Depressed Heart Rate Variability and Arterial Baroreflex in Conscious Transgenic Mice With Overexpression of Cardiac $G_{\alpha}$


Abstract—Recently, we developed a transgenic mouse with cardiac-specific $G_{\alpha}$ overexpression (TG mouse), which exhibits enhanced postsynaptic $\beta$-adrenergic receptor signaling, ultimately developing a cardiomyopathy. The goal of the present study was to determine whether cardiac $G_{\alpha}$ overexpression alters autonomic cardiovascular control, which could shed light on the mechanism responsible for the later development of cardiomyopathy. Mean arterial pressure was increased ($P<.05$) in conscious, chronically instrumented TG mice (123±1 mm Hg) compared with age-matched wild-type (WT) control mice (103±1 mm Hg). Respiratory frequency was increased ($P<.05$) in TG mice (269±26/min) compared with WT mice (210±20/min). By use of telemetric techniques, baseline heart rate (HR) was elevated ($P<.05$) in conscious, untethered TG mice (696±13 bpm) compared with WT mice (568±28 bpm). Intrinsic HR, after propranolol and atropine or after ganglionic blockade with hexamethonium, was not different between TG and WT mice. Both the normal minute-to-minute and circadian variations of HR observed in WT mice were markedly blunted in TG mice. HR variability was assessed by the time-domain and frequency-domain methods. At baseline, time-domain analysis indices were reduced ($P<.05$) in TG mice compared with WT mice. Although the low frequency (LF) component was higher ($P<.05$) than the high frequency (HF) component in WT mice, the LF component was less ($P<.05$) than the HF component in TG mice. In addition, arterial baroreflex regulation of HR was markedly blunted in TG mice in response to both nitroglycerin-induced hypotension and phenylephrine-induced hypertension. The reduced LF/HF ratio in TG mice was surprising in view of enhanced $\beta$-adrenergic signaling and may be due to reduced neural tone secondary to the elevated arterial pressure or alterations in arterial baroreflex control. Dobutamine infusion in WT mice also resulted in depressed HR variability. The combination of elevated baseline HR, arterial pressure, and respiratory frequency suggests that enhanced $\beta$-adrenergic signaling in TG mice results in reduced HR variability, in terms of both minute-to-minute variability and the lack of circadian variations in HR. The lack of normal HR variability in general and the failure of HR to decline, even during sleep, may actually be critical mechanisms contributing to the ultimate development of cardiomyopathy in these animals. (Circ Res. 1998;82:416–423.)

Key Words: spectral analysis ■ sympathetic nervous system ■ $\beta$-adrenergic receptor ■ circadian rhythm ■ arterial baroreflex
both time- and frequency-domain analyses. Studying these mice in the conscious state was particularly important, because anesthesia influences heart rate control in general and heart rate variability in particular. This was verified in the present study by examining heart rate variability before and after general anesthesia. Studies were also conducted using telemetric techniques to avoid the effects of restraints and tethers on autonomic control. To further understand heart rate control, additional experiments measuring arterial pressure and its effect on heart rate were conducted in conscious, chronically instrumented TG mice and age-matched controls, since arterial baroreflexes are critical to heart rate control. Additional studies on respiratory control were also conducted in the TG mice and wild-type (WT) controls. The effects of sympathetic and parasympathetic blockade with propranolol and atropine as well as ganglionic blockade with hexamethonium were also examined. Finally, we wished to determine whether administration of a sympathomimetic amine, dobutamine, to WT controls would result in a picture of heart rate variability mimicking that in TG mice.

Materials and Methods

Animals

Twenty-four male TG mice (5.2 months old) and 26 male WT littermates (5.3 months old) from the same genetic background were studied. Briefly, the transgene consists of a rat α-myosin heavy chain promoter linked to a $G_{a_1}$ DNA coding for the short isoform of $G_{a_1}$. Animals used in the present study were maintained in accordance with the Guide for Care and Use of Laboratory Animals (Department of Health and Human Services publication No. [NIH] 83–23, revised 1985).

Effects of Autonomic Blockade on Heart Rate

The mice were anesthetized with ketamine (0.065 mg/g), acepromazine (0.002 mg/g), and xylazine (0.013 mg/g) injected intraperitoneally. In seven TG mice and nine WT controls, three electrodes were placed subcutaneously, two on either side of the thorax and one on the midback, and a PE-10 catheter was inserted into the right jugular vein. The catheters and electrodes were tunneled subcutaneously to the back, externalized, and secured in a plastic cap. All mice were treated with 30 mg of cephalothin subcutaneously for 3 days after surgery. Experiments were initiated 3 to 6 days after recovery from surgical instrumentation. In the interim, the mice were trained daily for 1 to 3 hours to rest quietly in a mouse holder (Kent Scientific Co). On the day of the study, each mouse was placed in the mouse holder, the jugular venous catheter was accessed and connected to a microliter syringe (Hamilton Co), and the three electrodes were connected to an ECG amplifier (Gould). All experiments were recorded with animals in the conscious state between 10:00 AM and 3:00 PM on a multichannel tape recorder (Metrum) (Fig 1).

Experiments were initiated 2 to 3 days after recovery from surgical instrumentation. Mice with implanted telemetric devices were housed in individual cages with free access to food and water and were exposed to 12-hour light/dark cycles (light, 6:00 AM to 6:00 PM; dark, 6:00 PM to 6:00 AM) in a thermostatically controlled (24°C to 26°C) room. ECGs were recorded from the untethered conscious mice for 48 hours by using a tape recorder (Metrum) (Fig 1).

Effects of $\beta$-Adrenergic Stimulation on Heart Rate in WT Controls

In five WT controls, surgical instrumentation was performed as described for autonomic blockade. After baseline recording, dobutamine (20 to 30 $\mu$g · kg$^{-1}$ · min$^{-1}$) was administered intravenously. After the heart rate response stabilized during dobutamine infusion, another ECG recording was taken, which was subjected to analysis of time and frequency domains.

Effects of Anesthesia on Heart Rate in WT Controls

Six WT controls were anesthetized as described above and positioned on a warming pad (DeltaPhase isothermal pad) to keep temperature constant at 37°C. ECG leads were attached to each limb using needle electrodes (Grass Instruments). After the administration of anesthesia, the ECG was recorded when the heart rate stabilized.

Arterial Pressure Measurement

Six TG mice and six WT controls were anesthetized, and ECG electrodes and a jugular venous catheter were implanted as described. In addition, a mechanically stretched PE-10 catheter was inserted into the femoral artery. The catheters and electrodes were tunneled subcutaneously to the back, externalized, and secured in a plastic cap. Experiments were initiated 2 to 3 days after recovery from surgical instrumentation. Each mouse was placed in the mouse holder, and the femoral artery catheter was connected to a 1.8F micromanometer catheter (Millar Instruments) via a 25-gauge needle. The jugular venous catheter was connected to a microliter syringe. After the mouse was allowed to rest in the mouse holder for 30 to 120 minutes and a stable heart rate was achieved, baseline ECG and arterial blood pressure were recorded for 20 minutes. After baseline recording, arterial baroreflex sensitivity was also assessed by pharmacologically manipulating arterial pressure. Hypotension was induced by nitroprusside (5 to 40 $\mu$g/kg), and hypertension was induced by phenylephrine (5 to 40 $\mu$g/kg). All experiments were recorded in the conscious mice between the hours of 9:00 AM and 12:00 noon on a multichannel tape recorder (Honeywell).

Respiratory Rate and Frequency Measurement

In nine TG mice and nine WT controls, piezoelectric ultrasonic dimension crystals were implanted on opposing left and right surfaces of the chest wall to measure respiratory rate and frequency. All


Quantification of Atrial Gsα Protein

The procedures for quantifying atrial Gsα protein were similar to those used previously in the ventricle in this model.1 Mouse atria (n=5 Gsα atrial samples and n=5 WT control atrial samples) were placed in cold Tris buffer (50 mmol/L Tris-HCl, 5 mmol/L EDTA, and 1 mmol/L MgCl2, pH 7.4) and homogenized with a Polytron (Brinkmann) at half speed three times for 5 seconds each. Samples were centrifuged at 14,000g for 30 minutes, and the pellet was resuspended in Tris buffer plus 250 mmol/L NaCl. The suspension was homogenized for 15 seconds and then placed on ice for 20 minutes. Samples were centrifuged at 14,000g for 30 minutes. The pellet was resuspended in Tris buffer, and protein was quantified. Tissue extracts were then lysed in Laemmli’s buffer (62.5 mmol/L Tris-HCl, 2% SDS, 1% β-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue) for Gsα immunoblotting. The lysate was boiled for 5 minutes, and 20 μg was subjected to 10% SDS-PAGE. The gels were transferred to Immobilon-P membranes (Millipore Corp) by use of a semidry apparatus (Emprotech). The membranes were blocked with 2.5% BSA (fraction V, Sigma Chemical Co) and 2.5% nonfat dry milk in TBST (50 mmol/L Tris-HCl, pH 8.0, 100 mmol/L NaCl, and 0.5 mL/L Tween 20) for 1 hour at room temperature. Blots were probed with Gsα-specific antisera (Santa Cruz Biotech) for 1 hour at room temperature. Unbound antibodies were eliminated by three 5-minute washes in TBST followed by incubation with anti-rabbit F(ab)2, coupled to horseradish peroxidase (Amersham Corp) for 20 minutes at room temperature. Unbound antibodies were washed as described above with an enhanced chemiluminescence detection kit (DuPont-NEN). The blots were exposed to x-ray film, and the bands were quantified by densitometry.

Data Analysis and Statistics

The ECG and respiratory signals were analyzed off-line on a PC computer (Hewlett Packard Vectra 133v). After antialias filtering at a cut-off frequency of 1 kHz with eight-pole Butterworth filters, all the signals were detected at 2 kHz. R waves were detected from the ECG, cut-off frequency of 1 kHz with eight-pole Butterworth filters, all the signals were detected at 2 kHz. R waves were detected from the ECG, and a 30-Hz heart rate tachogram was constructed.10 Maximum inspiration was used to detect respiratory cycle, and a 30-Hz respiration rate tachogram was constructed. The power spectra of the heart, as (beats/min)2, and respiration rate, as (breaths/min)2, were analyzed for a 30-second segment by autoregressive spectral analysis. The degree of the autoregressive model was determined by optimizing Akaike’s theoretic information criterion.11 We defined the low frequency (LF) and high frequency (HF) bands as 0.1 to 1.75 Hz and 1.75 to 3.0 Hz. To normalize the power spectra, LF and HF energy levels, which represent the spectral components of the LF band and HF band, respectively, were divided by the total power, defined as the amplitude of the spectral component between 0.03 and 7.5 Hz.

All data were reported as mean±SE. Comparisons between TG mice and WT controls were made by using Student’s t test for group data. The responses to autonomic blockades were analyzed by one-way ANOVA for repeated measurements. If the ANOVA indicated the presence of significant differences, individual comparisons were made by contrast analysis. A value of P<.05 was taken as the minimal level of significance.

Results

Baseline Heart Rate in Conscious State

The resting heart rates of restrained, conscious TG mice (736±15 bpm, n=12) were higher (P<.05) than those of WT controls (580±18 bpm, n=13) (Table 1). In conscious, unrestrained mice, average heart rates derived using telemetry over 48 hours were also elevated (P<.05) in TG mice (696±13 bpm, n=5) compared with WT controls (568±28 bpm, n=6).

TABLE 1. Time-Domain Heart Rate Variability in Restrainted Mice

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mHR, bpm</th>
<th>mRR, ms</th>
<th>SDRR, ms</th>
<th>CVRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>13</td>
<td>580±18</td>
<td>105±4</td>
<td>7.3±1.4</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td>Transgenic</td>
<td>12</td>
<td>736±15*</td>
<td>82±2*</td>
<td>0.6±0.1*</td>
<td>0.01±0.00*</td>
</tr>
</tbody>
</table>

n indicates the number of mice; mHR, mean heart rate; mRR, mean R-R interval; SDRR, standard deviation of R-R interval; CVRR, coefficient variance of R-R interval.

*P<.05 vs wild type.

Interestingly, in conscious mice, heart rates were not different in conscious restrained versus unrestrained WT controls (580±18 versus 568±28 bpm) but were significantly higher (P<.05) in the conscious restrained overexpressed Gsα mice compared with conscious unrestrained TG mice (736±15 versus 696±13 bpm). These differences may be due to the postsynaptic amplification of sympathetic effects in the TG mice, associated with restraint.

Circadian Variation of Heart Rate in Unrestrained Mice

Heart rates were elevated during both daytime and nighttime in TG mice compared with the WT controls. However, the variance of the 24-hour heart rate recordings was reduced (P<.05) in TG mice (1335±757 bpm2) compared with WT controls (4776±2099 bpm2). Furthermore, the normal circadian variation observed in WT controls, ie, a significantly higher heart rate during nighttime than during daytime, was markedly blunted in TG mice (Figs 2 and 3).

Time-Domain and Frequency-Domain Indices of Variation in Restrainted and Unrestrained Mice

The time-domain indices of variation, the standard deviation of the R-R interval, and the coefficient of variation of the R-R interval were reduced (P<.05) in TG compared with WT restrained (Table 1) and unrestrained (data not shown) mice. The frequency-domain indices, both in restrained (Table 2) and unrestrained (Figs 3 and 4) mice, ie, the LF component, HF component, and total power, were also decreased (P<.05) in TG mice compared with WT controls. In WT controls, the normalized LF component (P<.05) was higher than the HF component. Conversely, in TG mice, the normalized LF
component was lower ($P<.05$) than the HF component. This difference, expressed as the LF/HF ratio, was reduced ($P<.05$) in TG mice compared with WT controls.

**Effects of Autonomic Blockade**

**Effects of Propranolol and Atropine**

Propranolol administration decreased heart rate to a greater degree ($P<.05$) in TG mice ($-142\pm10$ bpm, $n=6$) than in WT controls ($-85\pm10$ bpm, $n=9$). Atropine administration increased ($P<.05$) heart rate less ($P<.05$), by $18\pm7$ bpm, in TG mice ($n=7$) than in WT controls ($94\pm21$ bpm, $n=7$). Intrinsic heart rate following combined autonomic blockade with propranolol and atropine was not different between TG mice ($588\pm34$ bpm, $n=6$) and WT controls ($532\pm14$ bpm, $n=8$) (Fig 5).

**Effects of Ganglionic Blockade**

After ganglionic blockade with hexamethonium, the baroreflex heart rate response to sodium nitroprusside was blocked by $90\%$ in WT controls and $88\%$ in TG mice, whereas the baroreflex heart rate response to phenylephrine was blocked by $82\%$ in WT controls and $86\%$ in TG mice. Hexamethonium increased heart rate in WT controls ($557\pm25$ to $632\pm32$ bpm). In contrast, hexamethonium decreased heart rate in TG mice (from $749\pm12$ to $656\pm37$ bpm). After hexamethonium, just like after combined propranolol and atropine, intrinsic heart rates were no longer different in TG mice compared with WT controls (Fig 5).

**Baroreflex Sensitivity**

Baseline mean arterial pressure was elevated ($P<.05$) in TG mice ($123\pm1$ mm Hg, $n=6$) compared with WT controls ($103\pm1$ mm Hg, $n=6$). Arterial baroreflex sensitivity (Fig 6) was assessed by pharmacologically manipulating arterial pressure. Nitroprusside-induced hypotension resulted in less ($P<.05$) of an increase in heart rate in TG mice ($+33\pm9$ bpm) than in WT controls ($+148\pm19$ bpm), despite a greater decrease in mean arterial pressure in TG mice ($-39\pm4$ mm Hg) than in WT controls ($-16\pm3$ mm Hg). Phenylephrine-induced hypertension also elicited less bradycardia in TG ($-83\pm17$ bpm) than in WT controls ($-186\pm20$ bpm), although increases in mean arterial pressure were similar in TG mice ($+28\pm4$ mm Hg) and WT controls ($+24\pm3$ mm Hg).

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**TABLE 2. Frequency-Domain Heart Rate Variability in Restrained Mice**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LF</th>
<th>HF</th>
<th>TP</th>
<th>nLF</th>
<th>nHF</th>
<th>LF/HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>13</td>
<td>125±47</td>
<td>25±7</td>
<td>179±56</td>
<td>0.70±0.08</td>
<td>0.18±0.04</td>
<td>6.9±2.4</td>
</tr>
<tr>
<td>Transgenic</td>
<td>12</td>
<td>3±1*</td>
<td>5±2*</td>
<td>11±5*</td>
<td>0.30±0.05*</td>
<td>0.51±0.07*</td>
<td>0.7±0.3*</td>
</tr>
</tbody>
</table>

$n$ indicates the number of mice; LF, low frequency component; HF, high frequency component; TP, total power; nLF, normalized LF, and nHF, normalized HF.

*P<.05 vs wild type.
Effects of Dobutamine
In WT controls, dobutamine increased heart rate (from $548 \pm 6 \text{ to } 697 \pm 6 \text{ bpm}$) and decreased markedly the power spectra (Fig 7). Indeed, the power spectra in WT controls in the presence of dobutamine were indistinguishable from those in TG mice under baseline conditions (Fig 7).

Effects of Anesthesia
In WT controls, anesthesia decreased heart rate (248 $\pm 6 \text{ bpm}$) and also decreased the power spectra (Fig 7).

Respiratory Frequency
Respiratory rate was higher ($P < .05$) in TG mice (269 $\pm 26 \text{ breaths/min}$) than in WT controls (210 $\pm 20 \text{ breaths/min}$) (Fig 8). The power spectra of respiratory variability are compared with heart rate variability in Fig 8. The power spectra of respiration was coupled closely with the HF component of the ECG power spectra.

Discussion
We have developed a TG mouse model with overexpression of cardiac $G_{o,2,3,9}$. This mouse heart, in vitro, is characterized by enhanced $G_{o}$ protein and by increased numbers of $\beta$-adrenergic receptors binding agonists with high affinity. $G_{o}$ protein is also overexpressed in the atria, ie, by 523 $\pm 79\%$ (Fig 9). In vivo, the TG mice are characterized by augmented responses of heart rate and myocardial contractility to $\beta$-adrenergic receptor stimulation as well as by elevated baseline heart rate.$^{2,3}$ Our hypothesis is that the $\beta$-adrenergic receptor stimulatory gain is enhanced in the TG mice. Thus, for any given amount of receptor occupancy the effects on cardiac inotropy and contractility are amplified.

The first goal of the present investigation was to determine whether heart rate measured using telemetric techniques in unrestrained, conscious TG mice was also elevated and whether the alterations in $\beta$-adrenergic signal transduction affected circadian rhythm and heart rate variability. The unrestrained TG mice had a significantly higher heart rate than did their WT littermates. Heart rates were higher in both restrained and unrestrained conscious TG mice. Interestingly, a comparison of restrained versus unrestrained heart rate measurements revealed no difference in WT controls but a significant increase in heart rate in restrained TG compared with unrestrained TG mice. This is most likely due to amplification of the consequences of restraint on sympathetic activity; ie, for any given increase in synaptic norepinephrine, a greater postsynaptic $\beta$-adrenergic receptor gain is observed in the TG mice. Enhanced respiratory activity was also observed in TG mice that was consistent with a state of augmented sympathetic activity. The increased baseline heart rate was most likely due to enhanced sympathetic activity, since after combined sympathetic and parasympathetic blockade or after hexamethonium, the resultant intrinsic heart rate was no longer enhanced in TG mice. Interestingly, hexamethonium

**TABLE 3. Frequency-Domain Heart Rate Variability in the Presence of Ganglionic Blockade**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LF</th>
<th>HF</th>
<th>TP</th>
<th>nLF</th>
<th>nHF</th>
<th>LF/HF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5</td>
<td>195±64</td>
<td>41±20</td>
<td>254±82</td>
<td>0.78±0.04</td>
<td>0.14±0.04</td>
<td>8.3±2.6</td>
</tr>
<tr>
<td>GB</td>
<td>5</td>
<td>1±*</td>
<td>6.0±3.4</td>
<td>9.7±5.8*</td>
<td>0.21±0.06*</td>
<td>0.63±0.05*</td>
<td>0.4±0.01*</td>
</tr>
<tr>
<td><strong>Transgenic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4</td>
<td>1.2±0.5†</td>
<td>3.0±1.0†</td>
<td>4.6±1.4†</td>
<td>0.24±0.05†</td>
<td>0.63±0.03†</td>
<td>0.4±0.1†</td>
</tr>
<tr>
<td>GB</td>
<td>4</td>
<td>0.2±0.1</td>
<td>1.4±0.5</td>
<td>2.8±1.4</td>
<td>0.12±0.03</td>
<td>0.61±0.09</td>
<td>0.2±0.0</td>
</tr>
</tbody>
</table>

n indicates the number of mice; LF, low frequency component; HF, high frequency component; TP, total power; nLF, normalized LF; nHF, normalized HF; and GB, ganglionic blockade with hexamethonium.

$^*P < .05$ vs baseline; $^†P < .05$ vs wild type.
elicited opposite effects on heart rates in WT and TG mice, ie, increasing the heart rate as expected in WT controls and actually decreasing heart rate in TG mice. An increase in heart rate would be predicted for normal conscious animals. The fall in heart rate in TG mice after ganglionic blockade supports the argument that the increased baseline heart rate in these animals is due to enhanced sympathetic activity. It could be, however, that sympathetic tone is similar and that the increased heart rate was directly due to augmented postsynaptic \( \beta \)-adrenergic receptor gain.

Surprisingly, both the normal fluctuations in heart rate that occur during daily life and circadian variability were attenuated in TG mice (Fig 2). In most mammalian species, heart rate is higher during the day.\(^{12,13}\) However, in mice the reverse is observed, most likely because these animals sleep during daylight hours. In the present study, both groups of animals demonstrated heart rates that were significantly higher during nighttime. These day and night differences were markedly blunted in the TG mice.

To examine further these attenuated heart rate fluctuations, we used the technique of heart rate variability analysis developed by Akserlod et al,\(^4\) which provided insight into the autonomic control of heart rate. The frequency components of the heart rate spectra have been shown to be affected by both the sympathetic and parasympathetic nervous systems.\(^4\) Two major components can be identified in the heart rate spectrum: an HF component, which is associated with respiratory fluctuations,\(^4,14\) and an LF component, which is associated with baroreceptor regulation of arterial blood pressure fluctuations and includes some part of the HF component.\(^15–17\) Time-domain analyses of heart rate variability have been used traditionally as indices of parasympathetic tone.\(^18\)

These techniques have been used extensively in larger mammals, including humans.\(^5,14,15,19\) Less data are available in mice,\(^20,21\) particularly conscious mice, because of the technical limitations associated with their high cardiac frequency. Accordingly, one important feature of the present study was the development of techniques required to assess heart rate variability in conscious mice. By using this method, a lack of heart rate variability in TG mice was observed; ie, standard deviation of heart rate and total power frequency were diminished (Tables 1 and 2).

We also used heart rate variability analysis to evaluate autonomic regulation of the heart in the TG mice. In the present study, we observed in the mouse an LF peak in the frequency range 0.1 to 1.75 Hz and an HF peak in the frequency range 1.75 to 5.0 Hz. These results are consistent with those of other studies in mice.\(^20,21\) In addition, the frequency range of the respiratory power spectra corresponded to the HF components of ECG power spectra in WT controls and TG mice.

Naively, one might expect that the TG mice would demonstrate an elevated LF component, corresponding to enhanced \( \beta \)-adrenergic signaling, compared with WT controls. However, the reverse was observed. The LF component was markedly reduced in the TG mice compared with the WT controls. The LF/HF ratio, which is commonly used as a measure of sympathetic/parasympathetic balance, was also reduced in TG compared with WT mice. There are several potential explanations that could reconcile these surprising results. It is possible that because they are acting above their normal operating point (arterial pressure is elevated above normal) in the TG mice, autonomically mediated reflex mechanisms (such as the baroreceptor) are unresponsive or reset and thus generate reduced variability in the levels of parasympathetic and sympathetic inputs to the heart. In support of this concept, the arterial baroreflex regulation of heart

![Figure 7](https://example.com/image7.png)  
**Figure 7.** The effects of dobutamine were examined in wild-type (WT) control mice. Dobutamine reduced heart rate spectra (middle left) such that WT mice with dobutamine were indistinguishable from transgenic mice at the baseline state in terms of power spectra (right panel). Anesthesia also reduced heart rate spectra (middle right). LF indicates low frequency; HF, high frequency; and TP, total power.

![Figure 8](https://example.com/image8.png)  
**Figure 8.** ECG and respiration traces from wild-type (WT) control mice (top left) and transgenic (TG) mice (bottom left). The power spectra for respiratory frequency (dotted line) are compared with heart rate spectra (solid line) in a WT control mouse (top right) and a TG mouse (bottom right). The power spectra of respiration were coupled with the high frequency component of the ECG power spectra.

![Figure 9](https://example.com/image9.png)  
**Figure 9.** Quantification of atria \( G_{s\alpha} \) protein using immunoblotting. There was approximately a 5-fold increase in transgenic (TG) \( G_{s\alpha} \) compared with wild-type (WT) \( G_{s\alpha} \).
rate was found to be markedly impaired. Reflex changes in heart rate in response to both baroreflex hypertension and hypotension were severely blunted in TG mice (Fig 6). Potentially, as a result, both the HF and LF peaks in the heart rate power spectrum and measures of total heart rate variability are markedly diminished in the TG mice. Interestingly, patients with chronic hypertension exhibit a pattern of heart rate variability similar to that of the TG mice, reduced HF and LF heart rate variability. Another possibility relates to the elevated respiratory rate. The respiratory frequency peaks coincided with the HF heart rate peak. This action could also tend to reverse the LF/HF ratio in the TG mice.

The elevated arterial and cardiac pressures in the conscious TG mice are most likely the result of enhanced postsynaptic \( \beta \)-adrenergic signal transduction. These hemodynamic factors, in combination with increased cardiac output, could trigger autonomic reflexes (eg, the baroreflex) to reduce sympathetic tone and enhance parasympathetic tone. Thus, there could be a dichotomy between depressed resting neural tone and enhanced postsynaptic neural activity. This reasoning could also explain why propranolol might reduce heart rate more in TG mice than in WT controls, despite potentially reduced neural activity. Nonetheless, the higher heart rates in TG mice were indeed autonomically mediated, since intrinsic heart rates were similar in TG mice and WT controls.

The heart rate variability data after autonomic blockade in WT controls as well as variability in response to a sympathomimetic amine or dobutamine and also in response to anesthesia provided clues to why heart rate power spectra were so depressed in TG mice. Since autonomic blockade and anesthesia impair autonomic control, both these interventions resulted in marked reductions in heart rate power spectra in WT controls, as expected. However, increasing postsynaptic sympathetic activity with dobutamine also resulted in marked reductions in heart rate power spectra in WT controls, such that their spectra were similar to those in TG mice at baseline. These data indicate that in mice either increasing sympathetic activity of the heart or abolishing autonomic tone reduces heart rate variability, a condition that is observed under baseline conditions in TG mice with overexpressed \( G_\alpha \).

A final note of caution is that there is not always a direct correlation between increased sympathetic activity on the one hand and an increased LF component on the other. Several studies have shown that interventions that increase sympathetic tone reflexly (eg, nitroglycerin or postural hypotension alone) increase the LF component. Recently Pagani et al. demonstrated a predominance of the LF component in normal human subjects under conditions in which sympathetic tone increased. Conversely, other studies, with either direct electrical nerve stimulation or physiological stimulation of the sympathetic system (eg, during exercise), failed to detect an increase in the LF component. Therefore, as noted above, it is not possible to correlate mean levels of autonomic tone and spectral analysis. This is important to keep in mind for clinical studies, in which spectral analysis of heart rate variability is often used. In this connection, a recent study in patients with heart failure demonstrated absent LF variability despite increased heart rate and neural sympathetic nerve activity. This is particularly relevant to the overexpressed \( G_\alpha \) animal model, in which reduced LF is observed in an animal model that ultimately developed cardiomyopathy.

In summary, the overexpression of cardiac \( G_\alpha \) results in enhanced postsynaptic \( \beta \)-adrenergic signal transduction. This leads to chronic elevation in ambient levels of \( \beta \)-adrenergic signaling, chronic elevations in heart rate, but depressed minute-to-minute and circadian variability and variability in frequency spectra. This was associated with concomitant elevations in arterial pressure and respiratory frequency. It is possible that the chronically elevated arterial pressure results in depressed sympathetic neural activity and arterial baroreflex control, as shown in Fig 6, or that the enhanced postsynaptic \( \beta \)-adrenergic receptor signaling resulted in depressed heart rate variability, as occurs with administration of a sympathomimetic amine. In either case, the unexpected findings of spectral frequency analysis highlight the limitations using this technique to predict levels of sympathetic activity. The combination of the enhanced \( \beta \)-adrenergic receptor signaling with chronically elevated heart rate and associated lack of normal variability and circadian rhythm and impaired arterial baroreflex control over the life of the TG mice may be important mechanisms mediating the subsequent development of cardiomyopathy in these animals. This latter point may be crucial; ie, the inability to reduce the frequency and force of cardiac contraction even during sleep may prevent the replenishment of energy stores and exacerbate the discrepancy between energy supply and demand, resulting in the development of cardiomyopathy over the life of the animals.

Acknowledgments

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Depressed Heart Rate Variability and Arterial Baroreflex in Conscious Transgenic Mice With Overexpression of Cardiac G \( \alpha_\text{1L} \)


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