Effect of Gender in Centrally Induced Angiotensin II Hypertension in Dogs

Marie-Françoise Doursout, Jacques E. Chelly, Patrick Wouters, Christine Lawrence, Yang-Yan Liang, and Joseph P. Buckley

This study was designed to investigate the relation between gender, an endogenous inhibitor of the Na⁺-K⁺ pump, and volume-dependent hypertension induced by stimulation of the brain renin-angiotensin system and increased salt intake. Angiotensin II (20 ng/min i.c.v.) was infused for 4 weeks in five dogs of each sex with saline as the drinking fluid. In male dogs, angiotensin II induced parallel pressor (30%) and dipsogenic responses (70%), whereas no hypertension and no increase in fluid intake were observed in females. In contrast, the activity of the Na⁺-K⁺ pump as assessed by ⁸⁶Rb uptake was independent of gender. Our data provide novel evidence that gender plays a determining role in the physiological properties of centrally administered angiotensin II. (Hypertension 1990;15(suppl I):I-117-I-120)

Evidence suggests that steroids modulate the brain renin-angiotensin system. Severs et al. demonstrated that the pressor responses induced by angiotensin (Ang) II acutely administered into a lateral ventricle were diminished in cats in a late stage of pregnancy or lactation. Chen et al. reported a correlation between pituitary Ang II binding sites and the estrous cycle and established that estradiol treatment resulted in a diminution of the number of Ang II binding sites in the anterior pituitary. Jonklaass and Bugly observed that estradiol administered intraventricularly decreased dipsogenic and pressor responses to acute doses of centrally injected Ang II. Testosterone has been demonstrated to stimulate the release of brain renin.

Presently, the role of gender in the hypertension induced by long-term stimulation of the brain renin-angiotensin system remains unknown.

We and Balda et al. have presented evidence that an endogenous inhibitor of the Na⁺-K⁺ pump might be implicated in the development of hypertension due to long-term stimulation of the brain renin-angiotensin system and an increase in salt intake.

This study investigated the role of gender in the development of hypertension resulting from long-term intracerebroventricular administration of Ang II to awake instrumented dogs receiving 0.9% sodium chloride solution as the drinking fluid. The relation between hypertension and the presence of an endogenous inhibitor of the Na⁺-K⁺ pump was also determined.

Methods

Ten dogs, five of each sex (seven mongrels and three beagles; males 12.8–20.2 kg, mean 14.85 kg; females 16.4–21.5 kg, mean 18.8 kg), were anesthetized with thiopental (30 mg/kg i.v.), intubated, and prepared for sterile surgery. Under aseptic conditions, a heparin-filled Tygon catheter (0.125 in. o.d., 0.0625 in. i.d.) was implanted into the abdominal aorta via the iliac artery. A left thoracotomy was performed through the fourth intercostal space and an electromagnetic flow probe (14–16 mm, Micron Inc., Los Angeles, California) was placed around the pulmonary artery. The catheter and transducer leads were tunneled subcutaneously to the dorsum of the neck and secured in place after closure of the thoracotomy. The dogs received ampicillin (20 mg/kg) and streptomycin (1 g) intramuscularly for 4–5 days after surgery. The aortic catheter was flushed daily with heparin solution (1,000 units/ml). None of the females were in estrus during the study.

Experimental Protocol

Animals were permitted to recover from surgery for approximately 10 days. After the recovery period, each dog received tap water as the drinking fluid for 5 days (control tap water), followed by
isotonic saline solution as the drinking fluid for another 5 days (control saline). After control measurements, a second surgery was performed under the same conditions. A blunt, sterile 18-gauge needle was inserted into the left lateral ventricle and secured with dental cement. An osmotic pump (2 ml, Alza Corporation, Palo Alto, California) was connected to the needle and implanted subcutaneously to infuse Ang II (20 ng/min) for 4 weeks. During the infusion period, dogs received isotonic saline as the drinking fluid for 5 days, there was an increase in the daily fluid intake of male dogs, from 0.6±0.1 to 1.3±0.1 1/24 hr. MAP, HR, and CO of both male and female dogs were unaltered.

### Hemodynamic variables, which included mean arterial pressure (MAP), heart rate (HR), and cardiac output (CO), were recorded before and at the end of each control period and at the end of each week during Ang II infusion. Additionally, fluid intake was recorded daily and expressed as mean of daily intake for each study period.

#### Evaluation of Na⁺-K⁺ Pump Activity

The method used was that of Pamnani et al. After the 4-week infusion period, approximately 200 ml arterial blood was collected into heparinized tubes and placed on ice. After centrifugation at 1,100 g at 4°C for 10 minutes, the plasma was separated and allowed to equilibrate at room temperature for 30 minutes, after which it was boiled for 5 minutes at 100°C. After centrifugation, the plasma supernate was collected and immediately frozen for future determination of ⁸⁶Rb uptake by rat tail arteries.

Tail arteries were excised from pentobarbital-anesthetized normotensive rats, opened longitudinally, cut into two segments, and placed in Krebs-Henseleit solution. Each segment was incubated with male or female plasma supernate. Each arterial segment was incubated in oxygenated K⁺-free Krebs-Henseleit solution, first at 0°C for 10 minutes and then at 37°C for 20 minutes in the presence of unlabeled rubidium chloride (RbCl) (2 mmol) plus trace amounts of ⁸⁶RbCl (approximately 100 nmol). Before the second incubation, each segment was divided into halves, one half was incubated in the presence and the other in the absence of ouabain (1.0 mmol). Next, the arteries were placed in a crystal scintillation counter (Pharmacia LKB, Gaithersburg, Maryland) to determine ⁸⁶Rb uptake. After counting, each segment was dried at 100°C for 24 hours and weighed. Each determination was made in duplicate. ⁸⁶Rb uptake was expressed as picomoles per 18 minutes per milligram dry weight.

### Statistical Analysis

In both sexes, the effects of Ang II on blood pressure and fluid intake were analyzed with a one-way analysis of variance. When significant, multiple paired comparisons were applied. The effects of gender on ⁸⁶Rb uptake were analyzed with an unpaired t test. Alpha was set at 0.05. Data are presented as mean±SEM.9

#### Results

When normal saline solution replaced water as the drinking fluid for 5 days, there was an increase in the daily fluid intake of male dogs, from 0.6±0.1 to 1.0±0.1 1/24 hr (p<0.05), whereas there was only a slight increase in fluid intake of female dogs, from 1.0±0.1 to 1.3±0.1 1/24 hr. MAP, HR, and CO of both male and female dogs were unaltered.

The hemodynamic effects of intracerebroventricular Ang II and effects on fluid intake of male and female dogs receiving saline as the drinking fluid are presented in Table 1. Ang II infused intracerebroventricularly to male dogs produced significant pressor effects at weeks 3 and 4. In male dogs, a significant and more uniform increase in fluid intake was recorded as early as week 1 and, then, throughout the study. In contrast, Ang II infused intracerebroventricularly for 4 weeks to female dogs did not produce significant hemodynamic or dipsogenic effects. There were no significant changes in HR or CO in either sex.

There was no significant difference between the ouabain-sensitive ⁸⁶Rb uptake by tail arteries from normotensive rats incubated with plasma supernate from male or female dogs receiving Ang II

### Table 1. Effects of Intracerebroventricular Angiotensin II on Hemodynamic Variables and Fluid Intake of Awake Male and Female Dogs Drinking Isotonic Saline Solution

<table>
<thead>
<tr>
<th>Time</th>
<th>Male (n=5)</th>
<th>Female (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>Control (tap water)</td>
<td>88±1</td>
<td>78±4</td>
</tr>
<tr>
<td>Control (saline as the drinking fluid for 5 days)</td>
<td>91±2</td>
<td>75±3</td>
</tr>
<tr>
<td>Ang II i.c.v. 1 week</td>
<td>110±7</td>
<td>66±5</td>
</tr>
<tr>
<td>2 weeks</td>
<td>115±14</td>
<td>68±7</td>
</tr>
<tr>
<td>3 weeks</td>
<td>113±6*</td>
<td>75±4</td>
</tr>
<tr>
<td>4 weeks</td>
<td>118±6*</td>
<td>77±3</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; i.c.v. intracerebroventricular.

*p<0.05 vs. control saline; †p<0.05 vs. female fluid intake at the corresponding week.
intracerebroventricularly and saline as the drinking fluid. The ouabain-sensitive $^{86}$Rb uptake by rat tail arteries incubated with plasma supernate from male dogs was 695.9±244 and from female dogs was 838.8±131 pmol/18 min/mg dry wt. In a separate study, the ouabain-sensitive $^{86}$Rb uptake by rat tail arteries incubated with plasma supernate from mongrel dogs receiving saline, 10 µl/hr, intracerebroventricularly for 4 days and isotonic saline solution as the drinking fluid was 1,129±55 pmol/18 min/mg dry wt (unpublished data). Ouabain-insensitive uptake from rat tail arteries incubated with plasma supernate from male dogs was 104.8±9 and from female dogs was 118.5±11 pmol/18 min/mg dry wt.

**Discussion**

Our data provide novel evidence that gender plays a determining role in the development of hypertension induced by long-term stimulation of the brain renin-angiotensin system and an increase in salt intake. It is particularly interesting that published studies concerning the role of the brain renin-angiotensin system in hypertension used dogs of both sexes as well as male rats. The consequences of our findings on previously accumulated data remain to be established.

Based on our data, it is possible to propose that estrogen prevented hypertension in females or that testosterone was a determining factor in the increase in blood pressure recorded in males. Additional studies are required to determine which steroids interfere with the centrally increased hypertension mediated by stimulation of the brain renin-angiotensin system and an increase in salt intake.

In both male and female dogs, the changes in blood pressure paralleled changes in fluid intake. Consequently, the hypertension might have been secondary to a saline-dependent volume expansion. Acute and long-term volume expansion led to a sustained increase in blood pressure. In this respect, our findings might suggest the concept that the primary physiological role of the brain renin-angiotensin system is volume homeostasis rather than control of blood pressure. Additional evidence includes 1) the threshold of the centrally mediated dipsogenic response to Ang II is lower than the pressor response, and 2) the brain renin-angiotensin system has been reported to control the release of atrial natriuretic factor, a hormone clearly implicated in volume regulation.

The effects of plasma supernate of dogs on the Na⁺-K⁺ pump activity of the rat tail artery was independent of gender. Stimulation of the brain renin-angiotensin system has been reported to stimulate the release of an endogenous inhibitor of the Na⁺-K⁺ pump. Such a factor has been implicated in the development of volume-dependent hypertension including one-kidney, one clip, one wrapped, reduced renal mass, and deoxycorticosterone acetate-salt models. Because male and female plasma supernate similarly affected $^{86}$Rb uptake in rat tail arteries, we do not have solid evidence that would support the role of an endogenous inhibitor in the development of hypertension unless estrogen in females counterbalanced the Ang II-mediated dipsogenic responses and the vasoconstriction mediated by inhibition of the vascular Na⁺-K⁺ pump. In spontaneously hypertensive rats, estrogen treatment attenuated the development of hypertension through an endothelium-dependent vasodilation. Additionally, it is established that essential hypertension is more frequent in males than in females before menopause and that this difference disappears after menopause.

Appropriate consideration should be given to the role of gender in the physiological properties of the brain renin-angiotensin system including its role in hypertension, homeostasis, and the release of an endogenous inhibitor of the Na⁺-K⁺ pump.

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**References**


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