysis. The blots were hybridized to a MLC-2v-specific probe, and MLC-2v mRNA was visualized by autoradiography. As shown in Fig. 5*C*, the steady-state level of MLC-2v mRNA was not increased in transgenic ventricles. Taken together with the lack of β-MHC gene induction, this result indicates that molecular markers of cardiac hypertrophy are not induced in the transgenic animals.

We also examined sarcomere integrity by electron microscopy. Hearts were fixed by perfusion, embedded, and sectioned. Transgenic LV samples were indistinguishable from their nontransgenic counterparts (Fig. 6). Visual examination of sarcomere structure and morphometric analysis of Z-line intervals revealed no differences between the groups (controls, transgenics, $n = 4$). Taken together, these results indicate that the myofibrillar and sarcomeric structures of the α_{1B}-AR overexpressors are not perturbed.

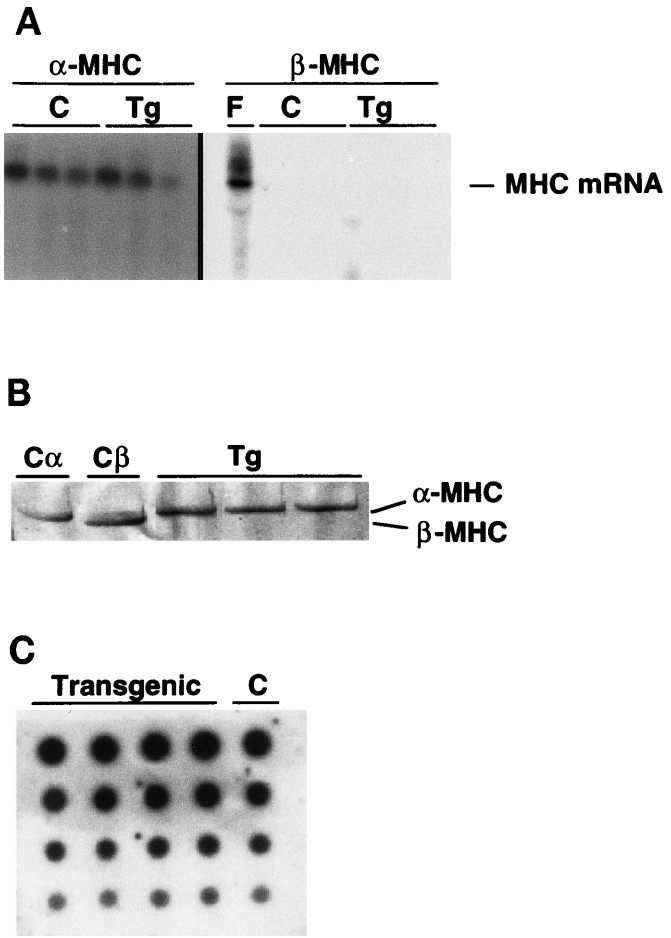

Fig. 5. Molecular analysis of hypertrophy markers. *A*: detection of myosin heavy chain (MHC) mRNA. Total ventricular RNA from 3 control (C) and 3 transgenic (Tg) animals was separated by formaldehyde gel electrophoresis and transferred to a nitrocellulose membrane by Northern blotting. Specific oligonucleotide probes were used to detect α- and β-isoforms, respectively, of MHC mRNA. F, fetal heart RNA (positive control for β-MHC). *B*: MHC protein content. α-Isoform and β-isoform of MHC present in ventricular protein extract were separated by SDS/glycerin gel electrophoresis and visualized by Coomassie brilliant blue staining. Ten micrograms of total protein were loaded per lane. Cα, normal control, containing α-MHC; Cβ, hypothyroid control, containing β-MHC; Tg, 3 transgenic samples. *C*: detection of myosin light chain-2 (MLC-2v) mRNA. Total ventricular RNA was subjected to RNA dot-blot analysis. Serial 2-fold dilutions were applied to a nitrocellulose membrane. A specific probe was used to detect MLC-2v mRNA. Transgenic, samples from 4 transgenic ventricles; C, nontransgenic ventricle.

Reduced contractility and Ca^{2+} transients in transgenic cardiomyocytes. The decrease in basal level contractility might be caused by a change in Ca^{2+} availability in the transgenic cardiomyocytes. To measure Ca^{2+} transients, hearts were perfused with collagenase-containing solution, and Ca^{2+}-tolerant cardiomyocytes were prepared as described. The cells were then loaded with the fluorescent Ca^{2+} chelator fura 2-AM and stimulated electrically at 15, 30, or 60 beats/min. Only intact cells that could sustain contractions under these conditions were considered for the recording of cell shortening and Ca^{2+} transients. The results obtained at the three stimulation frequencies were comparable, and findings at 15 beats/min are demonstrated. As shown in Fig. 7, the amplitude of the Ca^{2+} transients in transgenic cells was reduced to ~56% of control. Similarly, cell shortening was decreased in transgenics (controls, 7.67 ± 0.34%, $n = 7$; transgenics, 5.01 ± 0.47%, $n = 6$, $P < 0.001$). The magnitude of this reduction is quite similar to the relative loss of contractility observed in the anesthetized closed-chest mouse and the working-heart perfusion preparation (see Tables 1 and 2). It is likely, therefore, that the decrease of Ca^{2+} availability in transgenic cardiomyocytes is responsible for the diminished basal level cardiac contractility of the α_{1B}-AR overexpressors.

Reversal of the transgenic phenotype by prazosin, an α-AR antagonist. The preceding data fit the hypothesis that the overexpression of α_{1B}-AR in cardiomyocytes is directly or indirectly responsible for the observed contractile deficiencies of the transgenic hearts. It can be reasoned that the blockade of the α_{1B}-AR should then lead to the attenuation or reversal of the hypodynamic

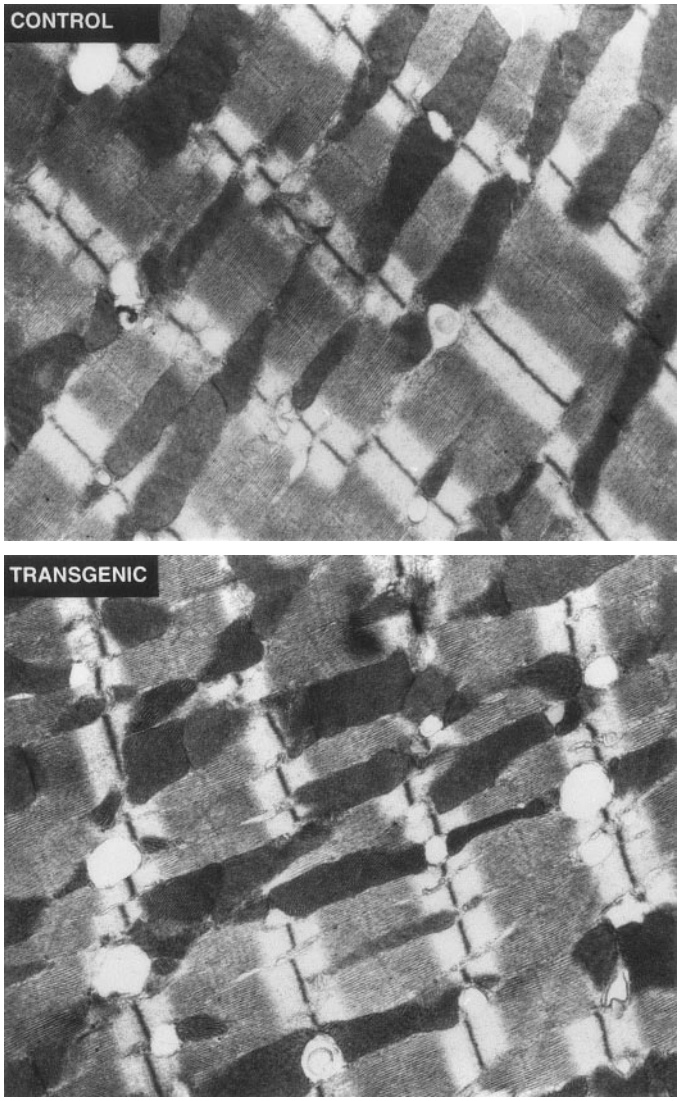

Fig. 6. Electron micrographs of LV muscle sections. Control and transgenic panels are labeled. Mouse hearts were perfusion-fixed and processed for electron microscopy. Representative sections are shown. Original microscopic magnification, ×10,000.

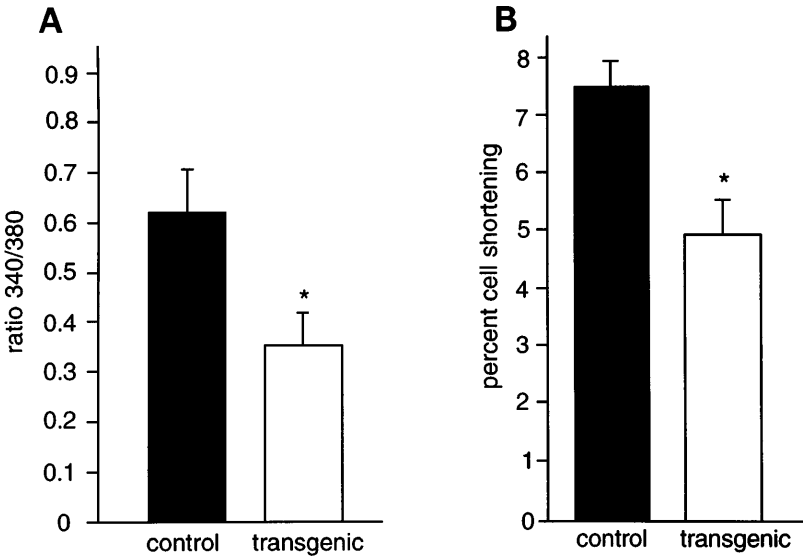

Fig. 7. *A*: Ca^{2+} transients in isolated cardiomyocytes. Cardiomyocytes were isolated by enzymatic digestion and loaded with the fluorescent Ca^{2+} chelator fura 2-AM. Ca^{2+} transients were recorded in electrically stimulated cells at 15 beats/min. *B*: contractility of isolated myocytes. Length changes during contraction were determined by video edge detection. Values per preparation between 3 and 6 cells were measured, and means of all measurements ± SE are shown for control and transgenic groups. *$P < 0.01$, control vs. transgenic.

phenotype. Acute perfusion of prazosin did not improve contractile functions in control ($n = 5$) or transgenic ($n = 4$) animals (data not shown). Therefore, mice were preinjected with 3 mg/kg body wt prazosin. Hearts were then subjected to work-performing perfusion, and contractility was determined. In control animals, prazosin did not significantly alter the contractile parameters studied (data not shown). The transgenic group, however, showed a dramatic improvement of performance (Fig. 8). Peak +dP/d*t* was increased from 2,600 ± 61 to 3,724 ± 53 mmHg/s. This value was not significantly different from control values, 3,780 ± 66 mmHg/s. Similar results were obtained for the maximal rate of relaxation. The value for −dP/d*t* increased from 1,906 ± 81 to 3,303 ± 111 mmHg/s in prazosin-treated animals, which was not significantly different from control values, 3,239 ± 101 mmHg/s. The measurements of the duration of contraction revealed the same trend. TPP and $RT_{1/2}$ were significantly shortened after prazosin treatment. The values for TPP were 0.64 ± 0.012 vs. 0.47 ± 0.006 ms/mmHg before and after antagonist treatment, respectively. Control values (nontransgenics) were 0.431 ± 0.007 ms/mmHg. Similarly, $RT_{1/2}$ was shortened from 0.797 ± 0.26 to 0.553 ± 0.018 ms/mmHg, with control values of 0.479 ± 0.014 ms/mmHg. In both cases, the duration was significantly shortened after prazosin treatment but not completely restored to control values. These data show that the blockade of α_1-AR in transgenic mice completely restores the rate of pressure development and significantly shortens the duration of contraction and relaxation. This strongly indicates that the decrease in cardiac performance in transgenic animals is indeed caused by α_{1B}-AR overexpression and is, at least in part, corrected by receptor blockade.

DISCUSSION

Depending on species and experimental conditions, the action of α_1-AR is linked to positive or negative inotropic effects. In our work-performing heart perfusion, α_1-AR stimulation by phenylephrine resulted in positive inotropic and chronotropic responses. To our knowledge, this is the first demonstration of the effects of an α_1-AR agonist on spontaneously beating, intact mouse hearts. Our results are similar to those obtained from rat and rabbit. Tanaka et al. (46) have described negative inotropism with very high doses of phenyleph-

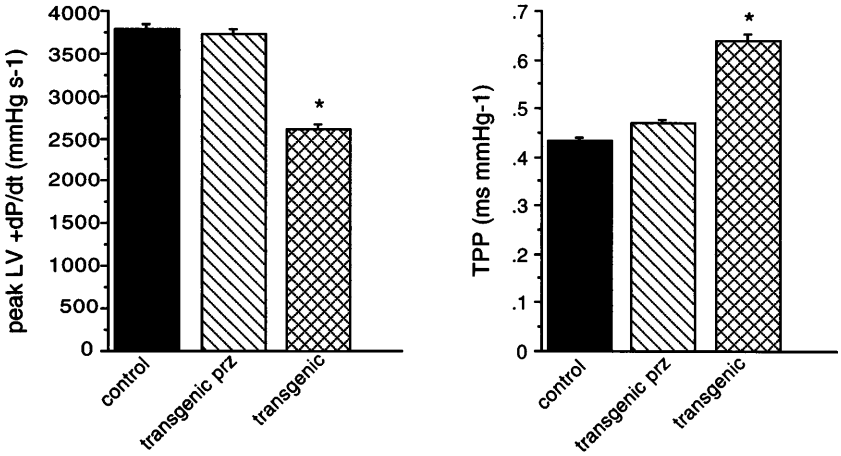

Fig. 8. Contractile parameters after α_1-AR antagonist treatment. Mice were injected intraperitoneally with 3 mg/kg body wt prazosin twice daily for 3 days before contractile parameters were measured in isolated work-performing hearts. Experimental values peak LV +dP/d*t* and TPP are shown for control (control, $n = 4$, 4 males), transgenic (transgenic, $n = 8$, 4 females, 4 males), and prazosin-treated transgenic (transgenic prz, $n = 4$, 1 female, 3 males) groups. *$P < 0.01$, transgenic vs. transgenic prz.

rine in mouse LV muscle strips. It is possible that the frequency dependence of the inotropic response in this system may explain their different findings.

In the present transgenic model, the overexpression of a wild-type α_{1B}-AR led to a receptor-mediated decrease in contractile function. Both contraction and relaxation phases were affected (Tables 1 and 2). These results indicate that basal level LV function is severely impaired in transgenic hearts. A causal relationship between depressed LV function and α_{1B}-AR overexpression is supported by the reversion of the contractile dysfunction by prazosin treatment. We therefore postulate that the chronic activation of α_{1B}-AR signaling creates a heart with depressed basal level contractile functions.

Using different experimental approaches, the anesthetized closed-chest mouse and the working-heart perfusion, we observed the same direction of functional changes in the transgenic hearts. This was further corroborated by the reduced cell shortening in isolated myocytes from transgenic hearts. The relative decrease in the rates of LV contraction and relaxation were quite similar in both systems, ranging between 32 and 42%. The actual values for the rates of pressure development were higher when measured in the closed-chest mouse. These differences in cardiac performance between the two preparations are due in part to the higher viscosity of blood and the closed pericardium. It has been shown previously that comparable rates are obtained when measuring peak LV dP/dt in the perfused preparation using the instrumentation of the closed-chest model (31). Similarly, in rat hearts, the rates observed vary considerably between Langendorff and closed-chest preparations (24, 27).

With the use of an open-chest methodology, contractile performance has been studied in the transgenic line Tg43 (1). There, no significant differences in basal level LVP and $+dP/dt$ and $-dP/dt$ were reported, although the values for dP/dt were lower in transgenics after isoproterenol stimulation. The reason for these differences to our data is unclear. Methodological approaches, e.g., open chest vs. closed chest, may account in part for the observed differences. A recent comparison of myocardial function showed that the type of preparation can indeed influence indexes of ventricular function (20). Also, in the open-chest measurements, transgenic heart rate was significantly reduced, which might directly influence contractility and relaxation. It is also possible that strain differences may affect the resulting cardiac phenotype.

The reduced amplitude of Ca^{2+} transients in electrically stimulated, isolated cardiomyocytes indicates that Ca^{2+} homeostasis is altered in the transgenic hearts. This is most likely the basis for the depression of baseline LV contractile function. The mechanism by which α_{1B}-AR overexpression negatively affects Ca^{2+} transients is unclear. Stimulation of α_1-AR is linked to the activation of phospholipase C and the generation of the second messengers, diacylglycerol and inositol 1,4,5-trisphosphate, which activate PKC and trigger Ca^{2+} influx from the sarcoplasmic reticulum, respectively. Previous analyses of line Tg43 have demonstrated an elevated level of diacylglycerol in myocardial extracts (1). Studies on isolated neonatal rat cardiomyocytes demonstrated an α_1-AR-dependent increase of L-type Ca^{2+} currents (28), although the mRNA for the α_1-subunit of the channel is downregulated after prolonged exposure to phenylephrine (33). In adult cells, however, L-type Ca^{2+} channels were negatively modulated, potentially reflecting a difference in G protein coupling at the two developmental stages (8, 29). In isolated rat hearts, α_1-AR stimulation resulted in a decrease of tissue cAMP levels (30). This may exert a negative effect on L-type Ca^{2+} channels that are stimulated by cAMP (42) and could potentially contribute to the decrease of contractile function in the α_{1B}-AR overexpressors.

Alternatively, one could speculate that the overexpression of α_{1B}-AR uncovers a functional interaction between the α_1-AR and the β-AR system. It is conceivable that various adrenergic inputs are integrated within the cardiomycyte, which requires a molecular communication between the receptors. Evidence for the contribution of both α_1-AR and β-AR to the inotropic response, as well as to the activity of ion channels, has been presented (8, 35, 36, 41). Similarly, the activity of other receptors acting in concert with the adrenergic system can be expected to modulate AR activity, an interaction that has been demonstrated for the δ-opioid receptors (37). The hypothesis of molecular cross-talk between AR is further supported by the finding that the β-AR high-affinity binding site for agonist is lost in α_{1B}-AR transgenics, indicating functional uncoupling of β-AR at the basal level (I. Lemire, H. Rindt, and T. E. Hebert, unpublished data). Such a mechanism could also explain the lower basal level contractile function observed in transgenic hearts both in the intact animal and the perfusion preparation. Interestingly, only chronic, but not acute, prazosin treatment restored contractile parameters. This suggests that the overexpression of α_{1B}-AR may alter the molecular cross talk between α_1-AR and β-AR in such a manner that a normal communication cannot be regained within the time frame of antagonist perfusion (minutes) but requires long-term treatment (hours to days). Prazosin is not a highly specific α_1-AR antagonist but can also block the activity of α_2-AR (18). This might result in an increase of norepinephrine release from presynaptic junctions that could potentially lead to the stimulation of cardiac contractility, thereby masking the effects of α_1-AR blockade in the transgenics. However, in control (nontransgenic) animals, prazosin did not increase contractile functions, indicating that potential α_2-AR effects do not play a discernible role in our perfusion protocol. Therefore, the observed recovery of contractility in the transgenic hearts after prazosin treatment is most likely due to the blockade of the overexpressed α_{1B}-AR.

The stimulation of β-AR with isoproterenol essentially restored contractility to control values, suggesting that the maximal inotropic response of the transgenic hearts was not affected. Similar results were

obtained in the anesthetized closed-chest animal (data not shown). Previous analyses (1) have demonstrated a reduced adenylyl cyclase activity at baseline in membrane preparations from transgenic hearts which presumably reflects a decrease in cAMP levels. Similarly, the maximal response to isoproterenol was attenuated. Both basal and stimulated cyclase activity were restored to control levels after pretreatment of animals with pertussis toxin, suggesting the involvement of a G_i-mediated mechanism (1). The depressed basal level contractility in the α_{1B}-AR transgenics may well be correlated with the lower basal cyclase activity. Surprisingly, the complete restoration of contractile function after β-AR stimulation with isoproterenol is paralled by only submaximal activation of adenylyl cyclase activity, suggesting lower cAMP levels. This may seem paradoxical; however, it has been shown that β-AR-mediated increases in contractility may at least in part be dissociated from cAMP levels (2, 53). In addition, Pepe et al. (37) recently described an example of receptor cross-talk, where the stimulation of δ-opioid receptors exerted a negative effect on β-AR-mediated inotropic actions. However, although increases in cAMP were blocked by a δ-opioid receptor agonist, β_2-AR subtype-specific stimulation still resulted in maximal positive inotropic effects in isolated perfused rat hearts. These findings again demonstrate that inotropic responses may be uncoupled from increases in cAMP. We believe that a similar mechanism most likely explains the observed positive inotropic response to β-AR stimulation by isoproterenol of the α_{1B}-AR transgenics in the absence of maximal activation of adenylyl cyclase.

Challenging the perfused hearts with an increase in minute work revealed a blunted response. Within a narrow range of work loads, the incremental increases in the rates of contraction and relaxation were comparable in control and transgenic groups. The transgenic hearts, however, could not be loaded to the same extent as their control counterparts. The parallel Starling curves for $+dP/dt$ and $-dP/dt$ at moderate work loads as well as electron microscopic analyses of LV wall muscle samples indicate the integrity of the sarcomeric structure. Also, at comparable minute work, LV end-diastolic pressure is elevated whereas dP/dt is diminished compared with controls, again indicating a state of depressed contractility. The α_{1B}-AR-overexpressing hearts are not completely unresponsive to work load, since they can increase contractility within a narrow range of imposed load. The lack of tolerance to higher work load indicates a contractile deficit that is also found in human congestive heart failure, and exercise tests are currently being performed to test stress tolerance in vivo. Surprisingly, with increasing afterload, the times of contraction and relaxation were shortened faster in transgenic hearts, approaching control values at maximal sustainable minute work. It is possible that this phenomenon is related to correction of part of the Ca^{2+} levels due to sarcomere stretch.

Cardiac hypertrophy was not induced in the α_{1B}-AR overexpressors. Heart weight-to-body weight ratio was not elevated, and typical hypertrophy marker genes, MLC-2v and β-MHC, were not upregulated. In addition, the phosphorylation status of MLC-2v is unchanged (data not shown). In a number of studies, the α_1-AR system has been implicated in myocyte hypertrophy. Treatment of neonatal rat cardiomyocytes in culture with α_1-AR agonists induced hypertrophy, as indicated by an increase in cell size and the upregulation of the β-MHC gene (22). These effects have been shown to be mediated by the α_{1A}-AR subtype (25). However, it has been suggested that the coupling specificity of α_1-AR changes during development (29), and the overexpression of a constitutively active mutant of the α_{1B}-AR subtype does indeed induce hypertrophy in the adult mouse heart (34). More recently, it was shown that the transgenic overexpression of G_q, an α_1-AR coupling partner, exhibited a hypertrophic phenotype (10). In addition, the overexpression of the PKC-β2 isoform in the myocardium resulted in hypertrophy and cardiomyopathy (52). In light of these data, the lack of hypertrophy in this model of wild-type α_{1B}-AR overexpression is somewhat surprising. It may be possible that higher levels of expression are required to initiate the hypertrophic response. However, α_{1B}-AR-dependent signaling does occur at the present level, as demonstrated by the contractile deficit and its correction by receptor blockade. Alternatively, one could speculate that not α_{1B}-AR but a different α_1-AR subtype is responsible for the induction of hypertrophy in the mouse heart. Another possibility may be the activation of a compensatory mechanism during development. Although the intrinsic α_1-AR density declines during early postnatal development, overexpression of the α_{1B}-AR begins in the neonate when the α-MHC transgene promoter becomes highly active in the mouse heart. This rapid increase in transgene activity may trigger a compensatory action preventing the onset of hypertrophy. Future studies are required to elucidate these potential mechanisms.

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REFERENCES

1. **Akhter, S. A., C. A. Milano, K. F. Shotwell, M.-C. Cho, H. A. Rockman, R. J. Lefkowitz, and W. J. Koch.** Transgenic mice with cardiac overexpression of α_{1B}-adrenergic receptors. In vivo α_1-adrenergic receptor-mediated regulation of β-adrenergic signaling. *J. Biol. Chem.* 272: 21253–21259, 1997.
2. **Altschuld, R. A., R. C. Starling, R. L. Hamlin, G. E. Billman, J. Hensley, L. Castillo, R. H. Fertel, C. M. Hohl, P.-M. Robitaille, L. R. Jones, R.-P. Xiao, and E. G. Lakatta.** Response of failing canine and human heart cells to β_2-adrenergic stimulation. *Circulation* 92: 1612–1618, 1995.
3. **Barrett, S., N. Honbo, and J. S. Karliner.** Alpha1-adrenoceptor-mediated inhibition of cellular cAMP accumulation in neona-

tal rat ventricular myocytes. *Naunyn Schmiedebergs Arch. Pharmacol.* 347: 384–393, 1993.

4. **Böhm, M.** Alterations of beta-adrenoceptor-G protein-regulated adenylyl cyclase in heart failure. *Mol. Cell. Biochem.* 147: 147–160, 1995.
5. **Bristow, M. R., R. Ginsburg, and R. E. Hershberger.** Heterogeneous regulatory changes in cell surface membrane receptors coupled to a positive inotropic response in the failing human heart. *Basic Res. Cardiol.* 82, *Suppl.* 2: 369–376, 1987.
6. **Bristow, M. R., W. Minobe, R. Rasmussen, R. E. Hershberger, and B. B. Hoffman.** Alpha-1 adrenergic receptors in the nonfailing and failing human heart. *J. Pharmacol. Exp. Ther.* 247: 1039–1045, 1988.
7. **Bruckner, R., W. Meyer, A. Mugge, W. Schmitz, and H. Scholz.** α-Adrenoceptor-mediated positive inotropic effect of phenylephrine in isolated human ventricular myocardium. *Eur. J. Pharmacol.* 99: 345–347, 1984.
8. **Chen, L., N. el-Sherif, and M. Boutjdir.** α_1-Adrenergic activation inhibits β-adrenergic-stimulated unitary Ca^{2+} currents in cardiac ventricular myocytes. *Circ. Res.* 79: 184–193, 1996.
9. **Corr, P. B., K. A. Yamada, and S. D. DaTorre.** Modulation of α-adrenergic receptors and their intracellular coupling in the ischemic heart. *Basic Res. Cardiol.* 85, *Suppl.* 1: 31–45, 1990.
10. **D'Angelo, D. D., Y. Sakata, J. N. Lorenz, G. P. Boivin, R. A. Walsh, S. B. Liggett, and G. W. Dorn II.** Transgenic $G\alpha_q$ overexpression induces cardiac contractile failure. *Proc. Natl. Acad. Sci. USA* 94: 8121–8126, 1997.
11. **Danziger, R. S., M. Sakai, E. G. Lakatta, and R. G. Hansford.** Interactive α- and β-adrenergic actions of norepinephrine in rat cardiac myocytes. *J. Mol. Cell. Cardiol.* 22: 111–123, 1990.
12. **Dorn, G. W., II, J. Robbins, N. Ball, and R. A. Walsh.** Myosin heavy chain regulation and myocyte contractile depression after LV hypertrophy in aortic-banded mice. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H400–H405, 1994.
13. **Endoh, M., and H. J. Schümann.** Frequency-dependence of the positive inotropic effect of methoxamine and naphazoline mediated by α-adrenoceptors in the isolated rabbit papillary muscle. *Naunyn Schmiedebergs Arch. Pharmacol.* 287: 377–389, 1975.
14. **Fedida, D., A. P. Braun, and W. R. Giles.** α_1-Adrenoceptors in myocardium: functional aspects and transmembrane signaling mechanisms. *Physiol. Rev.* 73: 469–487, 1993.
15. **Graham, R. M., D. M. Perez, J. Hwa, and M. T. Piascik.** α_1-Adrenergic receptor subtypes. Molecular structure, function and signaling. *Circ. Res.* 78: 737–749, 1996.
16. **Grupp, I. L., A. Subramaniam, T. E. Hewett, J. Robbins, and G. Grupp.** Comparison of normal, hypodynamic, and hyperdynamic mouse hearts using isolated work-performing heart preparations. *Am. J. Physiol.* 265 (*Heart Circ. Physiol.* 34): H1401–H1410, 1993.
17. **Guse, A. H., I. Berg, and G. Gercken.** Inhibition of alpha1-adrenoceptor-mediated inositol phosphate accumulation in cultured cardiac myocytes by cyclic AMP-generating compounds. *J. Mol. Cell. Cardiol.* 23: 1375–1382, 1991.
18. **Hancock, A. H.** α_1-Adrenoceptor subtypes: a synopsis of their pharmacology and molecular biology. *Drug Dev. Res.* 39: 54–107, 1996.
19. **Henkel, R. D., C. M. Kammerer, L. V. Escobedo, J. L. VandeBerg, and R. A. Walsh.** Correlated expression of atrial myosin heavy chain and regulatory light chain isoforms with pressure overload hypertrophy in the non-human primate. *Cardiovasc. Res.* 27: 416–422, 1993.
20. **Hoit, B. D., N. Ball, and R. A. Walsh.** Invasive hemodynamics and force-frequency relations in open versus closed chest mice. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H2528–H2533, 1997.
21. **Kadambi, V. J., S. Ponniah, J. M. Harrer, B. D. Hoit, G. W. Dorn II, R. A. Walsh, and E. G. Kranias.** Cardiac-specific overexpression of phospholamban alters calcium kinetics and resultant cardiomyocyte mechanics in transgenic mice. *J. Clin. Invest.* 97: 533–539, 1996.
22. **Kariya, K., L. R. Karns, and P. C. Simpson.** Expression of a constitutively activated mutant of the beta-isozyme of protein kinase C in cardiac myocytes stimulates the promoter of the beta-myosin heavy chain isogene. *J. Biol. Chem.* 266: 10023–10026, 1991.
24. **Klabunde, R. E., N. D. Kimber, J. E. Kuk, M. C. Helgren, and U. Förstermann.** N^G-methyl-L-arginine decreases contractility, cGMP, and cAMP in isoproterenol-stimulated rat hearts in vitro. *Eur. J. Pharmacol.* 223: 1–7, 1992.
25. **Knowlton, K. U., M. C. Michel, M. Itani, H. E. Shubeita, K. Ishikara, J. H. Brown, and K. R. Chien.** The α_{1A}-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. *J. Biol. Chem.* 268: 15374–15380, 1993.
26. **Landzberg, J. S., J. D. Parker, D. F. Gauthier, and W. S. Colucci.** Effects of myocardial α_1-adrenergic stimulation and blockade on contractility in humans. *Circulation* 84: 1608–1615, 1991.
27. **Li, P., X. Zhang, J. M. Capasso, L. G. Meggs, E. H. Sonnenblick, and P. Anversa.** Myocyte loss and left ventricular failure characterize long term effects of coronary artery narrowing or renal hypertension in rats. *Cardiovasc. Res.* 27: 1066–1075, 1993.
28. **Liu, Q. Y., E. Karpinski, and P. K. Pang.** The L-type calcium channel current is increased by α-1-adrenoceptor activation in neonatal rat ventricular cells. *J. Pharmacol. Exp. Ther.* 271: 935–943, 1994.
29. **Liu, Q. Y., E. Karpinski, and P. K. Pang.** Changes in α_1-adrenoceptor coupling to Ca^{2+} channels during development in rat heart. *FEBS Lett.* 338: 234–238, 1994.
30. **Lochner, A., E. Tromp, and R. Mouton.** Signal transduction in myocardial ischemia and reperfusion. *Mol. Cell. Biochem.* 160: 129–136, 1996.
31. **Lorenz, J. N., and J. Robbins.** Measurement of intraventricular pressure and cardiac performance in the intact closed-chest anesthetized mouse. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H1137–H1146, 1997.
32. **Lyons, I., L. M. Parsons, L. Hartley, R. Li, J. E. Andrews, L. Robb, and R. P. Harvey.** Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2–5. *Genes Dev.* 9: 1654–1666, 1995.
33. **Maki, T., E. J. Gruver, E. J. Davodoff, N. Izzo, D. Toupin, W. S. Colucci, A. R. Marks, and J. D. Marsh.** Regulation of calcium channel expression in neonatal myocytes by catecholamines. *J. Clin. Invest.* 97: 656–663, 1996.
34. **Milano, C. A., P. C. Dolber, H. A. Rockman, R. A. Bond, M. E. Venable, L. F. Allen, and R. J. Lefkowitz.** Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* 91: 10109–10113, 1994.

34a. **Mügge, A., C. Reupcke, and H. Scholz.** α_1-Adrenoceptor density in rats chronically treated with propranolol. *Eur. J. Pharmacol.* 112: 249–252, 1985.

35. **Oleksa, L. M., L. C. Hool, and R. D. Harvey.** α_1-Adrenergic inhibition of the β-adrenergically activated Cl^- current in guinea pig ventricular myocytes. *Circ. Res.* 78: 1090–1099, 1996.
36. **Osnes, J. B., H. Aass, and T. Skomedal.** Adrenoceptors in myocardial regulation: concomitant contribution from both alpha- and beta-adrenoceptor stimulation to the inotropic response. *Basic Res. Cardiol.* 84, *Suppl.* 1: 9–17, 1989.
37. **Pepe, S., R.-P. Xiao, C. Hohl, R. Altschuld, and E. G. Lakatta.** "Cross talk" between opioid peptide and adrenergic receptor signaling in isolated rat hearts. *Circulation* 95: 2122–2129, 1997.
38. **Rybin, V., H.-M. Han, and S. F. Steinberg.** G protein dependence of α-1-adrenergic receptor subtype action in cardiac myocytes. *Methods Neurosci.* 29: 344–361, 1996.
39. **Schümann, H. J., J. Wagner, A. Knorr, J. C. Reidemeister, V. Sadony, and G. Schramm.** Demonstration in human atrial preparations of α-adrenoceptors mediating positive inotropic effects. *Naunyn Schmiedebergs Arch. Pharmacol.* 302: 333–336, 1978.
40. **Shen, Y. T., D. E. Vatner, H. E. Gagnon, and S. F. Vatner.** Species differences in regulation of α-adrenergic receptor function. *Am. J. Physiol.* 257 (*Regulatory Integrative Comp. Physiol.* 26): R1110–R1116, 1989.
41. **Skomedal, T., I. G. Schiander, and B. Osnes.** Both alpha- and beta-adrenoceptor-mediated components contribute to final ino-

tropic response to norepinephrine in rat heart. *J. Pharmacol. Exp. Ther.* 247: 1204–1210, 1988.
42. **Sperelakis, N.** Regulation of calcium slow channels of heart by cyclic nucleotides and effects of ischemia. *Adv. Pharmacol.* 31: 1–24, 1994.
43. **Stewart, A. F. R., D. G. Rokosh, B. A. Bailey, L. R. Karns, K. C. Chang, C. S. Long, K. Kariya, and P. C. Simpson.** Cloning of the rat alpha 1C-adrenergic receptor from cardiac myocytes. Alpha 1C, alpha 1B, and alpha 1D mRNAs are present in cardiac myocytes but not in cardiac fibroblasts. *Circ. Res.* 75: 796–802, 1994.
44. **Strang, K. T., and R. L. Moss.** α_1-Adrenergic receptor stimulation decreases maximum shortening velocity of skinned single ventricular myocytes from rats. *Circ. Res.* 77: 114–120, 1995.
45. **Subramaniam, A., W. K. Jones, J. Gulick, S. Wert, J. Neumann, and J. Robbins.** Tissue-specific regulation of the α-myosin heavy chain gene promoter in transgenic mice. *J. Biol. Chem.* 266: 24613–24620, 1991.
46. **Tanaka, H., S. Manita, T. Matsuda, M. Adachi, and K. Shigenobu.** Sustained negative inotropism mediated by α-adrenoceptors in adult mouse myocardia: developmental conversion from positive response in the neonate. *Br. J. Pharmacol.* 114: 673–677, 1995.
47. **Terzic, A., M. Puceat, G. Vassort, and S. M. Vogel.** Cardiac α_1-adrenoceptors: an overview. *Pharmacol. Rev.* 45: 147–175, 1993.
48. **Thorburn, A., J. Thorburn, S.-Y. Chen, S. Powers, H. E. Shubeita, J. R. Feramisco, and K. R. Chien.** HRas-dependent pathways can activate morphological and genetic markers of cardiac muscle cell hypertrophy. *J. Biol. Chem.* 268: 2244–2249, 1993.
49. **Turki, J., J. N. Lorenz, S. A. Green, E. T. Donnely, M. Jacinto, and S. B. Liggett.** Myocardial signaling defects and impaired cardiac function of a human β_2-adrenergic receptor polymorphism expressed in transgenic mice. *Proc. Natl. Acad. Sci. USA* 93: 10483–10488, 1996.
50. **Vago, T., M. Bevilacqua, G. Norbiato, G. Baldi, E. Chebat, P. Bertora, G. Baroldi, and R. Accinni.** Identification of α-1-adrenergic receptors on sarcolemma from normal subjects and patients idiopathic dilated cardiomyopathy: characteristics and linkage to GTP-binding proteins. *Circ. Res.* 64: 474–481, 1989.
51. **Viko, H., J. B. Osnes, and T. Skomedal.** Alpha1- and beta-adrenoceptor-mediated increase in ^{86}Rb-uptake in isolated cardiomyocytes from adult rat heart: evidence for interaction betwen the two receptor systems. *Pharmacol. Toxicol.* 79: 287–292, 1996.
52. **Wakasaki, H., K. Daisuke, F. J. Schoen, M. R. Jirousek, D. K. Ways, B. D. Hoit, R. A. Walsh, and G. L. King.** Targeted overexpression of protein kinase Cβ2 isoform in myocardium causes cardiomyopathy. *Proc. Natl. Acad. Sci. USA* 94: 9320–9325, 1997.
53. **Xiao, R.-P., C. Hohl, R. Altschuld, L. Jones, B. Livingston, B. Ziman, B. Tantini, and E. G. Lakatta.** β_2-Adrenergic receptor-stimulated increase in cAMP in rat heart cells is not coupled to changes in Ca^{2+} dynamics, contractility, or phospholamban phosphorylation. *J. Biol. Chem.* 269: 19151–19156, 1994.