Enhanced isoproterenol-induced cardiac hypertrophy in transgenic rats with low brain angiotensinogen

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THE RENIN-ANGIOTENSIN SYSTEM (RAS) is acknowledged to interact with the autonomic nervous system (ANS) at several levels, namely at postganglionic nerve terminals (13), sympathetic ganglia (11, 27), and within the central nervous system (43). Central mechanisms involved in the long-term regulation of the cardiovascular system by correlated actions of the RAS and the sympathetic nervous system (SNS) have been described in both homeostatic and pathological conditions (1, 10, 14, 23). Several studies (3, 5, 18, 29) employing transgenic models indicate that the brain RAS may affect the ANS. Also, it has been shown that a transgenic manipulation of glial angiotensinogen may alter baroreflex control of heart rate (HR) (5, 32) through the SNS (32). A reduced SNS activity may lead to an increased sensitivity of hearts to catecholamines (12, 39). Previous data suggest that the transgenic rats with low levels of brain angiotensinogen [TGR(ASrAOGEN)] have a decreased sympathetic outflow (3, 18, 41) and a deficient sympathetic hyperactivity after myocardial infarction (41). This is why we investigated the hypothesis that cardiac responses to β-adrenoceptor (β-AR) stimulation are increased in TGR(ASrAOGEN). Thus we studied the cardiac inotropic and hypertrophic effects of isoproterenol in these transgenic rats.

METHODS

Rat strains. Adult (age, 5 mo) male transgenic rats [TGR(ASrAOGEN)] and age-matched Hanover Sprague-Dawley (SD, parent strain used as normal controls) rats were used. The rats were housed under a 12-h:12-h light-dark schedule at 24 ± 2°C and given free access to a standard rat diet and tap water. All experimental protocols were performed in accordance with the guidelines of the American Physiological Society and approved in advance by the local Animal Ethics Committees.

Isolated retrograde perfused heart preparation. A group of rats maintained under basal conditions was used to study the functional parameters of isolated hearts [5 SD and 5 TGR(ASrAOGEN)]. Left ventricular (LV) contractile function was investigated using the isovolumetric Langendorff technique adapted by Strömer et al. (20, 36) for accurate comparison of hearts of different sizes. The animals were weighed and anesthetized with 4% chloral hydrate (350 mg/kg body wt ip). The hearts were excised and immersed in ice-cold Krebs-Henseleit solution containing (in mmol/l) 4.74 KCl, 25 NaHCO3, 1.19 MgCl2, 1.19 KH2PO4, 11 glucose, 118 NaCl, and 2.5 CaCl2. After being weighed, the hearts were mounted on a cannula inserted into the ascending aorta just below the aortic arch and attached to a perfusion apparatus. Retrograde perfusion of the heart was carried out at a constant coronary perfusion pressure (60 mmHg). The heart was perfused with Krebs-Henseleit solution oxygenated with 95% O2-5% CO2 (resulting in a pH 7.4) at 37°C. Coronary flow was measured with an electromagnetic flow probe. LV isovolumetric pressure was measured through a water-filled compliant balloon (inducing a ventricular preload fixed at 12 mmHg) connected to a pressure transducer. Heart rate was fixed at 340 beats/min using pericardial electrostimulation. All experimental protocols were performed in accordance with the guidelines of the American Physiological Society and approved in advance by the local Animal Ethics Committees.

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rates, and coronary flow were calculated as percentages of initial basal values.

Isoproterenol-induced cardiac hypertrophy. A group of rats received isoproterenol (β-sympathomimetic, 5 mg/kg body wt/day sc) [5 SD and 5 TGR(ASrAOGEN)] or vehicle [6 SD and 6 TGR(ASrAOGEN)] for 7 days. This dose was selected on the basis of pilot experiments showing that it was well tolerated (low mortality rate) (33), had no effect on mean arterial pressure (MAP), and was sufficient to induce cardiac hypertrophy (17). After 7 days, the rats were euthanized and the heart was washed in 0.9% saline and weighed. The LV was then carefully separated from the right ventricle and atri, weighed, and snap-frozen in liquid nitrogen for subsequent RNA extraction and gene expression studies.

Cardiovascular effects of β₁-adrenoceptor or parasympathetic blockade. The measurements of MAP and HR were performed in conscious rats [6 SD and 6 TGR(ASrAOGEN)]. After anesthesia with 4% chloral hydrate (350 mg/kg ip), polyethylene catheters [PE-10 (ID, 0.28 mm, and OD, 0.61 mm)] were fused with a PE-50 (ID, 0.38 mm, and OD, 0.96 mm), and then filled with 10 IU/ml heparinized saline] were inserted into the femoral artery and vein for blood pressure recordings and intravenous drug injections, respectively. The catheters were exteriorized in the interscapular area. Two days were permitted for recovery after the catheters were implanted, before starting the experimental protocols. The arterial catheter was connected to a standard blood pressure transducer (model SP884, Sensoron), which was connected to a data acquisition and analysis system (PowerLab, ADInstruments). After 30 min of stabilization, arterial blood pressure was recorded for at least 30 min. After that, drugs were injected intravenously, and the arterial blood pressure continued to be monitored for at least 60 min. Each rat received a single injection of drugs per day. The experimental protocol for drug administration was as follows: day 1, methyl-atropine (muscarinic acetylcholine receptor antagonist, 0.5 mg/kg); and day 2, metoprolol (β₁-adrenoceptor blocker, 1 mg/kg). For cardiovascular analysis, MAP and HR were extracted as follows: mean value over a 30-min interval or mean value over a 30-min interval starting 20 min after drug administration represented baseline level or the drug effect, respectively.

β₁-AR and β₁-AR kinase-1 mRNA determination. Total RNA was isolated from LVs using the TRIZol Reagent (Life Technologies, Eggenstein, Germany), followed by chloroform-isopropanol extraction, according to the protocol of the manufacturer. Quantification of β₁-AR and β₁-AR kinase-1 (β₁-ARK1) mRNA was performed with a real-time RT-PCR assay (2) on an Icycler real-time PCR detection system (Bio-Rad, Munich, Germany). The forward and reverse primers and the fluorogenic probes used were as follows: for β₁-AR (GenBank accession number NM_012701), CGC TCA CCA ACC TCT TCA TCA, AAG GCA CCA CCA GCA GTC C, TGG CCA CGG CCG ATC TGG TC; for β₁-ARK1 (GenBank accession number M87854), CGC CAG CAA GAA GAT CCT G, CCT CTA GAT ACT TCT GCA TGA CGC, TGC CAG AGC CCA GCA TCC GC; and for β-actin (accession number V01217), CCT CTC AAG CTC AAG GCC AA, AGG CTT GAT GAC TAC GTA CA, TGA CCC AGA TCA TGT TGT AGA CCT TCA AC. The fluorogenic probes were labeled at the 5′ end with 6-carboxy-fluorescein (6-FAM, reporter fluorochrome) and at the 3′ end with 6-carboxy-tetramethyl-rhodamine (TAMRA, quencher fluorochrome). Oligonucleotides were synthesized by BioTec (Berlin-Buch, Germany). The cDNA obtained by reverse transcription of the total RNA was used for each PCR with the following time course: 2 min at 50°C and 10 min of initial denaturation at 95°C to activate SuperTag real-time polymerase (Ambion, Huntingdon, Cambridgeshire, UK), followed by 20 cycles of 2-step PCR consisting of 15 s at 95°C and 1 min at the annealing temperature specific for each primer pair. Each sample was tested in triplicate. Expression levels were normalized to β-actin expression using the 2^{-ΔΔCt}, where Ct is cycle threshold (26) or Pfaffl method (30), because they are considered as reliable to analyze relative changes in gene expression of real-time RT-PCR experiments.

Statistical analysis. Comparisons for multigroup and multifactorial analysis were realized with a two-way ANOVA and the Student-Newman-Keuls method for multiple comparison procedures. Changes versus control values (before intravenous drug administrations) were also studied by statistical analysis with the Student’s paired t-test. The criterion for significant differences between groups of study was P < 0.05. Data are presented as means ± SE.

RESULTS

Enhanced reactivity to isoproterenol of hearts isolated from TGR(ASrAOGEN). In isolated heart preparations, the heart weight-to-body weight ratio and the levels of LVPd, +dP/dt max, and −dP/dt max were not different between TGR(ASrAOGEN) and SD rats (Table 1). Isoproterenol induced a significantly greater increase in LVPd and +dP/dt max in TGR(ASrAOGEN) compared with SD rats (Fig. 1 and Table 1). The isoproterenol-induced −dP/dt max was not different between TGR(ASrAOGEN) and SD rats (Fig. 1 and Table 1). Coronary flow was higher at baseline in TGR(ASrAOGEN) than in SD rats and was not altered by the highest concentration of isoproterenol in either strain of rats.

Enhanced isoproterenol-induced cardiac hypertrophy in TGR(ASrAOGEN). The LV-to-body weight ratio (LV, index, in g/100 g body wt) was significantly increased by isoproterenol treatment in both TGR(ASrAOGEN) and SD rats (Fig. 2). The LV index increase in TGR(ASrAOGEN) was significantly higher than in SD rats (39.9% vs. 29.1%, respectively; Fig. 2). In untreated animals, there were no differences in LV-to-body weight ratio between the TGR(ASrAOGEN) and SD rats.

Table 1. Functional parameters of hearts isolated from TGR(ASrAOGEN) and SD rats: effect of isoproterenol treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SD</th>
<th>TGR(ASrAOGEN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight/body weight, g</td>
<td>0.37±0.02</td>
<td>0.36±0.01</td>
</tr>
<tr>
<td>LVPd, ℴHg</td>
<td>52.2±1.2</td>
<td>51.0±1.6</td>
</tr>
<tr>
<td>Isoproterenol, % of basal</td>
<td>335.3±6.0</td>
<td>382.8±4.8*</td>
</tr>
<tr>
<td>Minimal effect pEC50</td>
<td>8.6±0.02</td>
<td>8.8±0.03*</td>
</tr>
<tr>
<td>+dP/dt max, ℴHg</td>
<td>30.5±6.0</td>
<td>1,757.6±60.8</td>
</tr>
<tr>
<td>Basal, mmHg/s</td>
<td>1,819.08±30.5</td>
<td>1,757.6±60.8</td>
</tr>
<tr>
<td>Isoproterenol, % of basal</td>
<td>481.4±4.6</td>
<td>560.6±17.4*</td>
</tr>
<tr>
<td>Minimal effect pEC50</td>
<td>8.4±0.02</td>
<td>8.5±0.07</td>
</tr>
<tr>
<td>−dP/dt max, ℴHg</td>
<td>−30.5±6.0</td>
<td>−927.0±28.4</td>
</tr>
<tr>
<td>Basal, mmHg/s</td>
<td>−1,014.0±25.5</td>
<td>−927.0±28.4</td>
</tr>
<tr>
<td>Isoproterenol, % of basal</td>
<td>526.4±9.8</td>
<td>553.2±27.7</td>
</tr>
<tr>
<td>Minimal effect pEC50</td>
<td>8.4±0.03</td>
<td>8.6±0.1</td>
</tr>
<tr>
<td>Coronary flow</td>
<td>21.12±1.07</td>
<td>17.7±0.357*</td>
</tr>
<tr>
<td>Basal, ml/min</td>
<td>100.54±6.36</td>
<td>101.14±0.628</td>
</tr>
<tr>
<td>Isoproterenol, % of basal</td>
<td>21.12±1.07</td>
<td>17.7±0.357*</td>
</tr>
<tr>
<td>Minimal effect pEC50</td>
<td>100.54±6.36</td>
<td>101.14±0.628</td>
</tr>
</tbody>
</table>

Values are means ± SE. Maximal effect is calculated as percentage of basal value, because basal values do not significantly differ between transgenic rats with low brain angiotensinogen [TGR(ASrAOGEN)] and Sprague-Dawley (SD) rats. LVPd, developed left ventricular pressure; +dP/dt max and −dP/dt max, maximal contraction and relaxation, respectively; pEC50, negative logarithm of molar concentration of agonist producing 50% of maximum response (calculated after data was converted to percentage of maximum response). *P < 0.001, †P < 0.05 vs. SD control.
Levels of LV β-AR mRNA were not significantly different between TGR(ASrAOGEN) and SD rats (Fig. 3A). Isoproterenol treatment induced a significantly greater decrease in β-AR mRNA levels in the TGR(ASrAOGEN) compared with SD rats. Contrary to this, the levels of LV β-ARK1 mRNA were significantly increased in TGR(ASrAOGEN) compared with SD rats after isoproterenol treatment (85.8% vs. 36.5% β-ARK1/β-actin mRNA, respectively; Fig. 3B).

Decreased cardiovascular responsiveness to sympathetic but not parasympathetic antagonists in TGR(ASrAOGEN). The β-AR antagonist metoprolol induced a decrease in HR, which was significantly smaller in TGR(ASrAOGEN) compared with SD rats (Fig. 4A). Baseline HR levels (before drug application) in TGR(ASrAOGEN) were not different from those of SD rats (361.6 ± 9.8 vs. 376.7 ± 7.4 beats/min, respectively). Baseline MAP was significantly lower in the TGR(ASrAOGEN) compared with SD rats (118.8 ± 3.2 vs. 129.2 ± 3.0 mmHg, respectively) but was not altered by metoprolol in either TGR(ASrAOGEN) or SD rats (Fig. 4B). The parasympathetic blocker methyl-atropine induced a sustained increase of HR, which was not different between TGR(ASrAOGEN) and SD rats (Fig. 4C). The baseline MAP [lower in the TGR(ASrAOGEN) than in SD rats] was not significantly altered by methyl-atropine either in TGR(ASrAOGEN) or in SD rats (Fig. 4D).
DISCUSSION

Neural loci within the brain that generate sympathetic activity include nuclei in the hypothalamus and medulla (10). Local administration of angiotensins or RAS antagonists in such nuclei, including the paraventricular nucleus, rostral or caudal ventrolateral medulla (VLM), and nucleus tractus solitarii, affects the SNS. By using TGR(ASrAOGEN), we have recently demonstrated that a permanently altered brain RAS can alter the cardiovascular reactivity of the rostral VLM (4, 15). Moreover, these rats have an altered autonomic sympathovagal balance, as assessed through evaluation of the sensitivity of the spontaneous baroreflex control of HR (5). The TGR(ASrAOGEN) have an exaggerated spontaneous baroreflex, possibly due to alterations in the reactivity of the nucleus tractus solitarii, as shown by local injection of angiotensins (9) and alterations in ANG II receptor levels (28). Altogether these data indicate that long-term alterations in the brain RAS may affect the ANS. Cardiac hypersensitivity to catecholamines after sympathectomy or denervation (24, 37) despite a normal overall postsynaptic β-AR density has been described (12, 31, 38–40, 42). Using the TGR(ASrAOGEN), Wang et al. (41) have observed that the brain RAS plays an important role in the development of myocardial infarction and the associated sympathetic hypersensitivity. Because a deficiency in brain AOGEN may cause a decreased sympathetic outflow, we hypothesized that the TGR(ASrAOGEN) may have cardiac hyperactivity to β-AR agonist. Indeed, the present experiments showed that the TGR(ASrAOGEN) have increased inotropic effects to the β-AR agonist isoproterenol. This indicates that a marked reduction in brain AOGEN may lead to β-AR hypertrophy activity despite normal LV mRNA levels of β-AR, similarly to the denervation-induced sympathetic hypersensitivity. Studies (42) showing that a central sympathetic inhibition also induces an augmented response to isoproterenol support this contention. TGR(ASrAOGEN) have a lower baseline coronary flow than SD rats, as assessed in the setting of isolated perfused heart. While the significance of this finding remains elusive, it is conceivable that alterations in sympathetic outflow, as observed in TGR(ASrAOGEN), may have a long-term impact on the regulation of coronary flow (34).

The decreases in HR induced by the β-AR antagonist metoprolol were attenuated in TGR(ASrAOGEN) compared with SD rats. Because the TGR(ASrAOGEN) have normal LV mRNA levels of β-AR, these data suggest that these rats have a decreased sympathetic outflow to the heart. Another reason for the decreased HR effects to β-AR antagonist could be an overactive parasympathetic system. However, the effect of parasympathetic blockade by methyl-atropine on HR was similar in both strains. These results suggest also that the brain RAS is rather modulating the sympathetic than the parasympathetic cardiac control, similar to the observations of Sakai et al. (32). Further extended studies employing direct nerve recordings are, however, required to further clarify the mechanisms involved in the SNS regulation by the brain RAS.
Chronic sympathetic stimulation of β-ARs by the nonselective full agonist isoproterenol can induce cardiac hypertrophy. This model of cardiac hypertrophy seems to require solely β-AR stimulation, without the involvement of circulatory or cardiac RAS (25). The isoproterenol-induced cardiac hypertrophy was significantly more pronounced in TGR(ASrAOGEn) than in SD rats, as measured by LV-to-body weight ratio. It is well known that chronic β1-AR stimulation induces receptor desensitization and downregulation (22). As a consequence, we determined the changes in LV β1-AR mRNA levels induced by isoproterenol. A significant decrease in TGR(ASrAOGEn) after isoproterenol treatment was detected, compared with that in the SD rats. The relative nature of mRNA quantification by real-time RT-PCR may account for a lower sensitivity in the detection of smaller changes of LV β1-AR mRNA levels in SD rats in the present experimental setting. The fact that the decrease in β1-AR mRNA levels in SD rats did not reach statistical significance may also suggest that the well-characterized β-AR downregulation in isoproterenol-induced cardiac hypertrophy is a result of posttranscriptional changes (16).

Because we did not determine whether the changes found in mRNA levels of β1-AR translated into changes in protein levels, the possibility that such posttranscriptional modifications occurred in our study cannot be excluded. Measurement of β1-AR expression at protein level would be necessary to provide further insight into the mechanisms of these β1-AR alterations. Nevertheless, our data indicate that downregulation of β1-AR mRNA in response to isoproterenol was more pronounced in TGR(ASrAOGEn) than in SD rats. The main mechanism of receptor desensitization after chronic β-AR stimulation appears to be accounted for by agonist-dependent β-ARK1-mediated phosphorylation of β-AR (6, 19). We observed in the present study that the TGR(ASrAOGEn) show an increase in LV β-ARK1 mRNA levels after isoproterenol treatment, paralleling the observed LV hypertrophy. Thus the data suggest that the increased isoproterenol-induced cardiac hypertrophy in TGR(ASrAOGEn) is due to an increased sensitivity of β-AR, which may occur as a consequence of decreased sympathetic tone.

In summary, the major finding of the present study is that a marked deficiency in brain AOGEN may induce a sympathetic cardiac hypersensitivity to β-AR agonist with an exaggerated inotropic and hypertrophic effect, supporting the hypothesis that the brain RAS is an important modulator of the sympathetic outflow. Further studies, involving direct measurement of sympathetic nerve activity, are therefore required to determine peripheral sympathetic activity. Also, these data support the concept that AOGEN, which is produced in glial cells (8, 21, 35), has actions on neuronal pathways (1, 7).

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REFERENCES


