Molecular and Cellular Endocrinology 447 (2017) 98–105

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Sex chromosome complement involvement in angiotensin receptor sexual dimorphism

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ARTICLE INFO

Article history:
Received 7 November 2016
Received in revised form
24 February 2017
Accepted 25 February 2017
Available online 27 February 2017

Keywords:
Angiotensin receptors
Gene expression
Four core genotype mouse model
Renin angiotensin system
Area postrema
Renal cortex

ABSTRACT

This study aimed to define whether sex chromosome complement (SCC) may differentially modulate sex differences in relative gene expression of basal Agtr1a, Agtr2, and Mas1 receptors at fore/hindbrain nuclei and at medulla/cortical kidney.

Samples were collected from gonadectomized male (XX and XY) and female (XX and XY) mice of the "four core genotypes" model. At brain level, a SCC effect at the area postrema was demonstrated. An increase in mRNA level of Agtr1a and Agtr1a/Agtr2 ratio in XY-SCC mice was associated with a decrease in Mas1 compared to XX-SCC mice. In the renal cortex, a SCC effect for Agtr2 and Mas1 was observed. Regardless of sex (male or female), XX-SCC mice expressed higher levels of mRNA Agtr2 and Mas1 than XY-SCC mice {$F(1,12) = 6{,}126, p < 0.05$; $F(1,21) = 5{,}143, p < 0.05$}. Furthermore, XX-female mice showed a significant increase in Mas1 expression compared to XY-female mice.

These results reveal a SCC modulatory effect at central and kidney level on angiotensin receptor expression, with an enhancement of the vasodilatory arm in XX-mice and an increase in the vasoconstriction arm in XY-mice, which may underlie sex differences in the regulation of arterial pressure.

1. Introduction

Although awareness of sex differences in cardiovascular disease is increasing, much of what we know about blood pressure regulation has been derived from studies in males. However, principles learned in male models do not necessarily apply to females, and thus it is important to study the basis of sex differences.

The renin angiotensin system (RAS) exerts both hormonal and paracrine effects, modulating blood pressure regulation, among others. Clinical and basic findings demonstrate major sex differences in the way males and females respond to stimulation and inhibition of the RAS under physiological and pathophysiological circumstances (Brown et al., 2012; Sullivan, 2008; Xue et al., 2005) Differences in angiotensin peptides and receptors in males and females have been hypothesized to be one of the potential mechanisms contributing to sex-specific differences in cardiovascular homeostasis; which highlights the importance of studying basal brain and kidney RAS complements (Sandberg and Ji, 2012).

Ang II effects include a wide range of actions on the kidney, heart, blood vessels and adrenal gland in physiological and pathophysiological states. Furthermore, angiotensin peptides exert modulatory effects on the central nervous system, both in brain areas lacking the blood-brain-barrier (circulating Ang II-responders) as well as in brain areas in which Ang II is locally produced. Ang II binds to two G protein-coupled receptor (GPCR) subtypes, AT1 and AT2, exerting opposing and counterbalancing effects on the cardiovascular system. The classic excitatory effects evoked by Ang II (vasoconstriction, aldosterone and vasopressin release, sodium reabsorption, increased sympathetic activity and vascular growth) result from AT1 stimulation, whereas AT2 activation causes vasodilation, natriuresis, and anti-proliferation effects, thus opposing the vasoconstrictor and antinatriuretic effects of AT1-Ang II mediated responses (Carey et al., 2001; Li et al., 2003; Kaschina and Unger, 2003; Sandberg and Ji, 2000). Furthermore, Ang(1–7) mediates vasodilation via the AT2 receptor or its own receptor, the Mas receptor (Kaschina and Unger, 2003; Santos et al., 2003).

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http://dx.doi.org/10.1016/j.mce.2017.02.041
0303-7207/

Earlier studies showed differences in the ratio of AT1 to AT2 and Mas receptor expression between males and females (Sampson et al., 2012a,b; Silva-Antonialli et al., 2004), which may account for some of the Ang II-related sex differences associated with vasoconstrictor/vasodilator balance of the RAS. This leads to the question of what makes males and females different?

Exposure to sex steroids during critical periods of development can induce organizational (long-lasting or permanent) effects on sexually dimorphic traits. Sex steroids can also impart (temporary or reversible) activational effects at different times of life (during neonatal and peripubertal development as well as in adulthood) to cause most of the known sex differences in phenotype (Arnold and Gorski, 1984; McCarthy et al., 2012; Morris et al, 2004). For a long time, the organizational-activational dichotomy was applied to the understanding of many sex differences, and hormones were the only factors discussed as proximate signals causing sex differences. However, males and females differ not only in their sex (males are born with testes- and females with ovaries-hormonal factors) but also carry different sex chromosome complements (SCC: XY and XX respectively) and thus are influenced throughout life by different genomes. Exciting new data indicate that some genes escape X-inactivation and are expressed from both the "active" and "inactive" X chromosome, which may cause functional sex differences intrinsic to male (XY) and female (XX) cells, potentially contributing to sex differences in traits (sex-biased genes) (Carrel and Willard, 2005; Wolstenholme et al., 2013; Yang et al., 2006).

Although sex hormones (activational effects) are known to directly interact with RAS (Baiardi et al., 2005; Miller et al., 1999; Silbiger and Neugarten, 1995), the potential contribution of organizational hormonal and SCC effects on physiological sex-based difference in the regulation of the RAS remains undefined.

Taking into account that two of the components of the vasodilatador arm of the RAS (AT2 receptor (Agtr2) and ACE2 genes) are located in the X chromosome (Koike et al., 1994; Oudit et al., 2003) and that some genes escape X-inactivation (Carrel and Willard, 2005; Wolstenholme et al., 2013; Yang et al., 2006), it is tempting to speculate that genes residing in the SCC (which are asymmetrically inherited between males and females) may serve as candidate regulators of sexually dimorphic phenotypes. In this study, we sought to assess whether genetic differences within the SCC may differentially modulate the basal angiotensin type 1a (Agtr1a), 2 (Agtr2) and Mas (Mas1) receptor gene expression at kidney level and in brain nuclei involved in blood pressure regulation. We tested our hypothesis that the mRNA expression of the vasoconstrictor component (Agtr1a) of RAS would predominate in XY-SCC mice, while the vasodilator RAS components (Agtr2 and Mas1) would be enhanced in XX-SCC mice at specific brain nuclei and renal cortex levels.

To test our hypotheses, in the four core genotypes (FCG) mouse model, we evaluated relative mRNA expression levels of the angiotensin Agtr1a, Agtr2 and Mas1 receptors at different brain levels involved in blood pressure regulation and bradycardic baroreflex responses. We also evaluated angiotensinergic receptor expression in the renal cortex and medulla. The study was designed to evaluate the role of SCC and organizational hormonal effects, and therefore adult FCG mice were gonadectomized to remove the sex differences caused by the acute (activational) effects. 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 sexually dimorphic traits.

2. Methods

2.1. Animals

"Four core genotypes" mice were used in the following experiments. This mouse model combines a deletion of the testis-determining Sry gene from the Y chromosome (Y^-) and the subsequent insertion of a Sry transgene into an autosome. Sry gene deletion in XY 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, and thus four types of offspring are produced by breeding XY^-Sry males with 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 individuals possessing the Sry transgene develop testes and have a male external phenotype regardless of their SCC, while individuals lacking the transgene have ovaries and external female secondary sex characteristics. Male and female are defined here according to the gonadal phenotype. Throughout the text, we will refer to XX and XY^- as XX and XY females, and to XXSry and XY^-Sry as XX and XY male mice respectively. By comparing these genotypes, it is possible to segregate the role of a) SCC (comparing mice with the same gonadal type but with different SCC: XX vs. XY) b) gonadal sex (males vs. females regardless of SCC), and c) the interaction of SCC and gonadal sex (Fig. 1).

MF1 transgenic mice, kindly provided by Dr. Paul Burgoyne from Medical Research Council National Institute for Medical Research, UK, were born and reared in the breeding facilities at the Instituto Ferreyra (Córdoba, Argentina). All 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. Genotyping was performed as previously described in Caeiro et al., 2011.

2.2. Brain and kidney microdissection, tissue collection, RNA extraction and gene expression studies

Four core genotype mice (aged 45–50 days old) were gonadectomized to remove any activational effect of sex hormones that might mask the modulatory action of SCC and the organizational hormonal effects. After a 15-day-recovery period, the mice were decapitated and brain and kidneys were immediately excised and stored at −80 °C for Agtr1a, Agtr2 and Mas1 mRNA determination. Coronal sections of 590 μm (organum vasculosum laminae terminalis, OVLT), 720 μm (subfornical organ, SFO), 640 μm (paraventricular nucleus, PVN) and 560 μm (nucleus of the solitary tract, NTS and AP) were obtained from the frozen brains in a microtome with a stainless steel punch needle. The brain nuclei were identified and delimited according to the mouse brain atlas (Paxinos and Frank, 2001). Likewise, renal cortex and medulla samples were obtained. Longitudinal sections of 1600 μm were excised from the frozen kidney and punches were immediately taken with a stainless steel punch (inner diameter 1.5 mm).

RNA was isolated from punches of specific brain and kidney areas using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as directed by the manufacturer with some modifications: RNA precipitation with isopropanol was performed overnight at −20 °C. The RNA was treated with DNase (Fermentas) and quantified using a NanoDrop 2000 UV-Vis Spectrophotometer, and was then reverse-transcribed into cDNA (enzyme RTM-MLV – Promega). Brain Agtr1a and Agtr2 as well as brain and kidney Mas1 gene expression were determined using Sybr Green Real-Time PCR Master Mixes (Applied Biosystems™). Kidney Agtr1a and Agtr2 were determined with (Applied Biosystems™) in the Step One

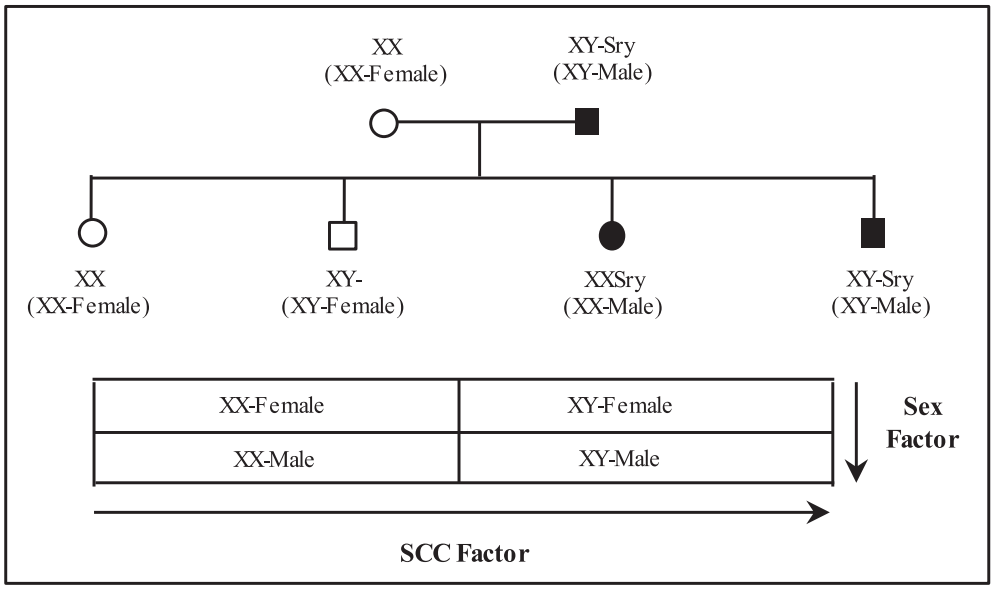

Fig. 1. Schematic representation of four core genotype mouse model (FCG). Four types of offspring are produced by breeding XY_*Sry* males to XX females: XX and XY_females (without *Sry* on the Y chromosome) and XX*Sry* and XY_*Sry* male mice (both with *Sry* in an autosome). All of the individuals possessing 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. XX and XY_ females are referred as XX and XY females and XX*Sry* and XY_*Sry* male mice as XX and XY male mice, respectively.

Real-Time equipment (Applied Biosystems). Primer sequences can be found in Table 1.

2.3. Calculations of relative gene expression

The relative quantification was determined by the ΔΔCt method where the fold change of mRNA content in the unknown sample relative to control group was determined by $2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct = \Delta Ct_{unknown} - \Delta Ct_{control}$). For each sample, the Ct was determined and normalized to the average of the housekeeping gene: Gapdh for the OVLT, NTS, AP, PVN and kidney (cortex and medulla) and 18s rRNA for SFO. All samples were run in duplicate with the average CT used for each sample. The Ct of the calibrator group (the mean Ct of the MF1 male mice for Agtr1a, Agtr2 and Mas1) was then subtracted from each sample to give a Ct value. Relative quantifications of the target gene (for Agtr1a, Agtr2 and Mas1) were normalized to wild-type MF1 male mice. Data are presented as mRNA expression relative to the control calibrator group.

2.4. Statistical analysis

Relative gene expression data were subjected to a 2-way mixed ANOVA with gonadal sex (male/female) and SCC (XY/XX) as independent factors. The loci of significant interactions or significant main effects were further analyzed using the Tukey test (type I error probability was set at 0.05). Results were expressed as group mean (M) ± standard error (SE).

3. Results

3.1. Effect of SCC on Agtr1a, Agtr2 and Mas1 gene expression at the brain level

3.1.1. Sensory circumventricular organs (OVLT, SFO, AP)

At the AP level, the angiotensin receptor gene expression showed the following pathern: regardless of sex (male or female), XY-SCC mice expressed higher levels of mRNA Agtr1a and a decrease in Mas1 gene expression compared with XX-SCC mice {$F(1,20) = 5.63$; $p < 0.05$ and $F(1,20) = 4,57$; $p < 0.05$, respectively}. Furthermore, XY-SCC mice exhibited a two-fold greater expression in the Agtr1a/Agtr2 ratio (2.71 ± 0.38) than in XX-SCC groups (1.36 ± 0.19) ($p < 0.01$){$F(1,20) = 10.31$; $p < 0.01$}. In contrast, no significant differences were observed in Agtr2 gene expression (Fig. 2, upper panel). Thus, at the AP we observed that XX-SCC mice showed an increase in gene expression levels of the vasodilatory arm (Mas1) while in XY-SCC mice an increase in the vasoconstrictor arm was evident (Agtr1a and Agtr1a/Agtr2 ratio gene expression).

In contrast, at SFO and OVLT level, no significant effect of either sex or SCC factors were found on the expression of any of the angiotensin gene receptors (Fig. 2, middle and lower panel).

3.1.2. Hypothalamic and brainstem nuclei (PVN and NTS)

Matching the OVLT and SFO gene expression profile, relative Agtr1a, Agtr2 and Mas1 gene expression showed no significant effect of either sex or SCC factors at either the PVN or the NTS (Fig. 3).

Table 1
Primer pairs for Agtr1a, Agtr2, Mas1, Gapdh mRNAs and 18s rRNA.

Gene	GenBank access number	Primer forward 5'-3'	Primer reverse 5'-3'	Product size (bp)	Annealing temp. (°C)
Agtr1a	NM_177322.3	GCTGCTCTCCCGGACTTAAC	GCATTGATCTGGTGATGGC	106	60 °C
Agtr2	NM_007429.4	TTACATCTCAGAGGCTGGCG	CCCGCATGCACTCCTTAAA	80	59 °C
Mas1	NM_008552.4	CTGACTAACGATGCCACCGA	GTCATTTTCTGGCTGGCAGG	115	59 °C
18s	M35283.1	AGAGCGGGTAAGAGAGGTG	GGTCCGACAAAACCCGTCC	95	60 °C
Gapdh	NM_008084.2	AGTGCCAGCCTCGTCCCGTAG	GTGCCGTTGAATTTGCCGTGAGTG	196	66 °C

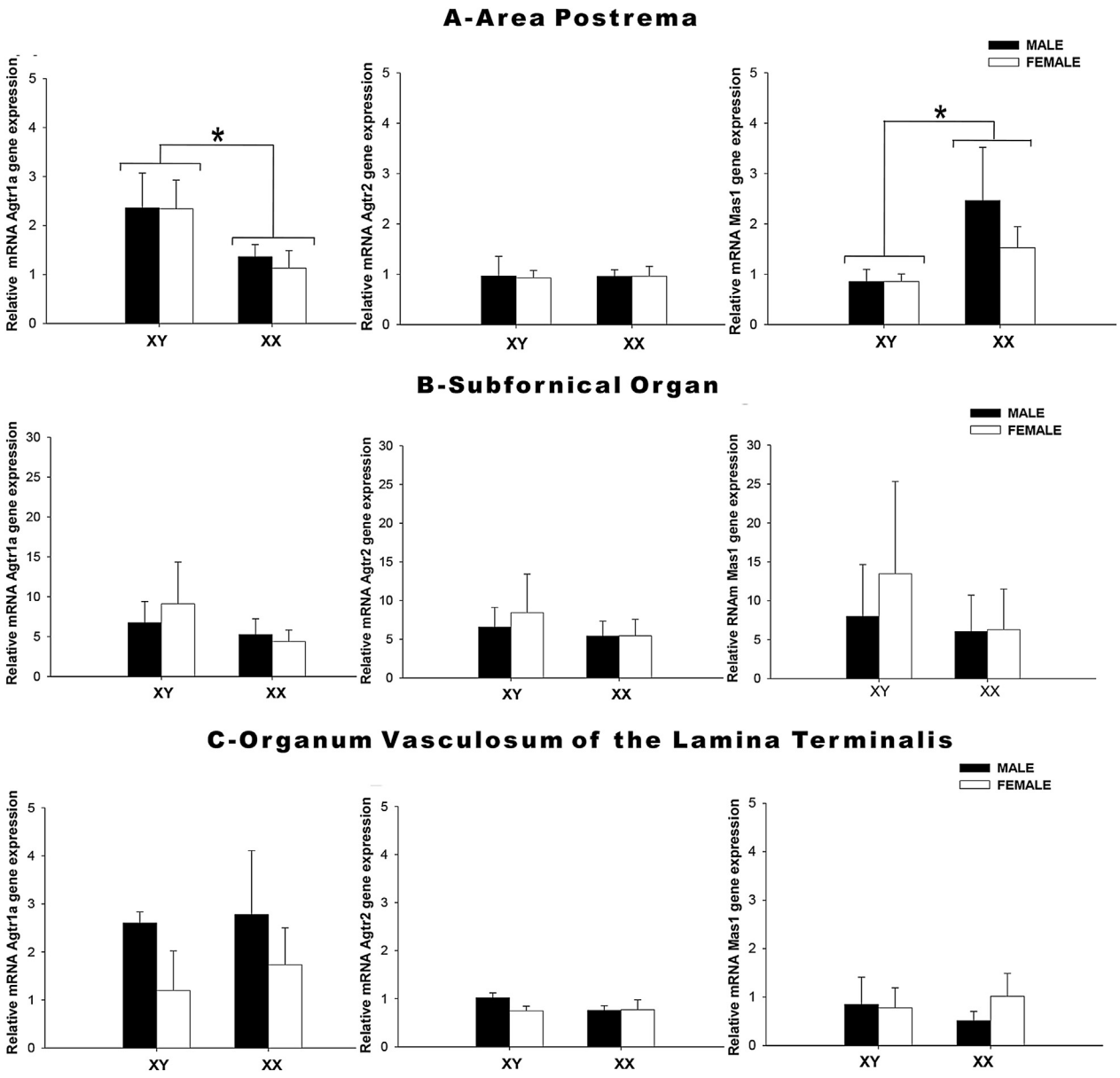

Fig. 2. Relative mRNA expression of angiotensin type 1a (Agtr1a), 2 (Agtr2) and Mas (Mas1) receptors (left, middle and right panels) at the area postrema , subfornical organ and organum vasculosum of the lamina terminalis (A, B and C respectively). Bar graphs show relative *Agtr1a, Agtr2* and *Mas1* mRNA gene expression in gonadectomized XY male and XX male mice [filled bars] and in XY female and XX female mice [open bars]. Values are mean ± SE, n=4–6/group. *$p < 0.05$.

3.2. Effect of SCC on Agtr1a, Agtr2 and Mas1 gene expression in the renal cortex and medulla

In the renal cortex data revealed a significant main of effect of SCC factor for both Agtr2 and Mas1 relative gene expression. As shown in Fig. 4, regardless of sex (male and female), XX-SCC mice expressed higher levels of mRNA Agtr2a and Mas1 genes than XY-SCC mice {$F(1,12) = 6{,}126$, $p < 0.05$; $F(1,21) = 5{,}143$, $p < 0.05$, respectively}. Mas1 gene profile also showed a significant effect of the interaction of SCC and gonadal sex {$F(1,12) = 4{,}59$; $p < 0.05$} with a significant increase in Mas1 expression in XX-female mice compared with XY-female mice. In contrast, no differences in mRNA Agtr1 levels were observed. Thus, at renal cortex a shift of the balance of the RAS to the depressor arm in XX-SCC mice was shown. Meanwhile, at the kidney medulla, no significant differences were attributable to phenotype, SCC or to the interaction of both factors for any of the angiotensinergic receptors tested.

4. Discussion

There were two major findings in the present study. First, at brain level we found a modulatory effect of SCC on AP angiotensin receptor gene expression, with increased mRNA levels of the vasodilatory arm (Mas1) in XX-SCC mice in association with a decrease of both Agtr1a and Agtr1a/Agtr2 ratio gene expression (the vasoconstrictor arm of the RAS) when compared with XY-SCC mice. Second, in the renal cortex, we consistently identified a sex chromosome effect for both Agtr2 and Mas1 gene expression. Regardless of sex (male or female), XX-SCC mice express higher levels of mRNA Agtr2 and Mas1 than XY-SCC mice, thus showing also at kidney level a shift of the balance of the RAS to the depressor arm in XX-SCC mice. The foregoing data provide new evidence of a SCC

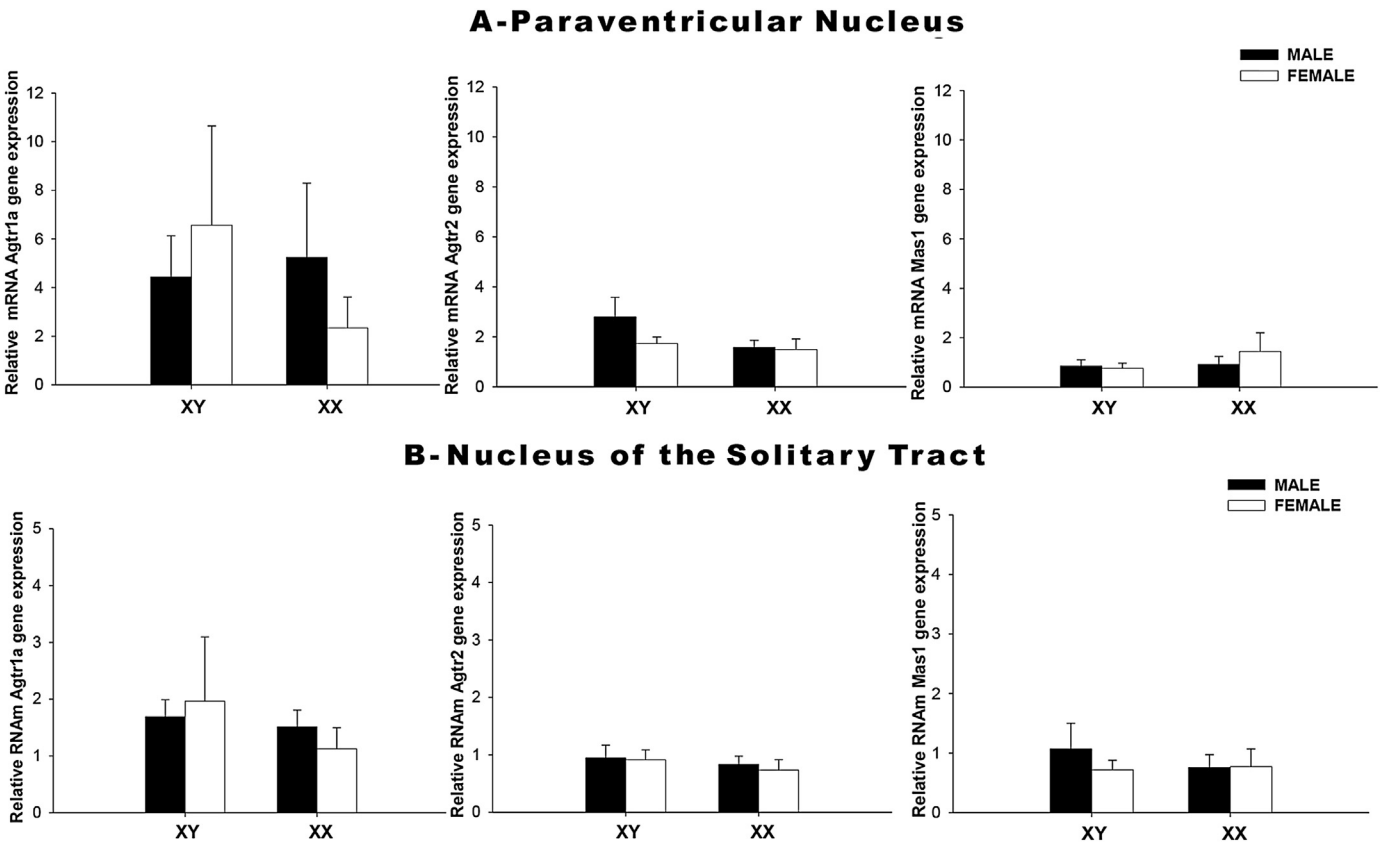

Fig. 3. Relative mRNA expression of angiotensin type 1a (Agtr1a), 2 (Agtr2) and Mas (Mas1) receptors (left, middle and right panels) at and paraventricular nucleus the nucleus of the solitary tract (A and B panels respectively). Bar graphs show relative *Agtr1a, Agtr2* and *Mas1* mRNA gene expression in gonadectomized XY male and XX male mice [filled bars] and in XY female and XX female mice [open bars]. Values are mean ± SE, n=4–6/group.

effect on the shift at central and kidney level in the vasoconstrictor/vasodilator ratio of angiotensin receptor expression toward vasoconstriction in XY-SCC and/or vasodilation in XX-SCC mice.

Although the mechanisms responsible for sexually dimorphic Ang II- bradycardic baroreflex responses are still under study, there is data indicating that the attenuation of the Ang II sexually dimorphic bradycardic baroreflex response in males may be due to central action of this peptide in the sensory circumventricular organs (CVOs). Changes in plasma and cerebrospinal fluid sodium concentration, osmolality, and Ang II/Ang 1–7 concentration are sensed by the brain, mainly in the sensory CVOs, the SFO and the OVLT in the wall of the third ventricle, and in the AP in the wall of the fourth ventricle. The fact that these nuclei are highly vascularized and contain an extensive network of fenestrated capillaries, receptor binding sites for circulating hormones like angiotensin II, atrial natriuretic peptides and vasopressin, is consistent with the proposal that these CVOs are sites at which blood-borne humoral agents exert central actions (McKinley et al., 2004).

At the AP, Ang II is involved in baroreceptor reflex and blood pressure regulation. The AP is subject to both neural and humoral control and sends projections to neural centers involved in cardiovascular regulation, including the NTS, dorsal vagal complex, the parabrachial nucleus and rostral ventrolateral medulla, thereby modulating sympathetic-parasympathetic activity and baroreflex response (Blessing et al., 1987; Shapiro and Miselis, 1985). Furthermore, injury at the AP blocks the Ang II-hypertensive response and, in males, it prevents the decrease in baroreflex sensitivity observed after acute administration of this peptide (Fink et al., 1987; Xue et al., 2003).

Ang II binds to AT1, inducing attenuation of the bradycardic baroreflex response, while both AT2 and Mas activation facilitate heart rate response to increases in blood pressure (Chaves et al., 2000; Gao et al., 2004; Sakima et al., 2005). Our present results on the effect of SCC on angiotensin gene expression match our previous physiological work in which we demonstrated that the bradycardic baroreflex sexual dimorphic response may be ascribed to differences in sex chromosomes (Caeiro et al., 2011). There we demonstrated that XX-SCC mice showed (irrespective of sex, XX-male and XX-female) a facilitatory bradycardic baroreflex control of heart rate and our present results consistently show an enhancement of the vasodilator component of RAS in the AP of XX-SCC mice, which may underlie the facilitation of the bradycardic baroreflex response. In contrast, the enhancement of the vasoconstrictor components of the RAS may bias XY-SCC mice towards attenuation of the bradycardic baroreflex response.

Long term blood pressure regulation is linked to renal function through the mechanisms of pressure natriuresis (Evans et al., 2005), which has been shown to be modulated by the RAS (Hall et al., 1999). Key components of the RAS are expressed throughout the kidney, and are implicated in renal excretory and hemodynamic function modulation (Sampson et al., 2012a,b). AT1 and AT2 Ang II receptors play significant roles in the regulation of renal blood flow. Well-known renal actions of Ang II mediated by the AT1 receptor include afferent and efferent arteriolar vasoconstriction, increased tubular sodium absorption at low doses, inhibition of reabsorption at higher doses, and constriction of renal vessels, which induces changes in renal blood flow, glomerular filtration rate and sodium excretion (Arendshorst et al., 1999; Navar

et al., 1996). Furthermore, the AT2 receptor has been reported to be expressed throughout the kidney (Armando et al., 2002; Cao et al., 2000; Miyata et al., 1999), importantly in the afferent arteriole and in the distal tubule, key locations for tubuloglomerular feedback regulation. Ang II, via the AT2 receptor, results in vasodilation of renal afferent arterioles (Arima et al., 1997), which activates the vasodilator/natriuretic cascade resulting in the increased production of bradykinin and nitric oxide. Furthermore, in vitro studies demonstrate that the proximal tubule AT2 receptor is linked not only to inhibition of sodium but also to bicarbonate absorption, an effect that opposes AT1 receptor-mediated responses (Haithcock et al., 1999). In line with this evidence a significant rightward shift in the pressure-natriuresis relationship in AT2 knockout mice has been reported; at similar perfusion pressure AT2 knockout mice excrete 3-fold less sodium and water than wild-type mice (Gross et al., 2000).

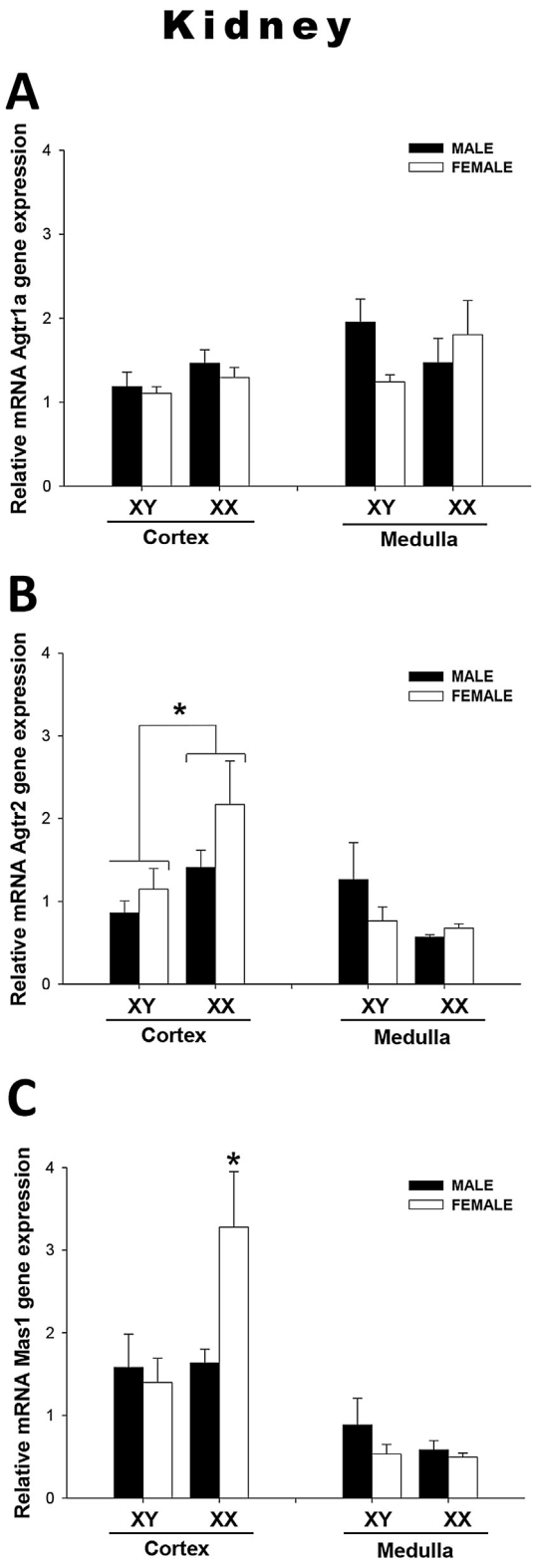

Fig. 4. Relative mRNA expression of angiotensin type 1a (*Agtr1a*), 2 (*Agtr2*) and Mas (*Mas1*) receptors at the renal medulla and cortex. Bar graphs show relative *Agtr1a, Agtr2 and Mas1* mRNA gene expression (panels A, B and C) in gonadectomized XY male and XX male mice [filled bars] and in XY female and XX female mice [open bars]. Values are mean ± SE, n=4–5/group. *$p < 0.05$.

In the pathophysiology of hypertension, it is clear that the kidneys' ability to achieve sodium and water homeostasis is compromised and that the set point for pressure-natriuresis is shifted to a higher blood pressure (Hall et al., 1999). Siragy et al. (1999) demonstrated that exogenous administration of Ang II in AT2 knockout mice led to sustained sodium retention and hypertension in contrast to wild-type control mice, highlighting the protective role of AT2 against the antinatriuretic and pressor action of Ang II. Furthermore, ACE inhibitors are clinically effective in the treatment of hypertension and associated end-organ disease (Robles et al., 2014). Ang(1–7) is degraded and inactivated by ACE, and thus the blood pressure-lowering effects of ACE inhibitors and AT1 antagonists may be attributable not only to direct inhibition of Ang II synthesis and direct AT1 receptor blockade, but also to an increase in vasodilator Ang(1–7), all of which produce favorable cardiovascular effects (Brosnihan et al., 1996; Chappell et al., 1998; Li et al., 1997).

The pressure-natriuresis relationship is sex-dependent. Studies in normotensive and hypertensive rats have shown that, compared to their male counterparts, females excrete the same amount of sodium as males at a lower arterial pressure and thus show a protective leftward shift in the pressure-natriuresis curve (Hilliard et al., 2011; Khraibi et al., 2001; Reckelhoff et al., 1998). Baiardi et al. (2005) also demonstrated that female renal vasculature show a greater renal AT2 expression. Matching this data, AT2 blockade has been shown to blunt the autoregulation of renal blood flow at low perfusion pressures in females but not in males (Hilliard et al., 2011). The impact of the Mas on renal hemodynamics appears to be sexually dimorphic, since Mas blockade decreases renal blood flow significantly in female but not in male rats (Safari et al., 2012). However, the entire mechanism underlying this sex difference is still unknown.

Numerous results demonstrate an activational hormonal effect on RAS components. Studies have shown a greater mRNA expression of AT2, Mas and ACE2 in adult female kidneys than in age-matched males (with a lower AT1/AT2 ratio in females), therefore shifting the balance of the RAS to the vasodilator arm (Baiardi et al., 2005; Hilliard et al., 2013a,b; Sampson et al., 2008). Studies in mice demonstrate that females express much higher numbers of renal AT2 receptors than males, and that treatment with estrogen increases renal AT2 receptor expression in male mice (Baiardi et al., 2005). Thus, estrogen treatment can decrease the AT1/AT2 receptor ratio, favoring vasodilation, a shift that may contribute to explain the sex differences in the response to Ang II (Miller et al., 1999; Silbiger and Neugarten, 1995). Furthermore, studies have demonstrated in female wild-type mice that the pressor responsiveness to Ang II was augmented with age and that vehicle-treated aged wild-type mouse had a lower renal AT2R/AT1R balance as compared to adult counterparts (Mirabito et al., 2014).

Studies have demonstrated that AT2 plays a greater role in modulating arterial pressure in females than in males. Chronic low-dose infusion of Ang II decreased blood pressure in female rats at a

dose that had negligible effects in males. Furthermore, AT2 blockade eliminates the depressor response to Ang II, confirming the vasodepressor role of the AT2 in females (Sampson et al., 2008). Similarly, while Ang II infusion results in a mild increase in blood pressure in WT male and AT2R-KO female mice, the Ang II pressor response in WT female mice is attenuated. In this group of mice, renal expression of AT2 was 3-fold greater in WT females than in WT males under basal conditions and after chronic Ang II. Furthermore, when renal sensitivity to Ang II was investigated, it was seen that Ang II increased tubuloglomerular feedback sensitivity in both male genotypes and the female AT2-KO group but had no apparent effect in female WT mice. This attenuated pressor response and resetting of tubuloglomerular feedback to Ang II in AT2 intact females gives support for a protective role of AT2 against the prohypertensive effects of Ang II in females. In line with this, greater renal cortical levels of Ang(1–7) have been identified in female versus male spontaneously hypertensive rats, both basally and after exogenous Ang II infusion. Furthermore, Sullivan et al. (2010) also demonstrated an increase in Mas expression in the renal cortex following Ang II infusion only in female spontaneously hypertensive rats. Hence, these results provide evidence of a shift in the renal vasoconstrictor/vasodilator ratio of the RAS toward vasodilation in females, with clear sex differences in the renal hemodynamic response to Ang II. However, are these differences among male and female due only to hormonal activational effects? Although previous data have indicated an undisputed impact of the circulating (activational) effects of sex hormones on sexually dimorphic cardiovascular responses (Hay et al., 2014; Sampson et al., 2012a,b; Xue et al., 2014), in the present study we found that SCC also modulates basal Agtr1a, Agtr2 and Mas1 receptor dimorphic gene expression in the renal cortex in isolation of organizational and activational hormonal effects. As previously shown by Brown et al. (2012), no differences in renal AT1R between male and female mice were observed either in XX-SCC or XY-SCC mice. However, our results identified a sex chromosome effect for both Agtr2 and Mas1 gene expression in the renal cortex. Regardless of sex (male or female), XX-SCC mice express higher levels of mRNA Agtr2 and Mas1 gene expression than XY-SCC mice, therefore showing a shift of the balance of the RAS to the depressor arm in XX-SCC mice. As AT2 and Mas receptors have been implicated as modulators of natriuresis (Gross et al., 2000; Siragy et al., 1999) and both receptors are upregulated in XX-SCC mice in the renal cortex (Sampson et al., 2008; Silva-Atonialli, 2004), this may result in a differential pressure-natriuresis relationship, contributing to sex-associated differences in long-term blood pressure regulation.

The approach of this study was focused on the analysis of angiotensin receptor gene expression, however some limitations should be taken into consideration. Mice were gonadectomized in late puberty and 15 days later gene expression of different brain and kidney tissue were analyzed. Although preliminary results demonstrate a dramatically reduction in estradiol and testosterone levels we cannot exclude a possible sex hormone effect in the analyzed phenotypes. Furthermore we were not able to confirm the translation to protein. Although this was intended, previous studies have tested available commercial AT1 and AT2 antibodies and have demonstrated to be nonspecific. The immunostaining patterns observed were different for every antibody tested, and were unrelated to the presence or absence of AT_1 receptor since identical bands were observed in wild-type mice and in AT_{1A} and AT2 knockout mice not expressing the target protein. Thus, from those studies it is conclude that none of the commercially available AT1 and AT_2 receptor antibodies tested upon the present meet the criteria for specificity and if used may lead to erroneous physiological interpretations and conclusions (Benicky et al., 2012; Hafko et al., 2013; Herrera et al., 2013).

To the best of our knowledge, this is the first study documenting the SCC modulatory effect at central and kidney level on the shift in the vasoconstrictor-vasodilator ratio of mRNA expression of angiotensin receptors toward vasoconstriction in XY-SCC and/or vasodilation in XX-SCC mice. From these results and those of other authors we can thus infer that both the activational hormonal effects (Baiardi et al., 2005; Miller et al., 1999; Silbiger and Neugarten, 1995) and the SCC factors are involved in sexually dimorphic mRNA expression of angiotensin receptors. This may constitute the basis of a shift in the functional sexually dimorphic angiotensin responses and in the incidence of cardiovascular diseases among males and females.

4.1. Perspectives

Considerable clinical and experimental data indicate that sex matters when it comes to blood pressure regulation and it is thus important to study brain-sex differences in blood pressure regulation in physiological and pathophysiological states such as hypertension. Addressing in more detail the sources of physiological disparity between sexes and in particular the contribution of the SCC factor to sex-related differences in cardiovascular homeostasis, may clarify some of the puzzling differences that have emerged between the sexes, not only in rates of cardiovascular disease, but also in terms of symptoms and risk factors. Thus, sex-detailed studies about the RAS components may provide a better understanding of the individual roles of the angiotensinergic receptor subtypes in the physiology of blood pressure regulation or pathophysiology of hypertension, which may have therapeutic relevance and thus offer important insights into designing improved sex-tailored therapeutic treatments in the future.

Sources of funding

This study was supported in part by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [N°2561], Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) [N° 1580]. International Society for Neurochemistry (ISN) [year 2015], Ministerio de Ciencia y Tecnología de la Provincia de Córdoba (MinCcyt) [N°113/2011] and Secretaria de Ciencia y Tecnología (SECyT-Universidad Nacional de Córdoba) [N°1565]. FAPESP [#2013/0799-1] and CNPq [N°301827/2011-7 and 401598/2014-4] to JAR; MJC, AG, LV and XEC are members of CONICET. FMD, CDC and AFM hold fellowships for CONICET.

Acknowledgments

We are grateful to Dr. Paul Burgoyne from the Medical Research Council National Institute for Medical Research, UK for providing the transgenic mice.

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