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SUMMARY The pattern of cardiac β-adrenergic receptor changes in different hypertrophy models varies according to the pathophysiology. In salt-sensitive Dahl rats, high dietary salt intake leads to a moderate degree of cardiac hypertrophy associated with increased numbers of cardiac β-adrenergic receptors but unchanged affinity for agonists. Isoproterenol-stimulated cardiac adenylyl cyclase is also higher in salt-loaded hypertensive rats without any change in basal or NaF-stimulated activities. In contrast, neither β-adrenergic receptors nor adenylyl cyclase activities are affected by variations in dietary salt in salt-resistant Dahl rats. The extent of isoproterenol-induced down regulation of β-adrenergic receptors on isolated cardiac myocytes as well as the recovery from this down regulation is not significantly different in either strain of Dahl rats and is not influenced by dietary salt. The enhancement of β-adrenergic pathways in salt-dependent genetic hypertension may be involved both in the initiation of cardiac hypertrophy and the preservation of contractile function.

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Key Words • adenylate cyclase • desensitization • hypertrophy • isoproterenol

Considerable evidence suggests that β-adrenergic mechanisms play a major role in the regulation of cardiac function. It is well known, for example, that β-agonists modulate the amount of activator Ca$^{2+}$ entering the cardiac cell following depolarization by opening sarcolemmal calcium channels. In addition, calcium transport by the sarcoplasmic reticulum-mediated relaxation of cardiac myocytes is also under β-adrenergic control. It is not surprising, therefore, that considerable attention has been focused on the relation between β-agonist and β-adrenergic receptor interactions and cardiac function in hypertrophy and failure. Indeed, several reports indicate that the inotropic responsiveness to catecholamines is diminished in the hypertrophied myocardium. The molecular basis for this decline is incompletely understood, however.

We have previously described a decline in the number of cardiac β-adrenergic receptors in spontaneously hypertensive rats of the Aoki-Okamoto strain. Subsequently, lower isoproterenol-stimulated adenylyl cyclase activity and contractile responses to β-agonists were reported for this model of genetic hypertension. It is not known, however, whether this reduction in β-adrenergic pathways is a consequence of cardiac hypertrophy or related to the pathogenesis of this particular model of hypertension. We have attempted to address this issue by studying cardiac β-adrenergic receptors and adenylyl cyclase in another model of genetic hypertension, the Dahl rat. Hypertension develops in the salt-sensitive Dahl rats (DS) in response to increased dietary salt while the salt-resistant Dahl rats (DR) remain normotensive irrespective of salt intake. Our results indicate substantial differences in cardiac β-adrenergic receptor regulation in Dahl rats compared with other models of hypertension.

Materials and Methods

Male DS and DR were purchased from Brookhaven National Laboratories, Upton, NY. At 5 weeks of age, the animals began receiving either a 0.6% or 8.0% NaCl diet, and they were killed 2 weeks later. Blood pressure measurements were obtained twice weekly using a tail cuff sphygmomanometer. At death, the heart to body weight ratios were determined and cardiac membranes were obtained as previously described for β-adrenergic receptor and adenylyl cyclase determinations. To study the effect of a more prolonged duration of high salt intake on cardiac β-adrenergic...


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receptors, DS and DR were randomized on the 0.6% or 8.0% NaCl diet at 5 weeks of age and animals (8 in each diet and strain subgroup) killed 6 weeks later.

For cardiac β-adrenergic receptor assays, the medium contained 25 mM Tris-hydrochloride (pH 7.5), 5 mM MgCl₂, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetate acid, 1 mM ethylenediaminetetraacetate acid, 10 mM theophylline, 5 mM phosphoenolpyruvate, 10 μg/ml pyruvate kinase, 0.5 mM [α-32P] ATP (specific activity, 33 Ci/mmol; New England Nuclear) and 20 to 30 μg of protein in a total volume of 0.1 ml. When appropriate, NaF (8 mM) or isoproterenol (0.1 mM) was added. The reaction was carried out at 37 °C for 15 minutes, and the amount of cyclic AMP formed was determined by the method of Salomon et al. 10

For adenylate cyclase assays, the medium contained 25 mM Tris-hydrochloride (pH 7.5), 5 mM MgCl₂, 1 M NaCl diet significantly increased the blood pressure of DS rats, whereas no difference in blood pressure was observed in DR rats.

**Results**

As expected, short-term administration of high salt diet significantly increased the blood pressure of DS rats (134 ± 5 mm Hg on 0.6% NaCl to 162 ± 6 mm Hg on 8.0% NaCl; p < 0.01) but not of DR (from 113 to 136 ± 7 mm Hg, p > 0.05). The slopes of the 2×2 factorial design indicate that diet significantly increased the blood pressure in DS, whereas no difference in blood pressure was observed in DR.
± 3 to 118 ± 3 mm Hg). A mild degree of cardiac hypertrophy developed in DS fed 8.0% NaCl as evidenced by an increase in heart to body weight ratios from 2.03 ± 0.04 mg/g to 2.25 ± 0.04 mg/g (p < 0.01) and heart weight from 0.45 ± 0.01 g to 0.49 ± 0.01 g (p < 0.01). There was, however, no correlation between the level of systolic blood pressure and heart to body weight ratio in any of the experimental groups. Significant differences between the two strains were noted in the response of the cardiac β-adrenergic receptor-adenylate cyclase system to the high salt intake. As shown in Figure 1, the number of the β-adrenergic receptors detected on cardiac membranes was similar in DR and DS fed 0.6% NaCl but increased in DS fed 8.0% NaCl while it remained unchanged in DR. This increase in adrenergic receptor numbers was not accompanied by any change in their affinities.

which were unaffected by strain or diet (Figure 2). The substantial increase in the number of cardiac β-adrenergic receptors in DS given a high salt diet is not a direct consequence of adrenergic receptor modification by NaCl because addition of NaCl to the assay medium failed to alter the number of cardiac β-adrenergic receptors assayable with [3H]dihydroalprenolol (data not shown).

The changes in cardiac adenylate cyclase activities are shown in Figure 3. In DR, there was no significant influence of diet on basal, NaF-stimulated, or isoproterenol-stimulated activities. In contrast, isoproterenol-stimulated adenylate cyclase was significantly higher in DS fed 8.0% NaCl (p < 0.05, compared with 0.6% NaCl) while basal and NaF-stimulated activities were unaffected. The concentration dependence of adenylate cyclase stimulation by isoproterenol is shown in Figure 4. The percent stimulation over basal activity was not altered by diet in DR over a wide range of isoproterenol concentrations in contrast to the significant increase seen for DS fed 8.0% NaCl (p < 0.01). The possibility that isoproterenol-induced down regulation is affected by salt intake in Dahl rats was then examined using isolated cardiac myocytes. As
shown in Figure 5, after in vitro incubation of cardiac myocytes with 1 μM (-)-isoproterenol, there was a rapid "loss" of β-adrenergic receptors detectable with [3H]CGP-12177, which labels only surface-bound receptors. As expected, this apparent loss was dependent on the concentration of isoproterenol (Figure 6), with half-maximal decline at 0.5 μM (-)-isoproterenol.

That isoproterenol-induced down regulation did not lead to irreversible loss of cardiac β-adrenergic recep-

![Figure 5](image_url)

**Figure 5.** Time course of cardiac β-adrenergic receptor decline during incubation of cardiac myocytes from DS or DR on either 0.6% or 8.0% NaCl diet with 1 μM (-)-isoproterenol at 37 °C. Results are expressed as percent of controls (incubated in the absence of isoproterenol).

![Figure 6](image_url)

**Figure 6.** Concentration dependence of isoproterenol-induced decline in cardiac β-adrenergic receptors detectable in myocytes from DS or DR on either 0.6% or 8.0% NaCl diet. Incubations were carried out at 37 °C for 20 minutes with the indicated concentrations of (-)-isoproterenol, and the myocytes were washed with excess buffer before the β-adrenergic receptor assay using 6 nM [3H]CGP-12177 as described in the text. Results are expressed as percent of controls (incubated in the absence of isoproterenol).

![Figure 7](image_url)

**Figure 7.** Time course of β-adrenergic receptor reappearance following (-)-isoproterenol-induced down regulation. Cardiac myocytes from DS and DR were incubated with 1 μM (-)-isoproterenol for 15 minutes at 37 °C. They were then washed and resuspended in sucrose, Tris-hydrochloride, Mg2+ buffer; incubation at 37 °C was carried out in the presence of 6 nM [3H]CGP-12177 for 2 to 20 minutes as described in the text. Results are expressed as percent recovery (binding over that of myocytes exposed to (-)-isoproterenol but not reincubated at 37 °C).

Isoproterenol induced a decline in the number of binding sites to 53 ± 6% of controls (without isoproterenol) in rats fed 0.6% NaCl and 52 ± 8% in those fed 8.0% NaCl. After incubating isoproterenol-treated, washed myocytes at 37 °C, 91 ± 5% of the β-adrenergic receptors reappeared in DS fed 0.6% NaCl compared with 87 ± 7% for the 8.0% NaCl group. Corresponding data for the DR are given in Figure 9. Again in agreement with the results obtained with [3H]dihydroalprenolol and isolated membranes, a high salt diet did not change the number of β-adrenergic receptors detectable on cardiac myocytes from DR. Similarly, the extent of isoproterenol-induced decline of β-adrenergic receptors (50 ± 7% of controls in DR fed 0.6% NaCl compared with 47 ± 6% in the 8.0% NaCl group) and their reappearance after removal of the agonist (86 ± 8% of controls in the 0.6% NaCl vs
To study the effect of more prolonged salt intake on cardiac β-adrenergic receptors, groups of DS and DR were randomized to a high (8.0%) or regular (0.6%) NaCl diet at 5 weeks of age and were killed 6 weeks later; eight animals were included in each diet and strain group. At the end of the study period, blood pressure had increased (to 189 ± 6 mm Hg) in the DS on 8.0% NaCl alone. Similarly, heart weights (1.10 ± 0.04 g on 8.0% NaCl vs 0.98 ± 0.02 g on 0.6% NaCl; p < 0.01) and heart to body weight ratios (3.36 ± 0.12 mg/g vs 2.5 ± 0.07 mg/g respectively; p < 0.001) were higher in the high salt DS while they were unchanged in DR. The number of β-adrenergic receptors was assayed on isolated cardiac myocytes using [3H]CGP-12177, as described in the Methods section. There was a significant increase in β-adrenergic receptor numbers in the high salt DS (85.2 ± 6.4 fmol/mg compared with 52.4 ± 5 fmol/mg on 0.6% NaCl; p < 0.01) with no significant change in the DR (48.2 ± 3 fmol/mg on 8.0% NaCl vs 50.1 ± 3.5 fmol/mg on 0.6% NaCl).

Discussion

Short-term administration of a high salt diet to Dahl rats resulted in a rise of systolic blood pressure in the salt-sensitive strain only. Although this rise was associated with a mild degree of cardiac hypertrophy, there was no correlation within the DS between levels of systolic blood pressure and the extent of hypertrophy. Such dissociation between severity of hypertension and cardiac hypertrophy has been observed by others and raises the possibility that other factors besides the functional overload imposed by increased blood pressure contribute to the development of hypertrophy. Since administration of catecholamines is known to induce cardiac hypertrophy and activation of the sympathetic nervous system accompanies the development of several models of cardiac hypertrophy, it has been suggested that adrenergic pathways are involved in the initiation of hypertrophy. In addition, they may contribute to the inotropic support of the hypertrophied myocardium and loss of their effectiveness may lead to deterioration of cardiac pump function.

It has not been established whether adrenergic pathways are altered in the myocardium of Dahl rats. Hemodynamic studies suggest that, despite development of marked hypertrophy, cardiac performance is well preserved in this hypertensive model; however, inotropic responsiveness to catecholamines has not been examined. Since the molecular basis for the physiological effects of catecholamines on the myocardium involves the interaction of these hormones with specific receptors on the cell surface and subsequent activation of adenylate cyclase, an examination of cardiac β-adrenergic receptors in Dahl rats is germane. A number of previous studies have indicated that hormone–adrenergic receptor interactions are altered as a result of hypertrophy and failure. In general, both experimental and human congestive heart failure is associated with decreased numbers of cardiac β-adrenergic receptors and low hormone-sensitive adenylate cyclase activity. In contrast, cardiac hypertrophy results in either high or low numbers of β-adrenergic receptors depending on its pathogenesis. In this respect, our demonstration of enhanced numbers of cardiac β-adrenergic receptors and isoproterenol-stimulated adenylate cyclase in salt-loaded DS is of interest because it differs dramatically from the reported decline in the spontaneously hypertensive rat as well as in induced hypertension. This variance is not

85 ± 7% in the 8.0% NaCl group) were not significantly different in the two diet groups.
simply due to differences in the severity of hypertension since low numbers of β-adrenergic receptors (as well as decreased inotropic responsiveness to β-agonists) is an early event in the spontaneously hypertensive rat. Furthermore, changes in the cardiac β-adrenergic receptor-adenylate cyclase system of DS occur in the presence of modest degrees of cardiac hypertrophy. The most likely explanation relates to possible differences in the pathophysiology of the hypertensive models.

The direct or indirect role of the high salt intake in mediating changes in the cardiac β-adrenergic receptor-adenylate cyclase system of Dahl rats should be considered. Such a role most likely is linked to the propensity to develop hypertension in response to dietary salt, since DR do not undergo any cardiac β-adrenergic receptor changes. However, it is not a direct influence on the β-adrenergic receptors since NaCl has no effect when added to the receptor assay mixture. A more likely pathogenetic pathway involves the changes in circulating catecholamines induced by variations in dietary salt intake. In humans, a high salt intake has been reported to result in enhanced cardiac β-responsiveness while sodium depletion by diuretics has the opposite effect. In the spontaneously hypertensive rat, MacPhee et al. have shown that a high salt diet attenuates the age-dependent decline in cardiac β-adrenergic receptors. The increase in cardiac β-adrenergic receptors of DS fed 8.0% NaCl may represent up regulation secondary to decreased sympathetic activity mediated through volume expansion. The lack of β-adrenergic receptor enhancement in DR would represent either hemodynamic adjustments, which minimize volume expansion in the face of salt loading, or failure of the sympathetic activity to decline despite volume expansion. This possibility is still speculative, however, since no major changes in plasma catecholamines have been observed during a high salt intake in either DS or DR. Furthermore, changes in dietary salt intake fail to affect cardiac norepinephrine concentration in either Dahl rat strain. Although an increase in cardiac output occurs in DS or DR in response to a high salt intake, this is transient and cardiac output returns to normal by the second week. The relation between cardiac β-adrenergic receptors, hemodynamic changes, and sympathetic activity in salt-dependent hypertension remains to be elucidated.

The interaction between plasma catecholamines (as a reflection of sympathetic nervous system activation) and cardiac β-adrenergic receptors is of considerable functional importance from another standpoint. It is known that occupancy of the β-adrenergic receptors by agonists leads to desensitization (i.e., loss of functional responsiveness to further agonist administration). This desensitization is characterized by a reversible loss of the β-adrenergic receptors (down regulation). We have recently examined some of the mechanisms involved in the cycling of the cardiac β-adrenergic receptors after exposure to isoproterenol. The apparent loss of cell surface-bound β-adrenergic receptors is related to their translocation to the cytoplasm and requires intact microtubule assembly. Reappearance of ostensibly lost β-adrenergic receptors is also rapid and energy dependent, and involves the lysosomes, Golgi apparatus, and microtubules. We compared this process in DS and DR, since this shuttling of the β-adrenergic receptors requires the participation of several organelles that may be altered in hypertension. Our results, reported herein, show that the intracellular traffic of the cardiac β-adrenergic receptors is intact in Dahl rats and is unaffected by diet. This finding is in striking contrast to the situation in the myocardium of spontaneously hypertensive rats, which demonstrates decreased susceptibility to isoproterenol-induced down regulation compared with that in age-matched Wistar-Kyoto rats. It is likely, therefore, that β-adrenergic receptor cycling is differentially affected in various models of hypertension. Since, in addition to the slow process of de novo synthesis, maintenance of a normal complement of membrane-bound β-adrenergic receptors depends on efficient recycling of internalized receptors, this intracellular traffic should be included in future studies of cardiac β-adrenergic receptor involvement in hypertrophy and failure.

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