Cardiac Adenylyl Cyclase, \( \beta \)-Adrenergic Receptors, and G Proteins in Salt-Sensitive Hypertension

Michael Böhm, Peter Gierschik, Andreas Knorr, Ulrich Schmidt, Korinna Weismann, Erland Erdmann

The present study investigated whether high salt intake (8%) in Dahl salt-sensitive and salt-resistant rats with and without hypertension produces a heterologous desensitization of cardiac adenylyl cyclase as observed in various types of hypertension and human heart failure. In membranes from Dahl salt-sensitive rats on a high-salt diet (8%) basal, isoproterenol-, 5'-guanylylimidodiphosphate-, and forskolin-stimulated adenylyl cyclase was reduced compared with the low-salt (0.4%) group and Dahl salt-resistant rats on either 0.4% or 8% sodium chloride. The activity of the catalyst was depressed, and the expression of the immunodetectable inhibitory G proteins \( G_{ia} \) was increased in Dahl salt-sensitive rats on 8% sodium chloride, whereas the density of \( \beta \)-adrenergic receptors and the activity of the stimulatory G protein \( G_{sa} \) reconstituted into \( G_{io} \)-deficient S49 cyc mouse lymphoma cell membranes were unchanged in any condition studied. We conclude that high salt intake in salt-sensitive hypertensive Dahl rats produces hypertension, cardiac hypertrophy, and heterologous desensitization of cardiac adenylyl cyclase. The latter alteration is due to an increase of \( G_{ia} \) proteins and a depressed catalyst activity of adenylyl cyclase. The results demonstrate that heterologous adenylyl cyclase desensitization can precede the development of contractile dysfunction in later stages and can occur independently of changes in \( \beta \)-adrenergic receptors. (Hypertension. 1993;22:715-727.)

KEY WORDS • hypertension, sodium-dependent • hypertrophy • adenylyl cyclase • G proteins • heart failure • cardiomyopathy, hypertrophic • receptors, adrenergic, \( \beta \) • sodium, dietary

An increase in cardiac adenylyl cyclase activity after stimulation of cardiac \( \beta \)-adrenergic receptors is accompanied by an increase in intracellular Ca\(^{2+}\) concentration during systole, thereby increasing force of contraction. \(^1\,^2\) After stimulation with agonists, adenylyl cyclase desensitizes. \(^3\) The desensitization has been suggested to protect the myocardium from sympathetic overstimulation. These desensitization mechanisms are important in a number of pathophysiological conditions and after pharmacologic interventions. In neonatal rat cardiomyocytes cultivated in norepinephrine, a downregulation of \( \beta \)-adrenergic receptors and an increased expression of the inhibitory guanine nucleotide binding protein \( (G_{ia}) \) was observed to accompany the heterologous desensitization of adenylyl cyclase. \(^4\) In adult rats in vivo, treatment with isoproterenol produced cardiac hypertrophy \(^5\,^6\) accompanied by a downregulation of \( \beta \)-adrenergic receptors, \(^5\) an increase of \( G_{ia} \) mRNA expression, \(^6\) and an increase of \( G_{ia} \) as judged from \( [\text{32P}] \) ADP ribosylation of \( G_{ia} \) by pertussis toxin, \(^5\) whereas stimulatory guanine nucleotide binding protein \((G_{sa})\) mRNA expression was unchanged. \(^6\) Similar changes have been observed in the failing human heart, in which a downregulation of \( \beta \)-adrenergic receptors, \(^7\,^8\) an increase in pertussis toxin substrates, \(^12\,^16\) and an increase in immunodetectable \( G_{ia} \) proteins \(^15\) in the presence of unchanged \( G_{sa} \) proteins \(^12\,^17\) accompany a heterologous desensitization of adenylyl cyclase. \(^12\,^15\,^16\)

In patients with heart failure, the sympathetic nervous system is activated, \(^18\) resulting in an increase of cardiac norepinephrine release \(^19\) and increased levels of circulating catecholamines \(^20\,^21\); therefore, the increased \( \beta \)-adrenergic drive imposed on the failing human myocardium has been suggested to play a central role in the genesis of heterologous adenylyl cyclase desensitization. \(^8\,^15\,^18\) However, these studies were performed in myocardium from patients with severe heart failure, and thus it remains unclear whether similar changes in the G protein–adenylyl cyclase complex occur also in hypertrophy, an adaptive process to reduce wall stress when an increase in pressure load is imposed on the myocardium, for instance, by the development of hypertension. Several reports are in favor of this suggestion. In experimental models of acquired hypertension and hypertrophy, a desensitization of adenylyl cyclase was observed. \(^22\,^23\) Recently, we reported an increase of \( G_{ia} \) without changes of the \( \beta \)-adrenergic receptors in rats with renal hypertension (one-kidney, one clip [1K1C]), with reduced renal mass, and after treatment with deoxycorticosterone acetate (DOCA) plus sodium chlo-
Tris-HCl, pH 7.5. The effluent was collected, and filtration were stopped by the addition of 500 mM NaCl for 20 minutes at the same temperature. Reactions were preincubated for 5 minutes at 37°C. The incubation time was 10 minutes at the same temperature. Reactions were performed for 12 hours at 4°C in a volume of 50 μL containing 100 mM Tris-HCl, pH 8.0 at 20°C; 25 mM dimethyl sulfoxide, 20 mM MgCl₂, 100 mM ATP, 10 mM GTP, 50 μM [³²P]NAD (800 Ci/mmol); and 20 μg/mL pertussis toxin that had been incubated overnight with 50 mM dimethyl sulfoxide at 4°C before incubation buffer. All experiments were performed in triplicate. Myocardial β-adrenergic receptors were probed using [³²P]cytopinopindoI (Cyp). Specific activity was 2000 Ci/mmol; 3 μCi/L (-)-propranolol was used to determine nonspecific binding.

**Immunoblotting Techniques**

Immunoblotting techniques were performed according to Gierschik et al. Retinal transducin α was purified from bovine rod outer segments as described elsewhere. The polyclonal antiserum (DS 4) was raised in rabbits against the C-terminal decapeptide of retinal transducin α (KENLKDCGLF) coupled to keyhole limpet hemocyanin as described by Goldsmidt et al. The antiserum recognized Gαs and Gαi, and only weakly or not Gαo and Gα (not shown). Blots were stained with an alkaline phosphatase–labeled goat anti-IgG antiserum.

**Iodination of KENLKDGLF Peptide**

The C-terminal synthetic peptide was iodinated by conjugation to the [³²P]labeled acetylating agent N-succinimidyl 3-(4-hydroxy, 5-[³²P]iodophenyl) propionate according to Bolton and Hunter. The iodinated peptide was purified by chromatography on a Sep-Pak C₁₈ cartridge (Waters Chromatography, Königstein, Germany). In brief, the iodination mixture (1 mL diluted in 0.1% [vol/vol] trifluoroacetic acid in water) was applied to the cartridge, which had been equilibrated before use. After washing with 5 mL of 0.1% [vol/vol] trifluoroacetic acid in water, the peptide was eluted from the matrix with a step gradient (10 steps, 1 mL each) starting with 0.1% [vol/vol] trifluoroacetic acid in water and ending with 0.1% [vol/vol] trifluoroacetic acid in acetonitrile. The material present in the third peak (20% to 40% [vol/vol] acetonitrile) bound more specifically to the antiserum DS 4 than material found in the other peaks.
The membranes were washed three times with a buffer containing sucrose (0.25 mol/L), Tris-HCl (20 mmol/L), EDTA (1 mmol/L), and dithiothreitol (1 mmol/L), benzo-

midine (3 mmol/L), phenylmethylsulfonyl fluoride (1 mmol/L), leupeptin (10 μmol/L), and soybean trypsin inhibitor (2 μg/mL) resuspended to 10 mg protein/mL with this buffer, and stored at -80°C. The yield of membrane protein was approximately 100 mg per 10^10 cells.

Reconstitution of Myocardial Gα Into S49 cyc^- Membranes

Reconstitution assays were performed according to Sternweis et al.39

Miscellaneous

Protein was determined according to Lowry et al.40 using bovine serum albumin as standard. SDS-PAGE was performed as described by Lammli.41 5^-Nucleotidase activity was analyzed with the method of Dixon and Purdom.42

Materials

Forskolin was donated by Dr Jürgen Metzger, Hoechst AG, Frankfurt, Germany. GTP, guanylimidodiphosphate [Gpp(NH)p], ATP, creatine phosphate, and creatine kinase were purchased from Boehringer-Mannheim (Germany) and isobutylmethylxanthine from EGAChemie, Steinheim, Germany. The ligand [32P]Cys was from Amersham-Buchler, Braunschweig, Germany. Dithiothreitol was from Serva, Heidelberg, Germany. Pertussis toxin was from List Biological Laboratories, Campbell, Calif. Bolton and Hunter reagent for peptide iodination [N-succinimidyl 3-(4-hydroxy, 5-[125I]iodophenyl) propionate; specific activity, 2000 Ci/mmol] was purchased from Amersham-Buchler. All other compounds used were of analytical grade or the best grade commercially available. Only deionized and twice-
distilled water were used throughout.

Statistics

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.43 A value of P<.05 was considered significant. Kd values were determined graphically in each individual experiment.

Results

Blood Pressure and Myocardial Hypertrophy

As shown in Fig 1, systolic blood pressure increased only slightly in Dahl R rats on the 8% sodium chloride diet, whereas in Dahl S rats on 8% sodium chloride, a marked increase of systolic blood pressure above 200 mm Hg developed. The development of hypertension on 8% sodium chloride was accompanied by an increase of heart weight and the ratio of heart to body weight. In contrast, in Dahl S rats on 0.4% sodium chloride and Dahl R rats on 8% sodium chloride, heart weights were only slightly increased over Dahl R rats on 0.4% sodium chloride. Blood pressure did not differ in Dahl R rats on 0.4% or 8% sodium chloride but was slightly increased in Dahl S rats on 0.4% sodium chloride. Body weights were similar in all groups.
Adenylyl Cyclase Activity

Concentration-response curves in Fig 2 summarize the effects of isoproterenol on adenylyl cyclase activity in cardiac membranes of Dahl S and Dahl R rats on either 0.4% or 8% sodium chloride. In Dahl R rats, the ability of isoproterenol to increase adenylyl cyclase was similar when rats were treated with 0.4% or 8% sodium chloride. In Dahl S rats, isoproterenol-stimulated adenylyl cyclase activity was reduced by approximately 50%. However, the isoproterenol effect on adenylyl cyclase was more efficacious in Dahl S rats on 0.4% sodium chloride than in Dahl R rats on either 0.4% or 8% sodium chloride. To investigate whether the reduced adenylyl cyclase in Dahl S rats on 8% sodium chloride was due to changes in the level of β-adrenergic receptors or G proteins, we investigated the effect of the poorly hydrolysable guanine nucleotide Gpp(NH)p (Fig 3). The effect of Gpp(NH)p was similar in Dahl R rats on 0.4% or 8% sodium chloride. As with isoproterenol, Gpp(NH)p elicited increased effects on adenylyl cyclase in Dahl S rats on 0.4% sodium chloride compared with Dahl R rats on 0.4% or 8% sodium chloride. When Dahl S rats were put on an 8% sodium chloride diet, the effects of Gpp(NH)p were markedly reduced. Thus, adenylyl cyclase activity in Dahl S rats on 8% sodium chloride was also reduced when adenylyl cyclase activity was stimulated at the level of the G proteins. Fig 4 shows the effects of forskolin, which directly stimulates the catalytic subunit of adenylyl cyclase. The effects of forskolin were similar in Dahl R rats on 0.4% or 8% sodium chloride diets. As with isoproterenol and Gpp(NH)p, the effects of forskolin on adenylyl cyclase activity were increased in Dahl S rats on 0.4% sodium chloride. When Dahl S rats were treated with 8% sodium chloride, the effect of forskolin was reduced by approximately 50%. Taken together, the results show that isoproterenol, Gpp(NH)p, and forskolin-stimulated adenylyl cyclase activities were reduced in Dahl S rats on 8% compared with 0.4% sodium chloride. Sodium chloride treatment had no effect on adenylyl cyclase activity in Dahl S rats on 0.4% sodium chloride compared with Dahl R rats on 0.4% or 8% sodium chloride.
Fig 3. Line graphs show effect of Gpp(NH)p on cardiac adenylyl cyclase in myocardial membranes from Dahl salt-resistant (Dahl R, left) and salt-sensitive (Dahl S, right) rats on 0.4% (open symbols) or 8% (closed symbols) sodium chloride diet. Basal adenylyl cyclase activities (in pmol cyclic AMP [cAMP]/mg protein x 20 minutes) were 60±8.6 (○, n=6), 48±4.1 (●, n=5), 78.8±11.4 (■, n=6), and 30.8±3.5 (▲, n=6).

Fig 4. Line graphs show effect of forskolin on cardiac adenylyl cyclase in myocardial membranes from Dahl salt-resistant (Dahl R, left) and salt-sensitive (Dahl S, right) rats on 0.4% (open symbols) or 8% (closed symbols) sodium chloride diet. Basal adenylyl cyclase activities (in pmol cyclic AMP [cAMP]/mg protein x 20 minutes) were 66.1±13.8 (○, n=6), 54±2.1 (●, n=6), 92.7±7.6 (■, n=6), and 37±5.6 (▲, n=6).

activity in Dahl R rats. The adenylyl cyclase activity of Dahl S rats on 0.4% sodium chloride was more sensitive to stimulation with isoproterenol, Gpp(NH)p, or forskolin compared with Dahl R rats of both treatment groups.

These data suggest that treatment of rats with 8% sodium chloride resulted in a reduction of β-adrenergic receptor- and G protein-regulated adenylyl cyclase activity in Dahl S rats. However, changes in the activity of the catalyst are also possible, as suggested by the reduced effect of forskolin in Dahl S rats on 8% sodium chloride. However, the effect of forskolin is influenced by guanine nucleotide–activated G proteins. Thus, an experimental condition was investigated that probes exclusively the effects of forskolin on the catalyst. Manganese ions have been reported to stimulate the catalyst and uncouple it from the regulatory influence of G proteins. Therefore, basal adenylyl cyclase activity and the effects of forskolin and forskolin plus Gpp(NH)p were investigated in Dahl S and Dahl R rats under either treatment condition in the presence of MnCl₂ (see Fig 5). In either group, forskolin stimulated adenylyl cyclase activity approximately fourfold. Gpp(NH)p plus forskolin did not alter adenylyl cyclase activity further compared with forskolin alone. This indicates that the effects of forskolin were independent of guanine nucleotide–activated G proteins and that MnCl₂ under this experimental condition completely uncouples the catalyst from G proteins. Basal adenylyl cyclase activity was similar in Dahl S and Dahl R rats on 0.4%
or 8% sodium chloride. The activity of the catalyst in Dahl S rats (0.4% sodium chloride) tended to be slightly higher than in the other groups, but the difference was not statistically significant. High-salt diet did not change the activity of the catalyst in Dahl R rats when measured at basal conditions, with forskolin, or with forskolin plus Gpp(NH)p in the presence of MnCl₂. In contrast, 8% sodium chloride reduced the activity of the catalyst in Dahl S rats. However, in the analysis of relative values, the decline of catalyst activity was less pronounced (approximately 35%) than the reduction of isoproterenol, Gpp(NH)p, or forskolin effects (approximately 50%).

A general problem inherent with studies of hypertrophied myocardium is the determination of the content of true membrane proteins in the whole tissue preparations. To address this problem, we related adenylyl cyclase activity to milligrams of protein as well as to 5'-nucleotidase activity (see Table 1). The effects were similar when related to protein or 5'-nucleotidase activity. Taken together, the results show that a high-sodium diet reduces adenylyl cyclase activity in Dahl S but not Dahl R rats. In this experimental condition, the reduction of adenylyl cyclase activity involves the catalyst. However, because the effects of isoproterenol, Gpp(NH)p, and forskolin were relatively more reduced than the activity of the catalyst, the latter observation is conceivable with the notion that another defect is also involved.

Cardiac 3-Adrenergic Receptors

Cardiac 3-adrenergic receptors were investigated using the radiolabeled ligand [¹²⁵I]Cyp. Fig 6 shows the density of 3-adrenergic receptors in cardiac membranes. There was no difference in the number of 3-adrenergic receptors between Dahl S and Dahl R rats when the rats received either 0.4% or 8% sodium chloride. The antagonist affinity did not differ in either group (not shown). There was no difference when the data were related to milligrams of membrane protein or to 5'-nucleotidase activity (Table 2), suggesting that changes in 3-adrenergic receptor densities do not play a role in changed adenylyl cyclase activity.

Stimulatory G Protein a-Subunits

To study the function of stimulatory G protein a-subunits, we solubilized Gₐ from rat heart membranes and functionally reconstituted it into murine lymphoma S49 cycl- cell membranes, which genetically lack Gₐ. Fig 7 summarizes adenylyl cyclase activity of S49 cycl- membranes under basal conditions (A) and following stimulation with isoproterenol (B) or Gpp(NH)p (C). Reconstitution of S49 cycl- membranes resulted in an increase of basal adenylyl cyclase by approximately 20% (Fig 7A). Reconstitution with Gₐ restored the stimulatory effect of isoproterenol (B) and Gpp(NH)p (C). The ability of isoproterenol and Gpp(NH)p to stimulate adenylyl cyclase was not different when S49 cycl- membranes were reconstituted with cardiac Gₐ from Dahl S or Dahl R rats on the 0.4% or 8% sodium chloride diet. These findings provide evidence that the activity of the stimulatory G protein a-subunits is not impaired under either condition studied.

Inhibitory G-Protein a-Subunits

To study whether a change of expressed inhibitory G protein a-subunits contributes to the altered adenylyl cyclase activity in Dahl S rats on 8% sodium chloride, we studied Gₐ proteins with a radioimmunoassay. DS 4 antisemum was raised in rabbits against the synthetic C-terminus of retinal transducin a (KENLKDCGLF). Retinal transducin a was purified from rod outer segments of bovine retinas and was used for constructing standard curves. First we set out to determine which G protein a-subtype is predominant in rat myocardial membranes. Therefore, high-resolution electrophoresis on SDS gradient gels in the presence of 4 mol/L urea was performed. A typical experiment is shown in Fig 8. In rat myocardial membranes, two pertussis toxin substrates were observed. The predominant substrate was detected at 40 kD and comigrated with the 40 kD
TABLE 1. Basal and Stimulated Adenylyl Cyclase Activity in Cardiac Membranes From Dahl Salt-Resistant and Salt-Sensitive Rats

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Related to milligrams of protein</th>
<th>0.4%</th>
<th>8%</th>
<th>0.4%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>63.08 ± 9.2</td>
<td>52.29 ± 4.9</td>
<td>89.9 ± 11.8*</td>
<td>34.19 ± 3.57†</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol, 10 μmol/L</td>
<td>950.17 ± 84.6</td>
<td>997.18 ± 44.1</td>
<td>1385.8 ± 74.1*</td>
<td>653.16 ± 76†</td>
<td></td>
</tr>
<tr>
<td>Gpp(NH)p, 100 μmol/L</td>
<td>503.97 ± 85.7</td>
<td>502.16 ± 26.4</td>
<td>715.11 ± 69.6*</td>
<td>404.83 ± 44.1†</td>
<td></td>
</tr>
<tr>
<td>Forskolin, 30 μmol/L</td>
<td>934.58 ± 72.3</td>
<td>1046.93 ± 98</td>
<td>1666.71 ± 122.6*</td>
<td>735.6 ± 66.6†</td>
<td></td>
</tr>
<tr>
<td>MnCl₂, 5 mmol/L</td>
<td>89.2 ± 9.9</td>
<td>83.97 ± 7.8</td>
<td>104.83 ± 11</td>
<td>76.79 ± 5.4†</td>
<td></td>
</tr>
<tr>
<td>MnCl₂+forskolin</td>
<td>1105.66 ± 72.6</td>
<td>1027.19 ± 92.1</td>
<td>1200.92 ± 119.7</td>
<td>861.9 ± 54.3†</td>
<td></td>
</tr>
<tr>
<td>MnCl₂+forskolin+Gpp(NH)p</td>
<td>1272.63 ± 71.9</td>
<td>1156.51 ± 139.4</td>
<td>1381.17 ± 186.8</td>
<td>929.9 ± 57.2†</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Related to 5'-nucleotidase, nmol Pi/mg protein×min</th>
<th>Dahl R</th>
<th>Dahl S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1.75 ± 0.25</td>
<td>1.63 ± 0.15</td>
</tr>
<tr>
<td>Isoproterenol, 10 μmol/L</td>
<td>27.15 ± 2.5</td>
<td>29.14 ± 2.2</td>
</tr>
<tr>
<td>Gpp(NH)p, 100 μmol/L</td>
<td>13.7 ± 3.4</td>
<td>14.25 ± 0.7</td>
</tr>
<tr>
<td>Forskolin, 30 μmol/L</td>
<td>25.44 ± 2.2</td>
<td>31.74 ± 3.74</td>
</tr>
<tr>
<td>MnCl₂, 5 mmol/L</td>
<td>2.47 ± 0.27</td>
<td>2.87 ± 0.27</td>
</tr>
<tr>
<td>MnCl₂+forskolin</td>
<td>30.63 ± 2</td>
<td>31.51 ± 3.3</td>
</tr>
<tr>
<td>MnCl₂+forskolin+Gpp(NH)p</td>
<td>35.25 ± 1.99</td>
<td>34.71 ± 4.28</td>
</tr>
</tbody>
</table>

Dahl R indicates Dahl salt-resistant rats; Dahl S, Dahl salt-sensitive rats; and Gpp(NH)p, guanylylimidodiphosphate. All experiments with MnCl₂ were performed in the absence of MgCl₂. Numbers in parentheses indicate n.

*P < 0.05 vs Dahl R.
†P < 0.05 vs 0.4% sodium chloride diet.

**pertussis toxin substrate in HL 60 cells and human heart known to represent Gαi.**15 The band at 41 kD probably represent Gαi but was very faint, suggesting that only traces of this G protein α-subunit are present in rat myocardial membranes. In addition, this substrate comigrated with recombinant Gαi isolated from E. coli. The antiserum DS 4, used to radioimmunochemically quantify Gαi protein, recognized the recombinant α-subunit rGαi and rGαo. Therefore, the predominant Gαi subtype was quantified by the following experiments. Fig 9 shows a representative immunoblot of Gαi in rat myocardial membranes from the experimental groups studied. The antiserum DS 4 recognized one 40-kD membrane protein comigrating with the DS 4-immunostained Gαi/Gαo α-subunits purified from bovine brain. To quantify Gαi, we used the radioimmunoassay. Fig 10 shows representative competition curves of retinal transducin α as standard and rat and human myocardial membrane extracts. The sensitivity of the radioimmunoassay was 0.25 μg/mL transducin α equivalents. Competition of DS 4 antiserum binding to [32P]-KENLKDCLGLF is shown for comparison. From the standard curve and competition of two concentrations of membrane extracts, the amount of Gαi was determined. The data of the immunochemical quantification of Gαi proteins in myocardial membranes are summarized in Fig 11. In both Dahl S...
and Dahl R rats, treatment with an 8% sodium chloride diet increased $G_{\alpha}$ by approximately 25% compared with the 0.4% sodium chloride groups ($P < .05$). In Dahl S rats, the amount of $G_{\alpha}$ was approximately 50% higher than in Dahl R rats in either treatment group ($P = .01$). Taken together, the results show that the immunodetectable amount of $G_{\alpha}$ proteins was increased in Dahl S compared with Dahl R rats. A high sodium intake increased CYP proteins in either group.

Another frequently used technique to quantify $G_{\alpha}$ proteins is the pertussis toxin-induced ADP ribosylation of a cysteine residue at the fourth position from the C-terminus. The incorporated radioactivity of $[^{32P}]$ADP into the 40-kD $G_{\alpha}$ proteins by pertussis toxin did not significantly differ. The mean values followed the same trend as the changes in the immunodetectable $G_{\alpha}$ proteins. However, none of the differences were significant (not shown). Taken together, the results show that, although mean values of pertussis toxin substrates followed the same trend as the immunochemical amount of $G_{\alpha}$ protein $\alpha$-subunits, the former technique failed to demonstrate a significant increase of $G_{\alpha}$ in Dahl S rats and in the group on the 8% sodium chloride diet.

**Discussion**

A high salt diet produced cardiac hypertrophy due to an increase of arterial blood pressure accompanied by a heterologous desensitization of adenylyl cyclase in Dahl S rats, whereas it had no effect on adenylyl cyclase activity in Dahl R rats. The desensitization in Dahl S rats on 8% sodium chloride is due to an increase of inhibitory G protein $\alpha$-subunits and an apparently dysfunctional catalyst of adenylyl cyclase. A slight but significant increase of immunodetectable $G_{\alpha}$ was also observed in Dahl R rats on 8% sodium chloride versus Dahl R rats on 0.4% sodium chloride and in Dahl S rats on 0.4% sodium chloride versus Dahl R rats on 0.4% sodium chloride. However, no differences in adenylyl cyclase activity were seen in Dahl S rats on 8% sodium chloride versus Dahl R rats on 0.4% sodium chloride.

**Table 2. Density of $\beta$-Adrenergic Receptors in Myocardial Membranes from Dahl Salt-Resistant and Salt-Sensitive Rats on 0.4% and 8% Sodium Chloride Diet**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Related to Membrane Protein, fmol $[^{125}\text{I}]$Cyp bound/mg protein</th>
<th>Related to 5'-Nucleotidase, fmol $[^{125}\text{I}]$Cyp bound/(\mu)mol P$_i$/mg protein x min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl R 0.4% (n=6)</td>
<td>26.64±2.7</td>
<td>0.89±0.1</td>
</tr>
<tr>
<td>Dahl R 8% (n=6)</td>
<td>32.35±0.78</td>
<td>1.16±0.15</td>
</tr>
<tr>
<td>Dahl S 0.4% (n=6)</td>
<td>30.13±2.15</td>
<td>1.05±0.2</td>
</tr>
<tr>
<td>Dahl S 8% (n=6)</td>
<td>31.39±1.99</td>
<td>0.92±0.2</td>
</tr>
</tbody>
</table>

$[^{125}\text{I}]$Cyp indicates $[^{125}\text{I}]$iodocyanopindolol; Dahl R, Dahl salt-resistant rats; and Dahl S, Dahl salt-sensitive rats.
Adenylyl cyclase activity were observed among these groups. Adenylyl cyclase activity was increased in Dahl S rats on 0.4% sodium chloride versus all other groups. The number of β-adrenergic receptors and the activity of the Gαi as judged from reconstitution experiments into S49 cell membranes did not differ significantly in either condition. Although the immunodetectable amount of Gα proteins was different in the groups, pertussis toxin labeling did not show any significant differences.

The activity of adenylyl cyclase is dually regulated by stimulatory and inhibitory receptors and G proteins.47 β-Adrenergic receptors couple through a heterotrimeric stimulatory guanine nucleotide binding protein (Gαβγ) to the catalyst of adenylyl cyclase, thereby stimulating the formation of cAMP from ATP.4748 Conversely, adenylyl cyclase activity is under inhibitory control of the inhibitory guanine nucleotide binding protein (Gai3βγ) that couples M2-muscarinic receptors and A1 adenosine receptors to the adenylyl cyclase.47 This family of G proteins is substrate for the ADP ribosyltransferase activity of pertussis toxin, which ADP-ribosylates a cysteine residue at the fourth position from the C-terminus.47 Pertussis toxin-induced ADP ribosylation of Gαi produces an uncoupling of receptors from the G-protein.47 Because in pertussis toxin–treated cells49 and membranes12 the adenylyl cyclase activity is increased, Gαi might possess a functional role in directly regulating adenylyl cyclase activity. After prolonged stimulation of receptor-regulated adenylyl cyclase with agonists, the adenylyl cyclase becomes desensitized.50 In explanted human hearts from patients with terminal heart failure, a downregulation of β-adrenergic receptors with a concomitant reduction in positive inotropic responses to β-adrenergic receptor agonists711 and cAMP-phosphodiesterase inhibitors10151 has been observed. These functional changes have been suggested to be due to a heterologous desensitization of adenylyl cyclase as measured by reduced basal, isoproterenol-, and Gpp(NH)p-stimulated activity.1215 On the level of the G proteins, an increase in Gαi1246 was observed, whereas Gαs was unchanged as measured immunologically46 as well as with functional reconstitution into S49 cell12 or with cholera toxin labeling.1217 These findings showed that in heart failure heterologous desensitization is due to a reduction of myocardial β-adrenergic receptors and an increased expression of Gαi.

Cardiac hypertrophy due to increased pressure load by hypertension is a compensatory mechanism to reduce wall stress5254 and is regarded to precede the development of heart failure.5556 Thus, the open question is whether in cardiac hypertrophy due to hypertension a similar desensitization of adenylyl cyclase occurs. Several reports are in favor of this suggestion. In SHR, a reduction of cardiac adenylyl cyclase activity has been reported to occur.2829 This desensitization was accompanied by a reduction in the number of β-adrenergic receptors2829 and an increase in Gαi as measured with mRNA expression,29 pertussis toxin labeling,2829 and specific antisera.28 In the present study, we set out to investigate whether a genetic predisposition to developing hypertension, after exogenous factors such as in-

Fig 8. Representative autoradiographs show pertussis toxin–catalyzed [32P]ADP ribosylation of G protein α-subunits in HL 60 cell membranes, human and rat cardiac membranes, and recombinant G protein α-subunits (rGα1-3, rGoa). Samples (HL 60: 6 μg/lane, human heart: 10 μg/lane, rat heart: 100 μg/lane) were [32P]ADP-ribosylated with pertussis toxin plus [32P]NAD and were separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis in the presence of a gradient of 4 to 8 mol/L deionized urea. Exposure time was 2 hours.

Fig 9. Western blot shows Gαi (M = 40 kD) by the antiserum DS 4 in myocardial membranes from Dahl salt-resistant (Dahl R) and salt-sensitive (Dahl S) rats on 0.4% or 8% sodium chloride diet. Immunostained material at approximately 40 kD comigrated with Gαi/Gαs purified from bovine brain. In membranes from the Dahl R strain, it appeared that slightly less Gαs was detected than in Dahl S membrane, particularly when rats were held on 0.4% sodium chloride.
increased salt intake were imposed, is capable of producing a heterologous desensitization of cardiac adenylyl cyclase. In the present study, a high-salt diet produced hypertension and cardiac hypertrophy in Dahl S but not Dahl R rats. The development of hypertension and hypertrophy was accompanied by a marked reduction of isoproterenol-, Gpp(NH)p-, and forskolin-stimulated adenylyl cyclase activity in Dahl S but not Dahl R rats. This finding suggests that exogenous factors leading to hypertension might also be involved in the genesis of heterologous adenylyl cyclase desensitization.

The reduced adenylyl cyclase activity in Dahl S rats on 8% sodium chloride was not accompanied by changes in the number of β-adrenergic receptors. Unchanged numbers of β-adrenergic receptors were obtained in renal hypertension of 1K1C rats,23 and DOCA-salt hypertensive rats.25 These findings show that heterologous desensitization in the rat heart with hypertensive cardiac hypertrophy of different genesis can occur without changes in β-adrenergic receptors. Thus, the findings in these models and the Dahl S rats on an 8% sodium chloride diet (this study) contrast not only with observations in the failing human heart but also with reports on SHR in which the β-adrenergic receptor numbers were also decreased.28-29 However, as in the failing human heart,12,17,58 functional reconstitution of Gαs into S49 cec cell membranes showed no difference in Gαs activity among the groups studied.

On the cellular level, an increase of Gαs content in myocardial membranes from Dahl salt-resistant (Dahl R) and salt-sensitive (Dahl S) rats on 0.4% or 8% sodium chloride diet. The following values were statistically significant: Dahl R 0.4% vs Dahl R 8%; Dahl S 0.4% vs Dahl S 8%; and Dahl S 8% vs Dahl R 0.4%.

![Fig 10. Line graph shows increasing concentration of transducin α (standard curve) and rat and human myocardial membranes competing for binding of 125I-KENLDCGLF to the antiserum DS 4.](image)

![Fig 11. Plot shows radioimmunologically quantified Gαs content in myocardial membranes from Dahl salt-resistant (Dahl R) and salt-sensitive (Dahl S) rats on 0.4% or 8% sodium chloride diet. The following values were statistically significant: Dahl R 0.4% vs Dahl R 8%; Dahl S 0.4% vs Dahl S 8%; and Dahl S 8% vs Dahl R 0.4%.](image)
Various Models of Hypertensive Cardiac Hypertrophy

<table>
<thead>
<tr>
<th>STUDIED PARAMETERS</th>
<th>SHR</th>
<th>1K1C, 2K1C Rats</th>
<th>DOCA Rats</th>
<th>Dahl S Rats (8% NaCl*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Adrenergic receptors</td>
<td>▼</td>
<td>(22.27,57,76.77)</td>
<td>▼</td>
<td>(78.79)</td>
</tr>
<tr>
<td></td>
<td>▼</td>
<td>(25,26)</td>
<td>▼</td>
<td>(26, this study)</td>
</tr>
<tr>
<td>Catalyst of adenyly cyclase</td>
<td>▼</td>
<td>(22)</td>
<td>▼</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>▼</td>
<td>(80)</td>
<td>▼</td>
<td>(This study)</td>
</tr>
<tr>
<td>Gα protein</td>
<td>▼</td>
<td>(22.81 [mRNA])</td>
<td>▼</td>
<td>(80 [cholera toxin labeling])</td>
</tr>
<tr>
<td></td>
<td>▼</td>
<td>(80 [functional])</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td>Gβ protein</td>
<td>▼</td>
<td>(28,29,82)</td>
<td>▼</td>
<td>(251)</td>
</tr>
<tr>
<td></td>
<td>▼</td>
<td>(81 [mRNA])</td>
<td>▼</td>
<td>(This study)</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; 1K1C, one-kidney, one clip; 2K1C, two-kidney, one clip; DOCA, deoxycorticosterone acetate; Dahl S, Dahl salt-sensitive; ▼, increase; and ▼, decrease.

*Compared with Dahl S on 0.4% NaCl

Weak increase.

The catalyst is depressed in Dahl S rats on 8% sodium chloride. However, in addition to or as a consequence of these genetic alterations, there are several indications for an enhanced sympathetic activity in Dahl S rats after sodium chloride loading. Although catecholamine plasma concentrations have been reported to be unchanged in Dahl S and R rats on a high- and low-salt diet, the response of plasma epinephrine concentrations to graded electrical foot shocks was greater in the high-salt than in the low-salt intake group in both Dahl R and S rats. In Dahl S rats, increased norepinephrine turnover was observed compared with Dahl R rats. High sodium chloride intake further increased norepinephrine turnover in the atrial appendages, sinus atrial node, and renal cortex in this strain. In addition to impaired cardiopulmonary baroreceptor reflex activity, enhanced adrenal norepinephrine synthesis, and failure to downregulate norepinephrine synthesis, sympathetic nerve activity after volume expansion with sodium chloride was observed in Dahl S rats. Thus, these changes in the activity of the sympathetic nervous system — ie, an aggravation of sympathetic activity in Dahl S rats after sodium chloride exposure— might induce the heterologous desensitization of adenyly cyclase and the increase of Gα in Dahl S rats on 8% sodium chloride. However, the mechanism by which the activity of the catalytic subunit is depressed cannot be explained at present. In addition, the increased adenyly cyclase activity in Dahl S rats on 0.4% sodium chloride could be due to an enhanced activity of the catalytic subunit. Furthermore, it is interesting that in Dahl S rats on 4% sodium chloride, stimulated adenyly cyclase activity was increased despite a relative increase of Gα compared with Dahl R rats on 0.4% sodium chloride. This observation could provide evidence that an increased activity of the catalytic subunit is required to overcome small increases of Gα. Alternatively, a threshold concentration of Gα could be necessary to produce inhibitory effects on adenyly cyclase activity. The exact mechanisms of these functional alterations have yet to be further elucidated.
but not in renal hypertensive rats and SHR; there-
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groups are summarized in Table 3, which gives data of
changes in adenylyl cyclase, β-adrenergic receptor, and
G protein in various models of hypertensive cardiac
hypertrophy. From the available studies, it appears most
likely that in the absence of any changes in
β-adrenergic receptors, receptor-independent changes
in the catalyt and inhibitory G protein α-subunits could play a general role in the regulation of myocardial
adenyl cyclase activity in pathological conditions such as hypertensive cardiac hypertrophy.

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