



Review

Sex differences in body fluid homeostasis: Sex chromosome complement influences on bradycardic baroreflex response and sodium depletion induced neural activity



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HIGHLIGHTS

- Sex chromosome complement influences on angiotensin-induced responses
- XX-sex chromosomes induce a facilitated baroreflex response to angiotensin II.
- Sexually dimorphic sodium appetite is in part a result of gonadal steroid action.
- XX-sex chromosomes' effect on sodium depletion-induced neural activity at SFO and AP

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ABSTRACT

Clinical and basic findings indicate that angiotensin II (ANG II) differentially modulates hydroelectrolyte and cardiovascular responses in male and female. But are only the activational and organizational hormonal effects to blame for such differences? Males and females not only differ in their sex (males are born with testes and females with ovaries) but also carry different sex chromosome complements and are thus influenced throughout life by different genomes. In this review, we discuss our recent studies in order to evaluate whether sex chromosome complement is in part responsible for gender differences previously observed in ANG II bradycardic-baroreflex response and sodium depletion-induced sodium appetite and neural activity. To test the hypothesis that XX or XY contributes to the dimorphic ANG II bradycardic-baroreflex response, we used the four core genotype mouse model, in which the effects of gonadal sex (testes or ovaries) and sex chromosome complement (XX or XY) are dissociated. The results indicate that ANG II bradycardic-baroreflex sexual dimorphic response may be ascribed to differences in sex chromosomes, indicating an XX-sex chromosome complement facilitatory bradycardic-baroreflex control of heart rate. Furthermore, we evaluated whether genetic differences within the sex chromosome complement may differentially modulate the known sexually dimorphic sodium appetite as well as basal or induced brain activity due to physiological stimulation of the renin-angiotensin system by furosemide and low-sodium treatment. Our studies demonstrate an organizational hormonal effect on sexually dimorphic induced sodium intake in mice, while at the brain level (subfornical organ and area postrema) we showed a sex chromosome complement effect in sodium-depleted mice, suggesting a sex chromosome gene participation in the modulation of neural pathways underlying regulatory response to renin-angiotensin stimulation.

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Differences between the sexes have been recognized at biochemical, cellular, and physiological levels, but historically most epidemiological and basic studies have been performed in male subjects, assuming that males and females are similar, differing only in the magnitude of the response. However, the principles learned in male models do not necessarily apply to females.

There is significant data indicating the participation of the activational and organizational effects of gonadal steroids in sexual dimorphism [1,2]; however, are gonadal steroids the only ones to blame for such differences? Although the role of gonadal steroids in sexual dimorphism is undeniable, a growing body of evidence indicates that some sexually dimorphic traits cannot be explained solely as a result of gonadal steroid action. Males and females differ not only in their sex (males are born with testes and females with ovaries) but they also carry different sex chromosome complements and are thus influenced throughout life by different genomes. In this way, genetic and/or hormone pathways may act independently or interact (synergistically/antagonistically) to modulate sexual dimorphic development [3,4,5, 6].

Angiotensin II (ANG II) in the central nervous system differentially modulates hydroelectrolyte and cardiovascular parameters in males and females [7,8]. Furthermore, a growing number of studies have shown that dysfunction of the brain renin–angiotensin system (RAS) is implicated in the development of hypertension, and that males and females do not respond equally to hypertensive treatment with RAS inhibitors, reflecting a sexually dimorphic cardiovascular response to angiotensin [7]. For example, clinical and basic findings indicate a sexually dimorphic baroreflex control of heart rate (HR). The acute administration of ANG II in normotensive male and female patients induces increases in blood pressure of similar magnitude; however, in men, the bradycardic baroreflex response is blunted relative to that observed in women [9]. Likewise, studies carried out by Pamidimukkala et al. [10] have shown that, in male mice, the slope of ANG II-induced baroreflex bradycardia is significantly less than that evoked by phenylephrine, whereas female mice show the same bradycardic response to ANG II and phenylephrine [10].

Although clinical and experimental studies have addressed the activational modulatory effect of gonadal steroids on the baroreflex control of HR [10,11], classic hormonal manipulations have failed to cause sex reversal of the differences observed in ANG II-bradycardic baroreflex response. Thus, one of the aims of our studies was to test the hypothesis that sex chromosome complement (XX or XY) contribute to ANG II baroreflex sexual dimorphism, modulating the bradycardic baroreflex response. To this end, we used the four core genotype mouse model, in which the effect of gonadal sex (testes or ovaries) and sex chromosome complement (XX or XY) is dissociated. To remove any activational effect of sex hormones that might mask effects of sex chromosomes, adult conscious gonadectomized (GDX) mice were used. 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 sex chromosome complement (XX versus XY) makes it possible to determine whether genes residing in the sex chromosome complement differentially influence sexually dimorphic traits (Fig. 1).

As shown in Fig. 2, ANG II acute infusion produced, irrespective of gonadal sex, a different baroreflex response depending on the genetic sex (significant effect of sex chromosome complement factor). Both male and female mice with XX-sex chromosomes showed a facilitated baroreflex response when compared with GDX-XY male and female mice ($P < 0.005$). A 30 mm Hg increase in blood pressure in GDX-XX mice was accompanied by a decrease in HR of -163.19 ± 11.92 beats \cdot min $^{-1}$, whereas in GDX-XY mice this response was attenuated (-76.82 ± 18.31 beats \cdot min $^{-1}$). Thus, these data may indicate that the sexually dimorphic ANG II bradycardic baroreflex response may be ascribed to differences in sex chromosomes, indicating an XX-sex

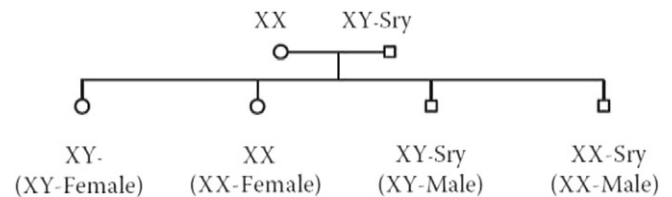


Fig. 1. Schematic representation of four core genotype mouse model. This mouse model combines a deletion of the testis-determining gene *Sry* from the Y chromosome (Y_{-}) with the subsequent insertion of an *Sry* transgene onto an autosome. The *Sry* gene deletion in male mice (XY_{-}) yields a female 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 sex chromosome complement, 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.

chromosome complement facilitatory bradycardic baroreflex control of HR [12].

In this animal model we also evaluated the effect of another pressor agent (phenylephrine) on the baroreflex function in comparison with AngII response. As it has been previously demonstrated for males the reflex inhibition of HR in response to ANG II in GDX-XY male mice induced a blunted bradycardic response when compared with the phenylephrine response, while in GDX-XX females showed the same bradycardic baroreflex response to both phenylephrine and ANG II (Fig. 3). The analysis of the bradycardic baroreflex response in mice with XX-sex chromosome complement but with different gonadal sex (GDX-XX male and GDX-XX female mice) showed the same bradycardic baroreflex response to both pressor agents.

Furthermore, the comparison of female mice with different sex chromosome complements (GDX-XX female versus GDX-XY female) showed that the administration of phenylephrine in GDX-XY females resulted in a significantly lower baroreflex response when compared with the other genotypes. Although changes in blood pressure were

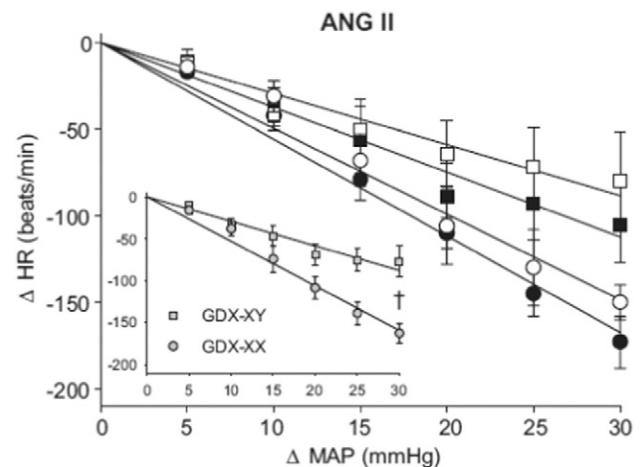


Fig. 2. Reflex bradycardic responses to angiotensin II (ANG II) infusion in MF1 gonadectomized (GDX) mice of the four core genotype. Graph shows mean relationship lines relating peak changes in heart rate (HR; delta HR) to increases in mean blood pressure (MAP; delta MAP) induced by Ang II. Black symbols and white symbols represent male and female mice respectively. Inset represents Ang II reflex bradycardic response in GDX-XY and GDX-XX mice, irrespective of gonadal sex. $\dagger P < 0.05$, 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.

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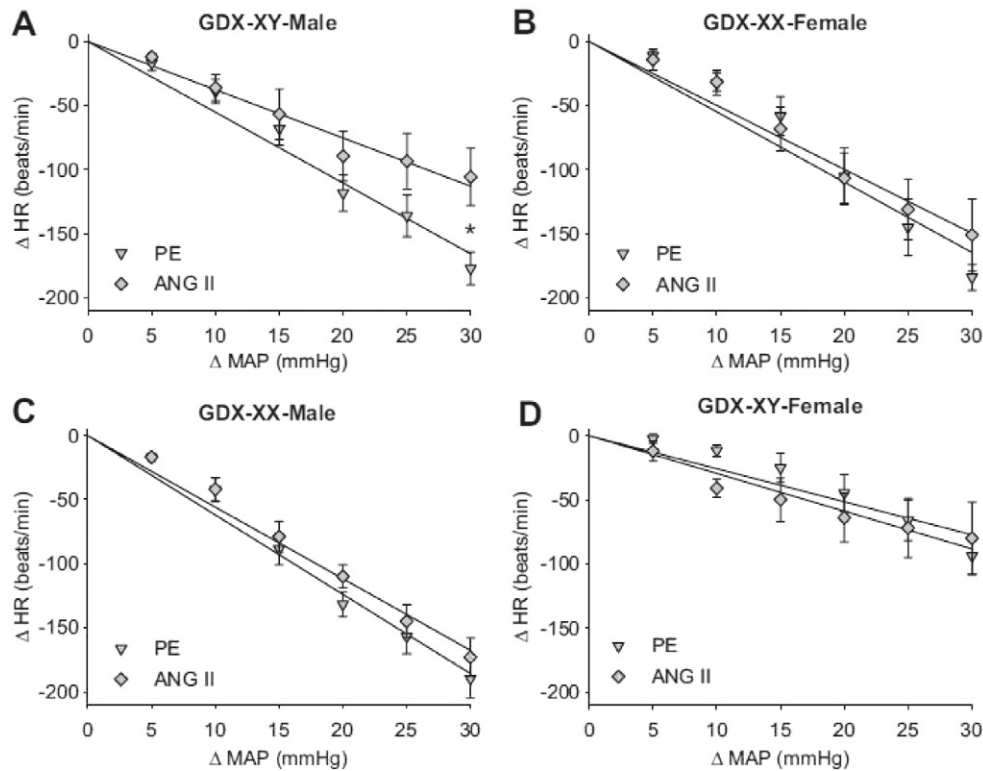


Fig. 3. Comparative reflex bradycardic baroreflex responses to phenylephrine (PE) and angiotensin II (ANG II) infusion in MF1 gonadectomized (GDX) mice of the four core genotype. Graphs show mean relationship lines relating peak changes in heart rate (HR; delta HR) to increases in blood pressure (BP) induced by both ANG II and PE. * $P < 0.05$, significant differences between the bradycardic response to ANG II and PE infusion; GDX-XY male (n₆), GDX-XX male (n₆), GDX-XY-female (n₆), and GDX-XX-female (n₅) mice.

identical, a phenylephrine 30-mm Hg increase in arterial pressure produced significantly smaller decreases in HR in GDX-XY female; thus the statistical analysis of phenylephrine-baroreflex bradycardic responses showed an effect of the interaction of the factors gonadal sex and sex chromosome complement ($P < 0.01$) [12].

Baroreceptor reflex acts as an effective buffer of short-term blood pressure fluctuations, and, in most experimental models of hypertension, a reduction in baroreflex sensitivity and/or resetting of the baroreflex curve toward higher blood pressure has been reported. Previous studies in the four core genotype 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 [13]. Although these results appear to be contradictory with our studies in which we found that acute ANG II infusion induces a blunted bradycardic response in GDX-XY compared with GDX-XX mice (regardless of the gonadal phenotype), it is important to note that chronic infusion of ANG II triggers regulatory responses associated with neuro-endocrine compensatory mechanisms. For example, 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 [14,15,16]. Studies carried out by Wei et al. [17] 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 (SFO) and paraventricular nucleus (PVN), although no significant effect on blood pressure is observed [17]. Moreover, increases in AT1R expression have been reported in pathophysiological states, such as hypertension [18]. Thus, changes in AT1R expressions in brain areas such as the SFO and area postrema (AP) attributed to chronic ANG II infusion may differentially influence the activity and responsiveness of the RAS system.

Sex has an important influence on hydroelectrolyte and cardiovascular regulation [19,20]. ANG II in the central nervous system differentially modulates cardiovascular parameters in males and females [7,8],

and sex chromosome complement is involved in both ANG II sexually dimorphic hypertensive and bradycardic baroreflex responses [12,13].

Taking into account the abovementioned data we sought to evaluate whether genetic differences within the sex chromosome complement may differentially modulate the known sexually dimorphic sodium appetite [20,21] as well as basal or induced brain activity due to physiological stimulation of the RAS.

To this end, furosemide-low sodium diet treatment (DEP group) was induced in transgenic mice of the four core genotype mouse model, and sodium appetite and brain FOS-immunoreactivity (FOS-ir) along the forebrain and brain stem levels were evaluated. It has been previously demonstrated that furosemide-low-sodium diet treatment leads to an increase in plasmatic renin and ANG II levels in association with sodium intake and specific brain FOS-ir [22].

As shown in Fig. 4, sodium depletion induced a significantly greater sodium appetite in males than in females regardless of sex chromosome complement, indicating that sex differences in mice in terms of induced sodium intake may be due to organizational hormonal effects rather than to intrinsic differences in the sex chromosome complement factor (XX and XY). Our results confirm the expected drinking response of sodium-depleted male mice, which had increased sodium consumption in comparison with depleted female mice. As predicted, furosemide/low sodium diet treatment resulted in a significant increase in both electrolyte excretion and diuresis, with a consequent decrease in urine osmolarity. As no differences were observed in diuretic/natriuretic effect between male and female mice, it follows that sexually dimorphic induced sodium intake could not be attributed to sex differences in sodium depletion treatment [23].

The intake profiles shown by depleted female mice are in agreement with those reported by Rowland and Fregly [24] in intact depleted female mice in which they showed that the mean sodium intake was about 60% of the urinary sodium loss and thus postulated that mice may require a longer period of time to replace their sodium losses. In agreement with their work, our results indicate that although this

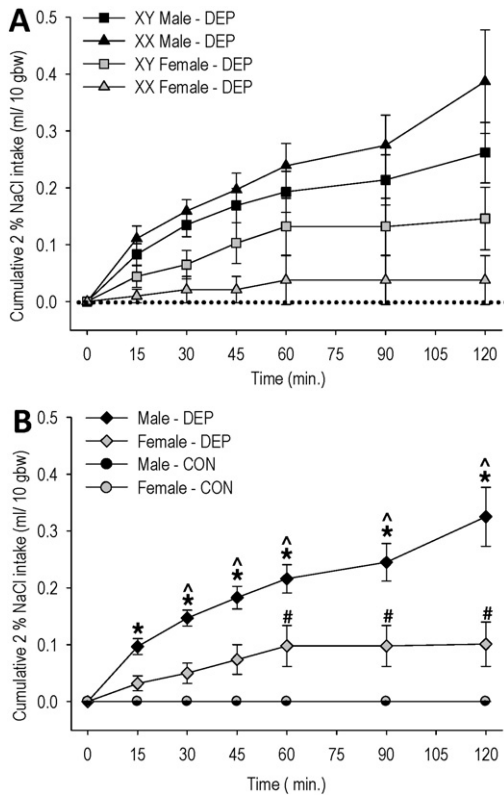


Fig. 4. Time course of cumulative 2% NaCl intake of mice subjected to furosemide low-sodium diet treatment. Panel A: cumulative intake of 2% NaCl per 10 g body wt in XY male, XX male, XY female, and XX female mice that were subjected to acute sodium depletion (DEP). Panel B: representation of the significant triple interaction (sex_treatment_time): cumulative 2% NaCl intake of male and female corresponding to DEP and control (CON) groups. **P* < 0.01 Male-DEP vs. CON groups, #*P* < 0.01 female-DEP vs CON groups. ^*P* < 0.01 male-DEP vs. female-DEP. Values are means ± SE, *n* = 4–9/group. (Reprinted with permission from Dadam et al., *Am J Physiol Regul Integr Comp Physiol* 306: R175–R184, 2014).

prediction may be accurate for DEP-female mice in which sodium intake represents $42.02 \pm 15.66\%$ of the urine sodium loss, DEP-male mice consume, irrespectively of sex chromosome complement, NaCl equal to or in excess of their furosemide induced-sodium deficit ($111.10 \pm 17.96\%$). Thus, the peritoneal dialysis and furosemide electrolyte data might indicate that, although female sodium intake is about half of that required in relation with urinary sodium loss, this does not indicate that female mice have decreased plasma and CSF water and sodium concentration, but instead it could be read as female mice may still have to recover their total body water and sodium content. The onset of specific sodium appetite as a result of body sodium loss is a slow and complex process. In rats it has been shown that acute sodium depletion by peritoneal dialysis produces a rapid and significant drop in both serum and CSF sodium concentration, followed by a relatively slow recovery [25]. Additionally, dialyzed animals exhibit a significant decrease in blood volume immediately after acute sodium depletion, which return to control values after 12 h. However, sodium appetite appears only 20–24 h after sodium depletion when serum and CSF sodium concentration and blood volume have already returned to normal values, even though the rats did not have access to sodium salts, supporting the hypothesis of the “sodium reservoir” [26]. Likewise, studies conducted in patients to whom furosemide was intravenously administered, showed that, despite the urinary losses, no significant changes in serum osmolality or electrolyte values occurred at any sampling time [27,28]. The “sodium “reservoir” hypothesis may explain how the initial drop in volemia and serum sodium concentration after acute sodium depletion stimulates body sodium release from reservoirs such as the bone, skin and skeletal muscle [26,29]. Furthermore, clinical studies

have shown an association of tissue Na⁺ storage with essential hypertension, thus addressing the important role of local physiological extrarenal homeostatic regulatory processes for systemic blood pressure control [29,30,31].

Hypotension and hyponatremia induced by sodium depletion result, among others, in the activation of the RAS. This system manages to compensate the generated hypotension restoring the extracellular volume space and inducing vasoconstriction. Increased plasma ANG II levels stimulate aldosterone secretion, which in turn increases sodium reabsorption by the kidney and also binds the SFO, organum vasculosum of the lamina terminalis (OVLT), and AP. The lack of blood–brain barrier allows these sensory circumventricular organs (CVOs) to be exposed to modulatory humoral factors, giving them the potential to integrate and modulate the homeostatic response [32].

Sodium depleted mice showed at the brain level a modulatory action (as shown in FOS-ir) of sex chromosome complement on neural activity along brain areas underlying fluid and electrolyte homeostasis. Particularly, the sex chromosome complement induced a differential pattern of cell activation within a diencephalic nucleus, the PVN and brain stem nuclei, the nucleus of the solitary tract (NTS) and lateral parabrachial nucleus. In addition, one of the main findings of this study showed an increased XX-sex chromosome complement effect on sodium depletion-induced activity at two CVOs, SFO and AP.

As shown in Fig. Fig. 5 at the SFO level, XX-DEP mice, irrespectively of the gonadal sex, showed a significant increase in the number of FOS-ir cells compared with XY-DEP as well as CON groups (XX and XY). Furthermore, the statistical analysis of brain activity in the AP showed an independent main effect of three analyzed factors (sex chromosome complement, sex and treatment), as well as a significant effect of the triple interaction, indicating that the XX male-DEP group showed a higher FOS-ir than the other DEP and CON groups. Thus these data together suggest a sex chromosome gene participation at brain level in the hydroelectrolyte responses triggered by sodium depletion [23].

The anatomical–functional substrate underlying the responses aimed at restoring body fluid homeostasis is composed of several fore-brain and brain stem neuronal groups. Changes in plasma and CSF sodium concentration, osmolality, and ANG II concentration are sensed by the brain mainly at the level of three CVOs, the SFO and the OVLT at the third brain ventricle, and the AP at the fourth ventricle [33].

At the SFO and AP level, ANG II is involved in water and sodium intake regulation, the bradycardic baroreceptor reflex and blood pressure response, modulating hydroelectrolyte and cardiovascular homeostasis. Although the cellular and molecular mechanisms underlying sex differences in the modulation of sodium appetite and ANG II hypertensive and bradycardic baroreflex response are still unknown, it is accepted that the efficiency of the ANG II sexually dimorphic phenotypes seems to be due to central actions of this peptide along the CVOs and in particular within the AP and SFO. Both the SFO and AP are subject to neural and humoral modulation and send projections to neural centers involved in cardiovascular regulation, including the NTS, dorsal vagal complex, the PVN, the parabrachial nucleus, and rostral ventrolateral medulla, thereby modulating sympathetic–parasympathetic activity and baroreflex response [34,35]. In addition, injury of the AP not only attenuates ANG II-mediated hypertension and abolishes the blunted bradycardic response in males, but also leads to an increase in spontaneous sodium intake in male rats [36,37,38,39,40].

Most of the well-known biological functions of ANG II (vasoconstriction, aldosterone and vasopressin release, sodium reabsorption and increased sympathetic activity) are mediated by the activation of ANG II type 1 receptor (AT1), whereas type 2 receptor (AT2) stimulation is responsible for vasodilatation and natriuresis, thus opposing the vasoconstrictor and antinatriuretic effects of ANG II mediated through the AT1 receptor [41,42,43]. Furthermore, ANG(1–7) via the AT2 receptor or its own receptor, the Mas receptor (Mas), induces vasodilation [41,44].

Differences in the angiotensinergic system between males and females have been hypothesized to account for some of the ANG II-related

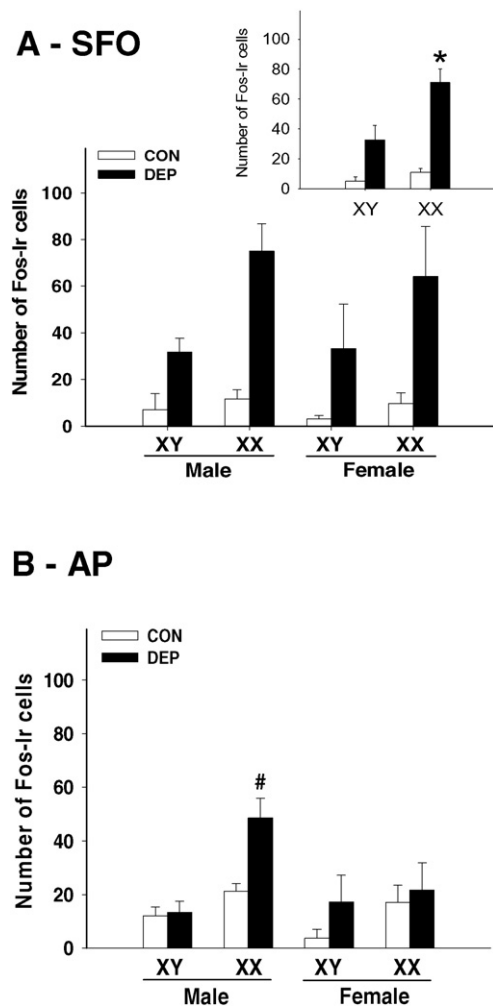


Fig. 5. Brain pattern of Fos-immunoreactivity (FOS-ir) following acute sodium depletion in subformal organ (SFO) and area postrema (AP). Panel A (SFO): Mean number of FOS-ir cells in XY male, XX male, XY female, and XX female mice corresponding to sodium depleted (DEP) and control (CON) groups (black and white bars, respectively). *Inset*: significant interaction of sex chromosome complement (SCC) \times treatment factors, $*P < 0.01$ XX-DEP vs. XY-DEP, XY-CON and XX-CON groups. Values are means \pm SE, $n = 3-5$ /group. Panel B (AP): Mean number of FOS-ir cells in XY male, XX male, XY female, and XX female mice corresponding to sodium depleted (DEP) and control (CON) groups (black and white bars, respectively). $\#P \leq 0.05$ XX male-DEP vs. the other DEP and CON groups. Values are means \pm SE, $n = 3-5$ /group. (Reprinted with permission from Dadam et al., *Am J Physiol Regul Integr Comp Physiol* 306: R175–R184, 2014).

sex differences associated with vasoconstrictor/vasodilator balance of the RAS, thus contributing to sex-specific differences in cardiovascular regulation. Studies have demonstrated that AT2 and Mas, mRNA expression levels are greater in adult female kidneys than in age-matched male kidneys, demonstrating an enhanced vasodilator arm of the RAS in female kidneys [45]. Moreover, 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 [46]. All this data together provide additional evidence of a shift in the vasoconstrictor:vasodilator ratio of the RAS in favor of vasodilation in females.

Exciting new data indicate that X chromosome inactivation is very far from the ‘all-or-none’ phenomenon that was initially described. Thus, some genes escape X-inactivation and are expressed from both the active and inactive X chromosome, which may lead to differences between XX cells and XY cells, potentially contributing to sex differences in traits (sex-biased genes) [47,48,49].

Taking into account that two of the components of the vasodilator arm of the RAS: AT2R receptor (Agtr2) and ACE2 genes are located in the X chromosome [50,51] and that some genes escape X-inactivation (and are thus expressed from both the active and the inactive X chromosome) [47,48,49], it is tempting to speculate that at the SFO and AP levels genes residing on the sex chromosome complement (which are asymmetrically inherited between males and females) may serve as candidate regulators of sexually dimorphic phenotypes [47]. Further investigation is needed, however, to assess the identity of the genes in the sex chromosomes responsible for the differential modulatory effect of sex chromosome complement on the angiotensin system in the brain areas involved in sexually dimorphic fluid and electrolyte balance control.

1. Perspectives and significance

In summary, our studies demonstrate sexually dimorphic induced sodium intake in mice, in which the organizational hormonal effect (but not the sex chromosome complement factor) modulates the sexually dimorphic profile. Moreover, we have also demonstrated that sex chromosome complement modulates both the sexually dimorphic ANG II-bradycardic baroreflex response and the activity of brain nuclei closely involved in the regulatory response to RAS stimulation, suggesting a sex chromosome gene participation in the modulation of neural pathways underlying fluid and electrolyte homeostasis.

Although awareness of sex differences in cardiovascular disease is increasing, the cardiovascular physiological and pathophysiological mechanisms behind these differences still require further study. Addressing in more detail the sources of physiological disparity between sexes and, in particular, the contribution of the sex chromosome complement factor to sex-related differences in cardiovascular homeostasis, may underline some of the puzzling differences that have emerged between the sexes, not only in the rates of cardiovascular disease, but also in terms of the symptoms and risk factors. Understanding in more detail the regulatory mechanisms underlying physiological differences between males and females (both at the peripheral and brain levels) may offer important insights into designing improved oriented sex-tailored therapeutic treatments in the future.

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