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J Clin Invest. 1998;101(6):1273-1282. <https://doi.org/10.1172/JCI1335>.

Research Article

While an age-associated diminution in myocardial contractile response to beta-adrenergic receptor (beta-AR) stimulation has been widely demonstrated to occur in the context of increased levels of plasma catecholamines, some critical mechanisms that govern beta-AR signaling must still be examined in aged hearts. Specifically, the contribution of beta-AR subtypes (beta1 versus beta2) to the overall reduction in contractile response with aging is unknown. Additionally, whether G protein-coupled receptor kinases (GRKs), which mediate receptor desensitization, or adenylyl cyclase inhibitory G proteins (Gi) are increased with aging has not been examined. Both these inhibitory mechanisms are upregulated in chronic heart failure, a condition also associated with diminished beta-AR responsiveness and increased circulatory catecholamines. In this study, the contractile responses to both beta1-AR and beta2-AR stimulation were examined in rat ventricular myocytes of a broad age range (2, 8, and 24 mo). A marked age-associated depression in contractile response to both beta-AR subtype stimulation was observed. This was associated with a nonselective reduction in the density of both beta-AR subtypes and a reduction in membrane adenylyl cyclase response to both beta-AR subtype agonists, NaF or forskolin. However, the age-associated diminutions in contractile responses to either beta1-AR or beta2-AR stimulation were not rescued by inhibiting Gi with pertussis toxin treatment. Further, the abundance or activity of beta-adrenergic receptor kinase, GRK5, or Gi did not significantly [...]

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Age-associated Reductions in Cardiac β_1 - and β_2 -adrenergic Responses Without Changes in Inhibitory G Proteins or Receptor Kinases

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Abstract

While an age-associated diminution in myocardial contractile response to β -adrenergic receptor (β -AR) stimulation has been widely demonstrated to occur in the context of increased levels of plasma catecholamines, some critical mechanisms that govern β -AR signaling must still be examined in aged hearts. Specifically, the contribution of β -AR subtypes (β_1 versus β_2) to the overall reduction in contractile response with aging is unknown. Additionally, whether G protein-coupled receptor kinases (GRKs), which mediate receptor desensitization, or adenylyl cyclase inhibitory G proteins (G_i) are increased with aging has not been examined. Both these inhibitory mechanisms are upregulated in chronic heart failure, a condition also associated with diminished β -AR responsiveness and increased circulatory catecholamines.

In this study, the contractile responses to both β_1 -AR and β_2 -AR stimulation were examined in rat ventricular myocytes of a broad age range (2, 8, and 24 mo). A marked age-associated depression in contractile response to both β -AR subtype stimulation was observed. This was associated with a nonselective reduction in the density of both β -AR subtypes and a reduction in membrane adenylyl cyclase response to both β -AR subtype agonists, NaF or forskolin. However, the age-associated diminutions in contractile responses to either β_1 -AR or β_2 -AR stimulation were not rescued by inhibiting G_i with pertussis toxin treatment. Further, the abundance or activity of β -adrenergic receptor kinase, GRK5, or G_i did not significantly change with aging. Thus, we conclude that the positive inotropic effects of both β_1 - and β_2 -AR stimulation are markedly decreased with aging in rat ventricular myocytes and this is accompanied by decreases in both β -AR subtype densities and a reduction in membrane adenylyl cyclase activity. Neither GRKs nor G_i proteins appear to contribute to the age-associated reduction in cardiac β -AR responsiveness. (*J. Clin. Invest.* 1998. 101:1273–1282.) **Key words:** β -adrenergic receptor subtype • β -adrenergic receptor kinase • aging • cardiac myocytes • inhibitory G proteins

Introduction

β -adrenergic receptor (β -AR)¹-mediated modulation of myocardial performance is a major component of cardiovascular reserve function. A large body of evidence has demonstrated that the cardiac response to β -AR stimulation decreases with aging (1–4). Previous studies in single rat ventricular myocytes have shown that the positive inotropic effects produced by CTP-cAMP (a membrane permeable cAMP analog), elevation of extracellular $[Ca^{2+}]_i$, or β AY-K 8644 (an activator of the sarcolemmal L-type calcium channel) are not altered by aging (3, 4). Thus, the age-associated reduction in response to β -AR stimulation is related to a specific deficit in β -AR signal transduction, whereas the intrinsic properties of excitation-contraction coupling machinery remain intact with aging. Over the last two decades many studies have focused on age-associated changes in the receptor- G_s -adenylyl cyclase-cAMP-dependent protein kinase (PKA) signaling cascade and usually do not discriminate β -AR subtypes (for review see reference 1). However, some cellular mechanisms that do not directly involve the β -AR signaling cascade, but are critical in regulating its signaling efficacy, have not yet been examined in aged hearts. For example, it is unknown whether aging causes increases in the abundance or activity of negative regulators of β -AR signaling including G protein-coupled receptor kinases (GRKs) and inhibitory G proteins (G_i).

In the mammalian heart, both the β_1 - and β_2 -AR subtypes are coupled to myocardial contractility (5–10). Recent studies from this laboratory have shown that β -AR subtype stimulation elicits distinct cellular responses in rat and canine ventricular myocytes (5, 11–14). While the contractile effect of β_1 -AR stimulation in rat cardiac cells is clearly mediated by the classic stimulatory G protein (G_s)-coupled cAMP-dependent signaling pathway (11, 14), β_2 -AR-stimulated increases in intracellular Ca^{2+} transients and contractility appear to be dissociated from the β_2 -AR-induced increase in cAMP (rat heart cells [11]), or occur without a detectable increase in cAMP content (canine myocytes [13]), and occur in the absence of an increase in cAMP-dependent phospholamban phosphorylation in both species (11, 13). Thus, β -AR subtype signaling cascades in rat heart cells differ from each other in multiple aspects and could be differentially affected by aging.

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Received for publication 29 July 1997 and accepted in revised form 6 January 1998.

The Journal of Clinical Investigation
Volume 101, Number 6, March 1998, 1273–1282
<http://www.jci.org>

1. **Abbreviations used in this paper:** β -AR, β -adrenergic receptor; β ARK1, β -adrenergic receptor kinase; G_i , inhibitory G proteins; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRKs, G protein-coupled receptor kinases; G_s , stimulatory G protein; ¹²⁵I-CYP, [¹²⁵I]-Cyanopindolol; LV, left ventricle; NE, norepinephrine; PTX, pertussis toxin; RT-PCR, reverse transcriptase-PCR.

As is true for most G protein-coupled receptors, prolonged exposure of β -ARs to an agonist leads to a rapid decrease in responsiveness, referred to as desensitization. Agonist-dependent desensitization can be initiated by phosphorylation of activated receptors by members of the GRK family (15, 16). The β -adrenergic receptor kinase (β ARK1) is a prototypic GRK that has been shown to phosphorylate activated β -ARs (both β_1 and β_2 subtypes) in vitro (16, 17). Phosphorylated receptors become binding substrates for a class of inhibitor proteins, β -arrestins, which sterically inhibit further G protein coupling (18). β ARK1 has also been shown to regulate β -ARs in vivo as increased expression (three- to fivefold) of β ARK1 in the hearts of transgenic mice leads to diminished β -AR agonist stimulated cardiac contractility in vivo (19) and in isolated ventricular myocytes (20). In addition, cardiac-specific expression of a β ARK1 inhibitor is accompanied by an enhanced basal contractility in vivo, suggesting that myocardial β -ARs are targets for tonic GRK regulation (19). Recently, we have demonstrated that overexpression of a second myocardial GRK, GRK5, in transgenic mice also leads to significant cardiac β -AR uncoupling (21). Thus, multiple mechanisms of β -AR uncoupling can exist in the myocardium and multiple GRKs may be responsible for promoting desensitization. Since the plasma levels of both norepinephrine (NE) and epinephrine, physiological ligands for both β_1 - and β_2 -ARs, are increased with aging (22, 23), it may be hypothesized that agonist-dependent β -AR desensitization may play a role in the age-associated diminution in cardiac β -AR responsiveness. While prior studies have addressed potential biochemical mechanisms for a reduction in myocardial β -AR signaling with aging (24–26), none has studied the specific roles of GRKs in aging. Interestingly, in chronic human heart failure, another condition characterized by diminished β -AR responsiveness and increased levels of catecholamines, the levels and enzymatic activity of β ARK1 have been shown to be elevated, which may be contributing to the β -AR uncoupling seen in this pathophysiological condition (27). Thus, β ARK1 and possibly other GRKs are important targets of study in the aged heart.

G_i proteins are pertussis toxin (PTX)-sensitive G proteins that are also negative regulators of β -AR signaling as they inhibit adenylyl cyclase and oppose β -AR- G_s coupling (28). In this regard, our recent studies have shown that in rat ventricular myocytes β_2 -ARs, but not β_1 -ARs, are simultaneously coupled to PTX-sensitive G proteins as well as to G_s . This novel pathway confers negative feedback on the positive inotropic effect of β_2 -AR stimulation (12, 14). Thus, any increase in PTX-sensitive G proteins could contribute to age-associated changes in contractile responses to both β -AR subtype stimulation in the rat heart, but via different mechanisms, as β_1 -ARs do not appear to directly couple to G_i in the heart (12).

The purpose of this study was to determine whether the contractile response to specific β -AR subtype stimulation in single rat ventricular myocytes decreases with aging, and to examine specific cellular mechanisms that are critical in regulating the β -AR signaling efficacy in hearts of a broad age range. Specifically, we measured (a) β -AR subtype densities in both isolated myocytes and heart tissues as well as the cellular contractile response to β -AR subtype stimulation, and (b) the abundance and activity of G_i proteins and GRKs. To provide a more comprehensive picture of β -AR signaling, in this study, we also determined age-associated changes in adenylyl cyclase activity.

Methods

Measurements of myocyte contraction characteristics. Single ventricular cardiac myocytes were isolated from 2, 8, and 24-mo-old Wistar rat hearts by a standard enzymatic technique (4). Cells were then placed on the stage of a modified inverted microscope (model IM-35; Carl Zeiss, Inc., Thornwood, NY) and perfused with Hepes buffer solution consisting of (in mM): CaCl_2 , 1.0; NaCl, 137; KCl, 5.0; dextrose, 15; MgSO_4 , 1.3; NaH_2PO_4 , 1.2; Hepes (5-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 20; pH 7.4, at 23°C. Cell length was monitored from the brightfield (650–750 nm) image of the cell by an optical edge tracking method using a photodiode array (Reticon Model 1024 SAQ) with a 3 ms time resolution (4). Cell contraction amplitude was indexed by the shortening of cell length (percentage of rest cell length) after electric field stimulation. Maximal contraction shortening velocity (micrometers per second) was also measured, as previously described (4).

Dose-response curves for the β -AR subtype agonists (NE and zinterol) were implemented in control or PTX-treated cells (see below) from hearts of different age groups. A given individual myocyte was exposed to only one drug concentration. All measurements were obtained under steady state conditions after a 10-min exposure to a specific agonist at room temperature (23°C). On each experimental day, a dose-response curve was usually constructed from cells of one heart and the EC_{50} (concentration of an agonist required to produce 50% of maximum effect on contraction amplitude) was estimated from each dose-response curve. Under our experimental conditions, NE in isolated rat ventricular myocytes is an exclusive β_1 -AR agonist, i.e., it does not evoke effects via α -adrenergic stimulation or β_2 -AR stimulation (4, 5). Zinterol, under our conditions, is a selective β_2 -AR agonist, since neither α_1 -adrenergic receptor blockers nor β_1 -AR antagonists significantly reduce the effects of zinterol on contraction in rat ventricular myocytes (5).

Pertussis toxin treatment. To examine the function of inhibitory G proteins (G_i/G_o), cells were incubated with PTX (1.5 $\mu\text{g}/\text{ml}$, at 37°C for at least 3 h) and compared with myocytes from the same heart which had been kept at 37°C in the absence of PTX for an equal time. Successful inactivation of inhibitory G proteins (G_i/G_o) in PTX-treated cells was routinely verified by a loss of the ability of adenosine or carbachol to reverse the positive inotropic effect of β -AR stimulation by NE, as previously described (12).

Preparation of tissue fractions. Frozen cell pellets or aliquots of left ventricle (LV) (200–300 mg) from rat hearts of varying ages were homogenized in ice-cold lysis buffer (5 mM Tris-Cl, pH 7.5/5 mM EDTA/10 μg per ml leupeptin/20 μg per ml aprotinin/1 mM phenylmethylsulfonylfluoride). The samples were centrifuged at 800 g for 15 min at 4°C to clear the homogenate of cellular debris and nuclei. Subsequent supernatants were filtered through cheesecloth and centrifuged at 25,000 g for 30 min at 4°C. The final supernatant was set aside for GRK analysis (see below) and the pelleted membranes were resuspended (~ 1 mg/ml) in ice-cold β -AR binding buffer (75 mM Tris-Cl, pH 7.5/12.5 mM $\text{MgCl}_2/2$ mM EDTA). Membranes were aliquoted and stored at -80°C until use in biochemical assays.

Receptor binding. Membrane fractions from LV or ventricular myocytes described above were used for β -AR radioligand binding studies using the nonselective β -AR antagonist ligand [^{125}I]-Cyanopindolol (^{125}I -CYP). Maximal binding was measured using a saturating amount of ^{125}I -CYP on 25 μg membrane protein from LV or from isolated ventricular myocytes. Binding was allowed to occur for 1 h at 37°C, as described previously (19, 21). Inclusion of 10 μM alprenolol defined nonspecific binding.

For competition isotherms, membranes (25 μg total protein) were incubated with 300 μM ^{125}I -CYP and increasing dilutions of ICI 118,551 (ICI), a selective β_2 -AR antagonist, as described previously (19). The percentage of β_2 -ARs was calculated from the high affinity binding subpopulation determined using GraphPad Prism (19).

Adenylyl cyclase assays. Cardiac membranes were prepared by homogenizing rat left ventricles in ice-cold lysis buffer (20 mM Tris-

HCl [pH 7.4], 250 mM sucrose, 1 mM EDTA, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 0.1 mM PMSF). The samples were centrifuged at 10,000 g for 10 min at 4°C. The supernatant was centrifuged at 100,000 g for 2 h at 4°C. The pellet was resuspended in a buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM EDTA up to final protein concentration of 4.5–5 mg/ml. Membranes were aliquoted and stored at –80°C. Aliquots of the cardiac membranes (20 µg total protein) were incubated for 15 min at 37°C (the incubation mixture containing: 1.6 mM [α -³²P]ATP (20–40 cpm/picomole), 0.5 mM ATP, 0.1 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724), 20 mM creatine phosphate, 100 U/ml creatine phosphokinase, 25 mM Tris-HCl buffer, pH 7.4, and agonists as indicated). Then, 250 µl of a solution containing 1.5 mM cyclic AMP and 0.05 µCi [³H]cyclic AMP in 10 mM Tris-HCl buffer, pH 7.4, was added and the reaction was terminated by placing the samples in boiling water for 3 min. The reaction mixture of each sample was applied to a column of neutral alumina (0.4 × 15 cm, prepared by pouring dry alumina into Pasteur pipets). Each column was washed with 3 ml 10 mM Tris-HCl buffer, pH 7.4. The effluent was collected in a scintillation vial. For the assay of column fractions, 100 µM forskolin and 5 mM MnCl₂ were added. In the various assays, 5 mM MgCl₂ was used. Blanks were prepared by incubating the samples in the absence of enzyme.

GRK activity assays. Aliquots of the myocardial supernatants prepared from left ventricular tissue (described above) were used for soluble GRK assays in which the primary GRK activity measured is β ARK1 (19). Samples containing equal protein amounts (3–5 mg) were adjusted to a NaCl concentration of 50 mM and partially purified using DEAE sephacel chromatography as we have described previously (19). Final concentrated eluate samples (50–60 µg total protein) were incubated in a volume of 100 µl of lysis buffer (see above) supplemented with 0.1 mM ATP (containing [γ -³²P]ATP), 10 mM MgCl₂ and rhodopsin-enriched rod outer segments (19, 21). After incubation in white light for 15 min at room temperature, the reactions were quenched with 300 µl of ice-cold lysis buffer and centrifuged for 15 min at 13,000 g. The sedimented proteins were resuspended in 25 µl of protein gel-loading dye and electrophoresed on 12% polyacrylamide/Tris-glycine gels which were then dried. Phosphorylated rhodopsin was quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Protein immunoblotting. Either membranes (GRK5, G_{ai}) or purified supernatants (for measurements of β ARK1) from rat LVs were used for protein determination by Western analysis. Equal protein amounts (50–75 µg) were electrophoresed under denaturing conditions on 12% polyacrylamide/Tris-glycine gels and transferred to nitrocellulose membranes, as described (19, 21). Protein immunoblotting was done with respective primary and secondary antibodies using standard chemiluminescence protocols (ECL[®]; Amersham Corp., Arlington Heights, IL). The primary GRK antibodies used were polyclonal antisera raised to the carboxyl termini of β ARK1 or GRK5 (19, 21). For G_{ai}, polyclonal antiserum were used (G_{ai}(1–3)) (Santa Cruz Biotechnology, Santa Cruz, CA).

Semi-quantitative RT-PCR. Total RNA from aged rat LV samples was isolated using Ultraspec[®] (Biotecx Laboratories, Inc., Houston, TX), a single step guanidinium-based RNA isolation solution (29). 1 µg of total RNA was then used for first strand cDNA synthesis using stratascript reverse transcriptase (Stratagene, Inc., La Jolla, CA). Aliquots equaling 1/10 of the cDNA product were then used for quantitative PCR reactions. Three sets of primer-pairs (sense and antisense sequences) were employed to amplify either β ARK1, GRK5, or the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sets were as follows: β ARK1 sense: 5'-AG-ATGGCCGACCTGGAGGC-3'; β ARK1 antisense: 5'-GGAGTCAAAGATCTCCCG-3'; GRK5 sense: 5'-GGAGCTGGAAACA-TCCGTGGCC-3'; GRK antisense: 5'-GCGATCAAAATATGCTG-TCC-3'; GAPDH sense: 5'-GGAAACCCATCACCATCTTCCAG-3'; and GAPDH antisense: 5'-AAGTCACAGGAGACAACCTG-GTCC-3'. The source of the primer sequences used and expected product sized were: rat β ARK1 (334 bp) (30); bovine GRK5 (590 bp)

(31); and rat GAPDH (617 bp). The β ARK1 and GRK5 primer pairs were tested for their specificity on plasmids for either β ARK1 or GRK5, and these studies indicated that under the PCR conditions described below, amplified products were specific for that primer pair.

Specific PCR conditions were determined for the individual primer-pairs using a variety of annealing temperatures to select the optimal temperature which resulted in the amplification of only the expected product. Individual annealing temperatures were as follows: β ARK1, 63°C; GRK5, 62°C; and GAPDH, 55°C. For quantitation, the reaction was titrated to identify an appropriate cycle number. Initially 10 µl of a 100 µl PCR reaction containing tracer [α -³²P]dCTP was removed every three cycles from 27–42 to generate a standard curve (see Fig. 6). The aliquots were electrophoresed on 1.5% agarose gels and products were excised and counted by liquid scintillation. Final cycle numbers were selected that represented the mid-region of the linear amplification of product.

For quantitation of individual mRNAs, cDNA aliquots of different aged rat hearts were added to the specific primer-pairs containing [α -³²P]dCTP (0.5 µCi) and 2 mM MgCl₂. The PCR protocol was as follows: (a) three cycles of 94°C × 3 min, annealing temperature × 3 min, 72°C extension × 3 min; (b) selected number of cycles (β ARK1, 36; GRK5, 36; GAPDH, 27) at 94°C × 1 min, annealing × 1 min, 72°C × 1.5 min. After completion of the PCR reactions, the products were electrophoresed on 1.5% agarose gels and the amplified products were excised and counted by liquid scintillation. The GAPDH product was used to normalize the quantity of starting material. RNA was extracted from six hearts of each age group and all reactions were done in triplicate. [³²P]-incorporated amplified products were excised from gels and counted by liquid scintillation.

Drugs. CGP 20712A (CGP) was kindly supplied by CIBA-GEIGY Corp. (Basel, Switzerland); ICI 118,551 (ICI) was kindly supplied by Imperial Chemical Industry (London, United Kingdom); and zinterol was kindly supplied by Bristol-Myers (Evansville, IN).

Statistical analysis. Data are reported as mean ± SEM. The effects of age and PTX treatment on the contractile dose-response to NE or zinterol were tested via one way ANOVA, two factor ANOVA, and repeated measures ANOVA when appropriate. Radioligand binding, RT-PCR quantities and adenylyl cyclase activities among specific age groups were compared by one way ANOVA.

Results

Contractile response to β_2 -AR stimulation with aging. There were no significant differences in either the contraction amplitude or maximum shortening velocity among cells of the three age groups (2, 8, and 24 mo) under basal conditions, while the

Table I. Baseline Contraction Parameters in Single Rat Ventricular Myocytes of Varying Age

Age	DL (µm)	CT (% DL)	VS (µm/s)
2 mo (–PTX) (n = 50)	112.15 ± 2.33*	6.83 ± 0.31	96.24 ± 3.97
2 mo (+PTX) (n = 43)	108.79 ± 2.52	6.63 ± 0.29	91.71 ± 4.60
8 mo (–PTX) (n = 36)	131.62 ± 2.68	6.45 ± 0.32	89.49 ± 4.89
24 mo (–PTX) (n = 60)	133.18 ± 2.77	6.78 ± 0.24	99.01 ± 4.33
24 mo (+PTX) (n = 50)	132.37 ± 2.92	6.86 ± 0.25	98.19 ± 3.85
Overall age effect (–PTX)	0.001	NS	NS
+PTX versus –PTX	NS	NS	NS

CT, contraction amplitude; DL, diastolic cell length; VS, maximum shortening velocity. *P < 0.01, 2 mo versus 8 mo, and 24 mo (one-way ANOVA).

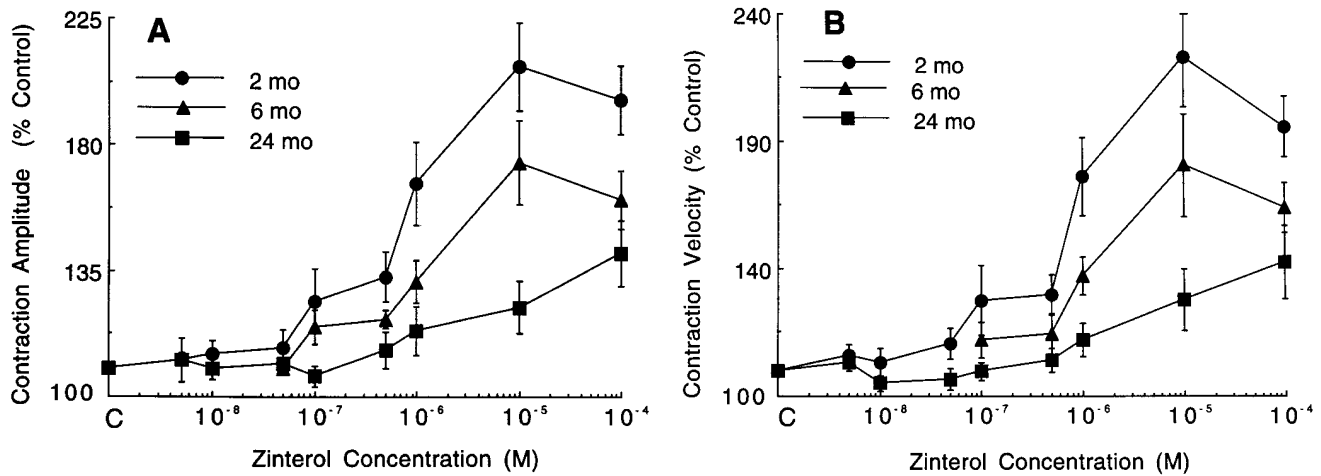


Figure 1. Dose response of the contraction amplitude (A) and the maximum shortening velocity (B) to a specific β_2 -adrenergic agonist, zinterol, in rat ventricular myocytes isolated from hearts of three age groups (2, 8, and 24 mo). The dose-response curves are shifted downward and rightward with aging. For both the contraction amplitude and velocity, the overall age effect is significant at $P < 0.001$ by analysis of two-factor ANOVA (note that two-factor ANOVA results are bar concentrations ranging from 10^{-7} to 10^{-4} M). Significant differences (by Bonferonni) are: 2 versus 8 mo; 2 versus 24 mo; and 8 versus 24 mo. Data are presented as percent of control value (see Table I). Each data point of the dose-response curves represents mean \pm SEM for 8–10 cells from eight hearts.

resting cell length (diastolic cell length) increased with aging, as noted previously (3, 4) (Table I). However, the responses of contraction amplitude and velocity to the β_2 -AR agonist, zinterol, decreased with aging (Fig. 1). The dose-response curves of contraction amplitude in cells from aged rats were shifted downward and rightward (Fig. 1 A). The EC_{50} was $8.0 \pm 0.6 \times 10^{-7}$ M, $1.5 \pm 0.1 \times 10^{-6}$ M and $6.0 \pm 0.4 \times 10^{-6}$ M for 2, 8, and 24 mo, respectively ($n = 8$ for each age group) ($P < 0.01$, 2 mo versus 8 mo and 24 mo; and 24 mo versus 8 mo). The zinterol-induced increase in contraction velocity was similarly blunted with increasing age (Fig. 1 B). These results indicate that both the potency and sensitivity of the cardiac myo-

cyte contractile response to β_2 -AR stimulation decreased with aging.

To compare the effect of age on the efficacy of β -AR subtype stimulation, we also measured contractile responses to β_1 -AR stimulation by NE in cells from the same hearts of 2- and 24-mo-old rats used for the β_2 -AR studies. Both the contraction amplitude and velocity responses to β_1 -AR stimulation by NE were significantly depressed with aging (Fig. 2). This is in agreement with our previous studies in which the ability of β_1 -AR stimulation by NE to increase cardiac calcium transients and contractility progressively diminished in aged hearts (3, 4).

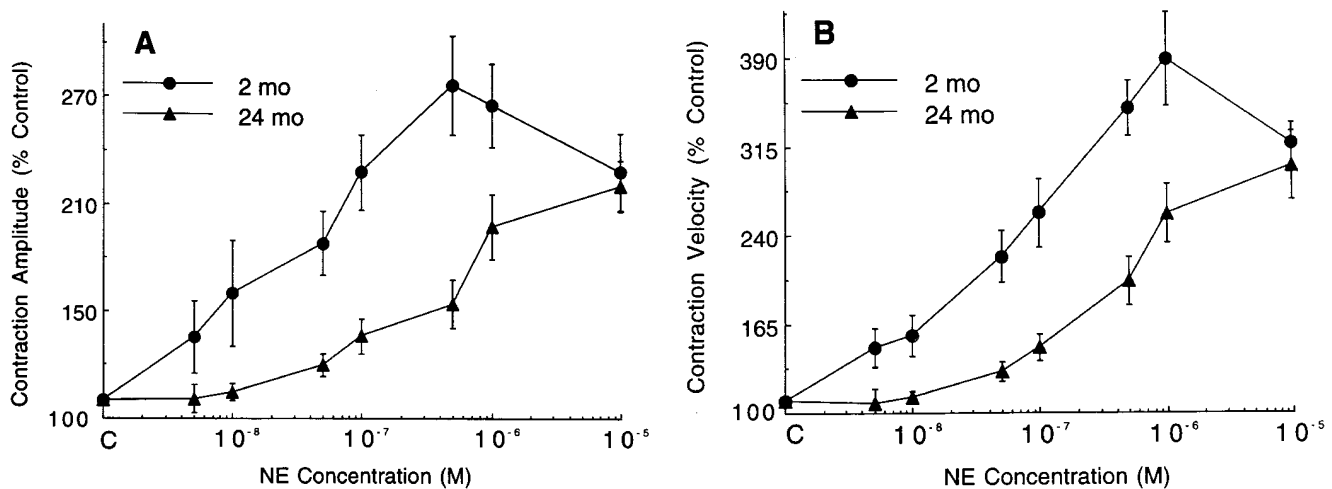


Figure 2. Dose response of the contraction amplitude (A) and the maximum shortening velocity (B) to a β_1 -AR agonist, NE, in rat ventricular myocytes isolated from young and old hearts (2 and 24 mo). For both the contraction amplitude and velocity, the age effect is significant at $P < 0.0001$ by analysis of two-factor ANOVA (note that two-factor ANOVA results are bar concentrations ranging from 5×10^{-9} to 10^{-6} M). Data are presented as percent of control value (see Table I). Each data point of the dose-response curves represents mean \pm SEM for 8–10 cells from eight hearts.

Table II. β -Adrenoceptor Radioligand Binding Properties in Left Ventricular Membranes Isolated from Rat Hearts of Varying Ages

Age	B _{max} (fmole per mg membrane protein)	Percent β 2ARs
2 mo	55.0±4.2	39.7±3.7
8 mo	45.2±2.9*	34.9±4.1
24 mo	39.4±2.2‡	36.3±4.1

n = 14 hearts for each age group; **P* < 0.05 compared to 2 mo (one way ANOVA); ‡*P* < 0.01 compared to 2 mo (one way ANOVA). IC₅₀s for ICI 118,551 competition binding are not different among three age groups. 2 mo: $\beta_2 = 1.50 \times 10^{-8}$ M, $\beta_1 = 2.78 \times 10^{-6}$ M; 8 mo: $\beta_2 = 1.48 \times 10^{-8}$ M, $\beta_1 = 3.58 \times 10^{-6}$ M; 24 mo: $\beta_2 = 1.33 \times 10^{-8}$ M, $\beta_1 = 3.86 \times 10^{-6}$ M.

β -adrenergic receptor density. Total β -AR density and β_1 and β_2 subpopulation studies were carried out on membranes from both LV and isolated, single ventricular myocytes of differing ages. Results for cardiac membranes are presented in Table II. Using saturating concentrations of the nonselective β -AR antagonist radioligand ¹²⁵I-CYP, a significant decrease (~20%) in the B_{max} for 8-mo-old rat hearts compared with 2-mo-old hearts (45.2±2.9 versus 55.0±4.2 fmol/mg membrane protein, respectively) was found. In 24-mo-old rat hearts, the B_{max} was 28% less than that of 2-mo-old rat hearts (Table II). A similar loss in β -AR density was observed in membranes purified from isolated ventricular myocytes (22.0±1.8 versus 15.3±1.0 fmol/mg membrane protein for 2 and 8-mo-old rat ventricular myocytes, respectively, *n* = 6, *P* < 0.01). An insufficient quantity of myocytes from 24-mo-old rat hearts precluded this measurement in this age group.

To determine the proportion of β_1 - and β_2 -ARs, competition isotherms with the β_2 -AR selective antagonist ICI 118,551 (ICI) were performed. Computer modeling indicated that a two-site model was preferred for these competition curves. The calculated β_2 -AR fraction remained constant (~35%) in all age groups (Table II) indicating that the loss in total β -AR density is due to losses in both β_1 -AR and β_2 -AR subtypes, as their relative proportions remain the same.

Adenylyl cyclase activity. While baseline adenylyl cyclase activity remained intact with aging, net maximum stimulation of adenylyl cyclase activity in response to β -AR agonists was severely blunted in 24-mo-old rat myocardium (Table III). The maximum absolute cyclase responses after a nonselective β -agonist, isoproterenol and the β_1 -AR selective agonist NE plus prazosin or the β_2 -AR selective agonist zinterol were reduced

Table III. Adenylyl Cyclase Activity in Left Ventricular Membranes Isolated from Rat Hearts of Varying Ages

Age	Basal	NE (10 ⁻⁵ M)	Zint (10 ⁻⁴ M)	ISO (10 ⁻⁴ M)	NaF (10 ⁻⁴ M)	Fors (10 ⁻⁴ M)
2 mo	3.83±0.36	16.86±2.63	6.27±0.22	8.72±1.36	94.32±3.23	99.5±6.99
8 mo	4.82±0.41	12.06±3.53	6.60±0.25	9.51±1.46	88.04±3.70	110.89±8.59
24 mo	3.05±0.38	8.94±0.47 [§]	3.31±0.53 [‡]	4.77±1.33*	49.09±11.92*	45.08±6.19 [‡]

Data expressed as pmol cAMP/mg/min (*n* = 4-7 hearts for each age group done in triplicate). Fors, forskolin; ISO, isoproterenol; NaF, sodium fluoride; Zint, zinterol. **P* < 0.05, ‡*P* < 0.01 compared to 2 mo and 8 mo samples (one way ANOVA); §*P* < 0.05 compared to 2 mo samples; ||*P* < 0.05 compared to 24 mo samples (one way ANOVA).

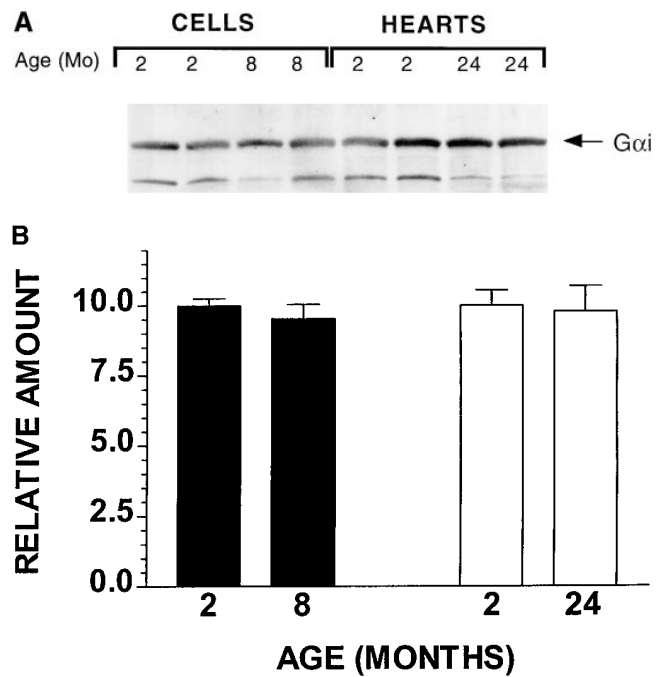


Figure 3. Abundance of G α_i . (A) Representative protein immunoblot for G α_i in membranes purified from isolated ventricular myocytes (2 and 8 mo) or heart tissue (2 and 24 mo). Duplicate samples of 40 μ g of membrane protein were electrophoresed and transferred to nitrocellulose as described in Methods. A commercially available polyclonal antiserum was used (G $\alpha_{i(1-3)}$, Santa Cruz Biotechnology) and the major immunoreactive band detected by standard chemiluminescence migrated at the expected size of G α_i (arrow, ~43 kD). (B) Histogram showing the mean + SEM of laser densitometry scans of four immunoblots from four individual hearts (open bars) or cell preparations (solid bars) of each age done in duplicate.

by ~50% in 24-mo-old hearts as compared with that in 2 and/or 8-mo-old hearts (Table III). Furthermore, the maximal post-receptor activation of adenylyl cyclase activity as measured by sodium fluoride, a G protein activator, or forskolin (an adenylyl cyclase activator) stimulation also significantly decreased with aging to a similar extent as did that after β -AR stimulation (Table III).

Abundance and physiologic activity of inhibitory G proteins. Since some G_i proteins work as negative regulators of β -AR signaling (28), we investigated whether G_i function or expression is enhanced with aging. Fig. 3A shows a representative Western blot of membrane proteins purified from 2 and

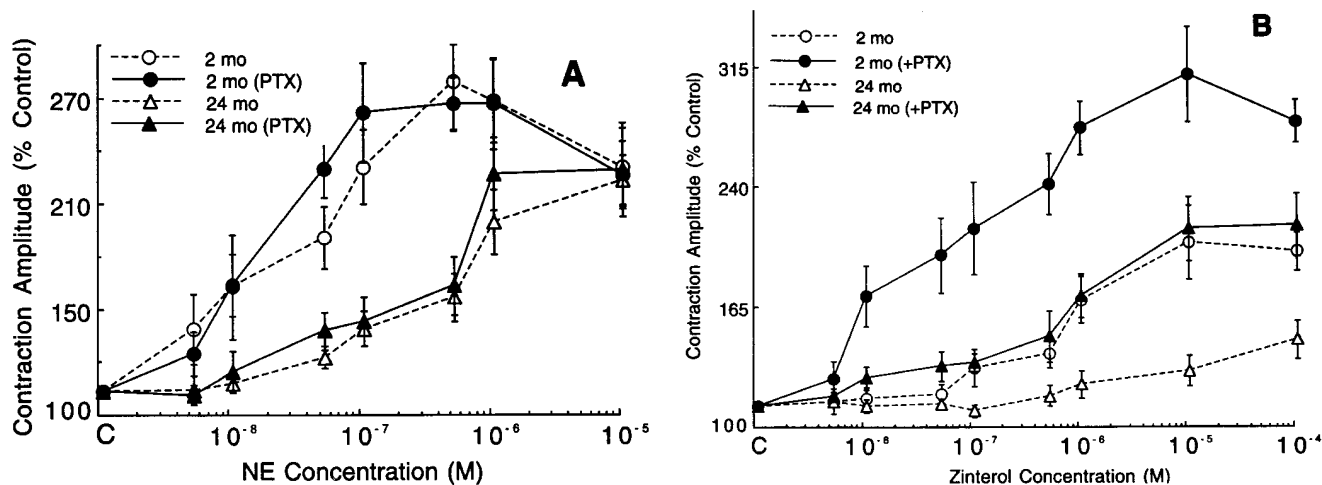


Figure 4. PTX pretreatment does not change the positive inotropic effect of β_1 -AR stimulation by NE (A), but specifically potentiates the effect of β_2 -AR stimulation (B) in both young and old groups (2 and 24 mo). However, PTX treatment does not reverse the age-associated reduction in contractile response to β_2 -AR agonist, zinterol (B). Data are presented as percent of control value (Table I). Each data point represents mean \pm SEM for 8–10 cells from eight hearts.

24-mo-old rat hearts or isolated cells from 2 and 8-mo-old hearts using G_{α_i} polyclonal antiserum (see Methods). No apparent age-associated change in G_{α_i} expression in rat heart membranes was observed (Fig. 3 B). Functional G_i activity was examined in single cardiac myocytes. Incubation of cells with PTX had no significant effects on the contraction under basal conditions (Table I). The positive inotropic effect of β_1 -AR stimulation was not significantly changed by PTX treatment in cells from 2 and 24-mo-old hearts (Fig. 4 A). In contrast, PTX pretreatment markedly enhanced the positive inotropic effect of β_2 -AR stimulation by zinterol, but equally in cells from both young and old groups (2 and 24 mo) (Fig. 4 B). Thus, the age-associated reduction in contractile response to β_2 -AR stimulation was not reversed by PTX pretreatment (Fig. 4 B) although the contractile response to zinterol in the PTX-treated 24-mo-old heart cells equaled non-PTX responses of 2-mo-old cells. Therefore, both our physiological and biochemical data indicate that the decreased positive inotropic effects of β_1 -AR or β_2 -AR stimulation with aging are not caused by an age-associated upregulation of PTX-sensitive G protein abundance or activity.

Activity and expression of GRKs. In principle, an increase in GRK-mediated β -AR desensitization could also account for the age-associated decline in the β -AR-induced contractile response. To test this hypothesis, the expression and activity of the myocardial GRKs, β ARK1 and GRK5, were examined. First, the enzymatic activity of soluble β ARK1 on the G protein-coupled receptor substrate rhodopsin (see Methods) was measured in cytosolic extracts purified from rat hearts of 2, 8, and 24 mo of age. Previous studies by our laboratory on myocardial extracts have shown that β ARK1 is the predominant GRK in soluble fractions while GRK5 is present exclusively in the membrane fraction (19, 21). As shown in Fig. 5, there were no apparent age-associated changes in soluble GRK activity, suggesting no enhanced activation or upregulation of β ARK1. Additionally, protein immunoblotting of myocardial cytosolic fractions revealed that the levels of β ARK1 do not change with age (data not shown). Likewise, protein immunoblots of

purified membrane fractions revealed no changes in the expression of GRK5 (data not shown).

Steady state mRNA levels of β ARK1 and GRK5 were also determined by semi-quantitative RT-PCR using the reference gene GAPDH as a control. Total RNA was isolated from the LV of rat hearts 2, 8, and 24 mo of age and specific products were amplified from reverse transcribed cDNA (see Methods). As shown in Fig. 6, no age-associated changes in the mRNA expression of β ARK1 or GRK5 was observed. Similar results were seen in RT-PCR experiments done with RNA isolated from single ventricular myocytes (data not shown).

Discussion

A reduction in the efficacy of cardiac β -AR stimulation with aging could, mechanistically, result from any of the following aspects in β -AR signal transduction: (a) defects in the excitation-contraction coupling machinery, the major effector system of β -AR modulation in the heart; (b) alterations in the β -AR-G protein-adenylyl cyclase-PKA signaling cascade per se; (c) a selective downregulation of β_1 -AR system, as manifested in human and canine heart failure; (d) an upregulation of GRK-mediated β -AR desensitization; and (e) an upregulation of the inhibitory G proteins, which might be particularly important for β_2 -AR signaling since it has been shown to specifically couple to G_i proteins as well as to G_s (12, 14). While previous studies of this laboratory and others have demonstrated multiple alterations in the β -AR signaling cascade with aging (1–4, 23–26) and ruled out an age-associated change in cardiac excitation-contraction coupling (3, 4), little is known regarding other potential age-associated alterations, e.g., changes in cardiac contractile response to β -AR subtype stimulation, expression or activity of GRKs and G_i proteins. The overall goal of this study was to examine the possible mechanisms of the age-associated diminution of cardiac response to β_2 -AR versus β_1 -AR stimulation and possible involvement of GRKs and G_i proteins.

Contribution of β_1 - and β_2 -AR to the overall age-associated

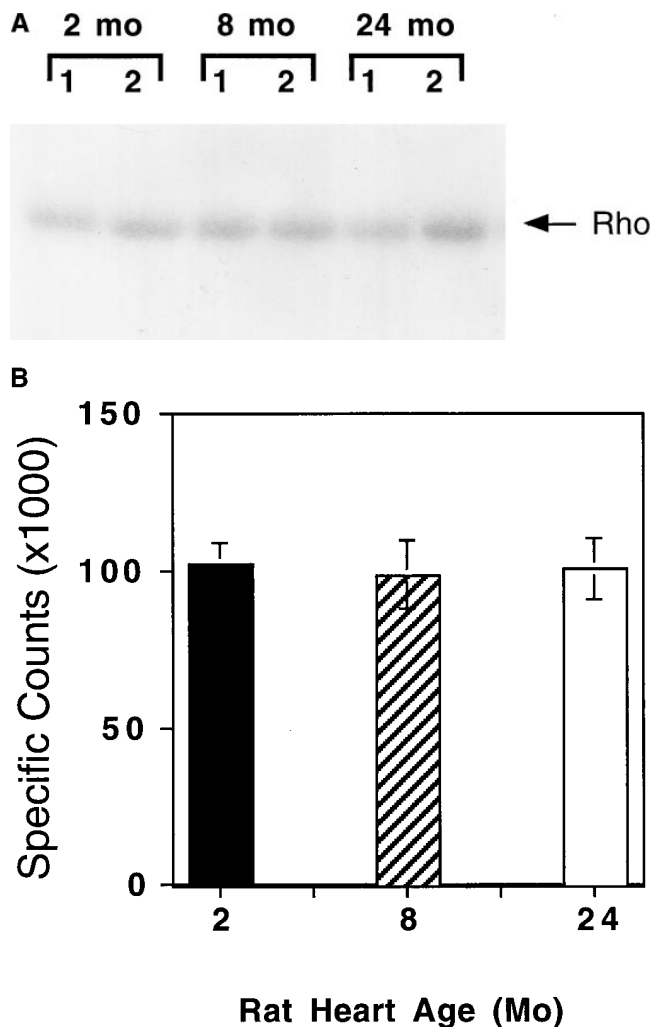


Figure 5. Soluble GRK (representing β ARK1) activity in myocardial left ventricular extracts from different aged rat hearts. (A) Representative autoradiogram of a dried gel showing phosphorylated rhodopsin (*Rho*, arrow), where duplicate samples (50 μ g protein) of each of three different aged hearts were assayed (see Methods). (B) Histogram of composite data of GRK activity results. Data shown is the mean \pm SEM of experiments of four separate left ventricles of each age done in duplicate expressed as specific counts determined using a Molecular Dynamics PhosphorImager (see Methods).

diminution in cardiac contractile response. First, in this study, we examined age-associated changes in cardiac contractile response to β_2 -AR as well as to β_1 -AR stimulation in the rat heart. Previous studies on age-associated changes in cardiac contractile response did not characterize the contributions of different β -AR subtypes. While a recent study in excised human myocardium has shown an age-associated reduction in the response of adenylyl cyclase to both β_1 - and β_2 -AR stimulation (26), the contractile response to β_2 -AR stimulation in human myocardium of different aged donors has to date not been examined. Since our recent studies have demonstrated that the β_1 -AR and β_2 -AR couple differently to cellular responses, at least in rat and canine ventricular cells (11, 13), measurements of adenylyl cyclase in response to β_2 -AR stimulation may be insufficient to characterize the functional effect

of the β_2 -AR signaling pathway. Thus, a direct assessment of the contractile response to β_2 -AR stimulation is essential to determine its functional consequence. Novel findings of this study are that β_2 -ARs, like β_1 -ARs, functionally exist in young and old rat ventricular myocytes and that the contractile responses to β_2 -AR stimulation, like that after β_1 -AR stimulation, are markedly decreased with aging (Figs. 1 and 2). This suggests that the decreased cardiac response to β -AR stimulation by mixed β -AR agonists, e.g., isoproterenol, as reported in previous studies both in human and animal hearts (23–26; for review see reference 1), reflects deficits in both β -AR subtype systems. Further, since the β_2 -AR stimulated increase in L-type Ca^{2+} currents remains the same in young and old rat ventricular myocytes (32), the diminished β_2 -AR contractile response suggests that the sarcoplasmic reticulum amplification function is reduced in the aged heart, as is the case in hypertrophied or failing hearts (33).

A nonselective age-associated reduction in β -AR density. Whether aging causes a selective downregulation of myocardial β -AR subtypes (β_1 versus β_2) has been controversial (26, 32, 34). Cerbai et al. (32) have shown recently that in rat ventricular myocytes the density of β_1 -AR, but not of β_2 -AR, decreases with aging, whereas Tumer et al. (34) have shown that both β_1 -AR and β_2 -AR are nonselectively decreased in aged rat hearts. The inconsistency might be due to the different experimental conditions, i.e., the former study was performed in isolated myocytes and the latter used heart tissues. Therefore, in this study, possible age effects on the densities of β -AR subtypes were determined in both isolated ventricular myocytes and myocardial tissues. Our results show that age nonselectively modulates the densities of β -AR subpopulations in rat myocardium or ventricular myocytes, as there was a significant ($\sim 30\%$) age-associated decline in both β_1 - and β_2 -ARs (Table II). However, the functional significance of a 30% decrease in β -AR number could be questionable, as rat hearts, unlike the human heart, are thought to have spare β -ARs (35). In this study, the nonselective decrease in β -AR density demonstrated contrasts to what has been found recently in older explanted human hearts where only β_1 -ARs showed a similar decrease in density (26). This might be caused by species difference or the presence of underlying disease in the explanted human hearts. The nonselective downregulation of both β -AR subtypes with aging is in sharp contrast to failing hearts, in which β_1 -AR is selectively downregulated with little or no change in β_2 -AR density (9, 27, 36, 37). This suggests that aging is different from heart failure in many important aspects, even though both are associated with diminished cardiac β -AR responsiveness and elevated plasma catecholamine levels.

Changes in cardiac adenylyl cyclase activity with aging. Our results indicate that the cardiac adenylyl cyclase responses to stimulation of both β -AR subtypes as well as to NaF (a G protein activator) or forskolin (an adenylyl cyclase activator) are markedly decreased with aging (Table III). This is in agreement with previous reports that a loss of catalytic unit number and/or the capacity to activate the catalytic units appears to be responsible for the age-associated decrease in the maximal β -AR-stimulated adenylyl cyclase activation (25, 38, 39). Because the contractile effect of β_1 -AR stimulation in isolated rat heart cells under these conditions is tightly controlled by the cAMP-dependent signaling pathway (11, 14), any age-associated change in signaling linked to a reduction in cAMP production would be expected to result in an age-associated

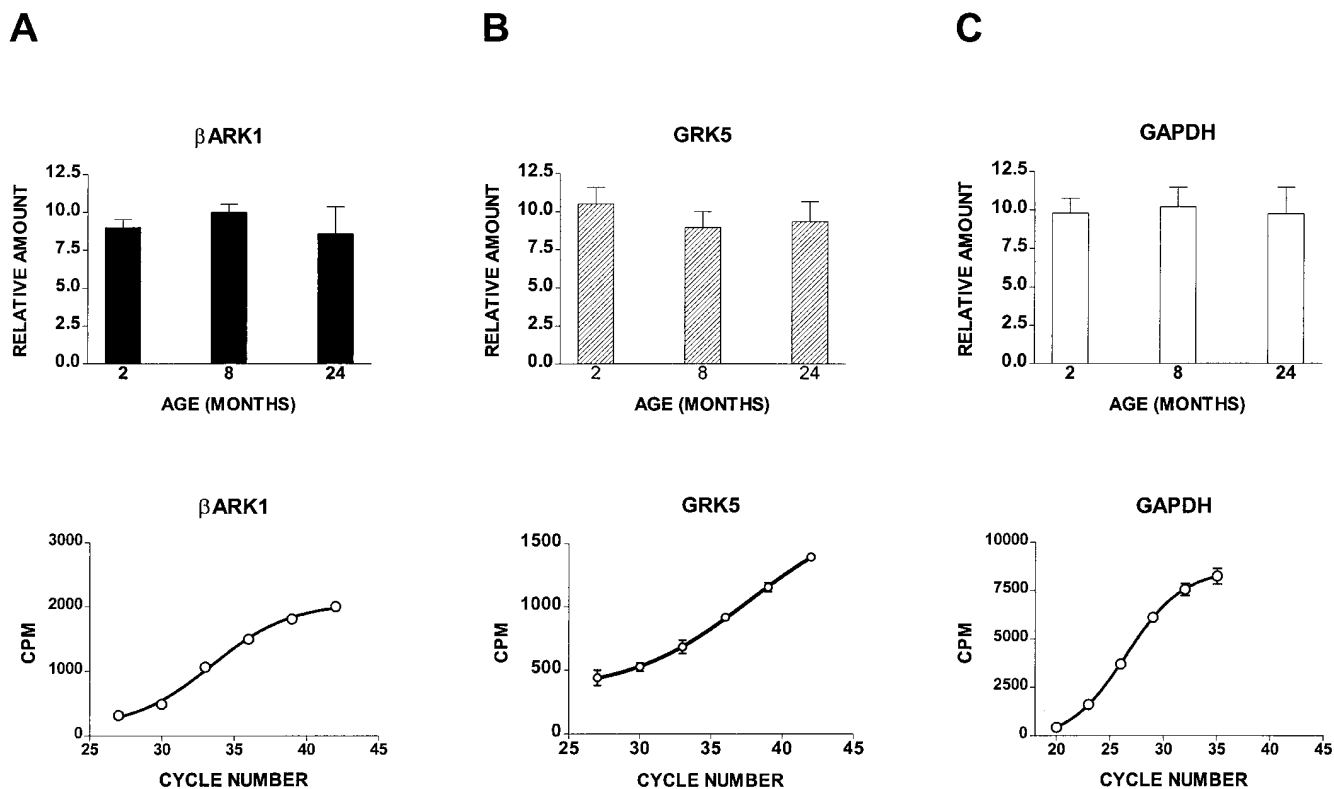


Figure 6. Semi-quantitative RT-PCR analysis of GRK mRNA. Histograms showing mRNA levels of β ARK1 (A); GRK5 (B); and GAPDH (C) in rat hearts (LV) of the indicated ages. RT-PCR on total RNA was performed as described in Methods. Levels of β ARK1 (A) and GRK5 (B) mRNA from individual samples were normalized to the specific counts of the control GAPDH mRNA (C). Levels of the 2-mo products were arbitrarily assigned the value of 10 and remaining products were normalized to this value (see Methods). Below each set of histograms is the linearity curve demonstrating that conditions for each specific primer pair were determined systematically (see Methods) by plotting product amplification by cycle number and choosing a final cycle number in the above reactions that were in the linear range. The linearity curves were generated with RNA isolated from 2-mo-old rat hearts.

decrease in cardiac β_1 -AR responsiveness. Therefore, in addition to a decrease in β_1 -AR number, changes in adenylyl cyclase activity may also contribute to the depressed β_1 -AR response in aged rat hearts.

While the β_2 -AR-stimulated positive inotropic effect initially appeared to be dissociated from cAMP production and cAMP-dependent protein phosphorylation in rat cardiac myocytes (11, 13), our more recent studies have demonstrated that the β_2 -AR stimulated increases in L-type calcium current, intracellular Ca^{2+} transient, and contractility are abolished by inhibition of PKA via inhibitory cAMP analogues, Rp-cAMP or Rp-CPT-cAMP (14), suggesting that a localized (compartmentalized) increase in adenylyl cyclase activity-cAMP within subcellular domains mediates the positive inotropic effect of β_2 -AR stimulation in cardiac myocytes (14). Thus, like the case of β_1 -AR stimulation, the age-associated reduction in cardiac membrane adenylyl cyclase activity, as well as the downregulation of β_2 -AR density, may contribute to the defect of cardiac β_2 -AR stimulation with aging.

No functional or biochemical changes of G_i proteins was revealed with aging. While some studies have shown that protein levels of G_i and PTX-mediated G_i ribosylation increase with aging in rat hearts (40), others have shown that G_i apparently is not altered with aging (26, 41). Our results not only

confirm that there is no age-associated difference in the levels of $G_{\alpha i}$ in the membranes of either rat ventricular myocytes or heart tissue (Fig. 3), but also extend the investigation to physiological responses, particularly, to the cell contractile response. PTX treatment cannot eliminate the age-associated reduction in the β -AR responsiveness while it markedly and specifically enhances the positive inotropic effect of β_2 -AR at all ages (Fig. 4). Additionally, PTX has no effect on the response to β_1 -AR stimulation in either young or old age group (Fig. 4). Thus, both the biochemical and physiological data indicate that the age effect on β -AR signaling does not result from an alteration in PTX-sensitive G protein (G_i/G_o) abundance or functional activity. Again, these results differ markedly from the observation in failing hearts, in which the amount and activity of G_i proteins are significantly elevated (42, 43), further suggesting that distinctly different mechanisms may be involved in heart failure versus normal aging.

No enhanced β ARK1 and GRK5 abundance or functional activity in aged hearts. Several characteristics are shared by both the aged and failing heart in human and animal models. Deficits in contractile β -AR responsiveness and adenylyl cyclase activity occur in both cases. Other similarities reported between the failing and aged heart include morphological, mechanical contractile, biochemical, and molecular changes (for

review see reference 44). In addition, in both chronic heart failure and aging in health, circulatory catecholamines are increased (22, 23, 26), and this can have profound effects on the myocardial β -AR system. Because of these striking similarities, we investigated specifically, in aged rat hearts, desensitization and inhibition mechanisms of β -ARs, which have been the focus of several studies in the failing heart (27, 36, 42, 43). In addition to β ARK1, we also measured the abundance and activity of GRK5, a second GRK that is found predominantly in the heart (21). Enzymatic GRK activity of soluble myocardial extracts revealed no difference among three age groups (Fig. 5), suggesting that β ARK1 activation is not altered. Protein and mRNA studies also revealed no molecular alterations of β ARK1 or GRK5 due to aging, suggesting that enhanced GRK-mediated β -AR desensitization is not involved in the diminished contractile response seen in aged ventricular myocytes. Our findings eliminate the possibility that GRKs or G_i proteins play a role in the diminished β -AR functional response in the aged rat heart. This is in contrast to the findings in human heart failure that increased sympathetic drive results in selective β_1 -AR downregulation (9, 27, 36, 37), increases in functional activity and levels of G_i (36, 42, 43) and β ARK1 (27). Although the data described above indicate that GRKs are not altered by aging, the increase in the ratio of GRKs to β -ARs, because of the nonselective decrease in β -AR density in aged hearts, might result in a relatively enhanced age-related β -AR desensitization. In this regard, the previous studies of Scarpace and Abrass (45) and Scarpace (46) have demonstrated that β -AR desensitization contributes, at least in part, to the age-dependent changes in β -AR signaling, since β -ARs in aged animals may be already partially desensitized under basal conditions. To address whether GRK- or PKA-mediated β -AR desensitization is an important determinant in the age effects, further studies are required. For example, it will be informative to determine whether β -ARs are more accessible for GRKs or PKA in aged hearts than in young ones, and whether inhibitors of these kinases can reverse the age-associated changes.

In summary, our results indicate a marked age-associated reduction in both β_1 -AR- and β_2 -AR-mediated positive inotropy in isolated rat cardiomyocytes. This loss of β -AR responsiveness is associated with a small but significant nonselective downregulation of both β_1 - and β_2 -ARs and a decrease in the agonist-stimulated adenylyl cyclase activity. In addition, for the first time, our results indicate that desensitization and inhibition mechanisms of the β -AR system, i.e., an increased abundance or activity of GRKs or G_i proteins, do not occur with aging. Therefore, while the current thought is that aging and heart failure have many features in common, this study showed that they are different in very important ways: the diminished β -AR responsiveness in failing hearts is associated with (a) a selective downregulation of β_1 -ARs; (b) an increase in β ARK1; and (c) an upregulation of G_i proteins. Thus, our findings not only provide insights for understanding normal senescence processes, but also have important implications for studying the pathogenesis of heart failure.

Acknowledgments

The authors wish to thank L.A. Smithwick, K. Shotwell, B. Ziman, and H. Spurgeon for their expert technical support, and M. Shiflett for excellent secretarial assistance.

References

- Xiao, R.-P., and E.G. Lakatta. 1992. Deterioration of β -adrenergic modulation of cardiovascular function with aging. In *Physiopathological Processes of Aging Towards a Multicausal Interpretation*. N. Fabris, D. Harman, D.L. Knook, E. Steinhagen-Thiessen, and I. Zs.-Nagy, editors. *Ann. NY Acad. Sci.* 673:293-310.
- Lakatta, E.G., G. Gerstenblith, C.S. Angell, W. Shock, and M.L. Weisfeldt. 1975. Diminished inotropic response of aged myocardium to catecholamines. *Circ. Res.* 36:262-269.
- Sakai, S., R.S., Danziger, R.-P. Xiao, H.A. Spurgeon, and E.G. Lakatta. 1992. Contractile response of individual ventricular cardiac myocytes to norepinephrine declines with senescence. *Am. J. Physiol.* 262: H184-H189.
- Xiao, R.-P., H.A. Spurgeon, F. O'Connor, and E.G. Lakatta. 1994. Age-associated changes in β -adrenergic modulation on rat cardiac excitation-contraction coupling. *J. Clin. Invest.* 94:2051-2059.
- Xiao, R.-P., and E.G. Lakatta. 1993. β_1 -adrenoceptor stimulation and β_2 -adrenoceptor stimulation differ in their effects on contraction, cytosolic calcium, and calcium current in single rat ventricular cells. *Circ. Res.* 73:286-300.
- Cerbai, E., I. Masini, and A. Mugelli. 1990. Electrophysiological characterization of cardiac β_2 -adrenoceptors in sheep Purkinje fibers. *J. Mol. Cell. Cardiol.* 22:859-870.
- Brodde, O.-E. 1988. The functional importance of β_1 and β_2 adrenoceptors in the human heart. *Am. J. Cardiol.* 62:24C-29C.
- Motomura, S., R.-H. Zerkowski, A. Daul, and O.E. Brodde. 1990. On the physiologic role of β_2 -adrenoceptors in the human heart: in vitro and in vivo studies. *Am. Heart J.* 119:608-619.
- Bristow, M.R., R. Ginsburg, V. Umans, M. Fowler, W. Minobe, R. Rasmussen, P. Zera, R. Menlove, P. Shah, S. Jamieson, and E.B. Stinson. 1986. β_1 - and β_2 -adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1 -receptor downregulation in heart failure. *Circ. Res.* 59: 297-309.
- Kuznetsov, V., E. Pak, P.B. Robinson, and S.F. Steinberg. 1995. β_2 -adrenergic receptor actions in neonatal and adult rat ventricular myocytes. *Circ. Res.* 76:40-52.
- Xiao, R.-P., C. Hohl, R. Altschuld, L. Jones, B. Livingston, B. Ziman, B. Tanti, and E.G. Lakatta. 1994. 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.
- Xiao, R.-P., X. Ji, and E.G. Lakatta. 1995. Functional coupling of the β_2 -adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. *Mol. Pharmacol.* 47:322-329.
- Altschuld, R.A., R.C. Starling, R.L. Hamlin, J. Hensley, L. Castillo, R.H. Fertel, C.M. Hohl, P.-M. Robitaille, L.R. Jones, R.-P. Xiao, and E.G. Lakatta. 1995. Response of failing canine and human heart cells to β_2 -adrenergic stimulation. *Circulation.* 92:1612-1618.
- Zhou, Y.-Y., H. Cheng, K. Bogdanov, C. Hohl, R. Altschuld, E.G. Lakatta, and R.-P. Xiao. 1997. Localized cAMP-dependent pathway mediates β_2 -adrenergic stimulation in rat ventricular myocytes. *Am. J. Physiol.* 273: H1611-H1618.
- Hausdorf, W.P., M.G. Caron, and R.J. Lefkowitz. 1990. Turning off the signal: Desensitization of β -adrenergic receptor function. *FASEB. J.* 4:2881-2889.
- Inglese, J., N.J. Freedman, W.J. Koch, and R.J. Lefkowitz. 1993. Structure and mechanism of the G protein-coupled receptor kinases. *J. Biol. Chem.* 268:23735-23738.
- Freedman, N.J., S.B. Liggett, D.E. Drachman, G. Pei, M.G. Caron, and R.J. Lefkowitz. 1995. Phosphorylation and desensitization of the human β_1 -adrenergic receptor: involvement of G protein-coupled protein kinase. *J. Biol. Chem.* 270:17953-17961.
- Lohse, M.J., J.L. Benovic, J. Codina, and M.G. Caron. 1990. β -arrestin: a protein that regulates β -adrenergic receptor function. *Science.* 248:1547-1550.
- Koch, W.J., H.A. Rockman, P. Samama, R. Hamilton, R.A. Bond, C.A. Milano, and R.J. Lefkowitz. 1995. Cardiac function in mice overexpressing the β -adrenergic receptor kinase or a β ARK inhibitor. *Science.* 268:1350-1353.
- Korzick, D.H., R.-P. Xiao, B.D. Ziman, W.J. Koch, R.J. Lefkowitz, and E.G. Lakatta. 1997. Transgenic manipulation of the β -adrenergic receptor kinase1 (β ARK1) modifies contractile responsiveness to β_1 -adrenergic stimulation in single cardiac myocytes. *Am. J. Physiol.* 41:H590-H596.
- Rockman, H.A., D.-J. Choi, N.U. Rahman, S.A. Akhter, R.J. Lefkowitz, and W.J. Koch. 1996. Receptor specific in vivo desensitization by the G protein-coupled receptor kinase-5 in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 93:9954-9959.
- Fleg, J.L., S.P. Tzankoff, and E.G. Lakatta. 1985. Age-related augmentation of plasma catecholamines during dynamic exercise in healthy males. *J. Appl. Physiol.* 59:1033-1039.
- Ng, A.V., R. Callister, D.G. Johnson, and D.R. Seals. 1993. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension (Dallas).* 21:498-503.
- Abrass, I.B., J.L. Davis, and P.J. Scarpace. 1982. Isoproterenol responsiveness and myocardial β -adrenergic receptors in young and old rats. *J. Gerontol.*

tol. 37:156–160.

25. Shu, Y., and P.J. Scarpace. 1994. Forskolin binding sites and G-protein immunoreactivity in rat hearts during aging. *J. Cardiovasc. Pharmacol.* 23:188–193.
26. White, M., R. Roden, W. Minobe, M.F. Khan, P. Larrabee, M. Wollmering, D. Port, F. Anderson, D. Campbell, A.M. Feldman, and M.R. Bristow. 1994. Age-related changes in β -adrenergic neuroeffector systems in the human heart. *Circulation.* 90:1225–1238.
27. Ungerer, M., M. Böhm, J.S. Elce, E. Erdman, and M.J. Lohse. 1993. Altered expression of β -adrenergic receptor kinase and β_1 -adrenergic receptors in the failing human heart. *Circulation.* 87:454–463.
28. Dohlmán, H.G., J. Thorner, M.G. Caron, and R.J. Lefkowitz. 1991. Model systems for the study of seven-transmembrane-segment receptors. *Annu. Rev. Biochem.* 60:653–688.
29. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
30. Ungerer, M., K. Kessebohm, K. Kronsbein, M.J. Lohse, and G. Richardt. 1996. Activation of β -adrenergic receptor kinase during myocardial ischemia. *Circ. Res.* 79:455–460.
31. Premont, R.T., W.J. Koch, J. Inglese, and R.J. Lefkowitz. 1994. Identification, purification and characterization of GRK5, a member of the family of G protein-coupled receptor kinases. *J. Biol. Chem.* 269:6832–6841.
32. Cerbai, E., L. Guerra, K. Varani, M. Barbieri, P.A. Borea, and A. Mugelli. 1995. β -adrenoceptor subtypes in young and old rat ventricular myocytes: a combined patch-clamp and binding study. *Br. J. Pharmacol.* 116:1835–1842.
33. Gómez, A.M., H.H. Valdivia, H. Cheng, M.R. Lederer, L.F. Santana, M.B. Cannell, S.A. McCune, R.A. Altschuld, and W.J. Lederer. 1997. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science.* 276:800–806.
34. Tümer, N., W.T. Houck, and J. Roberts. 1989. Upregulation of adrenergic beta receptor subtypes in the senescent rat heart. *Mech. Ageing Dev.* 49:235–243.
35. Brown, L., N.M. Deighton, S. Bals, W. Sohlmann, H.R. Zerkowski, M.C. Michel, and O.-E. Brodde. 1992. Spare receptors for β -adrenoceptor-mediated positive inotropic effects of catecholamines in the human heart. *J. Cardiovasc. Pharmacol.* 19:222–232.
36. Kiuchi, K., R.P. Shannon, K. Komamura, D.J. Cohen, C. Bianchi, C.J. Homcy, S.F. Vatner, and D.E. Vatner. 1993. Myocardial β -adrenergic receptor function during the development of pacing-induced heart failure. *J. Clin. Invest.* 91:907–914.
37. Bristow, M.R., W. Minobe, M.V. Reynolds, J.D. Port, R. Rasmussen, P.E. Ray, and A.M. Feldman. 1993. Reduced β_1 receptor messenger RNA abundance in the failing human heart. *J. Clin. Invest.* 92:2737–2745.
38. Narayanan, N., and J.A. Derby. 1982. Alterations in the properties of β -adrenergic receptors of myocardial membranes in aging: impairments in agonist-receptor interactions and guanine nucleotide regulation accompany diminished catecholamine responsiveness of adenylate cyclase. *Mech. Ageing Dev.* 19:127–139.
39. Scarpace, P.J. 1990. Forskolin activation of adenylate cyclase in rat myocardium with age: effects of guanine nucleotide analogs. *Mech. Ageing Dev.* 52:169–178.
40. Böhm, M., H. Dorner, P. Htun, H. Lensche, D. Platt, and E. Erdmann. 1993. Effects of exercise on myocardial adenylate cyclase and $G_{i\alpha}$ expression in senescence. *Am. J. Physiol.* 264:H805–H814.
41. Chin, J.H., A.N. Hiremath, and B.B. Hoffman. 1996. cAMP signaling mechanisms with aging in rats. *Mech. Ageing Dev.* 86:11–26.
42. Feldman, A.M. 1993. Modulation of adrenergic receptors and G-transduction protein in failing human ventricular myocardium. *Circulation.* 87:IV27–IV34.
43. Eschennhagen, T., U. Mende, M. Nose, W. Schmitz, H. Schole, A. Haverich, S. Hirt, P. DorKalmar, W. Hoppner, and H.J. Seitz. 1992. Increased messenger RNA level of the inhibitory G protein α subunit $G_{i\alpha 2}$ in human end-stage heart failure. *Circ. Res.* 70:688–696.
44. Lakatta, E.G. 1993. Cardiovascular regulatory mechanisms in advanced age. *Physiol. Rev.* 73:413–467.
45. Scarpace, P.J., and I.B. Abrass. 1986. β -adrenergic agonist-mediated desensitization in senescent rats. *Mech. Ageing Dev.* 35:255–264.
46. Scarpace, P.J. 1988. Decreased receptor activation with age. Can it be explained by desensitization? *J. Am. Geriatr. Soc.* 36:1067–1071.