Sex Differences in the Pressor and Tubuloglomerular Feedback Response to Angiotensin II

Russell D. Brown, Lucinda M. Hilliard, Geoffrey A. Head, Emma S. Jones, Robert E. Widdop, Kate M. Denton

Abstract—Awareness of sex differences in the pathology of cardiovascular disease is increasing. Previously, we have shown a role for the angiotensin type 2 receptor (AT$_2$R) in the sex differences in the arterial pressure response to Ang II. Tubuloglomerular feedback (TGF) contributes in setting pressure-natriuresis properties, and its responsiveness is closely coupled to renal Ang II levels. We hypothesize that, in females, the attenuated pressor response to Ang II is mediated via an enhanced AT$_2$R mechanism that, in part, offsets Ang II–induced sensitization of the TGF mechanism. Mean arterial pressure was measured via telemetry in male and female wild-type (WT) and AT$_2$R knockout (AT$_2$R-KO) mice receiving Ang II (600 ng/kg per minute SC). Basal 24-hour mean arterial pressure did not differ among the 4 groups. After 10 days of Ang II infusion, mean arterial pressure increased in the male WT (28±6 mm Hg), male AT$_2$R-KO (26±2 mm Hg), and female AT$_2$R-KO (26±4 mm Hg) mice, however, the response was attenuated in female WT mice (12±4 mm Hg; P between sex and genotype=0.016). TGF characteristics were determined before and during acute subpressor Ang II infusion (100 ng/kg per minute IV). Basal TGF responses did not differ between groups. The expected increase in maximal change in stop-flow pressure and enhancement of TGF sensitivity in response to Ang II was observed in the male WT, male AT$_2$R-KO, and female AT$_2$R-KO but not in the female WT mice (P between sex and genotype <0.05; both). In conclusion, these data indicate that an enhanced AT$_2$R-mediated pathway counterbalances the hypertensive effects of Ang II and attenuates the Ang II–dependent resetting of TGF activity in females. Thus, the enhancement of the AT$_2$R may, in part, underlie the protection that premenopausal women demonstrate against cardiovascular disease. (Hypertension. 2012;59:129-135.) • Online Data Supplement

Key Words: sex differences • renin-angiotensin system • Ang II • mean arterial pressure • hypertension • renal hemodynamics • tubuloglomerular feedback

Men have a greater prevalence for hypertension and cardiovascular disease than premenopausal women of the same age. The mechanisms underlying these differences in the pathophysiology of cardiovascular disease between men and women remain unclear. One of the suggested mechanisms underlying this sexual dimorphism is differences in the function of the renin-angiotensin system (RAS).2–4

Angiotensin II (Ang II), the main effector peptide of the RAS, exerts its effects through 2 main receptor subtypes. Stimulation of the angiotensin type 1 receptor (AT$_1$R) causes vasoconstriction, sodium reabsorption, and cell proliferation. The angiotensin type 2 receptor (AT$_2$R) opposes the AT$_1$R, causing vasodilation, sodium excretion, and apoptosis.5,6 Earlier studies have shown that females have a greater AT$_2$R:AT$_1$R ratio.7,8 We, and others, have demonstrated previously that the rise in arterial pressure in response to Ang II is blunted in females compared with males.7,9–11 Furthermore, we have provided evidence to suggest that arterial pressure is differentially regulated by the AT$_1$R in females as compared with males.7,12

There is considerable evidence that the kidney plays a crucial role in the regulation of long-term blood pressure and in the development of hypertension. Thus, renal mechanisms may potentially contribute to the sexual dimorphism of hypertension. During hypertension the set point for pressure-natriuresis is shifted to a higher pressure because of increased renal vascular resistance and sodium reabsorption. Afferent and efferent arteriole tones predominantly account for renal vascular resistance and thereby determine glomerular filtration rate and peritubular pressure. Afferent and efferent arteriolar tones are regulated by similar factors to those in other arterioles and additionally by tubuloglomerular feedback (TGF). The TGF mechanism is a negative feedback system, operating via the macula densa (MD) in the distal tubule. An increase in flow-dependent electrolyte load to the MD activates arteriolar constriction and subsequently decreases glomerular filtration rate. Hence, the TGF mechanism is important in the regulation of glomerular filtration rate, fluid and electrolyte homeostasis and thus, maintenance of fluid and electrolyte homeostasis.

Received June 29, 2011; first decision July 14, 2011; revision accepted November 1, 2011.
From the Departments of Physiology (R.D.B., L.M.H., K.M.D.) and Pharmacology (E.S.J., R.E.W.), Monash University, Melbourne, Victoria, Australia; Baker IDI Heart and Diabetes Institute (G.A.H.), Melbourne, Victoria, Australia.
Correspondence to Russell D. Brown, Department of Physiology, Building 13F, Monash University, Melbourne, Victoria 3800, Australia. E-mail russell.brown@monash.edu
© 2011 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.111.178715

129
long-term blood pressure. Ang II, in addition to its direct actions on the renal vasculature, also augments the TGF response.13,14 A heightened or sensitized TGF response has been implicated in the development of hypertension.15,16 Importantly, AT2Rs have been localized to the distal tubules and afferent arterioles where they can potentially modulate TGF response.17,18 Moreover, it has been demonstrated that AT2R expression is greater in the kidney in females.7,8

It is our hypothesis that the attenuated pressor response to Ang II is mediated via an AT2R mechanism in females. Furthermore, we hypothesize that enhanced renal AT2R expression in females offsets the Ang II–induced sensitization of the TGF mechanism, reducing renal sensitivity to Ang II. In this study, the pressor response to chronic Ang II was investigated in conscious male and female mice wild-type (WT) and AT2R knockout (AT2R-KO) mice via radiotelemetry. Sex differences in renal sensitivity to Ang II were also investigated by determining TGF response to acute administration of subpressor Ang II.

Methods

Animals

Ten- to 12-week-old FVB/N male and female AT2R-KOs were obtained from Monash University Mouseworks, initially established by Hein et al.19 Aged-matched male and female mice of the same genetic background were used as WT controls. The animals were fed a sodium-controlled diet (0.25% NaCl,AIN93M, Specialty Feeds, Western Australia, Australia) and water ad libitum and were housed in a temperature-controlled room (25°C) on a 12-hour light/dark cycle. Experiments were approved by the Monash University Standing Committee on Ethics in Animal Experimentation and in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Chronic Ang II Infusion

Mice were implanted with a radiotelemetry blood pressure device (TA11PA-C10; Data Sciences International, St Paul, MN), as described previously.20 Animals were allowed 10 days of recovery before commencing measurements. Arterial pressure was recorded as 10-second averages every 10 minutes using Dataquest ART data acquisition system (Data Sciences International). Basal mean arterial pressure (MAP) was measured for 3 consecutive days. Thereafter, the mice were anesthetized, and an osmotic minipump (ALZET model 1002, Durect Corp, Cupertino, CA) containing Ang II (600 ng/kg per minute) via an osmotic minipump for 10 days as described above before the animals were euthanized and the kidneys were collected, weighed, and snap frozen. RNA was extracted from the kidney using the RNeasy Midi kit (Qiagen, Doncaster, Victoria, Australia). AT1aR, AT1bR, and AT2R gene expressions were analyzed by real-time quantitative RT-PCR using as described previously.7 Samples were run in duplicate using 18S rRNA as the internal housekeeping gene, and calculations of relative expression were carried out using the comparative Ct method, as described previously.7

Tubuloglomerular Feedback

Mice were anesthetized and prepared for renal micropuncture, and TGF characteristics were determined by the stop-flow technique, as described previously.21 TGF responses were determined before and during acute subpressor Ang II infusion (100 ng/kg per minute IV).

Statistical Analysis

Data are presented as mean±SEM. For chronic Ang II responses, differences in MAP were analyzed using a 2-way ANOVA with repeated measures using factors sex (Psex; male or female) and genotype (Pgenotype; WT or AT2R-KO) and the interaction between sex and genotype (Psg). Gene expression and micropuncture data were analyzed using a 2-way ANOVA using factors treatment (Ptreatment; saline or Ang II) and Pgenotype or Ps indicate the interactions Psex or Psg. Bonferroni post hoc testing was performed for multiple comparisons where appropriate. Statistical significance was defined as P<0.05.

Results

Response to Chronic Ang II Infusion

There were no differences in basal 24-hour MAP or heart rate between the male WT (94±3 mm Hg; 499±11 bpm; n=6) and AT2R-KO (96±2 mm Hg; 526±15 bpm; n=7) and the female WT (95±1 mm Hg; 518±13 bpm; n=7) and AT2R-KO (95±1 mm Hg; 526±15 bpm; n=8) mice. Chronic Ang II infusion increased arterial blood pressure in all of the groups (Figure 1). The 10-day course of chronic Ang II treatment caused 24-hour MAP to increase by the same extent in the male WT, male AT2R-KO, and female AT2R-KO mice (28±6; 26±2 and 26±4 mm Hg, respectively). However, in the female WT mice, with intact AT2R, the pressor response to Ang II was significantly attenuated, and blood pressure increased by only 12±3 mm Hg (Psg=0.016). All of the groups exhibited a biphasic pressure response in response to the chronic Ang II infusion (Figure 1).

Renal AT1aR and AT1bR mRNA expressions were not different between the vehicle-treated male and female mice (Figure 2). AT1aR (P<0.0001) and AT1bR (P=0.001) expressions increased significantly after 10 days of Ang II in all of the groups (Figure 2). Renal AT2R expression levels were found to be 3-fold greater in WT females compared with males (P<0.001). Chronic Ang II infusion resulted in increased AT2R expression in both male and female WT groups (P<0.05; Figure 3).

TGF Response to Acute Subpressor Ang II Infusion

In a separate group of animals, TGF measurements were performed in male and female WT (n=5–6) and AT2R-KO
The main findings of this study demonstrate that the AT2R plays a greater role in modulating arterial pressure in females compared with males. Although basal arterial pressure was not different irrespective of genotype or sex, chronic Ang II infusion resulted in an immediate elevation of arterial pressure in the male WT and AT2R-KO mice. Female AT2R-KO mice responded to Ang II by the same extent as the males. Both the magnitude of the TGF response was increased (from 7.5 ± 0.7 to 10.2 ± 1.3 mm Hg) and TGF sensitivity was reset (from 19.4 ± 1.2 to 14.7 ± 1.0 nL/min) after subpressor Ang II in the female AT2R-KO mice (Figure 5).

**Discussion**

The main findings of this study demonstrate that the AT2R plays a greater role in modulating arterial pressure in females compared with males. Although basal arterial pressure was not different irrespective of genotype or sex, chronic Ang II infusion resulted in an immediate elevation of arterial pressure in the male WT and AT2R-KO mice. Female AT2R-KO mice responded to Ang II by the same extent as the males. However, the pressor response to Ang II was attenuated in the female WT mice compared with the other groups. Renal expression of AT2R was 3-fold greater in WT females compared with WT males under basal conditions and after chronic Ang II. To investigate renal sensitivity to Ang II, TGF characteristics were assessed in response to acute subpressor Ang II. Ang II increased TGF sensitivity in both male and female WT mice and in females by either ovariectomy or inhibition/deletion of the prohypertensive effects of Ang II in women.

In humans, several studies have shown that women have lower arterial pressure than men of the same age.22–24 In rodent models, differences in basal arterial pressure have not been uniformly reported,7,10,25 nor was a difference observed in the present study. However, in genetic models of hypertension, the spontaneous hypertensive rat and Dahl salt-sensitive rat strains, males have higher blood pressure than their female counterparts.26,27 In the Ang II–induced model of hypertension, we and others have also demonstrated that the pressor response to Ang II infusion is less in females than that in males.7,9,28 Xue et al10 demonstrated that testosterone and estrogen play important roles in the development of Ang II–induced hypertension, because the rise in arterial pressure was attenuated in males by castration and augmented in females by either ovariectomy or inhibition/deletion of the...
estrogen receptors. From several recent studies it has become clear that the components of the RAS are differentially regulated in males and females. The AT2R, part of the vasodepressor arm of the RAS, is upregulated by estrogen and has been shown to oppose the cardiovascular actions of the AT1R, causing vasodilation and decreased renal sodium reabsorption.29 Previously, we have shown that a chronic low-dose infusion of Ang II decreased blood pressure in female rats at a dose that had negligible effect on males.7 AT2R blockade, with PD123319, eliminated the depressor response to Ang II, confirming the vasodepressor role of the AT2R in females.7 In the current study, AT2R intact females exhibited an attenuated pressor response compared with the AT2R-KO female mice to chronic Ang II infusion, confirming earlier studies and suggesting that the AT2R plays a significant role in modulating arterial pressure in response to activation of the RAS in females.

There is growing evidence that the AT2R plays a pronounced role in arterial pressure regulation in premenopausal women. Polymorphism of the AT2R receptor has been associated with hypertension in premenopausal women but not in men.30 Furthermore, on a background of AT1R blockade, the renal response to Ang II infusion was attenuated more in women than in men, suggesting a role for the AT2R.31 Supporting these findings, we found that the female WT mice had a 3-fold higher basal AT2R expression compared with males (Figure 3). Importantly, in the current study, basal mRNA expression levels of the AT1aR and the AT1bR were not different between the males and females and increased by the same magnitude in response to Ang II and, thus, do not seem to contribute to the attenuated pressor response to Ang II in the WT females.

Previous studies on male mice lacking the AT2R have reported normal or elevated basal MAP.19,32–34 In the present study, despite differences in AT2R expression, we found no difference of basal MAP between the AT2R-intact and KO animals. The discrepancies between our study and others may be attributed to the technique used to measure blood pressure and the different mouse strains. Studies involving AT2R-KO males with the same FVB/N genetic background, as the mice used in the present study, exhibited no difference in basal blood pressure.19 Moreover, these studies were performed only in male AT2R-KO mice. In the present study we also used young female AT2R-KO mice with age-matched males

### Table. MAP, Pff, and PSF During Control and Subpressor Ang II Infusion in Anesthetized Mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>Genotype</th>
<th>MAP, mm Hg</th>
<th>Pff, mm Hg</th>
<th>n</th>
<th>PSF, mm Hg</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>WT</td>
<td>94.6±2.4</td>
<td>11.3±0.3</td>
<td>14</td>
<td>36.5±0.3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>95.5±1.6</td>
<td>12.7±0.6</td>
<td>13</td>
<td>38.6±0.9*</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>AT2R-KO</td>
<td>94.3±1.0</td>
<td>11.0±0.3</td>
<td>19</td>
<td>37.0±0.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>94.7±1.5</td>
<td>11.9±0.4</td>
<td>16</td>
<td>38.8±0.8*</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>WT</td>
<td>94.3±2.1</td>
<td>10.0±0.3</td>
<td>15</td>
<td>36.4±0.4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>91.9±1.7</td>
<td>10.2±0.3</td>
<td>20</td>
<td>36.6±0.4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>AT2R-KO</td>
<td>93.6±2.4</td>
<td>10.5±0.3</td>
<td>19</td>
<td>36.2±0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>92.2±2.0</td>
<td>11.4±0.4</td>
<td>19</td>
<td>38.4±0.9*</td>
<td>8</td>
</tr>
</tbody>
</table>

WT indicates wild-type; MAP, mean arterial pressure; Pff, proximal tubular free-flow pressure; PSF, proximal tubular stop-flow pressure; Ang II, angiotensin II; AT2R-KO, Ang II type 2 receptor knockout.

*P<0.05 vs control.

Figure 4. Tubuloglomerular feedback response shown as stop-flow pressure (PSF) at different rates of tubular perfusion. Curves are results of fitting normalized data from male (left) and female (right) mice under control conditions (solid lines) and after infusion of subpressor angiotensin II (Ang II; dashed lines).
Compensatory mechanisms, such as resetting of baroreceptors, increased production of atrial natriuretic peptide, and release of vasodilatory prostaglandins, take place in response to elevated blood pressure and may account for the decrease in blood pressure at this time. Welch et al\textsuperscript{15} showed that mice receiving a high pressor infusion of Ang II (1000 ng/kg per minute) also exhibit a further increase in arterial pressure that took place 1–1 week after infusion was started, corresponding with the second rise in arterial pressure seen in the present study. Furthermore, in the slow-pressor model of hypertension, arterial pressure also starts to rise after 7 to 8 days of Ang II infusion.\textsuperscript{15} The development of slow-pressor hypertension has been attributed to increased reactive oxygen species, structural vascular changes induced by Ang II, and increased activity of neurogenic pathways.\textsuperscript{10,35,37,38} Furthermore, a similar biphasic MAP response has been observed in response to chronic norepinephrine.\textsuperscript{19} Thus, the exact mechanisms responsible for the biphasic pressor response are complex and remain to be substantiated.

The RAS plays an integral role in regulating renal function. Ang II exerts several intrarenal effects, which include its direct constrictive effects on the renal vasculature, stimulation of tubular epithelial transport, and augmentation of TGF activity.\textsuperscript{40} Several studies have demonstrated that interfering with the formation of Ang II or its actions, such as angiotensin-converting enzyme inhibitors or AT\textsubscript{1}R blockers, greatly reduces or abolishes TGF response.\textsuperscript{41–46} Ang II potentiates TGF response through 2 pathways by activating AT\textsubscript{1}R; on the afferent arterioles, causing increased vascular reactivity, and on the luminal membrane of the MD, increasing the sensitivity to distal tubular load.\textsuperscript{41,47,48} Ang II has also been shown to restore TGF response during volume expansion, indicating that Ang II is an important modulator of TGF sensitivity.\textsuperscript{13} Notably, in genetic and experimental models of hypertension, enhanced TGF sensitivity has been associated with the developmental phase of hypertension.\textsuperscript{15,16,49}

The AT\textsubscript{2}R has been reported to be expressed throughout the kidney.\textsuperscript{17,50,51} Importantly, the AT\textsubscript{2}R has been localized in the afferent arteriole\textsuperscript{17} and in the distal tubule,\textsuperscript{50} key locations for TGF regulation. Despite this, there have been few studies investigating the involvement of the AT\textsubscript{2}R in modulating TGF response. Wang et al\textsuperscript{44} found that, in male rabbits, AT\textsubscript{2}R inhibition did not influence Ang II–induced changes in TGF response. Indeed, these findings are supported in the male mice in the present study, where, under control conditions, there were no observed differences in TGF response between the male WT and AT\textsubscript{2}R-KO. Subpressor Ang II infusion caused a sensitization of the TGF, seen as a leftward shift in the TGF response curve, to the same extent in both male genotypes, indicating an increase in MD sensitivity to distal tubular load. Although AT\textsubscript{2}R expression has been found throughout the renal vasculature, there has been no direct evidence for AT\textsubscript{2}R expression in the efferent arterioles. Our findings also suggest a greater postglomerular than preglomerular expression of AT\textsubscript{2}R in females, because Ang II increased glomerular hydrostatic pressure in all of the groups except for the AT\textsubscript{2}R-intact females. Miller et al\textsuperscript{52} found that, in response to acute Ang II infusion, glomerular filtration rate was maintained in men and markedly decreased

and, as with the males, found no difference in basal MAP. Basal MAP was not different between the WT and the AT2R-KO animals of both sexes, indicating that the AT2R does not play a significant role in determining blood pressure under basal conditions, with the mice on a controlled sodium intake.

In the present study, we examined changes in arterial pressure during chronic infusion of a moderately pressor dose of Ang II. The response to chronic Ang II infusion followed a distinctive biphasic pattern, suggesting that there are multiple pathways involved in response to prolonged Ang II infusion. As illustrated in Figure 1, Ang II caused a rapid increase in 24-hour MAP during the first 3 to 4 days, which then decreased toward the preinfusion levels before rising again. Mice receiving a chronic infusion of a high dose of Ang II (800–1000 ng/kg per minute) display a sustained elevated arterial pressure, whereas mice receiving a low dose of Ang II (400 ng/kg per minute) develop slow-pressor hypertension, where arterial pressure starts to increase after several days.\textsuperscript{10,35,36} The moderate dose of Ang II (600 ng/kg per minute) given in the present study was at a level to cause an immediate rise in arterial pressure, whereas not at a high enough level to override endogenous compensatory mechanisms. The initial increase in arterial pressure may be attributed to the direct vasoconstrictive effects of Ang II, causing a shift in the pressure-natriuresis curve to higher pressures.

![Graph showing the change in arterial pressure and turning point during Ang II infusion](image-url)
in women. Moreover, men exhibited an increase in filtration fraction that was blunted in women, despite renal blood flow decreasing to the same extent in both sexes. Our data also support earlier findings that Ang II–dependent resetting of TGF activity is not dependent on the presence of the AT2R in males.14

Ang II has been shown to stimulate renal production of NO through the AT2R,53 which is known to attenuate TGF response.21,54 Thus, an increased AT2R-dependent NO production could blunt TGF resetting in response to Ang II in the female WT mice. The resetting of TGF activity by Ang II contributes to modulating the pressure-natriuresis relationship,12 causing increased sodium retention and extracellular fluid volume and subsequent elevation of arterial pressure. The blunted resetting of TGF response in females would attenuate the sodium- and fluid-retaining effects of Ang II, thus preventing a rise in arterial pressure.

There were no differences in TGF response under basal conditions between the male and female mice, irrespective of genotype, indicating that the AT2R does not have a major influence on basal renal function. However, subpressor Ang II infusion augmented the TGF responsiveness in female AT2R-KO mice to the same extent as seen in the males but failed to shift the TGF response in the AT2R-intact female mice. The blunted increase in TGF response to Ang II in the female WT mice suggests that both enhanced renal tubular and vascular AT2R pathways are involved in opposing the Ang II–induced enhancement of the TGF response. Because the distal load is sensed by the MD cells, an attenuated sensitization of the TGF suggests the presence of AT2R at the MD site. In addition, AT2R-derived NO from the MD and/or arterioles may be responsible for the attenuated maximum response (ΔPsf max) in the female AT2R-intact mice. The attenuated Ang II–dependent sensitization of the TGF response supports the findings from the present study and others of a greater renal AT2R expression in females.7,8

In conclusion, these data indicate that the actions of the AT2R are augmented when the RAS is activated. The enhanced AT2R-mediated pathway in females counterbalances the hypertensive effects of Ang II and attenuates the Ang II–dependent resetting of TGF activity. Thus, the enhancement of the AT2R may, in part, underlie the protection that premenopausal women demonstrate against cardiovascular disease.

Perspectives

There is increasing evidence that there are marked sex differences in the contribution the RAS makes to the regulation of arterial pressure. The enhancement of the AT2R, in conjunction with other components of the vasodilator RAS axis, may underlie the protection that premenopausal women demonstrate against cardiovascular disease. AT2R-mediated modulation of the ability of Ang II to reset TGF activity in females is one powerful renal mechanism that may contribute to this effect. A greater understanding of the components of the vasodilatory arm of the RAS is required to aid in the design of therapeutic targets for treatment of cardiovascular disease in both men and women.

Acknowledgments

AT2R-deficient mice used in the present study were kindly provided by Lutz Hein, University of Freiburg (Freiburg, Germany).

Sources of Funding

This study was supported by the National Health and Medical Research Council of Australia (project grant 490919 and fellowship to K.M.D. 490918).

Disclosures

None.

References


Sex Differences in the Pressor and Tubuloglomerular Feedback Response to Angiotensin II
Russell D. Brown, Lucinda M. Hilliard, Geoffrey A. Head, Emma S. Jones, Robert E. Widdop and Kate M. Denton

_Hypertension_. 2012;59:129-135; originally published online November 28, 2011; doi: 10.1161/HYPERTENSIONAHA.111.178715
_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/59/1/129

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2011/11/29/HYPERTENSIONAHA.111.178715.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org/subscriptions/
ONLINE SUPPLEMENT

SEX DIFFERENCES IN THE PRESSOR AND TUBULOGLOMERULAR FEEDBACK RESPONSE TO ANGII

Russell D Brown, Lucinda M Hilliard, Geoffrey A Head, Emma S Jones, Robert E Widdop, Kate M Denton

Departments of Physiology and Pharmacology, Monash University, Melbourne, Australia

Baker IDI Heart and Diabetes Institute, Melbourne, Australia.

Telephone: +61-3-9905 2503
Email: russell.brown@monash.edu

Short title: AT2R and sex dependent AngII-response
**Methods**

**Tubuloglomerular feedback measurements**

Mice were anesthetized by spontaneous inhalation of isoflurane and placed on a servo-regulated heating pad to maintain body temperature at 37.5°C. Catheters were inserted into the carotid artery and the jugular vein for blood pressure measurements and infusion of maintenance fluid (0.9% NaCl, 0.35 ml/h), respectively. The left kidney was exposed through a sub-costal flank incision, dissected free from surrounding tissue, placed in a Lucite cup and fixed in a 3% agar solution. Tubuloglomerular feedback characteristics were determined by the stop-flow technique as described previously. Proximal tubular free-flow pressure (P\text{FF}) was measured in randomly chosen superficial segments with a pipette filled with 1 mol/L NaCL solution and connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT, USA). In nephrons in which three or more tubular segments were identified, tubular flow was interrupted with a wax block. A pipette containing artificial tubular ultrafiltrate and connected to a microperfusion pump (Hampel, Frankfurt, Germany) was inserted into the last accessible tubular segment. Stop-flow pressure (P\text{SF}) was determined upstream to the block at various perfusion rates between 0 and 35 nl/min by changing the perfusion rate by 2.5 to 5 nl increments. The maximal change in stop-flow pressure (\Delta P\text{SF}_{\text{max}}) was determined as the difference in P\text{SF} when the perfusion rate was increased from 0 to 35nl/min and was used as a measure of TGF reactivity. The tubular perfusion rate eliciting half-maximal \Delta P\text{SF}, the turning point (TP), was determined and served to indicate TGF sensitivity. TGF measurements were first performed under basal conditions, and 15 minutes after the initiation of a subpressor infusion of AngII (100ng/kg/min). The response curves in Figure 2 were plotted using a previously described normalization method. Normalized data were fitted to the following equation by means of a nonlinear least-squares curve-fitting program (Minuit, Cern):

\[
P_{\text{SF}} = P_{\text{SF}_{\text{min}}} + \frac{\Delta P_{\text{SF}}}{1 + \exp \left( w \left( P_{\text{R}} - \text{TP} \right) \right)}
\]

where P_{\text{SF}} is the stop-flow pressure, \Delta P_{\text{SF}} is the average maximal stop-flow response, and P_{\text{SF}_{\text{min}}} is the average minimum stop-flow pressure when the distal perfusion is increased. TP is the turning point, PR is the end-proximal perfusion rate and w is the factor determining the width of the perfusion interval during which the P_{\text{SF}} responded.

**References**
