Role of angiotensin in body fluid homeostasis of mice: fluid intake, plasma hormones, and brain Fos

Neil E. Rowland, Bradley E. Goldstein, and Kimberly L. Robertson

University of Florida, Department of Psychology, Gainesville, Florida 32611-2250

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Rowland, Neil E., Bradley E. Goldstein, and Kimberly L. Robertson. Role of angiotensin in body fluid homeostasis of mice: fluid intake, plasma hormones, and brain Fos. Am J Physiol Regul Integr Comp Physiol 284: R1586–R1594, 2003. First published February 20, 2003; 10.1152/ajpregu.00730.2002.—CD1 mice injected peripherally with either ANG I or ANG II failed to drink substantial amounts of water or NaCl, yet showed strong Fos immunoreactivity (ir) in subfornical organ (SFO). Mice injected with furosemide showed modest stimulation of NaCl intake either 3 or 24 h later, were hypovolemic, and showed elevated plasma renin activity (PRA). The pattern of Fos-ir in the brain after furosemide was similar to that seen after peripheral injection of ANG II. Mice became hypovolemic after subcutaneous injection of polyethylene glycol (PEG), showed large increases in PRA, aldosterone, and water intake, but did not show sodium appetite. PEG-treated mice had strong activation of SFO as well as other brain regions previously shown to be related to ANG-associated drinking in rats. ANG II appears to have a modified role in the behavioral response to fluid loss in mice compared with rats.

hypovolemia; aldosterone; subfornical organ; thirst; sodium appetite

BODY FLUID CONTENT and solute concentrations of mammals are maintained within narrow limits by sensitive homeostatic devices. When the concentrations of solutes in the extracellular fluid (including blood plasma) increase, the attendant rise in osmotic pressure activates osmoreceptors in several parts of the body, including gut and brain, to trigger physiological responses such as vasopressin secretion and behavioral actions such as seeking and drinking water. When the volume of extracellular fluid declines, baroreceptors reflexively trigger physiological events to support blood pressure, including release of renin from the kidney; this in turn generates the potent vasoconstrictor peptide, ANG II. Loss of body sodium also results in volume loss and similar physiological responses. Fluid seeking and ingestion are also stimulated, but in this case both water (thirst) and sodium solutions (salt appetite) are consumed (see Refs. 7, 10, 14 for reviews).

Fitzsimons (7) was among the first to show the same peptide (ANG II) that supports blood pressure in hypovolemia also is dipsogenic when administered to water-replete rats and other species. In rats, it is widely accepted that ANG II contributes to but is not the exclusive cause of hypovolemic thirst and sodium appetite. Sodium appetite is facilitated by several hormonal factors, primary among which is most likely aldosterone, whose synthesis and release is stimulated by ANG II (see Refs. 7, 10, 14 for reviews).

Advances in molecular genetics have focused interest on mice as the mammal of choice for many types of investigation. There are few studies on fluid balance in mice, and those include unresolved questions concerning the role of ANG II. Kobayashi et al. (12) first reported that neither C57BL/6 nor BALB/c mice drank after subcutaneous injections of ANG II up to 1 mg/kg. We were able to substantiate this result almost a decade later, using CD1 mice (16), and Denton et al. (4) showed that subcutaneous infusion of ANG II for 7 days had no effect on water intake in BALB/c mice. However, less direct evidence (1, 3, 11, 16, 22) suggests that ANG II may play a role in drinking in mice, at least under some conditions, so it is important to understand the limits of that role.

Two alternate hypotheses have guided our work. The first is that circulating ANG II, which is known to cross from blood into brain at circumventricular structures such as subfornical organ (SFO) in rats, may penetrate less well in mice. The second is that ANG II does cross into these brain regions in mice and produce similar cellular actions as in rats, but that some potent inhibitory mechanism on thirst may be present in mice. Initially, we attempt to distinguish these hypotheses by mapping brain activation in mice after either administration or generation of ANG II. In rats, immunohistochemical detection of the transcription factor Fos (or Fos-like factors) has proven to be a useful marker of activation in regions of the brain related to ANG action and fluid balance (13). The present studies report regional induction of Fos immunoreactivity (ir) in mouse brain after exogenous treatment with ANG II and compare this with the effects of the diuretic agent, furosemide, and the hypovolemic agent, polyethylene glycol (PEG). The behavioral and physiological effects of these agents have been documented well in rats (9, 10, 13, 14, 18–20).

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Address for reprint requests and other correspondence: N. Rowland, Psychology, PO Box 112250, Univ of Florida, Gainesville, FL 32611-2250 (E-mail: nrowland@ufl.edu).
METHODS

Animals. Male and female mice of the ICR:CD1 strain (Charles River, Newton, MA) between 3 and 9 mo of age were used in these studies. For at least 1 wk before and during the studies, mice were housed individually in polycarbonate tub cages (13 × 10 × 10 cm) with stainless steel wire mesh lids. A 1- to 2-cm depth of Sani-Chips bedding (Teklad-Harlan, Madison WI) was present in each cage and was changed before each experiment. The vivarium was maintained at 23 ± 1°C with a 12:12-h light-dark cycle (on 0600–1800). All acute treatments were performed during the middle of the light phase. Purina laboratory chow pellets (#5001) and tap water were available ad libitum, except as noted and for ~1 h before and during all acute tests. Mice were handled frequently before studies to minimize nonspecific stress responses after injections. All experiments conformed to the American Physiological Society’s guidelines on the care and use of animals.

Drinking studies. Tests were performed in the home cages from which food was removed for the duration of acute tests. Animals were given the designated treatment and, either immediately or after a delay, given access to either tap water, NaCl solution, or both. These fluids were contained in short cylinders graduated with an accuracy of 0.1 ml and fitted with a standard sipper tube. Tubes were inserted through the slots in the cage lid and intakes were measured volumetrically.

Blood sampling and assays. In some studies, mice were sedated by inhalation of isoflurane (Aerrane, Henry Schein, Melville, NY) for ~10 s in a closed container. Blood was collected from the retroorbital sinus using a capillary tube. About 50 µl was collected into ice-cold EDTA-coated tubes for subsequent determination of plasma renin activity (PRA), one capillary tube was filled with blood for direct determination of hematocrit ratio (Clay-Adams reader) and plasma protein concentration (Atago hand refractometer), and the remainder of the sample was collected into plain tubes for subsequent assay of aldosterone. The hormone samples were centrifuged, and plasma was frozen at −60°C for later radioimmunoassay. PRA was determined by incubating 10 µl plasma at 37°C for 1 h and measuring the ANG I generated using a double antibody kit (Perkin Elmer-New England Nuclear). The use of a 10-fold lower plasma volume than specified in the kit protocol allows us to read high PRAs but with loss of sensitivity at low values (range thus becomes ~1–100 ng ANG 1·ml−1·h−1). Plasma aldosterone concentration was determined using a coated tube radioimmunoassay (Diagnostic Products, Los Angeles, CA) with some samples diluted with zero standard to allow reading values >1,200 pg/ml. In mice from which all of the above plasma samples were obtained, the PRA sample was taken first. No blood was taken before or during a behavioral study, and no animal was sampled more than once a week. In a preliminary study to determine whether this sampling procedure progressively elevates PRA, we took five consecutive capillary tubes of blood (over a period of ~45 s) from two isoflurane-sedated mice and assayed them for PRA. No systematic increase was seen across the serial samples.

Brain Fos-ir. At a designated interval after treatment during which neither food nor fluid was available, except as noted, mice were deeply anesthetized (pentobarbital sodium 200 mg/kg intraperitoneally) and perfused intracardially with heparinized saline followed by 4% paraformaldehyde. Brains were removed and placed into paraformaldehyde solution overnight. On the following day, brains were sliced coronally (75 µm) using a vibratome. Free-floating sections were treated with borohydride to destroy endoperoxidases, then were incubated with primary antibody against Fos (SC-52; Santa Cruz Biotechnology, 1:20,000) at 4°C for 48 h. After secondary antibody (Zymed Labs) and ABC (Vector Labs) treatments, slices were stained using diaminobenzidine as described before (19). Sections were mounted on gelatin-coated slides for viewing under a microscope with a television viewer. The numbers of Fos-ir cells in template-delineated brain regions were counted by two observers, and their results were averaged; for regions that spanned more than one section, the slide with the most Fos-ir cells was included for statistical analysis.

Drinking and Fos-ir after peripheral injection of ANG II. To confirm and extend our previous finding of lack of drinking to ANG II in mice (16), 12 male mice were injected subcutaneously with ANG II (0.2 mg/kg; Sigma, St. Louis, MO); this dose produces robust drinking in rats (15). Four control mice were injected with water (1 ml/kg). Mice were given immediate access to water and 0.45 M NaCl; intakes were measured 1 h later.

In two follow-up studies, groups of six mice (3 of each sex) were injected with ANG I (0.25 or 0.5 mg/kg sc), with the ANG-converting enzyme inhibitor enalapril maleate (0.5 or 1 mg/kg intraperitoneally), or with enalapril followed 30 min later with ANG I. Controls received isotonic saline (2 ml/kg sc). Water was presented after ANG I injection, and intakes were recorded after 1 h.

To examine induction of Fos-ir in brain after ANG II, six male and five female mice received subcutaneous injection of ANG II (0.2 mg/kg) and, after 1 h without fluids, were anesthetized and perfused.

Sodium depletion: behavioral, hormonal and Fos-ir effects of furosemide. In a first behavioral study, 12 male mice were injected with furosemide (40 mg/kg sc; Abbott Labs, Chicago, IL), and 12 controls were injected with water (4 ml/kg). One-half of the mice were studied 3 h after injection and the other one-half after 24 h. After this interval (during which food and water were not present), they were given a single tube of 0.15 M NaCl and intake was measured for 1 h. In a second study, designed as a more stringent test of sodium appetite, six male mice were injected with furosemide and six with water, as above. Food and fluid were withheld for 24 h, and then a two-bottle choice of water and 0.45 M NaCl was given for 1 h.

Twenty-four mice, 12 of each sex, were used for blood assays. One group was sampled 3 h after injection and the other after 24 h. In both groups, six mice were injected with furosemide (40 mg/kg sc) and six with distilled water (4 ml/kg). Food and water were withheld between the time of injection and sampling. An additional 24 male mice were used for determination of Fos-ir, 12 studied after 3 h and the other 12 after 24 h. In each group, seven received furosemide injection and five were water-injected controls. Blood was collected for assay of PRA using isoflurane, and the mice were then immediately anesthetized for perfusion.

Hypovolemia: behavioral, hormonal, and Fos-ir effects of PEG. Mice were studied 2, 6, or 24 h after injection of PEG. PEG (formula weight 20,000; Fisher Scientific) was administered subcutaneously using an 18-gauge needle in a volume of 1 ml/mouse (~20–40 ml/kg for the range of weights in these studies) and either as a 25 or 40% wt/vol solution in distilled water. The PEG solution was prepared to spread from the site of injection in the nape of the neck. Control mice received a sham injection in which only a needle was inserted.

In the first study, six female mice received injection of 25% PEG and six received a sham injection. Water and 0.45 M
NaCl were presented 2 h later, and intakes were recorded after 1 and 4 h. Another four mice received PEG but no fluid. At the end of 6 h, blood samples were taken from all 15 mice. Another five mice received 25% PEG and four were sham injected and killed 6 h later, with no fluid available in the interim, for determination of brain Fos-ir.

To examine whether a higher dose of PEG might produce a more reliable hypovolemia, 12 mice were injected with 1 ml 40% PEG; six were given both water and 0.45 M NaCl to drink after 2 h while the other six had no fluid. All were sampled for blood after 6 h. The data bore out our expectation that the physiological results from the 25 and 40% doses would yield a continuous range, and so their data have been combined for presentation.

To determine whether a longer delay between injection and test would reveal a larger behavioral or physiological response, groups of 12 mice were injected with either 25 or 40% PEG or were given a sham injection and killed 6 h later, with no fluid available in the interim, for determination of brain Fos-ir.

To examine whether a higher dose of PEG might produce a more reliable hypovolemia, 12 mice were injected with 1 ml 40% PEG; six were given both water and 0.45 M NaCl to drink after 2 h while the other six had no fluid. All were sampled for blood after 6 h. The data bore out our expectation that the physiological results from the 25 and 40% doses would yield a continuous range, and so their data have been combined for presentation.

To determine whether a longer delay between injection and test would reveal a larger behavioral or physiological response, groups of 12 mice were injected with either 25 or 40% PEG or were given a sham injection. After 24 h without food or fluid, one-half of the mice in each group were given water and 0.45 M NaCl to drink for 1 h. The remaining mice had blood sampled at the time the others were given fluids. In this and the next study, one-half of the mice in each group were male and one-half were female. No sex differences were evident, so the data have been combined for presentation.

To determine whether a preexisting mild sodium depletion might potentiate or accelerate sodium appetite after injection of PEG, as is the case in rats (20), two groups of 12 mice were fed a sodium-deficient natural ingredient diet (Teklad; TD90228, −0.015% sodium) for either 1 or 3 days. At the end of this time, six mice in each group were injected with PEG (1 ml, 25%) and the other six received a sham injection. An additional six chow-fed mice received PEG. Water and 0.45 M NaCl were presented immediately, and intakes were recorded after 1 and 4 h. One week later, the treatments were repeated but this time no fluids were presented and blood was sampled 2 h later and assayed as above. An additional six untreated mice were added as baseline controls in this phase of the study.

Statistics. All data were analyzed using SigmaStat (SPSS) software. Group comparisons were made using either t-tests or 1-way ANOVAs with post hoc Newman-Keuls tests. Significance value was set at $P < 0.05$. Regression analyses used nontransformed data and a linear model.

RESULTS

Drinking and Fos-ir after peripheral injection of ANG II. Control mice injected with distilled water drank very little water or 0.45 M NaCl (means ± SE: 0.13 ± 0.05 and 0.03 ± 0.03 ml in 1 h, respectively). Those injected with ANG II consumed means of 0.07 ± 0.02 and 0.08 ± 0.02 ml, respectively; neither amount nor total intake differed significantly from the control group.

Fos-ir data are shown in Fig. 1 for SFO, area postrema (AP), median preoptic nucleus (MnPO), and supraoptic nucleus (SON). Compared with baseline data from the furosemide study (see below; these data are typical of other baseline data in our lab), significant increases were produced by ANG II in both males and females in SFO and AP, and in the SON in males only. Of other areas examined, significant increases above baseline were observed in the dorsolateral bed nucleus of stria terminals (BSTld) in both sexes and a marginal ($P < 0.07$) increase in the posterolateral (magnocellular) division of the paraventricular hypothalamus (PVNm) in males.

Administration of ANG I alone produced a very small drinking response that was significant in one study but not another and was not dose related (Table 1). Enalapril injection alone did not cause drinking at either dose, nor did its combination with ANG I potentiate the effect of ANG I.

Sodium depletion: behavioral, hormonal, and Fos-ir effects of furosemide. In the groups tested 3 h after injection, mice injected with furosemide drank significantly more 0.15 M NaCl than controls ($P < 0.01$; Fig. 2). In groups tested 24 h (without food or fluid) after injection, total intake was higher in both the furosemide and control groups, and there was no significant group difference. In the follow-up study in which mice were given a choice between water and 0.45 M NaCl after 24 h, salt but not water intake was significantly higher in the furosemide group (Fig. 2).

Table 1. Water intakes after treatment of mice with ANG I, enalapril, or their combination

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-h Water Intake, ml</th>
</tr>
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<tbody>
<tr>
<td><strong>Study A</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>ANG I (0.25 mg/kg)</td>
<td>0.25 ± 0.08*</td>
</tr>
<tr>
<td>Enalapril (0.5 mg/kg)</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>Enalapril + ANG I</td>
<td>0.23 ± 0.08*</td>
</tr>
<tr>
<td><strong>Study B</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>ANG I (0.5 mg/kg)</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>Enalapril (1 mg/kg)</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>Enalapril + ANG I</td>
<td>0.18 ± 0.06</td>
</tr>
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</table>

Values are means ± SE. *$P < 0.05$ vs. saline-injected control group.

Fig. 1. Mean (±SE) numbers of Fos immunoreactive (ir) cells per section in the subfornical organ (SFO), area postrema (AP), median preoptic nucleus (MnPO), and supraoptic nucleus (SON) of male and female CD1 mice 1 h after subcutaneous injection of ANG II (0.2 mg/kg). Horizontal lines show the mean Fos-ir in untreated control mice (see also Fig. 2). *$P < 0.05$ above control.
Three hours after furosemide injection, mice were hypovolemic compared with controls, shown by significantly higher hematocrit ratio and plasma protein concentration (Fig. 3). PRA was also increased three- to fourfold at this time \( (P < 0.05) \), but the rise in aldosterone was smaller and not significant. Twenty-four hours after injection, plasma protein concentration and PRA remained similar to the 3 h levels. Although hematocrit ratio increased slightly between 3 and 24 h in the furosemide group, it increased even more in the controls so that the difference between the groups was no longer significant. Plasma aldosterone levels were not increased above control after 24 h.

Brain Fos-ir after treatment with furosemide was greater than in water-injected control mice after both 3 and 24 h in SFO and after 24 h in MnPO (Fig. 4). PRAs measured in these mice when they were perfused were similar to those obtained in the previous study shown in Fig. 3.

**Hypovolemia: behavioral, hormonal, and Fos-ir effects of PEG.** The mean (±SE) intakes in milliliters between hours 2 and 6 in 25% PEG-treated mice were...
0.9 ± 0.3 (water) and 0.1 ± 0.1 (0.45 M NaCl). Although this water intake in the PEG group was significantly higher than the corresponding intake of sham-injected mice (0.1 ml, \( P < 0.05 \)), salt intake was slightly lower than that of the sham group (mean 0.3 ml). The total fluid intake by individual mice after PEG showed a large range (0 to 2.3 ml), and there was also a large range in the physiological measurements. Thus, whereas some mice showed extremely high values on all four measures, others fell in or near the range of the sham-treated group. Indeed, at the time the blood was sampled, we had noted a substantial range in size of the visible edema from quite large to negligible.

Thus, in an attempt to produce a more uniform hypovolemia, we added groups treated with 40% PEG. The mice allowed to drink all showed a robust water intake (1.9 ± 0.3 ml during the 3rd hour after PEG; 2.5 ± 0.4 ml total after 6 h), both significantly larger than the sham-treated mice in the previous study and substantial in absolute terms, amounting to a mean 5.4% of their body weight (46 g). Only one of these mice consumed any 0.45 M NaCl. This 40% PEG group again showed large individual variability in the physiological measures.

The data from the 25 and 40% PEG treatments were initially analyzed separately and yielded similar conclusions, so they were combined for final analysis and presentation in Fig. 5. Figure 5A shows that plasma protein and hematocrit ratio were highly correlated; notice that the mean values in the sham-treated group fall on the line and indicate that ~50% of the PEG-treated mice became hypovolemic by more than ~10%. Figure 5B shows that plasma aldosterone was reasonably correlated with hematocrit ratio (aldosterone in sham-treated animals was 306 ± 92 pg/ml). Similar regression lines were obtained between aldosterone and plasma protein or between PRA and either hematocrit or plasma protein (not shown). Figure 5D shows a
good correlation between aldosterone and PRA (PRA in sham-treated animals was 4.5 ± 0.6 ng ANG I·ml⁻¹·h⁻¹). In contrast, there was no correlation between any of the physiological measures and the amount consumed; Fig. 5C shows the scatterplot of fluid intake with PRA. It was expected that consumption of water without salt would not substantially alleviate the hypovolemic state, and this proved to be the case. The above scatterplots of physiological data include both fluid-deprived and water-drinking mice, and, although not differentiated specifically on those graphs, their data were indistinguishable.

In groups tested after 24 h, total fluid intake was elevated in the combined PEG groups (1.5 ± 0.1 ml) compared with the controls (0.9 ± 0.2; P < 0.05) in both groups, with ~25% of the total intake as NaCl. Plasma measures were more different from controls in the 25% group at this time (data not shown), indicating that any hypovolemia had dissipated. In contrast, four of the six mice given 40% PEG 24 h previously were grossly hypovolemic, with obvious edemas (mean hematocrit 59.6%, protein 11.6 g/dl, aldosterone 4,180 pg/ml, PRA 62 ng ANG I·ml⁻¹·h⁻¹) and were extremely lethargic.

Brain Fos-ir 6 h after 25% PEG was increased in each region examined relative to sham-injected controls (Fig. 6). As was the case with the physiological measures, there was some variability in the individual cell counts in PEG-treated mice (e.g., the range was 30–140 cells in the SFO), but all were above the range of values in the controls. Unlike after ANG II or furosemide, PEG-treated mice had strong induction of Fos-ir in the anteromedial (parvocellular) paraventricular nucleus (PVNp). In the mice perfused 24 h after PEG, Fos-ir had returned to near baseline levels in each of the above regions (data not shown).

The results of the study in which mice were fed low-sodium diet before PEG are shown in Table 2. In the chow-fed group, PEG again stimulated robust water intake but negligible NaCl appetite. The groups fed the low-sodium diet drank significantly less water after PEG than the chow-fed controls, and their intakes were not significantly elevated above those of corresponding sham-injected controls. NaCl intakes were again very low in all groups, with no significant differences, although the 3-day low-salt group did consume about threefold more than the chow-fed controls. The plasma measures corresponding to these behavioral data (Table 2) indicate that plasma protein was elevated by PEG treatment (P < 0.001), by diet (P < 0.05), but with no interaction. It is evident that plasma protein was not changed by feeding 3 days of low-sodium diet but that the injection of PEG caused greater hypovolemia in the low-sodium diet groups. In contrast, PRA was elevated in sham-injected mice by feeding low-sodium diet for 1 or 3 days, but PRA was comparably elevated in all three PEG-treated groups. Note that the PRA values after PEG were four- to fivefold higher than those induced by low-sodium diet alone. Aldosterone levels in general paralleled PRA: they were elevated about threefold on the low-sodium diet but that the injection of PEG caused greater hypovolemia in the low-sodium diet groups.

The above scatterplots of physiological data in-
this baseline by PEG. In the PEG-treated groups, in which PRA was the same, aldosterone was significantly higher in the low-sodium diet compared with the chow-fed groups.

**DISCUSSION**

In the first experiment, we confirmed previous observations (12, 16) that mice do not drink after acute peripheral injection of ANG II. The effect of ANG II was small, inconsistent, and unaffected by enalapril. In rats, both of these agents at these doses are potently dipsogenic, and the effect of ANG II is potentiated by enalapril (15). Despite the absence of a prominent behavioral response, we showed that Fos-ir was induced in several brain regions by treatment with ANG II. Regions showing the clearest activation were the circumventricular organs, in the forebrain, the SFO and PVNm (13). The activation of the latter structures seems to be less marked in mice compared with rats. Males but not females showed Fos-ir in the SON after ANG II (see Ref. 13 for a summary of sex differences in rats). However, in the experiments with furosemide and PEG, no physiological or behavioral sex differences were evident. These data allow us to reject our first working hypothesis that circulating ANG II does not penetrate the brain, at least at circumventricular structures, of mice.

We do not believe that these data are limited by either the dose of ANG II or the strain of mouse. In a preliminary study, at least two male and two female mice of the C57BL/6, DBA/2, and CD1 strains were injected subcutaneously with either low (0.2 mg/kg) or high (1.0 mg/kg) doses of ANG II and brain Fos determined. We found no strain differences and only a small increment in Fos-ir cells after high compared with low doses. Together, these results suggest that peripherally injected ANG II does penetrate the circumventricular organs in mice and causes cellular activation. The SFO of mice, like rats, contains a high concentration of ANG II type 1 receptors (AT1R) (8). It is thus likely, although we do not prove, that circulating ANG II engages AT1R in the SFO of mice. The relatively low activation of other regions in mice suggests that the functional influence of ANG II action in SFO on downstream regions of the brain may be reduced in mice compared with rats.

Despite this conclusion, there is evidence that both peripheral and central administrations of ANG II do have effects on fluid intake in mice under some conditions. Although peripheral infusion of ANG II is ineffective in stimulating fluid intake, it was reported to reverse the inhibitory effect of captopril on NaCl intake after furosemide in BALB/c mice (22). Both the elevation in PRA during cold exposure (3) and sodium depletion (22; see below) correlated with the observation of sodium appetite in these conditions in mice, and water deprivation induced Fos-ir in regions with ANG II receptors including SFO (21). Direct central manipulations of ANG II systems have profound effects. Cerebroventricular administration of ANG II to mice stimulates water intake after acute injection (11, and unpublished work) and a large but relatively slow onset (>24 h) increase in water and salt intakes during chronic infusion (4). Central infusion of the AT1R antagonist losartan blocked thirst induced in mice by cerebral hypernatremia (2), and the nonselective antagonist saralasin reduced water intake in a polydipsic strain (11). Studies on the neuronal effects of central administration of ANG II and its antagonists will be needed to understand fully and integrate these diverse observations.

In our second experiment, we examined whether acute sodium depletion using the loop diuretic furosemide, known to stimulate sodium appetite in mice (1, 22), activated similar brain regions. A similar dose of furosemide has been shown to cause a short-term loss of ~10 meq/kg Na+ (1, 16) in mice, which is comparable to or greater than body weight-adjusted losses in rats (9). Consistent with previous results (1, 16, 22), we found a modest stimulation of NaCl intake after 3 h in mice, and an appetite for hypertonic NaCl after 24 h. This result is not unlike that seen in rats, in which sodium appetite usually does not develop for several hours after the initial natriuresis is completed (9, 10, 18). We found that, as in rats, furosemide caused hypovolemia and stimulated PRA; plasma aldosterone was modestly elevated. Furthermore, Fos-ir was stimulated in the SFO, but not significantly in SON and PVNm, in a pattern similar to that seen after ANG II in the first study. This is consistent with our observations in rats previously fed chow and examined 24 h after furosemide: Fos-ir was increased in SFO of all rats, but in only one of five rats in SON and PVN (17). In that study in rats, we also showed that the salt appetite was unaffected by either the presence or absence of water during the 24-h delay. Thus, in the present studies in mice, we chose to withhold water for this 24-h period, thereby avoiding individual differences in intake as a potential source of variance. The increases in plasma protein and PRA in mice 24 h after furosemide are similar or slightly larger than those we have reported in rats under comparable conditions (17).

We reported previously that PEG increased intake of water and, when available, 0.15 M NaCl in mice (16). In the third experiment, we confirmed that PEG induced significant water intake, but without stimulation of sodium appetite using the more stringent test of appetite with 0.45 M NaCl. Sodium appetite also was not increased either by waiting a longer time after injection or by prior mild salt depletion by feeding low-sodium diet. In the latter study, not only was salt intake not stimulated but the normal dipsogenic effect of PEG was significantly impaired despite comparable physiological indexes of hypovolemia.
While the physiological indexes of hypovolemia such as plasma protein, hematocrit ratio, PRA, and aldosterone all were positively correlated, water intake did not appear to be related directly to the extent of hypovolemia. Compared with effects in rat (21), large doses of PEG were needed to produce hypovolemia in mice, and the failure of PEG to produce reliable edemas in some mice may reflect faster lymphatic drainage from the subcutaneous space. In contrast to the effect of exogenous ANG II, PEG induced Fos-ir in many brain regions, quite comparable to effects in rats (13, 20). In rats, PEG-induced Fos-ir in SFO and SON was reduced by peripheral treatment with losartan (20), so we suspect that the activity in SFO of mice is also a result of penetration of circulating ANG II. Notably, the highest PRA and aldosterone levels that we observed after PEG were considerably higher than those observed in the furosemide study. Compared with rats, the levels of hypovolemia achieved in this study are very high, and blood of the mice with the greatest depletions had become so viscous that sampling through the capillary tube was difficult.

PEG-induced drinking in rats has been shown to be partly or wholly independent of peripheral generation of ANG II (e.g., 13, 19, 20). The implication is that neural signals from cardiopulmonary baroreceptors, the most likely transducers of non-hypotensive hypovolemia, must be sufficient to stimulate thirst (10, 14). Peripheral administration of doses of ANG converting enzyme inhibitors often increase thirst or sodium appetite in rats by enhancing the availability of peripheral ANG I to brain and its conversion to ANG II (6, 7, 15); high doses of these inhibitors also apparently penetrate the brain and so the behavioral effects are absent. In mice, the enhancing effects of a “low” dose of a converting enzyme inhibitor (enalapril) on ANG I drinking were not evident in the present work, and neither captopril nor enalapril enhanced sodium appetite induced acutely by sodium depletion (4) or after chronic administration (4, 16). As in rats, this low dose of enalapril increased PRA by ~10-fold (unpublished observation). Furthermore, in studies not reported here, we have been unable to repeat our previous observation (16) of a rather small (up to 0.4 ml) water intake in mice after administration of the hypotensive agent isoproterenol either alone or after enalapril treatment. In summary, in these protocols the combination of high PRA and normal or low blood pressure did not stimulate reliable or increased fluid intake. Another unexpected observation for which we have no explanation is the inhibition rather than potentiation of hypovolemic thirst or sodium appetite in mice prefed low-sodium diet.

Perspectives

Collectively, the present observations indicate that the interactions among blood pressure, ANG II signaling, and drinking differ between rats and mice. This conclusion is consistent with our second working hypothesis, that blood-borne ANG II does have central effects in mice but that concurrent and as yet unidentified inhibitory mechanisms are more potent than in rats.

Although ANG II has effects on fluid homeostasis in a wide range of species (7, 12), it is probably true to say that its effects in rats are the most spectacular. If James Fitzsimons had originally studied mice instead of rats, we still might be unaware of the behavioral actions of ANG II. This in turn points to the pivotal but neglected role for comparative studies in the field. “The rat,” long dominant as a model species, now is being displaced by “the mouse.” It is evident from studies such as the present, as well as in other areas of neurobiology, that a mouse is not simply a small rat. Likewise, we study only a few select strains within these species. The coming-of-age of the powerful methods of molecular genetics requires that we embrace and understand species and strain differences that previously have drawn scant attention from the field and funding agencies.

REFERENCES

15. Rowland NE and Fregly MJ. Comparison of the effects of the dipeptidyl peptidase inhibitors captopril, ramipril, and enalapril...


