Sex Differences

Sex Chromosome Complement Contributes to Sex Differences in Bradycardic Baroreflex Response

Ximena E. Caeiro, Franco R. Mir, Laura M. Vivas, Hugo F. Carrer, María J. Cambiasso

Abstract—To investigate whether sex chromosome complement modulates bradycardic baroreflex response and contributes to the angiotensin II–bradycardic baroreflex sex differences, we used the four core genotype mice model in which the effect of gonadal sex and sex chromosome complement is dissociated, allowing comparisons of sexually dimorphic traits among XX and XY females, as well as in XX and XY males. In conscious gonadectomized (GDX) MFI transgenic mice we evaluated baroreflex regulation of heart rate in response to changes in blood pressure evoked by phenylephrine (1 mg/mL), angiotensin II (100 µg/mL), and sodium nitroprusside (1 mg/mL). The administration of phenylephrine in GDX-XY females resulted in a significantly lower baroreflex response when compared with the other genotypes (in beats·min⁻¹·mm Hg⁻¹ [slopes of regression lines for GDX-XY females −3.56±0.37 versus −6.06±0.38, −6.37±0.54 and −6.70±0.34 for GDX-XY male, GDX-XX female, and GDX-XX male mice, respectively]) \( F(1,19)=9.63; P<0.01 \). In addition, in both GDX-XY males and females, the angiotensin II-bradycardic baroreflex response was attenuated when compared with heart rate changes in GDX-XX male and female mice (in beats·min⁻¹·mm Hg⁻¹ [slopes of regression lines: −2.83±0.28 versus −5.76±0.26 in GDX-XY and GDX-XX mice, respectively]) \( F(1,19)=13.91; P<0.005 \). In contrast, reflex tachycardic responses to sodium nitroprusside were comparable in all of the genotypes. These data support the hypothesis that sex chromosome complement modulates reflex inhibition of heart rate to phenylephrine and angiotensin II. Elucidating the foundational sources of sexually dimorphic traits in the regulation of baroreceptor reflex may enable the design of more appropriate sex-tailored therapeutic treatments in the future. (Hypertension. 2011;58:505-511.) ● Online Data Supplement

Key Words: bradycardic baroreflex response ■ sex chromosome complement ■ Ang II ■ phenylephrine ■ sex differences ■ four core genotype

Historically, most epidemiological and basic studies were performed in male subjects and, if both sexes were included, no sex differentiation was taken into account during data analysis, assuming that males and females are similar, differing only in the magnitude of the response. Nonetheless, principles learned in male models do not necessarily apply to females.

Although the role of gonadal steroids in sexual dimorphism is undeniable, a growing body of evidence indicates that some sexually dimorphic traits cannot be entirely explained solely as a result of gonadal steroid action but may also be ascribed to differences in sex chromosome complement (SCC). Males and females carry a different complement of sex chromosome genes and are influenced throughout life by different genomes. Some X and Y genes act in a sex-specific manner on the gonads and other tissues to cause sex differences in XX and XY cells. The Y-linked Sry gene plays a dominant role by setting up the lifelong sex difference in secretion of gonadal hormones, which have organizational and activational effects on the brain and other tissues. The genetic and/or hormone pathways could, thus, act independently or interact (synergistically/antagonistically) in modulating sexual dimorphic development.1-4

Angiotensin II (Ang II) in the central nervous system differentially modulates cardiovascular parameters in males and females.5,8 In addition to its vasoconstrictor action at the peripheral level, it increases sympathetic while decreasing parasympathetic activity and modulates baroreceptor reflex sensitivity.7,8 The arterial baroreceptor reflex is a major negative feedback mechanism involved in stabilization of perfusion pressure, and changes in baroreflex control of heart rate (HR) have been described in physiological and pathophysiological states. Clinical and basic findings indicate a sexually dimorphic baroreflex control of HR. The acute administration of Ang II in normotensive male and female patients induces increases in blood pressure (BP) of similar magnitude; however, in men, bradycardic baroreflex response is blunted relative to that observed in women.8 Likewise, studies carried out by Pamidimukkala et al9 have shown that, in intact male mice, the slope of Ang II–induced baroreflex...
bradycardia is significantly less than that evoked by phenylephrine (PE), whereas no differences are observed in the response to both pressor agents in gonadectomized female mice. Moreover, they showed that estrogen replacement in ovariectomized female mice facilitates equally both bradycardic baroreflex response to Ang II and PE administration.8 A facilitatory role of testosterone in PE bradycardic response in male gonadectomized rats has also been reported.9 Although previous studies have addressed gonadal steroid modulatory action on the baroreflex control of HR, classic hormonal manipulations have failed to cause sex reversal of the Ang II-bradycardic baroreflex response.

The aim of the present study was to test the hypothesis that SCC contributes to Ang II baroreflex sexual dimorphism, modulating the bradycardic baroreflex response. To achieve this, we used the four core genotype (FCG) mouse model, which allows the effect of gonadal sex to be dissociated from that of SCC on sexually dimorphic phenotype, comparing mice with different SCCs (XX versus XY) but with the same gonadal type (ovaries or testis). To remove any activational effect of sex hormones that might mask effects of sex chromosomes, we used adult gonadectomized (GDX) mice. Baroreflex responses, relating changes in HR attributed to increases in BP evoked by pressor agents (PE and Ang II) and decreases in BP with the depressor drug sodium nitroprusside (SNP), were evaluated in conscious free-moving mice of the FCG mouse model.

**Methods**

An expanded Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.

**Animals**

The FCG mouse model combines a deletion of the testis-determining gene Sry from the Y chromosome (Y+) with the subsequent insertion of an Sry transgene onto an autosome.10,11 The Sry gene deletion in male mice (XY-) yields a female phenotype (ovaries). When the Sry transgene is inserted into an autosome of these mice, they have testes and are fully fertile (XY+Sry). The Y+ chromosome and the Sry transgene segregate independently; thus, four types of offspring are produced by breeding XY+Sry males to XX females: XX and XY- females (without Sry on the Y chromosome) and XX+Sry and XY+Sry male mice (both with Sry in an autosome). All of the individuals possessing the Sry transgene develop testes and have a male external phenotype, regardless of their SCC, whereas individuals lacking the transgene have ovaries and external female secondary sex characteristics. “Male” and “female” are defined here according to the gonadal phenotype: we refer throughout to XX and XY- as XX and XY females and to XX+Sry and XY+Sry as XX and XY male mice, respectively. By comparing these genotypes, it is possible to segregate the role of SCC (comparing mice with the same gonadal type but with different SCC; XX versus XY), gonadal phenotype (males versus females regardless of the SCC), and their interaction.4

MF1 transgenic mice, kindly provided by Dr Paul Burgoyne, were born and reared in the breeding facilities at the Instituto de Investigación Médica M. y M. Ferreyra (Córdoba, Argentina). Mice were housed in groups of 5 to 8 per cage in a temperature-controlled environment, maintained on a 12-hour light/dark cycle, and fed and watered ad libitum. All of the experimental protocols were approved by the appropriate animal care and use committees at our institute, following the National Institutes of Health guidelines for the care and use of laboratory animals.

**Experimental Design**

MF1 male (XX and XY) and female (XX and XY) mice aged 45 to 50 days were GDX, and 10 days later they were submitted to the surgical cannulation procedure. After a 5-day recovery period, mice were placed in individual recording cages, and the arterial catheter was connected through polyethylene tubing (50 µL, 0.039 inch OD, 0.023 inch ID, Clay Adams) covered with a stainless steel spring to a strain gauge transducer (BLPR, WPI) coupled to a data acquisition system (Power Laboratory, ADInstruments). The venous catheter was connected to a Braun calibrated pump modified for infusion of small volumes. A period of 45 minutes was allowed to elapse until baseline recordings were obtained.

As described by Xue et al.,13 assessments of cardiac baroreflex response stimulated by increases in BP induced by intravenous PE (Sigma, 1.0 mg/mL) and Ang II (Sigma, 100 µg/mL) were conducted in random order. Immediately after PE or Ang II administration, the venous catheter was flushed with saline solution to infuse the second pressor agent. Mice were allowed to recover for 45 to 60 minutes before subsequent drug administration: care was taken that basal BP and HR had returned to baseline. In a separate group of mice (represented by GDX mice of the 4 genotypes), the HR response to a progressive decrease in BP induced by intravenous infusion of SNP (Sigma, 1 mg/mL) was evaluated. Infusion rates (0.003 mL/min) were monitored to produce increases/decreases in BP within the range of 30 to 40 mm Hg over a period of 45 to 60 seconds.14 Baroreflex reactivity was assessed determining the relationship between peak changes in mean arterial pressure and HR (delta BP and HR, respectively). The studies were undertaken in conscious freely moving mice to avoid the confounding effects of anesthesia on the measured hemodynamic parameters. All of the protocols were carried out between 8:00 AM and 2:00 PM.

**Statistical Analysis**

Baroreflex function relating increases/decreases in HR to changes in mean arterial pressure were analyzed with linear regression, and ANOVA analysis was performed at the end point. Baroreflex responses to PE, Ang II, and SNP infusion were separately analyzed and submitted to a 2-way ANOVA, with gonadal sex (male/female) and SCC (XY/XX) as independent factors. Baroreflex responses to Ang II compared with PE in each genotype were analyzed by 1-way ANOVA with pressor agents as the independent factor. Planned comparisons among genotypes were performed with gonadal sex or SCC and pressor drug (PE/Ang II) as independent factors. The loci of significant interactions or significant main effects were further analyzed using the Tukey test (type I error P set at 0.05). Results were expressed as mean±SE.

**Results**

Baseline values of HR and BP (mean, diastolic, and systolic) measured at the day of the experiment were not statistically different among genotypes (Table 1).

**Bradycardic Baroreflex Response to PE**

The linear regressions of HR baroreflex response to intravenous infusion of PE in the 4 genotypes are shown in Figure 1A and Table 2. The statistical analysis of PE-baroreflex bradycardic responses showed an effect of the interaction of the factors gonadal sex and SCC (F(1,19)=9.63; P<0.01). GDX-XY female mice showed a significantly lower reflex inhibition of HR than that reported for the other genotypes (P<0.001; regression slopes for GDX-XY females: −3.56±0.37 beats·min⁻¹·mm Hg⁻¹ versus −6.06±0.38, −6.37±0.54, and −6.70±0.34 beats·min⁻¹·mm Hg⁻¹ for GDX-XY male, GDX-XX female, and GDX-XX male mice, respectively). Although changes in BP were identical, a PE 30-mm Hg increase in arterial pressure produced significantly smaller decreases in HR in GDX-XY female...
Figure 1. Reflex bradycardic responses to phenylephrine (PE) and angiotensin II (Ang II) infusion in MF1 gonadectomized (GDX) mice of the four core genotype. Graphs show mean relationship lines relating peak changes in heart rate (HR; delta HR) to increases in mean blood pressure (MAP; delta MAP) induced by both PE and Ang II (A and B, respectively). Inset in B represents Ang II reflex bradycardic response in GDX-XY and GDX-XX mice, irrespective of gonadal sex. *P<0.01, significant differences between the PE-bradycardic response in GDX-XY female mice versus the other genotypes. †P<0.05, significant bradycardic baroreflex differences between GDX-XX and GDX-XY mice independent of gonadal sex; GDX-XY male (n=6), GDX-XX male (n=6), GDX-XY female (n=6), and GDX-XX female (n=5) mice.

Bradyergic Baroreflex Response to Ang II
The analysis of bradyergic baroreflex response induced by Ang II infusion showed a significant effect because of SCC (F(1,19)=13.91; *P<0.005), indicating that the administration of Ang II produced, irrespective of gonadal sex, a different baroreflex response depending on the genetic sex (Figure 1B and Table 2). Both male and female mice with XX sex chromosomes showed a facilitated baroreflex response when compared with GDX-XY male and female mice (*P<0.005). Regression slopes for GDX-XX and GDX-XY mice are as follows: −5.76±0.26 and −2.83±0.28 beats · min⁻¹ · mm Hg⁻¹, respectively (inset in Figure 1B). Although an Ang II 30-mm Hg increase in BP in GDX-XX mice was accompanied by a decrease in HR of −163.19±11.92 beats · min⁻¹, a blunted bradycardic response in GDX-XY mice was observed (−76.82±18.31 beats · min⁻¹).

Tachycardic Response to SNP Administration
No differential responses in HR changes induced by acute administration of SNP were observed in mice from different genotypes. The regression slopes for GDX-XY males, GDX-XX males, GDX-XY females, and GDX-XX females were as follows: −1.80±0.11, −1.70±0.14, −2.55±0.06, and −1.69±0.12 beats · min⁻¹ · mm Hg⁻¹, respectively (Figure 2 and Table 2).

Comparative Bradyergic Baroreflex Responses to PE and Ang II
The statistical analysis of the reflex inhibition of HR in response to Ang II and PE infusion in GDX-XY male mice revealed significant differences (F(1,10)=9.25; *P<0.05).

Table 1. Resting Values of Blood Pressure and Heart Rate in Conscious Free-Moving Gonadectomized Mice of the Four Core Genotype Mouse Model

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Parameter</th>
<th>GDX-XY Male (n=11)</th>
<th>GDX-XX Male (n=12)</th>
<th>GDX-XX Female (n=10)</th>
<th>GDX-XY Female (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP, mm Hg</td>
<td>111.4±4.9</td>
<td>111.2±5.9</td>
<td>108.3±7.7</td>
<td>112.6±8.5</td>
</tr>
<tr>
<td></td>
<td>DAP, mm Hg</td>
<td>95.0±4.2</td>
<td>97.1±5.8</td>
<td>92.3±6.9</td>
<td>94.3±7.0</td>
</tr>
<tr>
<td></td>
<td>SAP, mm Hg</td>
<td>130.6±5.3</td>
<td>129.6±6.6</td>
<td>128.4±8.0</td>
<td>134.9±8.3</td>
</tr>
<tr>
<td></td>
<td>HR, beats · min⁻¹</td>
<td>580.5±25.6</td>
<td>575.8±19.0</td>
<td>600.2±46.3</td>
<td>660.9±43.5</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; DAP, diastolic arterial pressure; SAP, systolic arterial pressure; HR, heart rate; GDX, gonadectomized. Data are expressed as mean±SE.

Table 2. Slopes of Baroreflex Responses (in beats · min⁻¹ · mm Hg⁻¹) to PE, Ang II, and SNP Infusion in MF1 GDX Mice of the Four Core Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PE</th>
<th>Ang II</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDX-XY male</td>
<td>−6.06±0.38†</td>
<td>−3.81±0.29</td>
<td>−1.80±0.11</td>
</tr>
<tr>
<td>GDX-XX male</td>
<td>−6.70±0.34</td>
<td>−6.02±0.24</td>
<td>−1.70±0.14</td>
</tr>
<tr>
<td>GDX-XX female</td>
<td>−6.37±0.54</td>
<td>−5.47±0.32</td>
<td>−1.69±0.12</td>
</tr>
<tr>
<td>GDX-XY female</td>
<td>−3.56±0.37*</td>
<td>−2.83±0.26</td>
<td>−2.55±0.06</td>
</tr>
</tbody>
</table>

Values are mean±SE. PE indicates phenylephrine; Ang II, angiotensin II; SNP, sodium nitroprusside; GDX, gonadectomized.

†Data are significantly different from all of the other PE-bradycardic responses.

*Data are significantly different from GDX-XY male Ang II response.

($−93.83±14.49$ beats · min⁻¹) than in GDX-XX female ($−183.74±10.06$ beats · min⁻¹), GDX-XY male ($−176.94±12.73$ beats · min⁻¹), and GDX-XX male ($189.77±15.27$ beats · min⁻¹) mice.
Although both pressor agents elicited equivalent elevations in mean arterial pressure, reflex inhibition of HR in response to Ang II infusion was significantly attenuated compared with the PE response (slope of regression line: $3.81 \pm 0.29$ versus $6.06 \pm 0.38$ beats·min$^{-1}$·mm Hg$^{-1}$ for Ang II and PE, respectively; Figure 3A). On the contrary, GDX-XX female, GDX-XX male, and GDX-XY female mice showed the same bradycardic response to both pressor agents (slope of regression lines for Ang II and PE in beats·min$^{-1}$·mm Hg$^{-1}$: GDX-XX female mice: $-5.47 \pm 0.32$ and $-6.37 \pm 0.54$; GDX-XX male mice: $-6.02 \pm 0.24$ and $-6.70 \pm 0.34$; and GDX-XY female mice: $-2.83 \pm 0.26$ and $-3.56 \pm 0.37$, respectively; Figure 3B through 3D).

The comparison of bradycardic baroreflex response in male mice with different SCCs showed a main effect of pressor drugs [$F(1,19)=9.08; P<0.001$] and SCC [$F(1,19)=7.57; P<0.05$] factors. The pressor drug factor analysis showed that, irrespective of the SCC, Ang II-bradycardic baroreflex response was blunted compared with PE-induced reflex inhibition of HR. In addition, the analysis of SCC factor showed that male mice with XX-SCC exhibited, when compared with GDX-XY male mice, a facilitated bradycardic baroreflex response to both pressor agents (Figure 3A and 3C).

Moreover, the comparison of the bradycardic baroreflex response in mice with XX-SSC but with different gonadal phenotype (GDX-XX males versus GDX-XX females) showed no differences in HR response to Ang II or PE (Figure 3B and 3C). However, when comparing Ang II/PE bradycardic baroreflex responses in female mice with different SCCs (GDX-XX and GDX-XY females), the analysis showed that, regardless of the drug administered, the reflex inhibition of HR in GDX-XY females was attenuated [$F(1,12)=13.47; P<0.005$]. A 30-mm Hg increase in arterial pressure produced significantly smaller decreases in HR in GDX-XY female mice ($-82.82 \pm 20.82$ beats·min$^{-1}$) than in GDX-XX female mice ($169.11 \pm 13.24$ beats·min$^{-1}$; Figure 3B and 3D).

**Figure 2.** Reflex tachycardic response to sodium nitroprusside (SNP) infusion in MF1 gonadectomized (GDX) mice of the four core genotype. Graph shows mean regression lines relating increments in heart rate (HR; delta HR) to decreases in mean blood pressure (MAP; delta MAP); GDX-XY male (n=6), GDX-XX male (n=8), GDX-XY female (n=7), and GDX-XX female (n=5) mice.

**Figure 3.** Comparative reflex bradycardic baroreflex responses to phenylephrine (PE) and angiotensin II (Ang II) infusion in MF1 gonadectomized (GDX) mice of the four core genotype (FCG). Graphs show mean relationship lines relating peak changes in heart rate (HR; delta HR) to increases in blood pressure (BP) induced by both Ang II and PE in mice of the FCG mouse model. *$P<0.05$, significant differences between the bradycardic response to Ang II and PE infusion; GDX-XY male (n=6), GDX-XX male (n=6), GDX-XY-female (n=6), and GDX-XX-female (n=5) mice.
Discussion

The main finding of the present study is that Ang II-bradycardic baroreflex sexual dimorphism response may be ascribed to differences in sex chromosomes, indicating an XX-SCC facilitatory bradycardic baroreflex control of HR. On the other hand, our results show that PE-baroreflex bradycardic response depends on the complex interaction between SCC and gonadal steroids during critical periods of development in fetal and neonatal life. Although many clinical and experimental studies have analyzed the contribution of gonadal hormones in bradycardic baroreflex control of HR, none has considered the contribution of SCCs isolated from steroid hormonal influence. Comparing gonadal males and females after gonadectomy can test whether having testes or ovaries causes long-lasting differences in the phenotype (organizational effect) while comparing mice with the same gonadal type but with different SCCs (XX versus XY) makes it possible to determine whether genes residing in the SCC differentially influence sexuologically dimorphic traits. This is the first study to demonstrate that SCC influences bradycardic baroreflex response to acute PE and Ang II infusion in an animal model in which genetic and phenotypic sex are dissociated.

Clinical and experimental evidence demonstrate Ang II-bradycardic sex differences among males and females. In male subjects, Ang II produces a significantly lower reflex inhibition of HR when compared with the response induced by PE administration, whereas, in females, PE and Ang II exert similar bradycardic baroreflex responses.8,9,14 In the present study, using MF1 GDX mice of the FCG mouse model, we have shown that Ang II infusion in GDX-XY male mice, when compared with PE response, induces a blunted bradycardic response, whereas GDX-XX female, GDX-XX male, and GDX-XY female mice show the same bradycardic baroreflex response to both PE and Ang II. Mice with XX-SCC but with different gonadal sex (GDX-XX male and GDX-XX female mice) show the same bradycardic baroreflex response. Moreover, the comparison of female mice with different SCCs (GDX-XX female versus GDX-XY female) shows an attenuated baroreflex response to both pressor agents in GDX-XY female mice, indicating an XX-SCC facilitatory bradycardic baroreflex effect.

The fact that the Ang II-bradycardic baroreflex response in MF1 GDX mice was driven primarily by differences in SCCs suggests that sex differences in genes residing in the sex chromosomes may influence this sexuologically dimorphic trait. Studies of the influence of sex on gene expression patterns in patients with end-stage heart failure have identified 1837 differentially expressed genes between male and female cohorts, with sex-biased genes overrepresented on the sex chromosomes, as well as in autosomes 3, 4, and 14.15

Baroreceptor reflex acts as an effective buffer of short-term BP fluctuations, and, in most experimental models of hypertension, a reduction in baroreflex sensitivity and/or resetting of the baroreflex curve toward higher BP has been reported. In hypertensive conditions, resetting of the operational point of the arterial baroreflex may, therefore, contribute to the maintenance of high BP rather than opposing it.16 A growing number of studies have shown that dysfunction of the brain renin-angiotensin system (RAS) is implicated in the development of hypertension. In addition, males and females do not equally respond to hypertensive treatment with RAS inhibitors, reflecting an angiotensinergic sexually dimorphic cardiovascular response.5

Although the cellular and molecular mechanisms underlying Ang II-bradycardic dimorphic responses are still not completely understood, it is accepted that Ang II modulates cardiovascular function at the circumventricular organs. The subformical organ and the area postrema (AP) are 2 brain nuclei located at the blood-brain interface and are extremely sensitive to Ang II action. In particular, the AP sends projections to a number of neural centers involved in cardiovascular regulation, including the nucleus of the solitary tract, dorsal vagal complex, the parabrachial nucleus, and rostral ventrolateral medulla, modulating sympathetic-parasympathetic activity, as well as baroreflex response.17,18 AP lesion not only attenuates Ang II-mediated hypertension but also abolishes the blunted bradycardic response. Moreover, male mice with AP ablation show an Ang II-bradycardic baroreflex response of equal magnitude to that evoked by PE administration.7,13,19,20

Most of the well-known biological functions of Ang II (vasoconstriction and sodium reabsorption) are mediated by the activation of Ang II type 1 receptor (AT1R). On the other hand, Ang II induces vasodilation through Ang II type 2 receptor (AT2R) activation, which involves an increase in bradykinin and NO production.21,22 An important number of studies indicate that sex differences in angiotensin-converting enzyme activity and AT1R/AT2R expression/sensitivity could account for some of the Ang II–related sex differences associated with vasoconstrictor/vasodilator balance of the RAS.5 An enhancement of the vasodilation component of RAS in females has also been described, because the infusion of low doses of Ang II potentially biases females in the direction of vasodilation and males toward vasoconstriction.23 In the brain of male mice, high densities of AT1R in cardiovascular regulatory nuclei have been reported (organum vasculosum of the lamina terminalis, median preoptic nucleus, paraventricular nucleus, rostral ventrolateral medulla, nucleus of the solitary tract, and AP), whereas AT2R expression is moderate and circumscribed to defined central brain areas among which the nucleus of the solitary tract is included.24

Previous studies conducted in patients and spontaneously hypertensive rats have demonstrated an association of the Y chromosome with high BP.25,26 More recently, Ji et al,27 using the FCG mouse model, have shown that, after 2 weeks of Ang II infusion, mean arterial pressure is greater in GDX-XX than in GDX-XY mice. In the current study, using the same mouse model, we have found that acute Ang II infusion induces a blunted bradycardic response in GDX-XY compared with GDX-XX mice, indicating that the acute administration of Ang II produced, regardless of the gonadal phenotype, a different baroreflex response depending on the genetic sex. Although these results appear to be contradictory, it is important to note that chronic infusion of Ang II triggers
regulatory responses associated with neuro-endocrine compensatory mechanisms. In particular, changes in Ang II receptor expression attributed to increases in Ang II levels have been reported. In vitro and in vivo studies have shown an upregulation of central AT1R expression in response to physiological increases in plasma Ang II levels induced by water deprivation and sodium depletion.\textsuperscript{28–30} Studies carried out by Wei et al\textsuperscript{11} have also demonstrated that the subcutaneous infusion of a low dose of Ang II for 4 weeks induces an increase in AT1R mRNA expression in the subfornical organ and paraventricular nucleus, although no significant effect on BP is observed. Moreover, increases in AT1R expression have been reported in pathophysiological states, such as hypertension.\textsuperscript{32} Thus, changes in AT1R expressions in central brain areas attributed to chronic Ang II infusion may differentially influence the activity and responsiveness of the RAS system.

It is important to point out that the AT1R gene (Agr2) is located in the X chromosome\textsuperscript{33,34} whereas the AT1R gene (Agr1) is localized in an autosome chromosome 3.\textsuperscript{35} Thus, it is tempting to speculate that genes residing on the sex chromosomes (which are asymmetrically inherited between males and females) could well be influential in eliciting and maintaining sex-bias phenotypes.\textsuperscript{36} If this is the case, differential transcription or expression of the AT1R/AT2R might be responsible for Ang II sex-biased differences.

**Perspectives**

This study demonstrates that SCC influences bradycardic baroreflex response, addressing the contribution of the SCC factor to sex-related differences in reflex inhibition of HR to PE and Ang II. The observation of the XX-SCC facilitatory response stresses the importance of sex chromosomes in cardiovascular sexual dimorphism. Furthermore, analysis of biological responses of mice in which gonadal sex is dissociated from sex chromosomes might contribute to our understanding of the physiology of disorders of sexual differentiation as 46, XY-Swyer, or -androgen insensitivity syndromes. Additional studies are needed, however, to determine the identity of SCC genes responsible for differentially modulating bradycardic baroreflex control of HR. Understanding in more detail the sources of physiological differences between males and females may offer important insights into designing improved oriented sex-tailored therapeutic treatments in the future.

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**Disclosures**

None.

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SUPPLEMENTARY DATA

Sex chromosome complement contributes to sex differences in bradycardic baroreflex response

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EXPANDED METHODS

Genotyping
Tail samples were obtained from mice and genomic DNA was isolated using standard procedures. Genotypes were determined by PCR analysis by the presence of the Sry transgene [primers SryF (forward): CCT ACA GCC ACA TGA TA; SryR (reverse): GTC TTG CCT GTA TGT GAT GG] (1) and of the Y long-arm gene family Ssty [primers StsyF (forward): CTG GAG CTC TAC AGT GAT GA; StsyR (reverse): CAG TTA CCA ATC AAC ACA TCA C] (2). The autosomal gene Myogenin [primers MYOF (forward): TTA CGT CCA TCG TGG ACA GCA T; MYOR (reverse): TGG GCT GGG TGT TAG TCT TAT] served as an amplification control (3) yielding a 245-bp product in all genotypes. Amplification of DNA yielded the following products according to the genotypes: for XY-males the 384-bp Sry and the 302-bp Ssty; for XY-females the 302-bp Ssty; for XX-males the 384-bp Sry; while in XX-females only the myogenin control product was amplified.

Surgical procedures

Gonadectomy
Adult male and female mice were anesthetized with ketamine/xylazine mixture (ip, xylazine, 1 mg/kg, König and ketamine 162 mg/kg Holliday-Scott, Argentina). Bilateral incision was made in the scrotum region for male mice and just below the rib cage in the female mice in order to be able to perform bilateral gonadectomy. Afterwards, the muscle layer and the incision were sutured in place.

At the time gonadectomy was performed, no differences were reported in testosterone levels in XX-male vs. XY-male mice, or in XX-female vs. XY-female in SJL (3) and C57BL/6J (4) mice strain. Furthermore, gonadectomy resulted in undetectable levels of 17-β-estradiol (female) and testosterone (male) in MF1 mice of the FCG mouse model (5).

Chronic catheterization

Under ketamine/xylazine anesthesia, mice were surgically instrumented with intra-arterial catheters for direct measurement of arterial pressure and jugular vein catheters for drug administration. A middle incision was made in the neck and an end-heated 9 and 5 mm micro-renathane tubing (MRE25, Braintree Sci., Boston, MA) welded to a silastic catheter (Dow corning, 0.020 I.D. x 0.037 O.D.) was inserted into the left carotid artery and jugular vein respectively. The catheters were then firmly sutured in place, tunneled subcutaneously between the shoulder blades and connected to a stainless steel “elbow” made of 23-gauge hypodermic tubing. The cannulas were filled with sterile heparinized saline (50 U/mL) to prevent clotting and the external end of the elbow was closed up with a plastic cap (6).

REFERENCES
2. Turner JM, Mahadevaiah SK, Benavente R, Offenberg HH, Heyting C, Burgoyne PS. Analysis of male meiotic “sex body” proteins during XY


