Cardiac G protein-coupled receptors that function through stimulatory G protein $\alpha_i$, such as $\beta_1$- and $\beta_2$-adrenergic receptors ($\beta_1$ARs and $\beta_2$ARs), play a key role in cardiac contractility. Recent data indicate that several $\alpha_i$-coupled receptors in heart also activate $\alpha_i$, including $\beta_2$ARs (but not $\beta_1$ARs). Coupling of cardiac $\beta_2$ARs to $\alpha_i$ inhibits adenyl cyclase and opposes $\beta_2$AR-mediated apoptosis. Dual coupling of $\beta_2$AR to both $\alpha_i$ and $\alpha_i$ is likely to alter $\beta_2$AR function in disease, such as congestive heart failure in which $\alpha_i$ levels are increased. Indeed, heart failure is characterized by reduced responsiveness of $\beta$ARs. Cardiac $\beta$AR-responsiveness is also decreased with aging. However, whether age increases cardiac $\alpha_i$ has been controversial, with some studies reporting an increase and others reporting no change. The present study examines $\alpha_i$ in left ventricular membranes from young and old Fisher 344 rats by employing a comprehensive battery of biochemical assays. Immunoblotting reveals significant increases with age in left ventricular $\alpha_i$ but no changes in $\alpha_i$, $\alpha_i$, $\alpha_i$, $\beta_1$, or $\beta_2$. Aging also increases ADP-ribosylation of pertussis toxin-sensitive G proteins. Consistent with these results, basal as well as receptor-mediated incorporation of photoaffinity label [$^{32}$P]azidoanilido-GTP indicates higher amounts of $\alpha_i$ in older left ventricular membranes. Moreover, both basal and receptor-mediated adenyl cyclase activities are lower in left ventricular membranes from older rats, and disabling of $\alpha_i$ with pertussis toxin increases both basal and receptor-stimulated adenyl cyclase activity. Finally, age produces small but significant increases in muscarinic potency for the inhibition of both $\beta_1$AR- and $\beta_2$AR-stimulated adenyl cyclase activity. The present study establishes that $\alpha_i$ increases with age and provides data indicating that this increase dampens adenyl cyclase activity.

Cardiac contractility is controlled by several G protein-coupled receptors (GPCRs), such as $\beta_1$- and $\beta_2$-adrenergic receptors ($\beta$ARs and $\beta_2$ARs), that function through stimulatory GTP-binding regulatory proteins ($\alpha_i$) and activate adenyl cyclase (AC). Activation of AC increases the formation of cAMP, which activates cAMP-dependent protein kinase A resulting in the phosphorylation of proteins controlling cardiac excitation-contraction (1). An important recent discovery is that $\beta_2$ARs (but not $\beta_1$ARs) in both rat (2, 3) and human heart (4) also activate $\alpha_i$, a G$\alpha$-subunit that inhibits AC (5). We also demonstrated that $\alpha_i$ couples to several other $\alpha_i$-coupled receptors in human heart, including receptors for histamine, glucagon, and serotonin (4). Coupling of $\beta_2$AR and other $\alpha_i$-coupled receptors to $\alpha_i$ is relevant to cardiac function because inactivation of $\alpha_i$ by pertussis toxin (PTX) increases myocyte contractility in rat heart (6) and increases both basal and receptor-mediated AC activity in human heart (4). In addition to inhibiting AC, the $\alpha_i$ pathway in heart is involved in anti-apoptotic effects (7–9).

The dual coupling of $\beta_2$AR to both $\alpha_i$ and $\alpha_i$ is likely to alter $\beta_2$AR function in diseases in which cardiac $\alpha_i$ levels are increased, such as congestive heart failure and hypertensive cardiac hypertrophy (10–13). Indeed, both congestive heart failure and cardiac hypertrophy are characterized by a reduced responsiveness of $\beta$ARs. Similarly, a decline in the responsiveness of cardiac $\beta$ARs due to aging has been demonstrated in both humans (14–17) and rodents (18–21). The age-induced decrease in $\beta$AR responsiveness is characterized at the molecular level by decreased stimulation of AC and at the whole organ level by a decrease in heart rate, ejection fraction, and cardiac output.

There have been conflicting reports about the effect of age on cardiac $\alpha_i$ levels in both humans and rodents. In one study of human heart, $\alpha_i$ levels were measured in atrial appendages received from surgical patients, and it was found that $\alpha_i$ expression increased with age (17). Another study examined $\alpha_i$ expression in human ventricles from hearts that were not suitable for transplant and found no change in $\alpha_i$ with age (16). Similar studies in rat heart have yielded inconsistent results even when the same strain of rat was used. In Fisher 344 rats, Roth et al. (21) reported an age-associated increase in cardiac $\alpha_i$, which is reduced by chronic dynamic exercise. Johnson et al. (22) found an increase in $\alpha_i$ mRNA but no change in $\alpha_i$ protein. Two other reports found no increase in cardiac $\alpha_i$ protein (23, 24). In Sprague-Dawley rats, Bohm et al. (19) found that age increases cardiac $\alpha_i$, and the increase in $\alpha_i$ is reduced by exercise. In Wistar rats, Bazan et al. (25) reported an increase in cardiac $\alpha_i$ with age, but Xiao et al. (6) and Miyamoto et al. (26) found no change. Some of the reported differences on the effect of age may be attributable to experimental design. For example, the finding of Chin et al. (24) that age does not increase cardiac $\alpha_i$ in Fisher 344 rats may be explained by the fact that these investigators used 16-month-old rats for their old age group versus 24-month-old rats used...
by others. Moreover, most studies examined Go-i expression by immunoblotting only, and different Go-i antibodies were used. Nevertheless, the available evidence favors an increase in Go-i with age, as indicated by the recent review of Roka et al. (27). However, two recent reviews by Lakatta (28, 29) on global changes in cardiovascular aging state that Go-i in heart does not increase with age. We believe that this conclusion is premature.

The importance of Go-i in cardiac function underscores the need to establish whether or not cardiac Go-i is affected with age. Therefore, we undertook a detailed study on the effect of age on Go-i expression in rat ventricular membranes. We used Fisher 344 rats because these rats have been the most widely used rat strain for aging studies (30), and age-induced changes in cardiac structure have been characterized (31). Expression of the predominant cardiac subtypes of Go-i, as well as of Go-i1, Go-i2, and the major subtypes of Gβi, was assessed by immunoblotting. Levels of PTX-sensitive Go-i/Go-1 proteins were also examined by radiolabeling through PTX-catalyzed ADP-ribosylation. In addition, Go-i2 activity was assessed using photoaffinity labeling with [32P]azidoanilido-GTP (AA-[32P]GTP). We show age-dependent increases in both Go-i2 expression levels and activation of Go-i2 by βAR and other G protein-coupled receptors in heart. The age-induced increase in Go-i2 has the functional effect of suppressing AC activity, which is restored by disrupting receptor-Go-i coupling with PTX.

**EXPERIMENTAL PROCEDURES**

**Materials**—[R-(−)]-isoproterenol (−)bittartrate, ICI 118,551, and CGP 20712A were obtained from Research Biochemicals International (Natick, MA). Glucagon was from Peninsula Laboratories (San Carlos, CA). Forskolin, carbobol, alpenrol, and Ponceau S were from Sigma. [α-32P]ATP, [α-32P]GTP, [3H]cAMP, [32P]cAMP, [(−)-32P]iodo-2-deoxyuridine (I2-deoxy-(−)32P), [3H]Glucagon, [125I]iodocyanopindolol (125I-CYP), RM/1 antibody specific for Go-i2, was raised against the peptide sequence RMHLRQYELL, and AS/7 antibody specific for Gβi was prepared by our laboratory and was characterized previously (32). Recombinant Go-i protein standards from Escherichia coli were from Calbiochem (La Jolla, CA). Protein A-Sepharose and [32P]nicotinamide adenine dinucleotide ([32P]NAD) were from Amersham Biosciences. PTX was from List Biological Laboratories (Campbell, CA). SuperSignal chemiluminescent reagent was from Pierce.

**Animals**—Sixteen Fisher 344 rats (eight 3-month-olds and eight 24-month-olds) were obtained from the National Institute on Aging under an Institutional Animal Care and Use Committee-approved protocol. Animals were sacrificed by decapitation, and left ventricles were extracted immediately, frozen in liquid nitrogen, and stored at −80 °C.

**Membrane Preparation**—Samples were thawed, then homogenized in a Polytron PT3000 (Brinkmann Instruments, Westbury, NY) at medium speed, in 20 mM Tris (pH 7.4), 2 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, and 5 μg/ml aprotinin. Membranes were pelleted by centrifugation at 100,000 g for 30 min in an Optima TL ultracentrifuge (Beckman, Fullerton, CA). Membranes were resuspended, repelleted, and then resuspended again in the appropriate final resuspension buffer (see below) at ~2–4 mg of protein/ml. For photoaffinity labeling assays, the final resuspension buffer contained 50 mM Hepes (pH 7.4), 1 mM EDTA, 50 mM NaCl, and 2 mM benzamidine. For immunoblotting, ADP-ribosylations, and AC assays, the final resuspension buffer contained 75 mM Tris (pH 7.4), 125 mM MgCl2, 2 mM EDTA, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, 5 μg/ml aprotinin.

**Immunoblot Analysis**—Immunoblotting was performed by separating 10 μg of membrane protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by electrophoretic transfer onto polyvinylidene difluoride membranes. The membranes were stained for 5 min with 0.5% Ponceau S in 1% acetic acid to check for equal protein loading and transfer. The membranes were then blocked for 1 h with 10% nonfat milk in 20 mM Tris (pH 7.4), 500 mM NaCl, and 0.1% Tween 20, probed with either a 1:6000 dilution of RM/1, AS/7, or Gβi1 antibody, a 1:2000 dilution of anti-Gαs, or a 1:5000 dilution of Gβi2 antibodies, then incubated with a 1:2000 dilution of goat-anti-rabbit secondary antibody coupled to horseradish peroxidase. The immunoreactive proteins were visualized by incubation with SuperSignal chemiluminescent reagent and exposure to x-ray film. The autoradiograms showed prominent bands below the 45-kDa molecular mass marker that represented Go-i2 because it was immunoprecipitated with AS/7 antibody.

**Pertussin Toxin Treatment of Ventricular Membranes for AC Assays**—PTX (50 ng/μl) was activated by incubation with 100 mM DTT and 0.25% SDS for 30 min at 30 °C as described (34). The activation reaction was stopped by the addition of four volumes of 1 mg/ml ice-cold bovine serum albumin (BSA). Activated PTX (1 ng/ml) was added to 10 μg of control or PTX-treated ventricular membranes (20 μg protein) were added into a total volume of 50 μl containing 37 mM Tris (pH 7.4), 6.25 mM MgCl2, 2 mM EDTA, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, and 5 μg/ml aprotinin, at ~0.5–1.5 μg of protein/ml. A second tube containing the same volumes of all constituents, with the exception of H2O in place of PTX, was treated in the same fashion and used as a control.

**ADP-ribosylation of PTX-sensitive G Proteins—PTX (50 ng/μl) was activated by incubation with 100 mM DTT and 0.25% SDS for 30 min at 30 °C as described (34). The activation reaction was stopped by the addition of four volumes of 1 mg/ml ice-cold BSA. Activated PTX (1 ng/ml) was added to membranes (1.0–1.5 mg/ml protein) in a buffer containing 6.25 mM MgCl2, 2.5 mM ATP, 4 mM GTP, 10 mM thymidine, 10 mM DTT, 0.005% SDS, 0.08 mg/ml BSA, 0.5 mM benzamidine, 5 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, and 2.5 μg/ml aprotinin. The mixture was incubated for 30 min at 30 °C, then an equal volume was added of ice-cold 50 mM Tris (pH 7.4) with 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, and 5 μg/ml aprotinin. Membranes were pelleted by centrifugation at 100,000 × g in an Optima TL ultracentrifuge and resuspended in the final resuspension buffer containing 75 mM Tris (pH 7.4), 12.5 mM MgCl2, 2 mM EDTA, 1 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, and 5 μg/ml aprotinin, at ~0.5–1.5 μg of protein/ml. A second tube containing the same volumes of all constituents, with the exception of H2O in place of PTX, was treated in the same fashion and used as a control.

**Photoaffinity Labeling with AA-[32P]GTP**—After incubating the samples for 15 min at 37 °C, the reaction was terminated with 900 μl of stop buffer (360 μM ATP, 285 μM cAMP, and 50,000 cpm/ml [3H]Glucagon), and cAMP was isolated by sequential chromatography over Dowex and alumina columns. 15 μl of Leukofluor scintillant was added to each tube, and samples were excited by a microprocessor-controlled light source (Leukofluor phosphorimager, Research Products International, Mount Prospect, IL), and counting in a liquid scintillation counter (Wallac 1409, EG&G Wallac, Gaithersburg, MD).

**Adenyl Cyclase Assays**—AC activity was measured according to the method of Salomon and coworkers (33, 34) as detailed previously (4). Briefly, 20 μl of control or PTX-treated ventricular membranes (20–30 μg protein) were added into a total volume of 50 μl containing 30 mM Tris (pH 7.4), 5 mM MgCl2, 0.8 mM EDTA, 0.12 mM ATP, 0.06 mM GTP, 10 mM thymidine, 10 mM DTT, 0.005% SDS, 50 μM NaF, 10 Ci/mmol [32P]PAP, 0.08 mg/ml BSA, 0.5 mM benzamidine, 5 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, and 2.5 μg/ml aprotinin. The mixture was incubated for 30 min at 30 °C, and then 100 μl of sample buffer (22.5 mM Tris (pH 6.8), 7.2% SDS, 9% glycerol, 0.01% bromphenol blue, and 10% 2-mercaptoethanol) was added to each tube. Samples were separated by SDS-PAGE and exposed to a autoradiographic film. A second tube containing the same volumes of all constituents, with the exception of H2O in place of PTX, was treated in the same fashion and used as a control. Specific phosphorylation bands were identified on autoradiograms of the dried gels. [32P] incorporation was quantified by excision of the radioactivity bands, addition of 7 ml of Leukofluor scintillant (Research Products International, Mount Prospect, IL), and counting in a liquid scintillation counter (Wallac 1409, EG&G Wallac, Gaithersburg, MD).

**Radioactivity Biodistribution—βAR density was determined by [3H]ICYP binding using a saturating concentration of 200 pM. Non-specific binding of [3H]ICYP was determined by the inclusion of 1 μM alprenolol. Ligand binding was performed in triplicate (15 μg of membrane protein per tube) in a final volume of 500 μl consisting of 75 mM

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imunoblots are shown. Each tissue was assayed at least twice, and representative ADP-ribosylation of PTX-sensitive G proteins (G

To confirm the increase in G

Older Hearts

Expression of G

RESULTS

Expression of Gα2 in Rat Left Ventricular Membranes Increases with Age—Fig. 1 shows immunoblots for several G

PTX-sensitive G Proteins in Left Ventricular Membranes Increase with Age—To confirm the increase in Gα2 observed by immunoblotting by another independent method, we examined ADP-ribosylation of PTX-sensitive G proteins (Gα/Gβ), in ventricles from young and old rats. This method has been used previously by Feldman et al. (10) to demonstrate a Gα increase in the failing human heart and by Bohm et al. (19) to demonstrate a Gα increase in the hearts of old Sprague-Dawley rats. As Fig. 2 shows, the amount of Gα/Gα labeling with [32P]NAD in PTX-treated membranes is significantly increased by 39 ± 8% in ventricles from older rats.

More Gα2 Is Activated in Left Ventricular Membranes from Older Hearts—We next determined whether the increased expression of Gα2 in older left ventricular membranes is accompanied by increased activation of Gα2. To this end, we assessed the effect of age on the ability of βAR and other GPCRs to stimulate photoaffinity labeling of Gα2 with AA-[32P]GTP. Activated GPCRs catalyze the exchange of GTP for GDP on α-subunits of G proteins associated with the GPCR, so the amount of AA-[32P]GTP incorporated into the α-subunit gives a direct measure of the extent of G protein activation. Fig. 3A shows that stimulations through β2ARs and glucagon receptors are significantly increased with age, from 102 ± 25% and 101 ± 31% above basal levels in young ventricles, to 226 ± 33% and 244 ± 40% above young basal levels, respectively. These results indicate that, as age increases, more Gα2 is activated upon stimulation of β2ARs and glucagon receptors. Basal labeling of Gα2 also significantly increases (by 83 ± 18%) in older membranes (Fig. 3A), consistent with the increased expression of Gα2 in older ventricles shown in Fig. 1. Photoaffinity labeling of stimulatory Gα2 is not altered with age (data not shown).

We next determined whether activation of Gα2 is also increased in aged heart through muscarinic acetylcholine receptor, a GPCR that interacts with Gα, but not Gβ. As shown in Fig. 3B, stimulation of muscarinic receptors in older left ventricular membranes produces greater photoaffinity labeling of Gα2 with AA-[32P]GTP than in young membranes.

AC Activity Is Decreased in Older Rat Ventricles—Figs. 1, 2, and 3 show increased Gα2 protein expression and activation in old rat heart. Because stimulation of Gα2-coupled receptors inhibits AC, we determined whether there are age-dependent increases in inhibition of cardiac AC production of cAMP. As shown in Table I, basal AC activity is significantly lower in old hearts, as are stimulations by β2ARs (isoproterenol + β2AR antagonist CGP 20712A) and glucagon receptors. Age-dependent decreases in stimulations by β2ARs (isoproterenol + β2AR antagonist ICI 118,551) and forskolin are not significant. To ascertain that the decrease in AC activity in older membranes was not because of a decrease in receptor number, we examined the expression level of βARs in both young and old ventricular membranes. We determined βAR density using a saturating antagonist ICI 118,551 and found no difference in the mean density of βARs between young and old rat ventricles (Table I). These results are similar to other reports on βAR density in Fisher 344 rat hearts (21, 38–42), though Mader et al. (43) did report a decrease in affinity. In other rat strains there may be moderate decreases in receptor number (6, 19, 25, 44).

We also examined whether there are age-dependent changes in the ability of muscarinic receptors to inhibit β2AR- and β2AR-stimulated AC in rat ventricle. As shown in Table I, stimulation of muscarinic receptors strongly inhibits βAR-stimulated AC in both young and old rat hearts. Because muscarinic receptors are able to inhibit AC stimulation almost completely in these membrane preparations, there is no significant difference between the maximal percent inhibition in young and old hearts, despite the increased coupling of muscarinic receptors to Gα2 shown in Fig. 3B. When the dose-response relationships were examined between muscarinic agonist concentration and inhibition of βAR-stimulated AC activity, small but significant increases were seen in the potency of muscarinic inhibition (Fig. 4). EC50s for muscarinic inhibition of β2AR- and β2AR-stimulated activity are 0.84 ± 0.10 and 0.25 ± 0.06 μM in young ventricles, respectively, and 0.48 ± 0.10 and 0.14 ± 0.04 μM in old ventricles. Previous
with our finding that more G1 levels achieved in the younger tissue. These data are consistent with PTX treatment. PTX ADP-ribosylates G1/G2 and muscarinic receptors (100 μM carbachol (Carb)). Direct activation of AC was tested with forskolin (50 μM). n = 6 for each age group, AC assays were performed in duplicate, and binding assays were performed in triplicate. *, p < 0.05 compared with AC stimulation by same condition in young membranes.

![Figure 3](image)

**Figure 3.** Increased activation of Gα1 proteins in old hearts. A, photofinity labeling of old rat ventricular membranes reveals greater basal, β2AR-stimulated, and glucagon receptor-stimulated incorporation of AA-[32P]GTP into Gα1 than in young ventricles. Membranes were photolabeled with AA-[32P]GTP in the presence of no drug (Basal), 100 μM isoproterenol (Iso), or 50 μM glucagon (Gluc), as described under “Experimental Procedures.” The photolabeled membranes were subjected to SDS-PAGE followed by autoradiography. Histogram data are expressed as percentage of basal incorporation in young atria. *, p < 0.05 compared with same condition in young samples. B, muscarinic receptor-induced photofinity labeling also reveals greater incorporation of AA-[32P]GTP into Gα1 in older ventricles. Membranes were photolabeled with AA-[32P]GTP in the presence of no drug (Basal), 100 μM isoproterenol (Iso), or 100 μM carbachol (Carb), as described under “Experimental Procedures.” The photolabeled membranes were subjected to SDS-PAGE followed by autoradiography. n = 4 for each age group. A representative autoradiogram is shown.

![Figure 4](image)

**Figure 4.** Age increases the potency of muscarinic inhibition of βAR-mediated stimulation of AC. Dose-response assays reveal small but significant decreases in the EC50 for carbachol-mediated inhibition of cAMP production stimulated by either β1ARs or β2ARs in old (open squares or circles, respectively) versus young (filled squares or circles, respectively) left ventricular membranes. β1AR stimulation was achieved with 100 μM isoproterenol + 100 μM ICI 118,551 (β1AR antagonist), and β2AR stimulation was achieved with 100 μM isoproterenol + 100 μM CGP 20712A (β2AR antagonist). n = 4 for each age group. Assays were performed in duplicate, and representative curves are shown.

**Table I**

| Receptor | Basal | β2AR | β2AR + Carboh | β1AR | β1AR + Carboh |
|----------|-------|------|---------------|------|---------------|
| Gα1      |       |      |               |      |               |
| Young    | 7.0 ± 0.5 | 11.4 ± 0.8 | 10.1 ± 0.3 | 9.7 ± 0.4 | 81.3 ± 18.5 |
| Old      | 5.0 ± 0.2 | 10.9 ± 1.2 | 7.5 ± 0.4 | 7.9 ± 0.4 | 69.6 ± 16.7 |

**DISCUSSION**

The main finding of the present study is that Gα1 expression in rat left ventricle increases with age. The increase in Gα1 expression is demonstrated using several biochemical techniques including immunoblotting, ADP-ribosylation, and photofinity labeling with AA-[32P]GTP. The increase in Gα1 results in enhanced coupling to Gα1-coupled receptors such as β2ARs and glucagon receptors. G1 inactivation in old membranes restores receptor-stimulated cAMP production to the levels achieved in the younger tissue. These data are consistent with our finding that more Gα1 is activated upon stimulation of β2ARs and other receptors in old heart (Fig. 3). β2ARs do not couple to Gα1. Accordingly, the increase in β2AR-stimulated stimulation of AC following PTX treatment was not signifi-

**Studies (45) in Fisher 344 rats also indicate that maximal muscarinic inhibition of βAR-stimulated AC activity is similar in young and old rats.**

**PTX Treatment Causes a Greater Increase in AC Activity in Old Hearts than in Young Hearts—**We further examined the relationship between increased Gα1 levels and decreased basal and receptor-stimulated AC activity by eliminating G1 activity through PTX treatment. PTX ADP-ribosylates Gα/Gβ and prevents their interaction with receptors, thereby removing the inhibition of AC by these G protein subtypes (46, 47). Our previous studies have shown that PTX treatment of human atrial membranes results in an increase in AC stimulation through β2AR and other Gα1-coupled receptors (4), and others have shown that PTX treatment of myocytes increases β2AR-mediated contractility (2, 6). As shown in Table II, the inactivation of Gα1/Gβ1 by PTX increases both basal and GPCR-stimulated AC activity, and this effect is greater in the old than in the young left ventricular membranes for the Gα1-coupled β2ARs and glucagon receptors. G1 inactivation in old membranes restores receptor-stimulated cAMP production to the levels achieved in the younger tissue. These data are consistent with our finding that more Gα1 is activated upon stimulation of β2ARs and other receptors in old heart (Fig. 3). β2ARs do not couple to Gα1. Accordingly, the increase in β2AR-stimulated stimulation of AC following PTX treatment was not signifi-

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βARs and glucagon receptors, as well as to Gαi-coupled muscarinic receptors. Thus, the net effect of the increase in Gαi2 expression with age is an increase in Gαi2 signaling. This results in reductions in both basal and receptor-mediated AC activities in aged heart, both of which are restored by disabling of Gαi with PTX.

The present study examined the effect of age on cardiac Gαi, an important issue about which conflicting data exist in the literature. Determination of the effects of age on Gαi has become increasingly important in light of the recent demonstrations that many cardiac Gαi-coupled receptors, including βAR, also couple to Gαi (2–4). The data in the present study clearly indicate an increased level of Gαi2 in old Fisher 344 rats, and importantly, also demonstrate an elevation in the receptor-stimulated activation of Gαi2. Prior studies in rats had not provided a consensus as to the effects of age on cardiac Gαi. There have been reports of increased Gαi expression in Fisher 344 (21), Sprague-Dawley (19) and Wistar rats (25), as well as an increase in Fisher 344 Gαi mRNAs (22). However, there also have been reports of no change in Gαi expression in Fisher 344 (22, 23) and Wistar rats (6, 26). These differences make it necessary to examine Gαi by multiple biochemical approaches. Our data indicating Gαi2 increases in immunoblotting, ADP-ribosylation and photoaffinity labeling lead us to conclude that there is a significant age-dependent elevation in Fisher 344 cardiac Gαi2. The increase in Gαi2 in aged hearts is likely not due to hypertrophy because in Fisher 344 rats hypertrophy is seen at senescence, which occurs at 27–30 months of age (31).

In agreement with previous reports in both human (15–17) and rat heart (21, 23, 48–51), we also demonstrate decreases in AC activity in old heart. To explain this phenomenon, some studies have implicated changes at the level of AC. There has been a report of a decrease in the number of forskolin binding sites in old rats (23), indicating a lower amount of AC in old tissue, though there have been no reports on AC mRNA levels in Fisher 344 rats. Although decreases in AC activity with age could be due to decreased amounts of AC or its targets, the fact that PTX treatment restores βAR- and glucagon receptor-stimulated AC signals in older hearts to the levels in younger hearts indicates that PTX-treatment sensitive proteins are responsible for the decreased receptor-stimulated enzyme activity. PTX treatment in both guinea pig heart and rat bladder results in similar reversals of age-induced decreases in AC (20, 52). We conclude that elevated Gαi is the main cause of reduced AC activity in aged rat ventricles.

Apparantly, an increase in cardiac Gαi in aged rat heart does not affect βAR-mediated contractility. Although coupling of βARs to stimulation of AC and contractility through Gαi is widely accepted, questions remain as to whether effects on contractility through βAR involve CAM. In humans, stimulation of contractility via βARs has been reported to occur through a CAM-dependent mechanism that results in protein kinase A-catalyzed phosphorylation of phospholamban, troponin I, and C-protein, as well as enhancement of both inotropy and lusitropy, in both non-failing (53) and failing human heart (54), as well as in non-failing myocardium from infants with Fallot tetralogy (55). Therefore, one would expect that in humans, an increase in the coupling of βAR to Gαi would lower contractility. In contrast, CAMP-independent pathways control βAR-mediated contractility in rat (2, 56–58), cat (59), sheep (60), and dog (61). Thus, in these species, an age-induced increase in Gαi would not decrease βAR-mediated contractility. Consistent with this notion is the finding of Jain et al. (62) who found no difference in basal contractile and relaxation function in mice lacking either Gαi2 or Gαi3.

An important functional consequence of an age-induced increase in the coupling of cardiac βAR to Gαi2 may be increased inhibition of apoptosis. It was shown recently that norepinephrine, acting through a Gαi pathway, increases cardiac apoptosis (63, 64). More recent data indicate that βARs cause apoptosis, whereas βARs acting through a Gαi pathway oppose apoptosis (7–9). In adult rat ventricular myocytes, stimulation of a PTX-sensitive Gαi-coupled pathway by βAR inhibits the number of apoptotic cells as measured by flow cytometry (7). Using a neonatal rat myocyte model, it was shown that βAR/Gαi2-mediated protection from apoptosis occurs through phosphatidylinositol 3-kinase (PI 3-kinase) and Akt/protein kinase B pathways (8). The βAR/Gαi2PI 3-kinase signaling mechanism also has been shown to mediate the stimulation of NO production (65), a key mechanism in the cardioprotection conferred by ischemic preconditioning (66). Finally, βAR-mediated protection from apoptosis recently has been reported to occur through a Gαi-dependent stimulation of p38 kinase (67), though another study reports that βAR activates p38 kinase through a protein kinase A-dependent pathway that does not involve Gαi (68). Thus, although the downstream signaling molecules involved have yet to be fully elucidated, an increase in cardiac Gαi seen in aging or failing heart may be an adaptive mechanism to protect the heart from apoptosis, because apoptosis has been shown to occur in both aged (69, 70) and failing heart (71, 72).

In summary, the present study provides evidence that age increases Gαi2 in older rat ventricles, and this results in more activated Gαi2 upon stimulation of various GPCRs.

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TABLE II

|                      | AC activity (pmol cAMP/mg protein/min) |
|----------------------|----------------------------------------|
|                      | Basal                                  |
|                      | +PTX                                  |
|                      | Percent change from control tissue     |
|                      | Old                                   |
|                      | +PTX                                  |
|                      | Percent change from control tissue     |
| Basal                | 8.4 ± 0.6                              |
| βAR                 | 13.6 ± 1.5                             |
| Glucagon            | 13.8 ± 0.7                             |
| Forskolin           | 57.4 ± 21.4                            |
|                   | 20                                    |
|                   | 19                                    |
|                   | 42                                    |
|                   | 7                                     |
|                   | 7.7 ± 0.8                             |
|                   | 14.7 ± 0.8                            |
|                   | 15.3 ± 0.7                            |
|                   | 75.1 ± 15.9                           |
|                   | 54*                                   |
|                   | 35                                    |
|                   | 94*                                   |
|                   | 8                                    |

Age Effects on Cardiac Gα Protein

31261
Age Increases Cardiac $\alpha_{2}$ Expression, Resulting in Enhanced Coupling to G Protein-coupled Receptors

Jason D. Kilts, Toshimasa Akazawa, Mark D. Richardson and Madan M. Kwatra

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