Osmoregulatory fluid intake but not hypovolemic thirst is intact in mice lacking angiotensin

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McKinley MJ, Walker LL, Alexiou T, Allen AM, Campbell DJ, Di Nicolantonio R, Oldfield BJ, Denton DA. Osmoregulatory fluid intake but not hypovolemic thirst is intact in mice lacking angiotensin. Am J Physiol Regul Integr Comp Physiol 294: R1533–R1543, 2008. First published February 20, 2008; doi:10.1152/ajpregu.00848.2007.—Water intakes in response to hypertonic, hypervolemic, and dehydrational stimuli were investigated in mice lacking angiotensin II as a result of deletion of the angiotensinogen gene (Agt/−/− mice), and in C57BL6 wild-type (WT) mice. Baseline daily water intake in Agt/−/− mice was approximately threefold that of WT mice because of a renal developmental disorder of the urinary concentrating mechanisms in Agt/−/− mice. Intraperitoneal injection of hypertonic saline (0.4 and 0.8 mol/L NaCl) caused a similar dose-dependent increase in water intake in both Agt/−/− and WT mice during the hour following injection. As well, Agt/−/− mice drank approximately volumes of water following water deprivation for 7 h. However, Agt/−/− mice did not increase water or 0.3 mol/L NaCl intake in the 8 h following administration of a hypovolemic stimulus (30% polyethylene glycol sc), whereas WT mice increased intakes of both solutions during this time. Osmoregulatory regions of the brain [hypothalamic paraventricular and supraoptic nuclei, median preoptic nucleus, organum vasculosum of the lamina terminalis (OVLT), and subfornical organ] showed an increased number of neurons exhibiting Fos-immunoreactivity in response to intraperitoneal hypertonic NaCl in both Agt/−/− mice and WT mice. Polyethylene glycol treatment increased Fos-immunoreactivity in the subfornical organ, OVLT, and supraoptic nuclei in WT mice but only increased Fos-immunoreactivity in the supraoptic nucleus in Agt/−/− mice. These data show that brain angiotensin is not essential for the adequate functioning of neural pathways mediating osmoregulatory thirst. However, angiotensin II of either peripheral or central origin is probably necessary for thirst and salt appetite that results from hypovolemia. angiotensinogen gene; hypertonicity; dehydration; hypovolemia; fos

While it was proposed more than 30 years ago that a brain renin-angiotensin system generated angiotensin in the brain (13, 17), the physiological roles and modus operandi of this system are still incomplete (10, 39, 40). All components of a renin-angiotensin system, including renin, angiotensinogen (Agt), angiotensin converting enzymes, angiotensin I, II, and III, AT1 and AT2 receptors, and angiotensinases, have been identified within the central nervous system (12, 23–25, 29, 35, 44). While these different components of the renin-angiotensin system may not all be colocalized within specific parts of the brain, it is likely that angiotensin peptides are generated within the brain. It is thought that the active peptides angiotensin II, III, or IV may have roles within the brain in functions as diverse as the regulation of cardiovascular and fluid homeostasis (2, 3, 11, 19, 35, 40, 41, 46), reproduction (15), thermoregulation (28), memory (49), cognition, emotional responses to stress and anxiety, cerebral blood flow regulation, and brain developmental processes (39). However, some studies have questioned whether angiotensin is a neuropeptide because of its very low abundance in brain (23) and have proposed that an alternative endogenous ligand may act on angiotensin receptors in the brain (6).

Centrally administered angiotensin stimulates water drinking, vasopressin secretion, and a brisk natriuresis that promote a positive fluid balance and reduce plasma osmolality (19, 29, 38). One of the postulated physiological functions of brain angiotensin II is that of a signaling molecule in osmoregulatory neural pathways, probably as a neurotransmitter or modulator (5). A wealth of evidence in support of such a function for brain angiotensin comes from many reports of the potent effect of centrally administered angiotensin antagonists, such as losartan, in blocking water drinking, vasopressin secretion, increased arterial pressure, and natriuresis in response to centrally administered hypertonic saline (5, 18, 27, 36, 50). The inhibitory influence on osmoregulatory function of central antisense oligonucleotides directed against the synthesis of Agt in the brain is also consistent with this idea (43). In the present investigation, we have tested the hypothesis that angiotensin has an essential role in osmoregulatory function.

Genetically modified strains of mice lacking the gene encoding Agt, the only known source of the biologically active angiotensin peptides, have been developed in two laboratories (22, 48). These mice exhibit lower arterial blood pressure, higher plasma renin levels, lower body weight, and an increased fluid turnover compared with wild-type (WT) mice (1, 21, 22, 26, 47, 48). The lower body weight results from reduced white adipose tissue mass (26). The increased fluid turnover is due to a renal urinary concentrating defect resulting from a lack of angiotensin during kidney development (21, 47). In the case of mice with a homozygous lack of the Agt gene (Agt/−/− mice), most do not survive the first postnatal week unless they are rescued by fluid and electrolyte replacement during the postnatal period or angiotensin treatment (47). We have observed that adult Agt/−/− mice rescued by perinatal saline treatment, as expected, lack any angiotensin peptides in

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Thus they offer an experimental model to test whether brain angiotensin is necessary for the operation of central osmoregulatory neural pathways.

The aims of this investigation were first to investigate the role of angiotensin in osmoregulatory fluid intake using Agt−/− mice by testing whether osmotically stimulated fluid intake is disrupted in this mouse strain. It is not clear whether angiotensin, either of peripheral or central origin is necessary for hypovolemic thirst in mice (20, 37). Therefore, we also investigated whether water drinking in response to hypovolemia, induced by subcutaneous colloid injection, is disrupted in angiotensin-deficient mice. A further aim was to compare the expression in the brain of the protooncogene c-fos, an indicator of neuronal activity (31), in Agt−/− and WT mice in response to hyperosmotic and hypovolemic challenges.

MATERIALS AND METHODS

Animals

Experiments were approved by the Animal Ethics Committee of the Howard Florey Institute, which adheres to the Code of Practice of the National Health and Medical Research Council of Australia for the care and welfare of experimental animals. Experiments were performed on mice of the C57BL6 strain in which the coding region of the Agt gene had been deleted by homologous recombination. C57BL6 mice, heterozygous for the Agt gene (Agt+/− mice; n = 6). Both cumulative (bottom) and noncumulative intakes (top) are shown. Top row: only the final total cumulative intakes at the midpoint of each period of observation. Significant differences from the WT value at the corresponding time are indicated by *P < 0.05, **P < 0.01. Bottom row: only the final total cumulative intakes during the 24 h were tested statistically. #P < 0.0001 (paired t-test).

procedure using primers to label both neomycin and Agt DNA sequences. Due to the defective renal concentrating mechanism, Agt−/− mice have a phenotype of high water intake (~3 times that of WT C57BL6 mice). Thus, we checked that daily water intakes of mice matched the appropriate Agt phenotype in all mice.

After 1–2 mo, mice of either sex were housed individually in cages with ad libitum access to pelleted food and water, except during experiments involving water or food deprivation. All female mice were nulliparous. Water and 0.3 mol/l NaCl solution was provided from glass drinking spouts attached to plastic syringes that allowed measurement of water intake and NaCl intake each day. Room temperature was kept at 22°C, and a 12:12-h light-dark cycle was maintained.

Experimental Protocols

Daily intakes of water. In six age- and sex-matched pairs of mice (2 males, 4 females, aged 8.8 ± 1.0 mo), water intake was measured
during the course of the day at 3 h intervals from 0900 h to 2100 h, and the overnight intake was measured again at 0900 h of the next morning. Lights were switched off at 1800 h and on again at 0600 h.

Water intake in response to intraperitoneal injection of hypertonic NaCl. WT and Agt−/− mice, matched for sex (3 males, 6 females) and aged 9.9 ± 1.5 and 12.1 ± 1.0 mo, respectively, were allowed access to water and food. They were injected intraperitoneally with hypertonic 0.4 or 0.8 mol/l NaCl solution (2% of body wt) or isotonic 0.15 mol/l NaCl (controls) at 1000–1030 h. Water intake during the following hour was measured. This period of observation was chosen, because in preliminary experiments, we observed that the drinking response to intraperitoneal hypertonic saline in WT mice was completed within 1 h of injection of hypertonic saline. As well, the high baseline water intake of Agt−/− mice necessitated that this period of observation be as short as possible.

Water and NaCl intakes in response to water deprivation. WT and Agt−/− mice (matched for sex; 4 males, 2 females) and aged 9.1 ± 2.6 and 11.2 ± 3.0 mo, respectively, were provided with water and 0.3 mol/l NaCl to drink for several days prior to the day of water deprivation. At 0900 h on this day, both solutions were removed, and the body weight of the mouse was measured. In the first experiment, the period of water deprivation was 24 h, and all mice were allowed access to food during this time. At 0900 h on the next day, mice were again weighed, food intake was measured, and then water and 0.3 mol/l NaCl solution were placed back on their cages. Water and NaCl intakes were then measured after 1 h. Because Agt−/− mice became more dehydrated than WT mice when deprived of water, another experiment was undertaken in an attempt to produce a similar degree of dehydration. In the next experiment, different groups of WT and Agt−/− mice were used (matched for age = 6.4 ± 0.9 mo and sex; 5 males, 1 female) that had been provided with food and water (but not NaCl solution) during the days prior to water deprivation. A similar protocol was employed, except that the period of deprivation from water was 7 h in Agt−/−, and 24 h in WT mice. When water was placed back on the cage at 1600 h, intake was measured after 1 h.

Effect of subcutaneous injection of polyethylene glycol on water intake. Age (6.5 ± 1.5 mo) and sex-matched (all males) Agt−/− and WT mice were provided with both water and 0.3 mol/l NaCl to drink several days prior to experiments. Daily water, NaCl, and food intakes were measured. On the experimental day, mice were weighed at 0930 h and injected subcutaneously with 30% polyethylene glycol (PEG; molecular weight 20,000) in 0.15 mol/l NaCl solution (0.2 ml/10 g body wt). Intakes of water, 0.3 mol/l NaCl and food were measured at 2, 4, 6, 8, 24, and 48 h after the injection.

Statistical analysis of intakes of water, food, and sodium solution. Results are expressed as means ± SE. Analysis of the intakes during each period of observation (noncumulative) by two-factor
analysis of variance for repeated measures followed by a post hoc multiple comparison test (Newman-Keuls) was used to evaluate differences between WT and Agt−/− mice. While cumulative intakes are shown for convenience (see Figs. 1 and 3), these were not tested statistically except for the final total values that were compared by either Student’s t-test or the Mann-Whitney U-test. Where appropriate, square root transformation of data was utilized to obtain homogeneity of variance.

Fos immunohistochemistry in response to hypertonicity, hypovolemia, or no treatment. Agt−/− and WT mice were injected either intraperitoneally with hypertonic 0.8 mol/l NaCl (0.2 ml/10 g, n = 5) or subcutaneously with 0.2 ml/10 g of 30% PEG (n = 4) and were then killed by intraperitoneal injection of pentobarbital sodium (1 mg/10 g) at either 2 h (hypertonicity) or 6 h (PEG treatment), respectively, after the stimuli were administered. In addition, control untreated Agt−/− mice (n = 4), WT mice (n = 4), or WT mice injected intraperitoneally with isotonic NaCl solution 2 h earlier (n = 5) were also killed with pentobarbital sodium (1 mg/10 g). Drinking water was provided on the cage during the period following treatments to prevent confounding effects of dehydration that could be additional to the PEG-induced hypovolemia, particularly in the Agt−/− mice. The brains of all these mice were then perfused via the heart with isotonic saline (30 ml) followed by 4% paraformaldehyde/0.1 M PBS, removed, postfixed in this solution for 1 h, and then immersed in 20% sucrose/PBS overnight. Serial coronal sections (40 μm) of the brain were then cut on a freezing microtome and subjected to standard immunohistochemical procedures for the detection of Fos protein by using a polyclonal antisera (Ab-5; Oncogene Science) as previously described (33). Diaminobenzidine was used as the chromogen to visualize the final reaction product. Counts were made of cell nuclei exhibiting Fos-immunoreactivity (Fos-IR) in five osmoregulatory regions of the brain (8, 30, 33), the supraoptic nucleus (SON), hypothalamic paraventricular nucleus (PVN), organum vasculosum laminae terminalis (OVLT), the subfornical organ (SFO), and the median preoptic nucleus (MnPO) in each brain. Using an eyepiece fitted with a graticule to avoid repeat counting, we averaged the counts of cell nuclei exhibiting Fos-IR in three sections for each specific region, using corresponding rostrocaudal stereotaxic levels for the C57BL6 mouse brain (16) for the particular nuclei in each brain. For the MnPO, we standardized the part from which counts were made by counting Fos-labeled nuclei within a window of 400 × 75 μm ventral from the midlevel of the anterior commissure in the midline immediately anterior to this structure (see Fig. 9).

Results are expressed as means ± SE. Comparisons of effect of treatments on Fos-IR counts for each brain site between WT and Agt−/− mice were made by two factor analysis of variance and subsequent Newman-Keuls multiple comparison test. Significance was assigned at P < 0.05.

Fig. 4. Daily intakes of water, NaCl, and food during the 2 days before and 2 days after subcutaneous injection of either NS (white bars, n = 5) or PEG (diagonal hatching, n = 5) in WT and Agt−/− mice. Significant difference from the corresponding values for the first 2 days is indicated by asterisks: *P < 0.05, **P < 0.01.
RESULTS

Normal Pattern of Fluid and Food Intake in Agt−/− and WT Mice

Water intake during 24 h was threefold greater in Agt−/− mice than in WTs matched for age and sex. Increased fluid intake was evident within 3 h of the commencement of measurements and continued throughout the 24 h of observations (Fig. 1). Food intake in the Agt−/− mice was not significantly different from WT mice (Fig. 1). Body weight was significantly lower in the Agt−/− mice than in the WT mice (23.8 ± 2.7 vs. 29.2 ± 7.6 g, P < 0.05, Wilcoxon paired sample test).

Comparison of Water Intake in Response to Systemic Injection of Hypertonic Saline in Agt−/− and WT Mice

Intraperitoneal injections of hypertonic NaCl (0.4 and 0.8 mol/l) caused a dose-dependent drinking response during the hour following the injection in both groups of mice compared with the intakes following control injections of isotonic NaCl (Fig. 2). There was a small but significantly greater intake of water in Agt−/− mice compared with the WT animals, consistent with the baseline intake of Agt−/− being greater than that of WT mice.

Water Intake in Response to Fluid Deprivation in Agt−/− and WT Mice

In the first experiment in which both strains of mice were deprived of water for 24 h, the amount of water drunk by the mice upon regaining access to water was much greater in the Agt−/− than WT mice (1.01 ± 0.11 vs. 0.59 ± 0.07 ml/10 g body wt, n = 6 pairs) during the first hour of access to water. Neither group consumed 0.3 mol/l NaCl solution. However, it was evident that Agt−/− mice had probably incurred a much greater fluid deficit than WT mice during the period of water deprivation because their loss of body weight (25.2 ± 1.3 to 19.1 ± 1.1 g) was ~25% compared with that of ~10% in WT mice (28.6 ± 2.0 to 25.8 ± 1.9 g). Therefore, in another group of Agt−/− mice (n = 6), we attempted to produce a similar weight deficit in response to water deprivation as occurred in their age- and sex-matched WT littermates deprived of water for 24 h. Thus, we observed that the body weight of Agt−/− mice fell by 2.0 ± 0.3 g in Agt−/− when deprived of water for 7 h. This weight loss was not significantly different from that of WT mice (2.7 ± 0.4 g, n = 6 pairs) during the first hour of access to water. Neither group consumed 0.3 mol/l NaCl solution.

Fig. 5. Photomicrographs of the effect of no treatment (Cont; A and B), intraperitoneal injection of 0.8 mol/l NaCl [hypertonic saline (HTS) C and D], or subcutaneous 30% PEG (E and F) on Fos-immunoreactivity (Fos-IR; seen as black dots) in the supraoptic nucleus of WT or Agt−/− mice. Bar = 100 µm.
different from that lost by WT mice (2.3 ± 0.3 g) when deprived of water for 24 h, although it was a marginally but significantly greater percentage weight loss than WTs (9.5 ± 1.3% in Agt−/− mice vs. 7.5 ± 0.9% in WT mice). When the 24-h water-deprived WT mice were given water to drink, they consumed 0.9 ± 0.3 ml during the following hour, which represented 43 ± 11% of the weight lost, while Agt−/− mice that had been deprived of water for 7 h drank 1.7 ± 0.5 ml in the ensuing hour, equivalent to 94 ± 41% of their weight loss incurred during the period of water deprivation.

Comparison of Water and Sodium Intake in Response to Hypovolemia Resulting From Subcutaneous Injection of PEG in Agt−/− and WT Mice

Subcutaneous injection of 30% PEG in WT mice caused a significant increase in water intake within 2 h compared with baseline intake of mice subcutaneously injected with isotonic saline (Fig. 3). There was a small but significant intake of 0.3 mol/l NaCl solution following PEG administration (Fig. 3). However, in the Agt−/− mice, no such effects were observed, and intakes of water and NaCl were similar to intakes observed during the 8 h following a control subcutaneous injection of isotonic saline solution (Fig. 3). PEG treatment did not have any effect on food intake in either group of mice during 8 h following PEG treatment. The dipsogenic and natriorexic effects of PEG treatment in WT mice were also reflected in increased water and NaCl intakes in the 24-h intakes following treatment. However, in Agt−/− mice, there was a pronounced reduction in the 24-h intakes of water, NaCl, and food following PEG treatment (Fig. 4). As well, three of the five Agt−/− mice died 24–48 h following PEG administration, whereas no deaths occurred in the WT mice during this period.

Expression of c-fos in the Brains of Agt−/− and WT Mice Normally, and in Response to Hypertonicity or PEG-Induced Hypovolemia

Fos-IR was investigated in regions of the brain known to have a role in body fluid homeostasis and cardiovascular regu-

Fig. 6. Photomicrographs of the effect of control (A and D), intraperitoneal injection of 0.8 mol/l NaCl (HTS; B and E), or subcutaneous 30% PEG (C and F) on Fos-IR (seen as black dots) in the hypothalamic paraventricular nucleus of WT or Agt−/− mice. LM, lateral magnocellular subnucleus; MM, medial magnocellular subnucleus; MP, medial parvocellular subnucleus. Bar = 150 μm.
lation. These regions were the SON (Fig. 5) and PVN (Fig. 6) in the hypothalamus, and the OVLT (Fig. 7), SFO (Fig. 8), and MnPO (Fig. 9) in the lamina terminalis. Many neurons exhibited basal Fos-IR in commissural and medial subnuclei of the nucleus of the solitary tract (NTS) and area postrema in the hindbrain of untreated Agt−/− mice (not shown), but not in WT mice. Because of the pronounced difference in baseline Fos-IR in NTS and area postrema between WT and Agt−/− mice, we did not make counts of these regions with hypertonic or hypovolemic stimuli. Baseline Fos-IR in the SON and PVN tended to be greater in Agt−/− mice than in WT mice; however, the differences were not statistically significant (Table 1). Although some Agt−/− mice exhibited considerable Fos-IR in the OVLT and MnPO, it was not significantly different from that in WT mice. Baseline Fos-IR was very low in the SFO of both Agt−/− and WT mice (Fig. 8).

Intraperitoneal injection of hypertonic 0.8 mol/l NaCl caused a large and significant increase in the number of neurons expressing Fos-IR in the SON, PVN (predominantly but not exclusively in its lateral and medial magnocellular subnuclei), OVLT, SFO, and OVLT in both Agt−/− mice and WT mice (Figs. 5–9). There were no significant differences in Fos-IR counts between the two strains of mice for any of these brain regions (Table 1).

Hypovolemia resulting from PEG treatment caused a large increase in the number of neurons expressing Fos-IR in the SON, OVLT, and SFO of WT mice compared with baseline counts of Fos-IR (Table 1, Figs. 5–9). Although the increased Fos-IR in the PVN did not reach significance, this may be due to the low number of mice (n = 4). However, isotonic saline control injection also appeared to cause a comparable increase in Fos-IR in WT mice, and handling may have contributed in part to the response of the PVN in WT mice to PEG. In Agt−/− mice, PEG treatment increased the number of neurons expressing Fos-IR in the SON (Figs. 5), but not in the OVLT, SFO, MnPO, or PVN (Table 1, Figs. 6–9). Water intakes over the 6 h from injection of PEG until mice were killed were similar in both groups being 1.5 ± 0.4 in WT mice and 1.3 ± 0.2 in Agt−/− mice.

**DISCUSSION**

Mice in which the Agt gene had been deleted exhibited a baseline level of daily water intake that was almost three times that of age- and sex-matched WT mice, consistent with earlier results of other studies in Agt−/− mice (22, 48). An increased water turnover occurs in Agt−/− mice because they have a partial nephrogenic diabetes insipidus resulting from a developmental impairment of renal concentrating mechanisms caused by a perinatal lack of angiotensin peptides (21, 47). The elevated daily water intake in Agt−/− mice is probably secondary to fluid loss from the kidney, which would have a dehydrating effect. This loss of body fluid should provide osmotic and nonangiotensin-mediated volmetric stimuli for thirst. The dipsogenic responses to hyperosmolar and hypovolemic stimuli that we report, are set against this high baseline level of water drinking that occurs in angiotensin-deficient Agt−/− mice.

The primary aim of these experiments was to determine whether angiotensinergic mechanisms in the brain are necessary for the function of osmoregulatory thirst mechanisms. Previously, we and others had shown that centrally administered angiotensin antagonist drugs were capable of blocking water drinking, vasopressin release, and natriuresis in response to intracerebroventricularly injected hypertonic saline, suggesting angiotensin signaling in osmoregulatory pathways (5, 18, 27, 29, 36, 48). The results we have obtained in Agt−/− mice show that angiotensin peptides are not necessary in the brain (or elsewhere) for central osmoregulatory thirst mechanisms to function adequately. These mice ingested as much water in excess of baseline intakes as did WT mice in response to intraperitoneal injections of hypertonic saline. As well, they appeared to ingest appropriate volumes of water in response to periods of water deprivation. As a consequence of a development disorder of their renal concentrating mechanisms (21), Agt−/− mice lose greater volumes of urine than do WT C57BL6 mice. Accordingly, we observed that Agt−/− mice deprived of water for 7 h, incurred approximately the same loss of body weight as did WT mice deprived of water for 24 h.
When water was returned to the mice, Agt−/− mice drank amounts of water during the next hour that were still more than the intakes of the WT mice that had been deprived of water for 24 h.

If angiotensinergic mechanisms within the central nervous system are unnecessary for osmotically stimulated drinking mechanisms, how then can the reported inhibitory effects that angiotensin antagonists exert on osmotically stimulated thirst...
Table 1. Number of neurons expressing Fos-immunoreactivity (Fos-positive cells per section) in selected osmoregulatory brain regions with no treatment or intraperitoneal (IP) injection of normal 0.15 mol/l NaCl (n sal), hypertonic 0.8 mol/l NaCl, or subcutaneous injection of polyethylene glycol (PEG)

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<th>No Treatment</th>
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<th>IP Hypertonic NaCl</th>
<th>Subcutaneous PEG</th>
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<td>WT</td>
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<td>SON</td>
<td>2±1</td>
<td>10±1</td>
<td>2±1</td>
<td>64±6*</td>
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<tr>
<td>PVN</td>
<td>9±3</td>
<td>25±8</td>
<td>42±14</td>
<td>121±6*</td>
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<tr>
<td>OVLT</td>
<td>5±3</td>
<td>17±3</td>
<td>5±1</td>
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<td>0±0</td>
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<td>MnPO</td>
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Values are means ± SE; n = 4 or 5 in each group. WT, wild type; SON, supraoptic nucleus; PVN, paraventricular nucleus; OVLT, organum vasculosum of the lamina terminalis; SFO, subfornical organ; MnPO, median preoptic nucleus. Statistical evaluation was by 2-factor ANOVA and subsequent Newman-Keuls test for multiple comparisons. The IP n. sal. WT Group was not included in the statistical analysis. *Significant difference (P < 0.01) from “No Treatment” value for the same genotype; †significant difference (P < 0.01) between WT and Agt−/− groups for a particular treatment.

It is possible that centrally administered losartan may be blocking another type of receptor that influences thirst and is independent of the brain angiotensin system, i.e., losartan, at the doses used, is not a specific angiotensin receptor antagonist. Another possibility is that a nonangiotensin ligand that could bind to AT1 receptors may be released upon stimulation of central osmoreceptors. Evidence of a nonangiotensin ligand partially purified from sheep hypothalamus that can bind to AT1 and AT2 receptors, shows the feasibility of this suggestion. It is also possible that the AT1 receptor could be activated without a ligand, for instance by physical changes. There are data showing that the AT1 receptor in mouse myocytes can be activated by mechanical stretch independent of angiotensin peptides (51). This mechanical activation of the AT1 receptor is blocked by an AT1 antagonist, candesartan. Thus, a possible explanation of our results is that activation of the AT1 receptor in osmoregulatory brain regions is caused by direct mechanical changes (cell shrinkage) associated with osmotically stimulated movement of fluids (osmosis), without the involvement of angiotensin II. While the results reported here show that brain angiotensin is not essential for adequate osmoregulatory thirst and fluid intake, the engagement of compensatory redundant mechanisms is often advanced as an explanation for a function being maintained in an animal with a deleted gene. This possibility cannot be discounted here.

Unlike the appropriate water drinking that occurred in Agt−/− mice in response to systemic hypertonicity or dehydration, we observed that hypovolemic thirst resulting from subcutaneous PEG treatment was severely inhibited in Agt−/− mice. Subcutaneous administration of PEG causes sequestration of extracellular fluid under the skin, with a reduction in blood and extracellular fluid volumes (14, 45). In mice, this treatment has been shown previously to stimulate water drinking (20, 37, 38) and sodium appetite (37). We observed that while water drinking and intake of 0.3 mol/l NaCl solution increased in PEG-treated WT mice during 8 h following treatment, there was no increase in water or salt intake in Agt−/− mice compared with controls with PEG treatment. This result implicates an angiotensin mechanism, either peripheral or central, in hypovolemic thirst and salt appetite subsequent to PEG administration. It suggests also that neural signals from low- or high-pressure baroreceptors do not mediate the PEG-induced water and salt intake, unless such signals act together in concert with angiotensin. This is because change in baroreceptor signals would be expected to be even greater in Agt−/− than in WT mice after PEG treatment, yet water and sodium intakes in Agt−/− were similar to the control saline-injected Agt−/− mice.

This result contrasts with that of Crews and Rowland (7) who observed that hypovolemic thirst was not blocked by losartan (100 mg/kg) treatment in mice. A possible explanation for this disparity in results is that PEG treatment caused a greater nonspecific debilitation in Agt−/− mice compared with WTs treated with PEG. Arterial pressure is already 20 mmHg lower in the Agt−/− mice than in WT mice (1, 22, 48) and could be expected to have fallen much further with PEG treatment in the null mice, because they lack a functioning renin-angiotensin system to support arterial pressure. Clearly there was a deleterious effect of PEG treatment in Agt−/− mice at some stage, because both water and NaCl intakes fell significantly below baseline in the subsequent 24 h, and three of the five Agt−/− mice died 24–48 h after treatment. Notwithstanding these observations, evidence of Agt−/− mice being behaviorally debilitated during the first 8 h following PEG administration was not apparent. Their intake of food over this period, although small, was not significantly different than that of saline-injected controls and appeared similar to that of WT mice during the initial 8 h following PEG treatment. As well, intakes of water and sodium were similar to those observed during the baseline day, levels that could have been expected if the dipsogenic signaling pathway for hypovolemic thirst had been disrupted in Agt−/− mice. Therefore, the possibility remains that angiotensin, acting either peripherally or centrally, has a significant role in mediating hypovolemic thirst in mice as it does in the rat. Similarly, our results show that sodium intake stimulated by PEG-induced hypovolemia in WT mice is also dependent on angiotensin II, either of peripheral or central origin. Contrasting with some earlier studies (7, 20, 38), we were able to observe increased sodium chloride intake in WT mice following PEG administration. The reasons for this difference are not clear; however, there is one other report of increased intake of NaCl solution (0.15 mol/l) in mice following PEG administration (37).

In regard to c-fos expression in the brains of Agt−/− and WT mice, baseline levels of Fos-IR tended to be low in osmoregulatory regions of the brain, such as the lamina terminals and hypothalamic SON and PVN. However, there was a tendency for some Agt−/− mice to exhibit marginally more Fos-IR in some of these regions. This probably reflects the high
water turnover in Agt−/− mice (a consequence of their partial nephrogenic diabetes insipidus) with some mice being killed for brain immunohistochemistry at a time when their water drinking had not totally replenished current losses in urine. A marked increase of baseline Fos-IR was observed in the commissural and medial parts of the NTS and also the area postrema in Agt−/− mice compared with WT mice. This is not surprising in view of the lower arterial blood pressure that has been reported to occur in Agt−/− mice (1, 22, 48), the importance of these two regions in cardiovascular control mechanisms (9), and previous reports of increased Fos expression in these regions caused by hypotensive stimuli (4, 34).

The pattern of Fos immunoreactivity observed in osmoregulatory regions (lamina terminalis, SON, PVN) of the brains of Agt−/− mice in response to hypertonicity was similar in Agt−/− and WT mice, consistent with the comparable osmoregulatory drinking responses observed in the two strains of mice. Although we did not measure blood levels of vasoressin, its secretion in response to hypertonicity probably occurred in Agt−/− as well as WT mice, because similar increases in Fos expression were observed in the magnocellular regions of the hypothalamic SON and PVN of both groups of mice with the hypertonic stimulus.

Interestingly, we did observe differences in Fos-IR in the lamina terminalis of Agt−/− mice subjected to PEG treatment compared with that in WT mice. Neither the SFO or OVLT showed any increase in Fos-IR in response to this hypovolemic stimulus in Agt−/− mice, whereas such hypovolemia resulted in a large increase in the number of cells (presumably neurons) expressing Fos-IR in these circumventricular organs in WT mice. These data show that activation of neurons in the SFO and OVLT of WT mice in response to hypovolemia is probably caused by the action of circulating angiotensin II that would increase in the circulation due to hypovolemic stimulation of the renin-angiotensin system by PEG treatment in WT mice, but not in Agt−/− mice. Our data are consistent with observations of Crews and Rowland (7), showing that peripheral administration of the AT1 receptor antagonist losartan prevents c-fos expression in the SFO and OVLT of PEG-treated C57BL6 mice. By contrast, the number of neurons exhibiting Fos-IR in response to hypovolemia increased in the SON of both Agt−/− and WT mice. Hypovolemia resulting from PEG treatment increases vasopressin secretion (42), and it is likely that a significant proportion of the Fos-IR in the SON is localized to vasopressin-containing magnocellular neurons. The Fos-IR observed in these magnocellular neurons in Agt−/− mice shows that activation of such neurons by hypovolemia may be independent of angiotensin mechanisms (either peripheral or central). Most likely, ascending neural pathways from either or both low- and high-pressure receptors in the thorax, that are relayed to the hypothalamus via the medulla oblongata, instigate hypovolemia-stimulated release of vasopressin from the neurohypophysial axons of magnocellular neurons.

Perspectives and Significance

Adequate water intake following hypertonic stimuli observed in mice totally lacking angiotensin peptides shows that angiotensinergic neural pathways are not essential for adequate osmoregulatory thirst. By contrast, angiotensin, either peripherally or centrally generated, is probably essential for the thirst and salt appetite that occurs in response to hypovolemia in mice.

Our results question whether there is any role at all for angiotensin as a signaling molecule for osmoregulatory mechanisms within the central nervous system. If there is not, it is difficult to explain why centrally administered AT1 antagonists inhibit drinking stimulated by intracerebroventricular injection of hypertonic saline in several species that include mice. As mentioned above, the explanation could be that either AT1 antagonists are nonspecific, blocking another receptor, or that they block the action of a nonangiotensin molecule, which could act at the AT1 receptor. Another possibility is that drinking stimulated by intracerebroventricular hypertonic saline is not comparable to normal osmotic thirst. We speculate that the dipsogenic response to intracerebroventricular hypertonic NaCl does not use the physiological neural pathway that is activated by systemic hypertonicity.

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