Desensitization of Adenylate Cyclase and Increase of $G_{i\alpha}$ in Cardiac Hypertrophy Due To Acquired Hypertension

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The present study investigated whether reduced adenylate cyclase activity and an increase in inhibitory guanine nucleotide binding proteins ($G_{i\alpha}$), which have been observed in the failing human heart, already occur in myocardial hypertrophy before the stage of heart failure. In membranes of hypertrophic hearts from rats with different forms of experimentally induced hypertension without heart failure (one-kidney, one clip rats, deoxycorticosterone-treated rats, and rats with reduced renal mass), basal as well as isoprenaline-, 5′-guanylylimidodiphosphate–, and forskolin-stimulated adenylate cyclase activity was reduced. The activity of the catalyst was depressed in deoxycorticosterone but unchanged in one-kidney, one clip and reduced renal mass compared with controls. The number of β-adrenergic receptors was similar in all groups. Radioimmunological quantification of $G_{i\alpha}$ proteins revealed an increase by 73% in one-kidney, one clip, 67% in reduced renal mass, but only 20% in deoxycorticosterone compared with sham-operated, age-matched control rats. The increase of $G_{i\alpha}$ was accompanied by smaller changes of pertussis toxin–induced [^{32}P]ADP-ribosylation of a 40-kd membrane protein. It is concluded that $G_{i\alpha}$ contributes to the reduced adenylate cyclase activity in cardiac hypertrophy in one-kidney, one clip and reduced renal mass and to a smaller extent in deoxycorticosterone. It is suggested that an enhanced expression of $G_{i\alpha}$ could occur not only in severe heart failure but also in cardiac hypertrophy and could, therefore, contribute to myocardial depression and progression of disease in heart failure. In addition, $G_{i\alpha}$ might represent an important regulatory mechanism for cardiac adenylate cyclase activity and thus, might play an important role in various cardiac diseases. (*Hypertension* 1992;20:103–112)

KEY WORDS • **experimental hypertension • hypertrophy • adenyl cyclase • guanine nucleotide regulatory protein • adrenergic receptors • cardiomyopathy • heart failure**

In the failing human heart desensitization of adenylate cyclase occurs, which leads to blunted effects of cyclic AMP (cAMP)–dependent positive inotropic agents and endogenous catecholamines.[1–4] This desensitization, which also impairs the effects of non–β-sympathomimetic cAMP-dependent positive inotropic agents,[2,4,6] has been attributed to a decline in the number of β-adrenergic receptors[1–6] and to an increase of pertussis toxin substrates.[3,4,7] However, these data were obtained in human myocardium from terminally failing hearts obtained at heart transplantation. Hitherto, it has been unknown whether an increase of inhibitory guanine nucleotide binding proteins ($G_{i\alpha}$) also occurs earlier during the development of cardiomyopathy, namely, in cardiac hypertrophy.

Myocardial hypertrophy has been regarded as an adaptive process to reduce wall stress when an increased pressure load is imposed on the myocardium.[8] Hypertrophy has been suggested to be one of the initial steps that reduces the contractility of myocardium in the development of heart failure.[9,10] However, it is unclear whether changes of β-adrenergic receptors or $G_{i\alpha}$ proteins occur in the hypertrophied human heart before the onset of heart failure. Since viable human myocardial tissue with hypertrophy is not available, we investigated different forms of experimentally induced hypertensive cardiomyopathy in rats. In renal hypertensive rats and in deoxycorticosterone (DOCA) rats, a reduction of β-adrenergic receptor–stimulated adenylate cyclase has been reported.[11–13] The data on the density of β-adrenergic receptors are controversial. In DOCA rats, some investigators observed a decline of the number of β-adrenergic receptors,[11] whereas others observed no changes.[14] In renal hypertensive rats, increases[15,16] as well as decreases[12,13] or no changes[14,17] of β-adrenergic receptors have been observed. Some investigators hypothesized that defects of G protein-effector coupling mechanisms (e.g., changes of $G_{s\alpha}$ proteins or the catalyst) could occur in cardiac hypertrophy due to acquired forms of hypertension and could be independent from β-adrenergic receptors.[14–19] However, experimental data on $G_{i\alpha}$ changes in support of this suggestion are lacking.

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The present study investigated whether changes of adenylate cyclase similar to those observed in the failing human heart, namely, heterologous adenylate cyclase desensitization involving $G_{i\alpha}$ proteins, occur in the hypertrophied myocardium of rats with different forms of acquired hypertension before the development of heart failure. Thus, we studied one-kidney, one clip (1K1C) renal hypertensive rats, DOCA-treated one-kidney rats, and rats with reduced renal mass (RRM). Changes of cardiac adenylate cyclase activity were characterized and related to the amount of a 40-kd pertussis toxin substrate and the amount of expressed $G_{i\alpha}$ proteins. $G_{i\alpha}$ proteins were studied using a novel radioimmunochemical method with the iodine-125–radiolabeled synthetic C-terminal decapeptide of retinal transducin α (KENLKDCGLF), which has been described recently.[20] Cardiac β-adrenergic receptors were studied for comparison.

Methods

Wistar-Hagemann rats ($n=62$) were purchased from Hagemann (Münster, Germany) and were divided into four groups. Rats in the 1K1C group underwent clipping of the right renal artery with a clip 0.18 mm in diameter. One week later nephrectomy of the left kidney was performed. In the second group (DOCA rats), DOCA pellets (50 mg) were implanted subcutaneously and the left kidney was extirpated. These animals were given 1% NaCl for drinking water. In the third group (RRM rats), the upper and lower pole of the left kidney was dissected. One week later, the animals underwent nephrectomy on the right side. These procedures resulted in a reduction of renal mass by about 68–70%. RRM rats were given 0.5% NaCl for drinking water. All control animals underwent sham operations. Experiments were conducted 4–7 weeks after the operations were performed. In all experimental animals, hypertension was established for 2 weeks. Blood pressure was measured with the tail-cuff method according to Pfeffer et al.[21] The last measurement was taken immediately before the rats were killed.

Adenylate Cyclase Determination

Particulate washed membrane fractions (10,000*g* sediment) were prepared according to the method of Kruse and Scholz[22] from homogenates of rat hearts. The activity of adenylate cyclase was determined in a reaction mixture containing 50 μmol/l [^{32}P]α-ATP (approximately 0.3 μCi/100 μl), 50 mmol/l triethanolamine-HCl, 5 mmol/l $MgCl_2$, 100 μmol/l EGTA, 1 mmol/l 3-isobutyl-1-methylxanthine, 5 mmol/l creatine phosphate, 0.4 mg/ml creatine kinase, and 0.1 mmol/l cAMP at pH 7.4 in a final volume of 100 μl. The mixture was preincubated for 5 minutes at 37°C. The reaction was started by addition of the membrane suspension (30 μg per tube) and was continued for another 20 minutes at the same temperature. Reactions were stopped by the addition of 500 μl of 120 mmol/l zinc acetate. cAMP was purified by chromatography on neutral alumina. After centrifugation for 5 minutes at 10,000*g*, 0.8 ml of the supernatant was applied on neutral alumina columns equilibrated with 0.1 mmol/l Tris/HCl, pH 7.5. The effluent was collected, and the [^{32}P]cAMP was determined by measuring radioactivity in a liquid scintillation spectrometer.

Radioligand Binding Experiments

Rat hearts were chilled in 30 ml ice-cold homogenization buffer (10 mmol/l Tris-HCl, 1 mmol/l EDTA, dithiothreitol 1 mmol/l, pH 7.4). Connective tissue was trimmed away, myocardial tissue was minced with scissors, and membranes were prepared with a motor-driven glass-Teflon homogenizer for 1 minute. Afterward, the membrane preparation was homogenized by hand for 1 minute with a glass-glass homogenizer. The homogenate was spun at 484*g* (rotor, Beckman JA 20) for 10 minutes. The supernatant was filtered through two layers of cheesecloth, diluted with an equal volume of ice-cold 1 mol/l KCl, and stored on ice for 10 minutes. This suspension then was centrifuged at 100,000*g* for 30 minutes. For radioligand binding experiments, the pellet was resuspended in 50 vol incubation buffer (50 mmol/l Tris-HCl, 10 mmol/l $MgCl_2$, pH 7.4) and homogenized for 1 minute with a glass-glass homogenizer. This suspension then was recentrifuged at 100,000*g* for 45 minutes. The final pellet was resuspended in incubation buffer (50 vol) and was stored at −70°C. Storage did not alter the results.

The radioligand binding assays were performed in a total volume of 250 μl incubation buffer. The incubation was carried out at 37°C for 60 minutes. These conditions allowed for complete equilibration of the receptors with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatman GF/C filters, and filters were immediately washed three times with 6 ml each of ice-cold incubation buffer. All experiments were performed in triplicate. Myocardial β-adrenergic receptors were studied using ^{125}I-cyanopindolol as radiolabeled ligand. Specific activity was 2,000 Ci/mmol; 3 μmol/l (-)-propranolol was used to determine nonspecific binding.

Pertussis Toxin–Induced [^{32}P]ADP Ribosylation

[^{32}P]ADP-ribosylation of $G_{i\alpha}$ by pertussis toxin was performed for 1 hour at 37°C in a volume of 50 μl containing 100 mmol/l Tris/HCl, pH 8.0 at 20°C, 25 mmol/l dithiothreitol, 2 mmol/l ATP, 1 mmol/l GTP, 50 nmol/l [^{32}P]NAD (800 Ci/mmol), and 20 μg/ml pertussis toxin that had been activated by incubation with 50 mmol/l dithiothreitol for 1 hour at 20°C before the labeling reaction. Optimal labeling conditions were achieved when 0.5% (vol/vol) Lubrol PX was present in the assay medium. Samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (10% [wt/vol] acrylamide, 16 cm total gel length). Gels were stained with Coomassie blue and dried before autoradiography was performed.

Immunoblotting Techniques

Immunoblotting techniques were performed according to Gierschik et al.[23] The retinal transducin α-subunits were purified from bovine rod outer segments as described elsewhere.[23,24] The polyclonal antiserum (DS 4) was raised in rabbits against the C-terminal decapeptide of retinal transducin α KENLKDCGLF coupled to keyhole limpet hemocyanine as described by Goldsmith et al.[25]. Under the conditions used, this serum was strongly reactive against α_1, α_{i1}, and α_{i2} but weakly reactive against α_{i3} and α_o. The electrophoretic transfer and immunostaining methods have been described previously.[20]

TABLE 1. Systolic Blood Pressure, Body Weight, and Cardiac Hypertrophy Expressed in Heart Weight and Heart Weight–to–Body Weight Ratio in One-Kidney, One Clip Rats, Deoxycorticosterone-Treated Rats, Rats With Reduced Renal Mass, and Control Rats

Measurements	Control (n=11)	1K1C (n=6)	DOCA (n=6)	RRM (n=6)
Systolic BP (mm Hg)	96.4±3.6	222±6.1*	204.8±6.8*	207.8±4.5*
Body weight (g)	260.9±10.8	235±9.7	213.3±11.0*	218.3±9.0*
Heart weight (mg)	604.8±19.0	1,032±21.0*	925.2±43.9*	770.0±44.0†
Heart weight/body weight ratio (mg/g)	2.3±0.1	4.4±0.1*	4.4±0.3*	3.6±0.2*

1K1C, *one-kidney, one clip rats*; DOCA, *deoxycorticosterone rats*; RRM, *reduced renal mass rats*; BP, *blood pressure*.
*$p<0.01$, †$p<0.05$, significantly different from control.

Iodination of C-Terminal Synthetic Peptide and Radioimmunoassay

The C-terminal synthetic peptide was iodinated by conjugation to the iodine-125–labeled acylating agent *N*-succinimidyl 3-(4-hydroxy, 5-[^{125}I]-iodophenyl) propionate according to Bolton and Hunter[26] and modified as described recently.[20] The assay was performed in a final volume of 80 μl, containing 20 μl radiolabeled peptide diluted to 10,000 cpm/tube in 50 mmol/l Na-phosphate buffer with 0.25% gelatine at pH 7.5, 20 μl antiserum dilution (1:100), and 40 μl solubilized membranes, transducin α in 50 mmol/l Na-phosphate buffer at pH 7.5 or solubilization buffer instead of membranes. Standard curves were constructed by using 0.25–25 μg/ml retinal transducin α. The assay was performed at 4°C for 18 hours. For precipitation of immune complexes, samples were supplemented with 30 μl staphylococcal protein A suspension and incubated for 30 minutes on ice. Details have been described elsewhere.[20]

Miscellaneous

Protein was determined according to Lowry et al[27] using bovine serum albumin as standard. SDS-PAGE was performed as described by Lämmli.[28] 5′-Nucleotidase activity was investigated with the method of Dixon and Purdom.[29] Human leukemia (HL-60) cells were grown in suspension culture and were induced to differentiate into mature myeloid forms by cultivation in the presence of 1.25% (vol/vol) dimethyl sulfoxide for 5 days. Details of cultivation and membrane preparations are described elsewhere.[30]

Materials

Forskolin was donated by Dr. Metzger (Hoechst AG, Frankfurt, Germany). Carbachol was from Sigma Chemical Co., Deisenhofen, Germany. Guanosine 5′-triphosphate, 5′-guanylylimidodiphosphate [Gpp(NH)p], adenosine 5′-triphosphate, creatine phosphate, and creatine kinase were purchased from Boehringer-Mannheim (Germany), and isobutylmethylxanthine was purchased from EGA-Chemie, Steinheim, Germany. The ligand ^{125}I-Cyp was from Amersham-Buchler, Braunschweig, Germany. Dithiothreitol was from Serva, Heidelberg, Germany. Pertussis toxin was from List Biological Laboratories, Campbell, Calif.). Antiserum (DS 4) was raised against the C-terminal decapeptide of bovine retinal transducin α. Bolton and Hunter reagent for peptide iodination (*N*-succinimidyl 3-(4-hydroxy, 5-[^{125}I]iodophenyl) propionate, specific activity 2,000 Ci/mmol) was purchased from Amersham-Buchler. The C-terminal decapeptide of transducin α was kindly provided by Prof. Dr. U. Weber, Tübingen, Germany. Immunoprecipitin (staphylococcal protein A adsorbant) suspension was from Gibco/BRL, Eggenstein, Germany. All other compounds used were of analytical or best grade commercially available. Only deionized and twice-distilled water was used throughout.

Statistics

The data shown are mean±SEM. Statistical significance was estimated with Student's t test for unpaired observations and analysis of variance according to Wallenstein et al.[31] A value of $p<0.05$ was considered significant. K_D values and the drug concentration producing 50% of the maximal effect (EC_{50}) were determined graphically in each individual experiment.

Results

Blood Pressure and Myocardial Hypertrophy

As shown in Table 1, systolic blood pressure was elevated similarly above 200 mm Hg in all experimental groups of acquired hypertension. Consistently, heart weight and heart weight–to–body weight ratio were increased in all groups with hypertension compared with controls. In all groups, there was a decrease of body weight. The decline in weight was significant and stronger in DOCA and RRM than in 1K1C rats.

Adenylate Cyclase Activity

Concentration–response curves for isoprenaline summarize the effects of β-adrenergic receptor stimulation on cardiac adenylate cyclase activity (Figure 1, left panel). In either group with experimentally acquired hypertension, the ability of isoprenaline to stimulate adenylate cyclase activity was reduced. This reduction was more pronounced in DOCA than in 1K1C or RRM rats. To investigate whether the effects of agents bypassing the β-adrenergic receptor–mediated stimulation of adenylate cyclase are sustained, we investigated the poorly hydrolyzable guanine nucleotide analogue Gpp(NH)p and the diterpene derivative forskolin. The effects of both Gpp(NH)p (Figure 1, middle panel) and forskolin (Figure 1, right panel) were reduced compared with control rats. As with isoprenaline, the decline of the effectiveness of Gpp(NH)p and forskolin was strongest in DOCA rats as compared with 1K1C and RRM rats. One general problem for the determination of adenylate cyclase activity in hypertrophied myocardium was to control the preparation for the amount of membranes. To overcome this potential

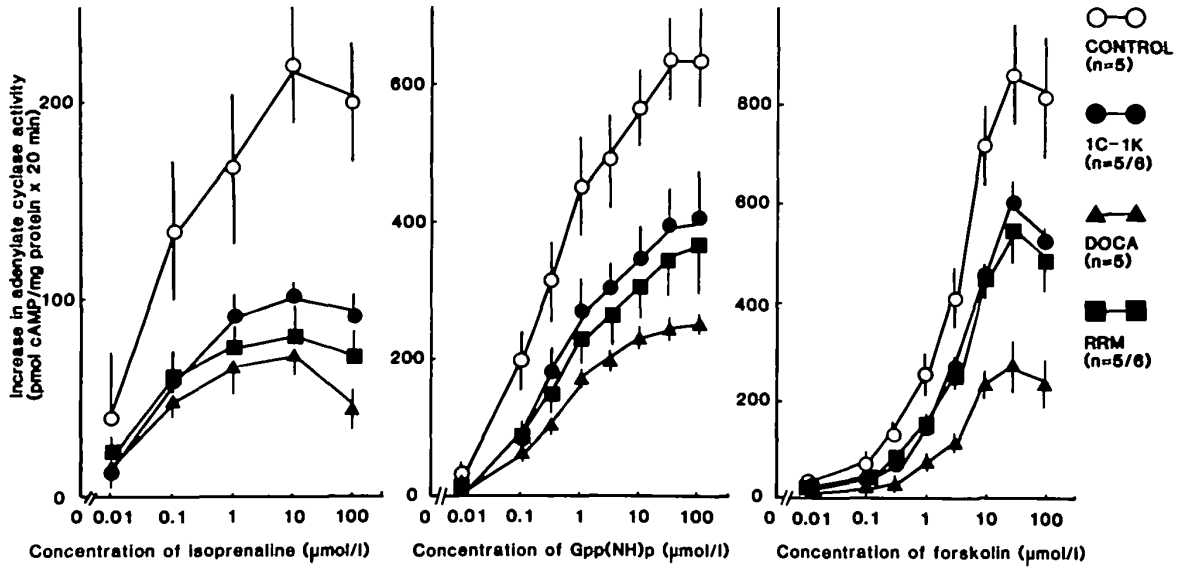

FIGURE 1. *Line plots show concentration-dependent effects of isoprenaline* (left panel, *0.01–100 μmol/l), 5'-guanylylimidodiphosphate [Gpp(NH)p]* (middle panel, *0.01–100 μmol/l), and forskolin* (right panel, *0.01–100 μmol/l) on cardiac adenylate cyclase activity in one-kidney, one clip (1C-1K) rats, deoxycorticosterone-treated rats (DOCA), and rats with reduced renal mass (RRM). Experiments in the control rats (control) are shown in all panels for comparison. cAMP, cyclic AMP.*

pitfall, we related adenylate cyclase activity to both membrane protein and 5'nucleotidase activity as membrane marker enzyme. As summarized in Table 2, basal adenylate cyclase activities were similarly reduced in the experimental hypertensive animals when the data are related to milligrams membrane protein or to 5'nucleotidase activity (Table 2).

To study whether the reduced adenylate cyclase activity is due to defective G protein–effector coupling, enzyme activity was studied in the presence of 5 mmol/l $MnCl_2$. Manganese ions are known to uncouple the catalytic subunit from guanine nucleotide regulatory sites.[32,33] Thus, under these conditions the activity of the catalyst itself can be predominantly studied. Figure 2 summarizes the data. In 1K1C and in RRM rats, adenylate cyclase activity in the presence of $MnCl_2$ was not different compared with controls. This holds true for basal adenylate cyclase activity, adenylate cyclase stimulated with forskolin, and forskolin plus Gpp(NH)p. Addition of Gpp(NH)p did not further increase the effect of forskolin, indicating that in the presence of $MnCl_2$, only the effect of forskolin on the catalyst is studied. In contrast, in DOCA rats adenylate cyclase activity assayed in the presence of $MnCl_2$ was reduced compared with control rats and also compared with 1K1C and RRM rats. Similar results were obtained when the data were related to milligrams membrane protein or to 5'nucleotidase activity (Table 2). In DOCA the adenylate cyclase activity in the presence of $MnCl_2$ was also not further increased by forskolin plus Gpp(NH)p compared with forskolin alone. These data show that the catalytic subunit was completely uncoupled from the influences of guanine nucleotide–activated G proteins. It is depressed in DOCA but not in RRM and 1K1C rats.

Cardiac β-Adrenergic Receptors

Saturation experiments for ^{125}I-Cyp binding to cardiac membranes showed that binding of the radiolabeled ligand was monophasic and saturable (not shown). Table 3 summarizes the data. There were no differences in the numbers of cardiac β-adrenergic receptors between 1K1C, DOCA, RRM, or control rats. The data were similar when the receptor density was related to milligrams membrane protein or to 5'nucleotidase activity.

$G_{i\alpha}$ Proteins

Figure 3 shows a typical autoradiogram for the pertussis toxin–induced [^{32}P]ADP ribosylation of a 40-kd protein in rat myocardial membranes. This protein comigrated with the 40-kd pertussis toxin substrate $G_{i\alpha 2}$, $G_{i\alpha 3}$ from human leukemia (HL-60) cells and with G_i/G_o purified from bovine brain. Pertussis toxin labeling was enhanced in myocardial membranes from 1K1C and RRM rats and only slightly increased in DOCA compared with control. Figure 4 summarizes the data for all groups studied. There was a significant increase of pertussis toxin substrates only in 1K1C and RRM rats, whereas no significant increase in DOCA rats was detected.

To study whether the increase of pertussis toxin substrates in 1K1C and RRM rats is due to an enhanced expression of $G_{i\alpha}$ proteins or whether it is due to other factors affecting the pertussis toxin–catalyzed ADP-ribosylation reaction, we developed a radioimmunoassay to directly quantify the amounts of $G_{i\alpha}$ proteins in rat cardiac membranes. DS 4 antiserum was raised in rabbits against the C-terminus of retinal transducin α. Figure 5 shows that the antiserum was capable to immunostain a 40-kd membrane protein in rat cardiac membranes. Immunostaining of the 40-kd band was concentration dependently inhibited by the synthetic

TABLE 2. Adenylate Cyclase Activity Under Basal and Stimulated Conditions in Cardiac Membranes From One-Kidney, One Clip Rats, Deoxycorticosterone-Treated Rats, Rats With Reduced Renal Mass, and Control Rats

Condition	Control	1K1C	DOCA	RRM
Related to mg protein				
Basal	47.0±4.7	30.8±6.8	18.5±2.7*	29.1±4.3†
	(8)	(9)	(8)	(6)
Iso (10 μM)	795±99.9	367.1±31.3†	226.2±22.2*	323.3±6.0†
	(5)	(5)	(5)	(5)
Gpp(NH)p (100 μM)	723.4±86.3	454.9±74.6†	272.3±20.6*	411.6±66.6†
	(5)	(6)	(5)	(6)
Forskolin (30 μM)	914.6±128.1	618.8±48.5	277.2±53.1*	570.2±77.4
	(5)	(5)	(5)	(5)
$MnCl_2$	96.6±19.8	87.0±24.6	55.5±12.0	91.6±26.4
	(6)	(6)	(6)	
$MnCl_2$+forskolin (30 μM)	1,025.4±61.3	969.5±36.1	529.0±60.3*	1,008.3±25.8
	(6)	(6)	(6)	(6)
$MnCl_2$+forskolin+Gpp(NH)p	1,086.0±71.6	984.6±38.1	594.8±64.9*	1,089.1±55.5
	(6)	(6)	(6)	(6)
Related to 5'nucleotidase (nmol Pi/mg protein · min)				
Basal	1.5±0.1	0.9±0.2†	0.5±0.1*	1.0±0.2†
	(8)	(9)	(8)	(6)
Iso (10 μM)	25.9±4.0	11.2±1.7†	6.2±0.9*	11.1±0.7†
	(5)	(5)	(5)	(5)
Gpp(NH)p (100 μM)	23.4±1.9	13.0±2.3†	7.2±0.6*	14.9±2.6†
	(5)	(6)	(5)	(6)
Forskolin (30 μM)	29.0±3.3	17.8±1.8†	7.5±1.4*	18.5±2.7
	(5)	(5)	(5)	(5)
$MnCl_2$	3.2±0.6	2.6±0.7	1.4±0.3†	3.3±0.9
	(6)	(6)	(6)	(6)
$MnCl_2$+forskolin (30 μM)	34.1±3.1	29.5±1.6	13.6±1.4*	33±1.6
	(6)	(6)	(6)	(6)
$MnCl_2$+forskolin+Gpp(NH)p	34.5±4.6	29.7±1.2	15.2±1.4*	33.2±0.8
	(6)	(5)	(6)	(5)

All experiments with $MnCl_2$ were performed without $MgCl_2$; 1K1C, one-kidney, one clip rats; DOCA, deoxycorticosterone rats; RRM, reduced renal mass rats; Iso, isoprenaline; Gpp(NH)p, 5'guanylylimidodiphosphate.
*$p<0.01$, †$p<0.05$.

C-terminus of retinal transducin α (KENLKDCGLF). DS 4 antiserum was used to quantify $G_{i\alpha}$ in a radioimmunoassay with iodinated retinal transducin α (^{125}I-KENLKDCGLF) and retinal transducin α as standard. Figure 6 shows competition curves of increasing concentrations of retinal transducin α and membrane extracts from human leukemia cells (HL-60) and rat myocardial membranes. Transducin α, human leukemia cells (HL-60), and rat myocardial membranes were capable to concentration dependently displace DS 4 antiserum binding to iodinated retinal transducin α (^{125}I-KENLKDCGLF). As can be seen from the standard curve with retinal transducin α, the sensitivity of the radioimmunoassay was 0.25 μg/ml transducin α. The interassay variation was less than 10%. Figure 7 summarizes the results in the different groups with experimental hypertension. In 1K1C and RRM rats, there was an increase of $G_{i\alpha}$ proteins by 73% and 67%, respectively; in DOCA rats, there was a small albeit significant increase by 20% compared with controls. Figure 8 shows the correlation of systolic blood pressure (left panel) and heart weight–to–body weight ratio (right panel) to the density of $G_{i\alpha}$ proteins in control and hypertensive animals. It can be seen that in DOCA rats, the increase of $G_{i\alpha}$ was less than in 1K1C or RRM rats, although elevation of blood pressure and cardiac hypertrophy were similar. Taken together, there is a substantial increase of expressed $G_{i\alpha}$ proteins in 1K1C and RRM rats and a more moderate but significant increase in DOCA rats. The increase in $G_{i\alpha}$ proteins poorly corresponds to the increase in pertussis toxin substrates, which was less pronounced and failed to detect the small increase of $G_{i\alpha}$ in DOCA rats.

Discussion

The results of the present study show that in the hypertrophied myocardium from 1K1C and RRM renal hypertensive rats as well as that from DOCA rats, there is a desensitization of adenylate cyclase activity. The desensitization not only affects β-adrenergic receptor–stimulated adenylate cyclase but also Gpp(NH)p- and forskolin-stimulated activities. Desensitization of adenylate cyclase was not accompanied by significant changes of myocardial β-adrenergic receptors. Uncou-

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