Influence of brain angiotensin on thermoregulation and hydromineral balance during pregnancy in rats

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DURING PREGNANCY, reversible changes occur in maternal homeostasis that alter body weight, temperature, and hydromineral balance (25). In several mammalian species that have been investigated, there is a 0.5–1°C reduction in maternal temperature toward the end of pregnancy (13, 19, 26). In late-pregnant rats studied in a thermoneutral environment (temperature gradient between 12 and 36°C), environmental temperature preference was unchanged between pregnant and nonpregnant animals (12), indicating that the reduction in maternal temperature is under thermoregulatory control rather than an inability to maintain a higher temperature. Similarly, the threshold temperature for cooling mechanisms such as skin vasodilation and salivary evaporative heat loss are reduced compared with nonpregnant rats (32), indicating that pregnant rats defend a lower body temperature. Fetal temperature in sheep has been shown to be higher than maternal temperature, which may indicate that the mother functions as a heat sink for its offspring to maintain optimal physiological temperature in utero (19). This reduction has been proposed to be both a neuroprotective mechanism against induction of hyperthermic stress in developing offspring (4) and a means of conserving oxygen availability by reducing thermogenic metabolic processes (7). The mechanism that mediates this reduction in maternal core temperature during late gestation remains unknown.

Similar to the decrease in core temperature that occurs during pregnancy, there is a regulated decrease in blood osmolality and an increase in water intake during gestation (2, 11). Relaxin, a hormone that is secreted by the ovary during the second half of pregnancy in the rat, has been shown to stimulate both water drinking and vasopressin release in this species (28, 29). Relaxin has been implicated in the increased water intake of pregnancy and the alteration of the osmoregulatory set point (31). Another dipsogenic hormone that could contribute to water drinking during pregnancy is circulating angiotensin II (ANG II), which is increased in pregnant rats and potentiates relaxin-induced drinking (28).

Distinct from its actions as a circulating hormone, ANG II may also be acting as a signaling molecule within the brain to alter body fluid homeostasis and also thermoregulation as a signaling molecule within the brain (23). There is considerable evidence that all components of the renin-angiotensin system are synthesized in the brain (15). Furthermore, extensive central angiotensinergic pathways have been mapped to regions controlling blood pressure and body fluid balance (20). Many investigators have shown that intracerebroventricular administration of angiotensin receptor antagonists inhibit water drinking in response to various dipsogenic stimuli (5, 30), including relaxin-induced drinking (29). As well, our laboratory has observed in rats that central administration of the angiotensin AT1 antagonist losartan augments the rise in core temperature during exposure to a hot environment (22). Thus it is possible that central angiotensinergic mechanisms may influence thermoregulation as well as body fluid homeostasis.

In the light of evidence that both thermoregulation and body fluid homeostasis are altered during pregnancy, we investigated a possible role for brain angiotensinergic mechanisms in the control of body temperature and fluid balance in pregnant rats. The aim of the present experiment was to test the effect of intracerebroventricular infusion of an angiotensin AT1 antagonist (candesartan) on core temperature, water intake, and plasma osmolality of pregnant rats.

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METHODS

Experimental protocols received prior approval from the Animal Ethics Committee of the Howard Florey Institute, which adheres to the guidelines of the National Health and Medical Research Council of Australia for the care and use of animals for scientific purposes.

Animals. Female Sprague-Dawley rats (200 g) were purchased from the Animal Resource Centre (Willetton, Western Australia). Animals were housed in a room with a constant temperature of 22 °C and a 12:12-h light-dark cycle (0700–1900). Food (standard rat fodder, GR® LW Alexander's Stockfeeds, Preston, Australia) and tap water were available ad libitum.

Surgical preparation. Rats were anesthetized with Equithesin at a dose of 3 ml/kg ip (formula described below) and placed in a small-animal stereotoxic instrument (model 900, David Kopf Instruments, Tujunga, CA). A midline sagittal skin incision was made on the dorsal surface of the head, and the periosteum was cleared. A small hole was drilled into the skull over the right ventricle at a point 1.5 mm lateral to the midline and 0.2 mm caudal to the bregma. A stainless steel cannula (23 gauge) was then inserted perpendicularly into the hole, 4.0 mm below the dura mater, and fixed in position by using skull screws and dental acrylic.

At day 10 of lactation (i.e., after all experimental data had been collected), 5 μl of Evans blue dye (5%) were injected into the ventricular cannula. Rats were anesthetized with pentobarbital sodium (60 mg/kg) and fixed by transcardiac perfusion of normal saline followed by 4% paraformaldehyde (in 0.1 M phosphate buffer). The brain was removed and immersed in 20% sucrose for 24 h. Coronal sections (40 μm) were cut through the lateral ventricles on a freezing microtome (model CM 1900, Leica, Wetzlar, Germany). Only data from rats showing staining of the lateral ventricle walls were included in this study.

Mini-osmotic pump. Solutions were administered into the brain by using a mini-osmotic pump (model 2004, Alzet Osmotic Pumps, Durect, Cupertino, CA), which pumps at a rate of 0.25 μl/h for 28 days. The pumps were filled by using a blunt-ended 23-gauge needle and 1-ml syringe with a 0.45-μm sterile filter (Millipore, Bedford, MA) with either the artificial cerebrospinal fluid (aCSF) or candesartan dissolved in aCSF. Polyvinyl tubing with an inside diameter of 0.76 mm was filled and secured around the flow modulator. Pumps were primed according to the manufacturer’s instructions.

The mini-osmotic pump was inserted subcutaneously between the scapulae, and the polyvinyl tubing was connected to the ventricular cannula. The skin incision was sutured, and then animals were placed in heated chambers at 25°C for 24 h of recovery, before being returned to metabolism cages. Water supplemented with 5% sucrose and aspirin (100 mg·kg⁻¹·day⁻¹) was supplied for a further 24 h to aid in recovery.

Drugs. The octapeptide ANG II was supplied in dry powder form at 80% purity (AusPep, Parkville, Australia).

Candesartan (2-ethyl-1'-[(1H-tetrazol-5-yl)[biphenyl]-4-yl]-methyl-1H-benzoimidazole-7-carboxylic acid; gift from AstraZeneca, Södertälje, Sweden), was dissolved in aCSF buffered at pH 7.8. Candesartan was infused into a lateral ventricle at 5 μg·kg⁻¹·day⁻¹ because this dose has been shown to block the pressor response to central but not peripheral administration of ANG II (6). Additionally, we performed a pilot study and showed that 5 μg·kg⁻¹·day⁻¹ candesartan blocked the dipsogenic response to an injection of ANG II (50 ng) into a lateral ventricle.

Equithesin was prepared according to the following protocol: 8.5 mg of chloral hydrate in 20 ml of 95% ethanol were added to 1.96 g of pentobarbital sodium (Nembutal, Rhone Merieux, Pinkenba, Australia) and 4.25 g of magnesium sulfate. Propylene glycol (60 ml) was added followed by water to a final volume of 200 ml.

Experimental design. Three groups of female Sprague-Dawley rats were monitored throughout pregnancy until the first week of lactation. One group received an intracerebroventricular infusion of the non-peptide AT₁ receptor antagonist, candesartan, while a control group received aCSF. The third group of pregnant rats remained untreated throughout the experimental period. Measurement of body fluid, weight, and temperature were made as described below.

Balance studies. Age- and weight-matched animals were housed individually in wire-mesh metabolism cages, which allowed the separate collection and/or measurement of urine, water, and food intake. Animals were acclimatized to the metabolism cages for a period of 3 days before experimentation. Baseline data were collected for a period of 4 days, and then the animals were mated and the onset of pregnancy determined by the presence of sperm in a vaginal smear. This was designated day 1 of pregnancy. Surgery for implantation of a cannula into a lateral cerebral ventricle and a mini-osmotic pump was performed on day 2. After surgery, balance data were collected from day 3 until the birth of the pups. At day 7 of lactation, the number and weight of the pups were recorded.

Measurements of body weight, food and water intake, and urinary output were made between 0900 and 1000, and metabolic cages were cleaned daily. Urine samples were collected overnight under 1 ml of mineral oil to prevent evaporation and stored at 4°C until analysis. Urine volume was measured daily, and a sample was retained for the measurement of Na⁺ and K⁺ by using a flame photometer (flame photometer IV, Technicon, Tarrytown, NY). A timeline for the balance studies is shown in Fig. 1.

Colonic temperature. Temperature was recorded during baseline; on days 4, 7, 10, 14, 17, and 20 of pregnancy; and day 5 of lactation. Colonic temperature was measured by using a flexible K-type thermocouple (0.2-mm Teflon-encased wire, RS Components, Melbourne, Australia) covered in Silicon tubing (2-mm outer diameter, Dow Corning, MI), connected to a dual-channel electronic thermometer (Fluke 52, John Fluke, Everett, WA). The tip of the thermocouple was coated with 5% lidocaine gel (Xylocaine, AstraZeneca, Sydney, Australia) as a local anesthetic and lubricant. The thermocouple was...
inserted 6 cm into the anal sphincter to achieve accurate measurements of colonic temperature. Once in position, the thermocouple was secured to the base of the tail with masking tape, and the animal was not restrained during temperature measurement. Our laboratory has previously shown that this procedure accurately measures colonic temperature in conscious rats, with little evidence of stress to the animal (22). Measurements were taken between 10 AM and 2 PM.

Core body temperature is often determined by inserting a thermocouple or thermistor probe into the rectum or colon. The average temperature for an unrestrained rat at an ambient temperature of 20–24°C ranges from 37 to 38°C, and it has been reported that handling animals results in an increase in body temperature (14). To minimize the effect of stress, animals were pretrained in the procedures for both the colonic measurement and the tail cuff technique for at least three trials before any measurements were recorded.

Blood samples. Blood samples were collected during baseline; days 5, 11, and 18 of pregnancy; and day 7 of lactation. The animals were heated under an infrared lamp for 5 min and then placed in a restrainer with the tail exposed. An area 5 cm caudal to the tail stump was sterilized by using a cotton ball soaked in 70% alcohol. Approximately 400 μl of blood were collected from the tail vein of each animal via a 25-gauge needle inserted into the tail vein. The samples were transferred to 1.5-ml conical tubes (Eppendorf, Hamburg, Germany) containing 50 units of heparin (Commonwealth Serum Laboratories, Parkville, Australia) to prevent clotting. A portion of the blood was removed into microhematocrit tubes (Becton Dickinson, Franklin Lakes, NJ) for the analysis of hematocrit. The hematocrit tubes underwent centrifugation at 3,000 rpm for 5 min in a Biofuge A Centrifuge (Heraeus-Sepatech, Hanau, Germany), and the hematocrit measured by using a graded template.

Remaining blood samples were then centrifuged at 3,000 g for 10 min (Sorvall RT7, Heraeus Sepatech, Hanau, Germany) and the supernatant plasma assayed for Na⁺, K⁺, osmolality, and total protein. Na⁺ and K⁺ assays were made with ion-selective electrodes by using a clinical analyzer (Synchron CX-5, Beckman Coulter, Foster, CA). Osmolality was measured by freezing-point depression by using an osmometer (model one-ten, Fiske Associates, Norwood, MA).

Statistical analysis of data. Sample sizes were calculated with an α of 0.05 and a power (1−β) of 80%. After collection, the data were tested for normality by using the Darling-Joiner test. If the data proved to be normal, a two-way ANOVA with repeated measures was used. Significant treatment effect was examined post hoc by using the Tukey multiple comparison procedure. All statistics were performed by using Sigmastat (version 4.0, SPSS Science, Chicago, IL).

RESULTS

Core temperature. Colonic temperature decreased in both the untreated (−0.8°C; P < 0.01) and the aCSF (−0.5°C; P < 0.01) groups (Fig. 2) over the course of gestation, reaching a nadir on day 20. After the birth of the pups, there was an increase in temperature at day 7 of lactation, which was significantly higher than during baseline measurements in these groups. By contrast, in the candesartan-treated group, colonic temperature increased relative to baseline after the first week of pregnancy, and it was higher than the aCSF and untreated groups on days 7 (P < 0.01), 17 (P < 0.05), and 20 (P < 0.001) of gestation. After the birth of the pups, temperature in the candesartan-treated animals remained elevated relative to baseline, but it was now at the same level as the control and aCSF-treated groups.

Food and water intake. Daily values of food intake and water intake are shown in Fig. 3, A and B, respectively. During the baseline acclimatization period, animals consumed an average of 20 g food/day. Intake increased gradually throughout gestation, reaching ~28 g/day by day 21 of gestation. Intake increased over threefold compared with baseline values in all groups by day 7 of lactation. There was no significant difference in food intake between the three experimental groups.

Water intake also showed a gradual increase throughout gestation in all groups, as did cumulative excretion between the groups. At baseline, animals drank 24–31 ml/day, and the amount increased to 45–50 ml/day by day 20 of gestation. Water intake escalated after parturition, reaching values of ~80 ml/day by day 7 of lactation.

Body weight. Body weights during gestation and lactation are shown in Fig. 3C. All groups gained a similar amount of weight by the end of gestation. Weight loss at parturition was also equivalent across groups (P = 0.90). Pup weights and litter numbers were not significantly different between groups (Table 1).

Plasma composition. Plasma osmolality and Na⁺ dropped significantly in all groups, reaching its lowest level on day 18 of pregnancy and then rising during lactation (Fig. 4, A and B). There was no significant difference between groups over the course of the study. Plasma hematocrit also tended to decrease in all treatment groups over pregnancy (Fig. 4C), becoming significant on day 18 and then returning to baseline levels by day 7 of lactation.

Urine composition. Cumulative Na⁺ and K⁺ outputs are shown in Fig. 5. Toward the end of pregnancy, a higher level of daily Na⁺ output was evident in the aCSF group compared with both control and candesartan groups, resulting in a significantly greater cumulative urinary Na⁺ excretion (P < 0.05). Urinary K⁺ output increased during pregnancy from baseline levels in all experimental groups, and there was no difference in cumulative excretion between the groups.

DISCUSSION

Brain ANG II has been implicated in the regulation of body temperature in the nonpregnant rat. During gestation, maternal adaptations occur in the regulation of fluid balance and tem-
The novel finding of this study was that central AT1 receptor antagonism prevented the normal decrease in maternal temperature during gestation. After parturition, the usual increase in maternal temperature associated with lactation was not affected. Body fluid regulation was not affected by central AT1 receptor blockade.

Candesartan-treated, untreated control, and aCSF-treated groups showed equivalent increases in food intake and water intake and an increase in body weight throughout gestation. An equivalent number and weights of offspring in each group indicated that there was no marked effect of treatment on fetal development. Pup weights were not measured at birth, but it is unlikely there was a difference because maternal weight decreased equivalently at parturition in all groups.

Thermoregulation. The relative hypothermia of late pregnancy and hyperthermia of lactation are well documented in the rat (12, 13, 17). In an elegant study, Eliason and Fewell (12) showed that the hypothermia of late gestation was a regulated decrease in temperature because rats selected the same ambient temperature (24–25°C) as nonpregnant females. This contrasted with lactation, where they selected much lower temperatures (~14°C) than nonlactating rats, showing that the hyperthermia was not a regulated increase and required a “forced” thermoregulatory response. Because candesartan has been proven to effectively block the AT1 receptor (6), our present results suggest that a central angiotensin AT1 receptor-mediated mechanism has an important role in the hypothermia of late pregnancy but not in the hyperthermia of lactation.

The effect of blocking central angiotensin AT1 receptors was evident by day 17 of gestation, when the expected reduction in temperature was measured in both the aCSF and the untreated control groups. In contrast, the candesartan-treated animals showed an increased colonic temperature. This result suggested that thermoregulatory cooling pathways utilize ANG II as a signaling molecule within the brain. Another possible explanation was that chronic angiotensin receptor blockade with candesartan activated a hyperthermic mechanism, which may have masked a non-angiotensin-mediated cooling mechanism in late gestation. We think that this possibility is unlikely for two reasons. First, continuous intracerebroventricular infusion of candesartan treatment began on day 2 of gestation, yet there was no increase in temperature on day 4. Second, during lactation, the expected increase in core temperature was to the same level in all groups, and there was no evidence of an added hyperthermic mechanism in the group that received candesartan. Therefore, the most likely explanation for our data is that there was a specific cooling mechanism mediated by brain angiotensin, which was initiated by day 7 of pregnancy, increased late in pregnancy, and was no longer evident by day 7 of lactation.

Our laboratory has shown previously in male rats that central AT1 receptor blockade by losartan did not inhibit tail skin vasodilation or salivation (evaporative heat loss) in response to hot ambient temperature (22). However, core temperature was increased relative to control animals, indicating that total heat defense was compromised by central ANG II blockade (22). An increase in core temperature during body heating has been associated with an increase in lumbar, renal, and splanchnic sympathetic nerve activity. Kregel et al. (18) showed that blocking central AT1 receptors during heat exposure prevented the usual increase in splanchnic nerve activity and suggested that this would prevent the redistribution of blood flow to the skin during heat exposure. Therefore, it is possible that AT1...

<table>
<thead>
<tr>
<th>Group</th>
<th>Litter Number</th>
<th>Pup Weight, g</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>13.0 ± 3.4</td>
<td>14.1 ± 3.6</td>
</tr>
<tr>
<td>aCSF</td>
<td>16.0 ± 2.0</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>Candesartan</td>
<td>15.5 ± 2.2</td>
<td>12.4 ± 0.6</td>
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Values are means ± SD. aCSF, artificial cerebrospinal fluid.
receptor antagonism may attenuate splanchnic sympathetic nerve activity, causing pooling of the blood in the viscera and reducing the efficacy of heat loss from the skin (18). While the exact location in the brain for ANG II mediation of thermoregulation remains to be elucidated, hypothalamic sites expressing AT1 receptors, such as the median preoptic nucleus or the paraventricular nucleus, could be possible sites of AT1 antagonist influences on thermoregulation (1).

The fact that candesartan-treated animals fail to cool themselves while pregnant, while reverting to a higher temperature during lactation, indicates that there is a specific cooling mechanism during pregnancy that is not activated or is impaired when central angiotensin is blocked. Studies in a thermocline would help to determine whether there is a coordinated drive to reduce maternal temperature during pregnancy (by recruiting another cooling mechanism to replace the impaired mechanism) or whether there is an increase in the tolerance range of core temperature so that animals do not activate cooling mechanisms despite the increase in core temperature.

Body fluid balance. Fluid and electrolyte data from this study confirm previous observations in rats regarding pregnancy-related changes in food and water intake, urine output and Na⁺ excretion (2) and in plasma osmolality (3). A similar pattern of changes in body fluid balance occurs during human pregnancy (9). Despite the Na⁺ retention that occurs in the latter stages of pregnancy, levels of Na⁺ and osmolality in maternal plasma remained depressed, possibly due to Na⁺ being sequestered by the fetuses (8). In our experiment, the aCSF group (control) displayed an unexpected increase in Na⁺ excretion during pregnancy compared with both the untreated and candesartan-treated groups. Additionally, these animals tended to drink more water compared with the other groups (not significant). This observation suggests that the central administration of aCSF had a natriuretic effect on the animals.

A possible explanation for this observation relates to the fact that plasma osmolality is reset at a lower level during gestation. The ionic composition of rat aCSF is designed to mimic that of a nonpregnant rat, and it has an osmolality of 287 mosmol/kgH₂O. Thus the osmoreceptors in the pregnant rat brain may have detected the exogenous aCSF as being hypertonic compared with maternal plasma.

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pared with blood osmolality and therefore responded with the small increase in Na⁺ excretion. Interestingly, the mechanisms by which the brain regulates the tonicity of the cerebrospinal fluid and controls natriuresis and diuresis are thought to be dependent on angiotensin signaling in the brain (21, 24). Consequently, candesartan-treated animals would not respond to the hypertonic (relative to plasma) aCSF due to blockade of AT₁ receptors. This may explain why they did not exhibit the same level of Na⁺ excretion as the aCSF-treated animals.

Chronic blockade of brain AT₁ receptors with candesartan failed to significantly alter any of the pregnancy-related adaptations in fluid balance. These findings suggest that mechanisms other than brain ANG II are responsible for the resetting of thresholds for fluid balance during pregnancy. Gestation alters the concentration of many hormones, which may mediate the changes in plasma osmolality and plasma volume. Daily fluid intake increases during pregnancy, despite the ambient hynoprotension and hyposmolality, and all the factors that drive this fluid intake remain to be elucidated. Studies by Davison et al. (10) showed that human chorionic gonadotropin decreased osmotic thresholds for arginine vasopressin release and drinking to the levels of pregnant women. Synthetic human relaxin administered to rats has been observed to be dipsogenic and result in a decrease in the osmotic threshold for arginine vasopressin release, whereas the “gain” of the system remains unchanged (29, 31). Although central effects of relaxin are blocked by intracerebroventricularly administered angiotensin antagonists (28), the present experiments show central AT₁ blockade did not affect fluid intake or plasma osmolality. These results suggest that central angiotensinergic pathways do not mediate the increased water intake and hynoprotension of pregnancy.

Perspective. The regulated decrease in maternal temperature may have evolved as a protective mechanism for the developing fetus. One possibility is that a lower maternal temperature and hence a lower fetal temperature during gestation may also decrease fetal oxygen requirements thereby reducing the risk of hypoxia. Metabolic rate decreases nearly 10% for each 1°C change in temperature (16). Therefore, a maternal decrease in temperature could decrease fetal demand for oxygen. A decrease in temperature has also been reported to increase the oxygen saturation of the blood by causing a leftward shift in the oxyhemoglobin dissociation curve. This may be advantageous in situations under which fetal oxygen is severely limited, such as asphyxia, because mild hypothermia has been reported to help reduce neuronal injury in hypoxic conditions (27). Furthermore, maternal hyperthermia during gestation has been shown to retard fetal growth and increase mortality in rats (4).

In conclusion, central AT₁ blockade in the pregnant rat by intracerebroventricular candesartan had no significant effect on fluid balance. This suggests that other factors are more important for the adaptations in these systems. In contrast, temperature regulation in the pregnant rat was significantly altered by central AT₁ blockade. Therefore, this study provides evidence of a novel role for brain ANG II in the decrease in maternal temperature during pregnancy.

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GRANTS

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