Brain-derived adrenomedullin controls blood volume through the regulation of arginine vasopressin production and release

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Taylor, Meghan M., Jennifer R. Baker, and Willis K. Samson. Brain-derived adrenomedullin controls blood volume through the regulation of arginine vasopressin production and release. Am J Physiol Regul Integr Comp Physiol 288: R000–R000, 2005.—Central nervous system-derived adrenomedullin (AM) has been shown to be a physiological regulator of thirst. Administration of AM into the lateral ventricle of the brain attenuated water intake, whereas a decrease in endogenous AM, induced by an AM-specific ribozyme, led to exaggerated water intake. We hypothesized that central AM may control fluid homeostasis, in part by regulating plasma arginine vasopressin (AVP) levels. To test this hypothesis, AM or a ribozyme specific to AM was administered intracerebroventricularly, and alterations in plasma AVP concentrations were examined under basal and stimulated (hypovolemic) conditions. Additionally, we examined changes in blood volume, kidney function, and plasma electrolyte and protein levels, as well as changes in plasma aldosterone concentrations. Intracerebroventricular administration of AM increased plasma AVP levels, whereas AM ribozyme treatment led to decreased plasma AVP levels under stimulated conditions. During hypovolemic challenges, AM ribozyme treatment led to an increased loss of plasma volume compared with control animals. Although overall plasma osmolality did not differ between treatment groups during hypovolemia, aldosterone levels were significantly higher and, consequently, plasma potassium concentrations were lower in AM ribozyme-treated rats than in controls. These data suggest that brain-derived AM is a physiological regulator of vasopressin secretion and, thereby, fluid homeostasis.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (200–250 g; Harlan, Indianapolis, IN) were housed individually with a 12:12-h light-dark cycle and given free access to food and water. All animal procedures were approved by the Saint Louis University Animal Care Committee. Right lateral cerebroventricular cannulas were implanted as previously described (31) in each animal under ketamine (60 mg/kg; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine anesthesia (8 mg/kg; TranquilVed, Vedco, St. Joseph, MO), and buprenorphine analgesia (0.05 mg/kg; Buprenex, Reckitt & Colman, Richmond, VA) was administered. Animals were allowed to recover for ≥5 days or until they returned to presurgery body weight. Cannula

POSTTRANSLATIONAL PROCESSING of the adrenomedullin (AM) gene product results in two biologically active proteins, proadrenomedullin NH2-terminal 20 peptide (PAMP) and AM. AM, a member of the calcitonin peptide family, is a vasoactive peptide that is produced in many peripheral tissues as well as in the central nervous system. The AM receptor calcitonin receptor-like receptor, in association with receptor activity-modifying protein 2 or 3 (1, 17), is also present in the central nervous system. The AM receptor calcitonin receptor-like receptor, in association with receptor activity-modifying protein 2 or 3 (1, 17), is also present in the central nervous system. The AM receptor calcitonin receptor-like receptor, in association with receptor activity-modifying protein 2 or 3 (1, 17), is also present in the central nervous system.

Here, the mechanisms by which brain-derived AM regulates fluid homeostasis are further investigated. During our thirst studies examining the effects of AM on water intake (40), the AM-specific ribozyme-treated rats tended to lose more body weight than controls during water restriction. One potential reason for the loss of more body weight by the AM ribozyme-treated animals is that they were less able than control animals to mount a compensatory response to the dehydration, leading to excess water loss. A major regulator of water homeostasis in the body is AVP; therefore, we hypothesized that central AM may control fluid homeostasis, in part by regulating plasma AVP levels. To examine this hypothesis, we administered AM or a ribozyme specific to AM intracerebroventricularly and examined alterations in plasma AVP under basal and stimulated conditions. Additionally, changes in blood volume, kidney function, plasma electrolyte and protein levels, and plasma aldosterone concentrations were examined under hypovolemic conditions.

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patency was tested by a dipsogenic response to 50 pmol of angiotensin II 2–3 days before experimentation.

**Peptides.** Rat AM, PAMP, and angiotensin II were purchased from Phoenix Pharmaceuticals (Belmont, CA). AVP and OT for RIA standards and iodination were also purchased from Phoenix Pharmaceuticals.

**Vasopressin.** Central AM-induced changes in plasma AVP levels were examined by intracerebroventricular administration of 2 μl of saline vehicle or vehicle containing 128, 256, or 768 pmol of AM or 256 pmol of PAMP. At 5, 10, 20, or 30 min after the injection, groups of rats were killed by rapid decapitation, and trunk blood was collected. Effects of decreased endogenous central AM content on plasma AVP levels were monitored by intracerebroventricular administration of a ribozyme specific to AM mRNA (40). Two treatment groups were employed: a group treated with 2-O-methylated control ribozyme (2 μg in 2 μl of saline) containing scrambled base antisense arms and a group treated with 2-O-methylated AM-specific ribozyme (2 μg in 2 μl of saline) (40). At 18 h after ribozyme administration, when hypothalamic AM protein levels were decreased by ~30% (40), rats were killed by decapitation, and trunk blood was collected as described above. Because decreased plasma levels of AVP were predicted after central AM ribozyme treatment, AVP levels were elevated in rats through two separate paradigms: 18 h of water restriction and administration of 15% (wt/vol) polyethylene glycol (PEG; Carbowax PEG 20,000, Fisher Scientific, Pittsburgh, PA) solution in saline subcutaneously in conjunction with 18 h of water and food restriction (37). PEG-treated rats were anesthetized by isoflurane gas (3% in O2 for induction, 2% in O2 for maintenance of anesthesia; IsoSol, Vedco) inhalation, a small area on their back was shaved and cleaned with surgical scrub, and the rats were injected subcutaneously with 5 ml of warmed (37°C) PEG solution. In each water-restriction paradigm, one group of rats was treated intracerebroventricularly with the control ribozyme and the other group was treated with the AM-specific ribozyme at the same time as PEG treatment and/or water bottle removal. At 18 h after ribozyme administration, rats were killed by decapitation, and trunk blood was collected into heparinized tubes. Plasma was obtained from all samples by centrifugation (3,000 g for 10 min at 4°C) of whole blood samples and frozen until AVP and OT levels were measured by RIA as previously described (26, 30). AVP and OT data are expressed as means ± SE. For the AM dose-response experiment and the analysis of significance between ribozyme experiments, significance was determined by ANOVA with Bonferroni’s post hoc test. AM time course (at each time point), PAMP, and within-treatment (i.e., euvoelmia, water restriction, or PEG) ribozyme experiments were analyzed by independent-sample t-tests, with P < 0.05 considered significant.

**Hypothalamoneurohypophysial system explants.** Rats (300–350 g) were killed by rapid decapitation, and a tissue explant containing the SON projection to the posterior lobe of the pituitary was dissected as previously described (27, 36). The rostral limit of the explant was the suprachiasmatic-supraoptic continuum. The caudal limit was the arcuate nucleus at the exit of the pituitary stalk. Included in the hypothalamoneurohypophysial system (HNS) explants are the SON, ventral portions of the anterior hypothalamus and medial preoptic area, and the rostral pole of the arcuate nucleus. The explants were immersed in 2 ml of Dulbecco’s minimum essential medium (Bio-Whittaker, Walkersville, MD) containing 0.1% BSA (Sigma, St. Louis, MO) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA) and incubated at 37°C in 5% CO2. The medium was changed every hour for 4 h before experimentation. Six consecutive 20-min test periods were conducted on each explant as follows: control medium (test period 1), control medium (test period 2), 100 nM AM in control medium (test period 3), control medium (test period 4), control medium (test period 5), and 60 mM KCl in control medium (test period 6). Medium from each test period was collected and frozen until measurement of AVP and OT content by RIA (27). At the end of testing, explants were weighed. Weights were similar among all explants (49.1 ± 3.1 mg, n = 8). Peptide release during test periods 2–6 was expressed as percentage of AVP or OT released in test period 1, and differences among the groups were determined by ANOVA with Bonferroni’s post hoc test.

**AVP mRNA quantitation.** The control ribozyme or AM ribozyme was administered, and the rats were allowed free access to water, permitted no water, or treated with PEG as described above. After 18 h, the rats were rapidly decapitated, and hypothalami were dissected from the brains. Hypothalamic tissue was homogenized in 1 ml of TRIZol Reagent (Invitrogen), and total RNA was collected according to product instructions. AVP mRNA levels were examined by RT-PCR. Equal total RNA amounts (determined by spectrophotometry) were used to amplify AVP or OT mRNA with the Superscript One-Step RT-PCR system (Invitrogen) using AVP- or OT-specific primers (34), and β-actin mRNA levels were used as a control (8). Expected product sizes are as follows: 417 bp (AVP), 422 bp (OT), and 380 bp (β-actin). The cDNA products of the RT-PCR were run on a 1.5% agarose gel for separation and stained with ethidium bromide for visualization and photography with a Kodak Gel Documentation System.

**Urinary output and water and food intake.** Rats (250–280 g) previously implanted with intracerebroventricular cannulas were housed in metabolic cages (Nalgene) and allowed to acclimate for 4 days. On the morning of experimentation, rats received an intracerebroventricular injection of control ribozyme or AM ribozyme (2 μg in 2 μl saline), and total ad libitum food and water intake was monitored hourly for 48 h. At 24 h after the initial ribozyme administration, rats received a second identical ribozyme injection to ensure that brain levels of AM remained decreased. Urine was collected in 12-h increments over the entire experimental period, and urine volume and urine osmolality (freezing-point depression; μOsmette, Precision Systems, Natick, MA) were measured for each sample. Total food and water intake were analyzed hourly by independent t-tests. Urine volume and osmolality were also analyzed by t-test, with P < 0.05 considered significant.

**Blood chemistry.** Rats were anesthetized by isoflurane gas inhalation (3% in O2 for induction, 2% in O2 for maintenance of anesthesia). A blood sample (0.5 ml) was removed from the tail artery and collected into serum separator tubes (Microtainer Brand Amber tubes with serum separator, Fisher Scientific). A microhematocrit tube was filled with blood and centrifuged for hematocrit determination, and the remainder of the blood sample was placed on ice for ~30 min. The animals were then treated with control or AM ribozyme, and one group was injected subcutaneously with PEG as described above. PEG- and non-PEG-treated animals were subjected to water restriction for 18 h (PEG-treated animals were also denied food) before a second blood sample was removed under isoflurane anesthesia. Blood samples were separated by centrifugation and stored at 4°C until they were assayed. Plasma protein, sodium, potassium, chloride, creatinine, and blood urea nitrogen levels were assayed by the Saint Louis University Department of Comparative Medicine Clinical Pathology Laboratory using a Roche Cobas Mira Plus CC. Plasma osmolality was assayed by freezing-point depression (μOsmette). Plasma aldosterone was assayed with a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Significance was analyzed by independent t-tests between control and ribozyme-treated groups and by paired t-tests within each group before and after water restriction or PEG treatment.

**RESULTS**

**Vasopressin.** Central administration of AM led to a dose-related increase in plasma AVP levels 10 min after AM administration (Fig. 1A), which was the time at which plasma AVP levels peaked (Fig. 1B). Central administration of PAMP did not significantly alter plasma AVP levels: 1.03 ± 0.30 (n = 10) and 0.78 ± 0.20 (n = 16) for control and PAMP, respec-
Plasma OT levels were not significantly different between animals treated with 256 pmol AM or 256 pmol PAMP or saline-treated controls at any time (data not shown). AVP (Fig. 2) or OT (data not shown) secretion rate was not altered in HNS explants in response to 100 nM AM; however, AVP and OT secretion was significantly increased in response to a depolarizing concentration of KCl (both $P < 0.001$), indicating that the SON/posterior pituitary projections were intact and functional.

Scrambled ribozyme treatment (Fig. 3) did not alter basal AVP levels compared with saline-treated controls (Fig. 1A), and AVP levels were similar to those previously reported (12, 28, 41). Ribozyme compromise of brain-derived AM in euvolemic rats tended to reduce circulating AVP levels ($P = 0.112$; Fig. 3). However, the effect of central AM reduction on plasma AVP was better seen under stimulated conditions. Intracerebroventricular administration of AM ribozyme followed by 18 h of water restriction significantly decreased plasma AVP content compared with scrambled riboyme-treated controls ($P < 0.05$; Fig. 3). Plasma AVP levels were similar in water-restricted rats treated with the scrambled ribozyme and in saline-treated, water-restricted rats (12). After 18 h of water restriction, AM ribozyme-treated rats tended to lose more weight than control ribozyme-treated rats ($-6.4 \pm 0.4\%$ and $-5.8 \pm 0.5\%$, respectively); however, the weight loss never attained significance. A second paradigm that elevates plasma AVP is PEG treatment in conjunction with food and water restriction, which induces an isotonic hypovolemia (37). At 18 h after 15% PEG and ribozyme administration, the AVP response was significantly lower in AM ribozyme-treated rats than in control animals ($P < 0.05$; Fig. 3). Again, scrambled ribozyme treatment did not alter the AVP response to PEG treatment (4, 14). AM ribozyme treatment did not alter plasma OT levels between treatment groups after water restriction or PEG treatment (data not shown).

AM ribozyme pretreatment did not alter AVP mRNA levels under basal or water-restricted conditions (Fig. 4). However, AVP mRNA levels were dramatically reduced in animals treated controls ($P < 0.05$; Fig. 3). Plasma AVP levels were similar in water-restricted rats treated with the scrambled ribozyme and in saline-treated, water-restricted rats (12). After 18 h of water restriction, AM ribozyme-treated rats tended to lose more weight than control ribozyme-treated rats ($-6.4 \pm 0.4\%$ and $-5.8 \pm 0.5\%$, respectively); however, the weight loss never attained significance. A second paradigm that elevates plasma AVP is PEG treatment in conjunction with food and water restriction, which induces an isotonic hypovolemia (37). At 18 h after 15% PEG and ribozyme administration, the AVP response was significantly lower in AM ribozyme-treated rats than in control animals ($P < 0.05$; Fig. 3). Again, scrambled ribozyme treatment did not alter the AVP response to PEG treatment (4, 14). AM ribozyme treatment did not alter plasma OT levels between treatment groups after water restriction or PEG treatment (data not shown).

AM ribozyme pretreatment did not alter AVP mRNA levels under basal or water-restricted conditions (Fig. 4). However, AVP mRNA levels were dramatically reduced in animals...
treated with AM ribozyme and then with PEG (Fig. 4). OT mRNA levels were unaltered by AM ribozyme treatment (Fig. 4).

**Urine output and water and food intake.** Urine volume was significantly increased in AM ribozyme-treated rats compared with controls (Fig. 5A), as would be predicted by the decreased plasma AVP levels. Additionally, urine osmolality tended to be lower in AM ribozyme-treated rats but was significantly lower only during the initial 12-h collection (Fig. 5B). AM ribozyme-treated rats drank significantly more water than control rats beginning 5 h after the initial ribozyme treatment, and water intake remained elevated over most of the remainder of the experiment (Fig. 5C). Food intake was also significantly higher in the AM ribozyme-treated rats than in controls but did not attain significance until 14 h after the initial ribozyme treatment (Fig. 5D).

**Blood chemistry.** Before AM ribozyme or control ribozyme treatment, there were no significant differences between the two groups in terms of hematocrits, plasma protein levels, or osmolalities (Table 1). Central ribozyme treatment in the euvoletic rat did not alter hematocrit, plasma protein levels, or plasma osmolality. After 18 h of water restriction, hematocrits, plasma protein levels, and plasma osmolalities were elevated in control and AM ribozyme-treated animals. However, AM ribozyme treatment led to even higher (P < 0.05) hematocrits and plasma protein levels than control treatment. Plasma osmolality was not significantly different between control and AM ribozyme-treated animals after water restriction (Table 1). Similarly, hematocrits, plasma protein levels, and osmolalities were elevated in both treatment groups after PEG administration, and hematocrits and plasma protein levels were higher still in the AM ribozyme than in the control animals (P < 0.05) with no differences in osmolalities between groups (Table 1). AM ribozyme treatment did not alter plasma sodium, chloride, or potassium levels in euvoletic rats (Table 2). Plasma sodium and chloride concentrations were similar between AM and control ribozyme treatment groups before and after water restriction and PEG treatment (Table 2). Plasma potassium levels, however, were significantly lower in the AM ribozyme group under water restriction and PEG treatment conditions (Table 2).

**Aldosterone and creatinine.** Basal aldosterone levels were similar in AM and control ribozyme treatment groups (Fig. 6). After water restriction, however, in AM ribozyme-treated rats, plasma aldosterone levels were significantly elevated compared with control ribozyme-treated rats and compared with

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**Fig. 4.** Alterations in hypothalamic AVP and oxytocin (OT) mRNA levels induced by ribozyme compromise of brain-derived adrenomedullin (AM). Co, control ribozyme. β-Actin levels are shown as a loading control.

**Fig. 5.** Ribozyme compromise of brain-derived AM increased urine excretion (A), decreased urine osmolality (B), and increased water (C) and food (D) intake in rats. Values are means ± SE; n = 12 (control) and 14 (AM ribozyme). *P < 0.05 vs. control ribozyme.
Central administration of AM caused a dose-related increase in plasma AVP concentrations (Fig. 1A) that was relatively short-lived, peaking at 10 min after injection and returning to baseline by 30 min (Fig. 1B). Our findings agree with those of Yokoi et al. (45), who showed no changes in plasma AVP levels at 30 min after intracerebroventricular administration of AM (1 μg). However, our findings contradict other findings of

Table 1. Differences in hematocrit, plasma protein, and plasma osmolality induced by loss of brain-derived AM

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<th>Before Treatment</th>
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<tr>
<td></td>
<td>Euvolemia</td>
<td>Water restriction</td>
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<tr>
<td>Hematocrit, %</td>
<td>Control 45.1±0.6</td>
<td>44.5±0.9</td>
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<tr>
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<td>AM ribozyme 44.3±0.6</td>
<td>43.8±1.1</td>
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<tr>
<td>Protein, g/dl</td>
<td>Control 5.7±0.1</td>
<td>5.5±0.2</td>
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<tr>
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<td>AM ribozyme 5.9±0.1</td>
<td>5.7±0.1</td>
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<tr>
<td>Osmolality, mOsm</td>
<td>Control 288.9±1.8</td>
<td>291.6±1.3</td>
</tr>
<tr>
<td></td>
<td>AM ribozyme 291.4±1.7</td>
<td>292.1±1.9</td>
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<tr>
<td></td>
<td>PEG treatment</td>
<td>PEG treatment</td>
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<tr>
<td>Hematocrit, %</td>
<td>Control 45.4±0.5</td>
<td>52.6±0.7‡</td>
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<td>AM ribozyme 45.6±0.5</td>
<td>54.4±0.5*‡</td>
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<tr>
<td>Protein, g/dl</td>
<td>Control 5.6±0.1</td>
<td>7.1±0.1‡</td>
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<td>AM ribozyme 5.8±0.1</td>
<td>7.3±0.1*‡</td>
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<tr>
<td>Osmolality, mOsm</td>
<td>Control 292.0±1.4</td>
<td>322.6±3.2‡</td>
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<td></td>
<td>AM ribozyme 293.3±2.5</td>
<td>323.6±3.3‡</td>
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Values are means ± SE; n = 10. A blood sample was taken from the tail artery of anesthetized rats before intracerebroventricular administration of a control ribozyme or an adrenomedullin (AM)-specific ribozyme. One group was left untreated, another was water restricted, and a third was given 5 ml of 15% (wt/vol) polyethylene glycol (PEG) subcutaneously with food and water restriction. After 18 h of treatment, a second blood sample was taken. Before treatment, there were no significant differences between groups in any parameter analyzed. *P < 0.05 vs. control after treatment. †P < 0.05 vs. control ribozyme.## wartości średnich ± SE; n = 10. Wzmacnianie zębia z leczenia przed wprawianiem jonu wody intracerebroventricular administration of a control ribozyme and an adrenomedullin (AM)-specific ribozyme. One group was left untreated, another was water restricted, and a third was given 5 ml of 15% (wt/vol) polyethylene glycol (PEG) subcutaneously with food and water restriction. After 18 h of treatment, a second blood sample was taken. Before treatment, there were no significant differences between groups in any parameter analyzed. *P < 0.05 vs. control after treatment. †P < 0.05 vs. control ribozyme.## wartości średnich ± SE; n = 10. Wzmacnianie zębia z leczenia przed wprawianiem jonu wody

Table 2. Differences in plasma electrolytes induced by loss of brain-derived AM

<table>
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<tr>
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<th>Before Treatment</th>
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<tr>
<td></td>
<td>Euvolemia</td>
<td>Water restriction</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>Control 138.9±1.0</td>
<td>138.9±1.0</td>
</tr>
<tr>
<td></td>
<td>AM ribozyme 140.2±0.2</td>
<td>139.1±0.5</td>
</tr>
<tr>
<td>Cl⁻, mmol/l</td>
<td>Control 102.6±0.4</td>
<td>101.6±0.4</td>
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<td></td>
<td>AM ribozyme 102.3±0.4</td>
<td>101.3±0.5</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>Control 5.7±0.3</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td></td>
<td>AM ribozyme 5.5±0.1</td>
<td>5.8±0.1</td>
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<tr>
<td></td>
<td>PEG treatment</td>
<td>PEG treatment</td>
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<tr>
<td>Na⁺, mmol/l</td>
<td>Control 136.8±0.6</td>
<td>137.6±0.8</td>
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<td></td>
<td>AM ribozyme 139.8±0.7</td>
<td>138.3±0.7</td>
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<tr>
<td>Cl⁻, mmol/l</td>
<td>Control 104.2±0.5</td>
<td>102.2±0.9</td>
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<td>AM ribozyme 105.8±0.5</td>
<td>103.7±0.5</td>
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<tr>
<td>K⁺, mmol/l</td>
<td>Control 6.0±0.2</td>
<td>6.7±0.2‡</td>
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<tr>
<td></td>
<td>AM ribozyme 5.7±0.2</td>
<td>5.8±0.2*</td>
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Values are means ± SE; n = 10. See table 1 footnote for procedure. Before treatment, there were no significant differences between groups in any parameter analyzed. *P < 0.005; †P < 0.001 vs. control after treatment. ‡P < 0.05 vs. before treatment.

their basal aldosterone level (Fig. 6). Aldosterone levels were not elevated after 18 h of water restriction in control ribozyme-treated rats. Plasma aldosterone levels were significantly elevated compared with basal aldosterone levels after PEG treatment in control and AM ribozyme-treated rats (P < 0.001; Fig. 6). Aldosterone levels were significantly (P < 0.05) higher in AM ribozyme-treated rats, however, than in control-treated rats after PEG treatment. Water restriction did not alter plasma creatinine concentration in the control or AM ribozyme-treated groups: 0.5 ± 0.02 and 0.5 ± 0.01 mg/dl, respectively, in euvolemic and 0.5 ± 0.02 and 0.5 ± 0.02 mg/dl, respectively, in water restriction (n = 10 each). PEG treatment significantly elevated plasma creatinine content (P < 0.001 vs. control euvolemic animals), but levels in control sample and AM ribozyme treatment groups did not differ: 0.9 ± 0.06 and 0.9 ± 0.07 mg/dl, respectively (n = 10 each).

DISCUSSION

Central administration of AM caused a dose-related increase in plasma AVP concentrations (Fig. 1A) that was relatively short-lived, peaking at 10 min after injection and returning to baseline by 30 min (Fig. 1B). Our findings agree with those of Yokoi et al. (45), who showed no changes in plasma AVP levels at 30 min after intracerebroventricular administration of AM (1 μg). However, our findings contradict other findings of
Yokoi et al., namely, that a single dose of AM (1 µg) inhibited AVP release in response to a hypovolemic challenge at a single time point (30 min). We believe that our findings represent a more physiologically relevant action of central AM, because we not only demonstrate time and dose dependency of the central AM stimulation of AVP (Fig. 1), but we were able to show that compromise of endogenous AM inhibited AVP release in response to a hypovolemic challenge (Fig. 3). Plasma OT levels were not significantly different from controls after central AM administration (data not shown), findings in agreement with those of Mimoto et al. (18). Although another group reported that intracerebroventricular administration of AM increased plasma OT concentrations, the effect was seen only when very high doses (10 µg) of AM were administered (32). Moreover, the effect of this high dose of AM was very transient, with OT levels returning to baseline by 15 min and probably reflected a general stress reaction caused by administration of a concentrated substance, rather than a specific action of AM on OT-containing neurons. Therefore, these results suggest that AM’s actions in neurohypophysial hormone secretion are specific to AVP. The effects of AM on AVP secretion are likely due to the activation of a series of neurons leading to depolarization of SON and PVN neurons projecting to the neural lobe. Evidence supporting this conclusion includes the fact that AM did not cause the release of AVP from HNS explants (Fig. 2) and that bath application of AM to brain slices led to a rapid hyperpolarization of magnocellular PVN neurons (6), which would cause an inhibition, and not a stimulation, of AVP release, as seen here.

We next used a ribozyme to compromise AM production and determine whether the actions of endogenous, brain-derived AM to stimulate AVP are physiologically relevant. We previously showed that a single intracerebroventricular injection of the AM ribozyme is able to reduce hypothalamic AM peptide content by 30%, and these ribozymes were used to establish the physiological relevance of AM to regulate thirst (40). Here, it was demonstrated that, under basal conditions, central AM ribozyme administration had a tendency to decrease plasma AVP levels (Fig. 3), a finding that complements the results of increased AVP release after intracerebroventricular administration of AM (Fig. 1). It is not surprising that the AM ribozyme-induced inhibition of AVP levels did not attain statistical significance in euvolemic rats, because normal plasma AVP levels are so low that it is nearly impossible to detect drops in normal plasma AVP levels with the current methods used to measure the hormone.

An inhibition of AVP release due to loss of central AM could be better seen under conditions that normally stimulate AVP release, such as hypovolemia. Water restriction for 18 h led to a mild-to-moderate dehydration in rats (as seen in increases in plasma protein levels and hematocrit, Table 1), thereby causing a significant elevation in plasma AVP levels (Fig. 3). A single injection of AM ribozyme almost completely attenuated this rise in plasma AVP. This suggested that, during overnight water restriction, AM ribozyme-treated rats may have lost more water through urine excretion than control rats because of lower AVP levels. In fact, AM ribozyme treatment did result in increased urine output, even in euvolemic rats. This would also explain the observation that AM ribozyme-treated rats tended to lose more body weight during overnight water restriction. A more severe intravascular fluid loss than that caused by water restriction alone can be obtained by subcutaneous administration of PEG in conjunction with food and water restriction. PEG is a colloid that draws isotonic plasma from the blood vessels and is, in this manner, similar to a hemorrhage (37). PEG treatment led to a dramatic increase in plasma AVP levels that was significantly attenuated by AM ribozyme pretreatment (Fig. 3).

Endogenous AM can control plasma AVP levels via two mechanisms: regulation of AVP secretion rate and regulation of AVP protein production. No differences were seen in AVP transcript levels between AM ribozyme-treated animals and controls under euvoletic or water-restricted conditions; however, AM ribozyme treatment reduced AVP mRNA levels after PEG treatment (Fig. 4). It may be that alterations in AVP mRNA levels after PEG treatment reflect the strength of the stimulus and, thereby, the significant upregulation of the AVP system (see control levels in Fig. 3). It would be predicted then that diminished AVP mRNA levels could eventually be seen under conditions of a more permanent compromise of central AM production in euvolemic and water-restricted rats, which have a theoretically lower production rate of AVP than PEG-treated rats. Data from HNS explants (Fig. 2) and single-cell recordings from magnocellular PVN neurons (6) indicate that the stimulatory effects of AM on AVP secretion are exerted indirectly through interneurons, suggesting that transcriptional control of AVP by AM may also be through indirect actions.

The decrease in plasma AVP levels after central AM compromise is likely the reason for the development of increased urine output (Fig. 5A), decreased urine osmolality (Fig. 5B), and increased water intake (Fig. 5C) in AM ribozyme-treated rats. Basal food intake was also elevated in AM ribozyme-treated animals (Fig. 5D); however, this is most likely a compensatory mechanism for the increase in water intake that would then maintain electrolyte balance. Alternatively, the trend toward altered food intake may reflect a physiological action of AM and PAMP to inhibit food intake, as is seen for both peptides when they are administered intracerebroventricularly in pharmacological doses (22, 39). This increased food intake may be the reason for the significant decrease in urine osmolality in AM ribozyme-treated rats only during the first 12 h of experimentation.

AVP is a major regulator of fluid excretion; therefore, alterations in this hormone can have significant effects on fluid homeostasis within the body. Water restriction and PEG treatment led to increases in hematocrit, plasma protein levels, and osmolality (Table 1), suggesting that these treatments decreased blood volume. Although PEG treatment is often thought to cause an isotonic hypovolemia, the treatment does in fact lead to alterations in plasma osmolality. The fluid withdrawn into the subcutaneous PEG depot is isotonic; however, the hypovolemic state of PEG-treated animals leads to a decrease in kidney function (see below). Therefore, the PEG-treated animals become oliguric, often anuric, and because of the attendant uremia, their plasma osmolality is elevated. Although water restriction and PEG treatment lead to apparently decreased blood volume in all animals, hematocrits and plasma protein levels were significantly higher in the AM ribozyme-treated rats than in controls, indicating an even greater loss of blood volume than in control ribozyme-treated rats (Table 1).
Even though overall osmolality did not differ between treatment groups (Table 1) when plasma concentrations of individual electrolytes were examined, significant changes were observed. Plasma sodium and chloride, the major electrolytes in plasma, did not significantly differ between AM and control ribozone-treated groups after water restriction or PEG (Table 2). However, AM ribozone treatment led to significantly decreased plasma potassium concentrations after water restriction and PEG treatment (Table 2). This prompted an examination of plasma aldosterone levels in these animals. The primary function of aldosterone, a hormone produced by the adrenal gland, is to regulate body fluid homeostasis by increasing sodium and, thus, water reabsorption from the kidney at the cost of potassium ions. Not surprisingly, plasma aldosterone levels were elevated in AM ribozone-treated rats after water restriction and PEG treatment (Fig. 6). It is not clear whether brain-derived AM directly regulates aldosterone secretion. However, a more likely explanation for the elevated aldosterone levels in AM ribozone-treated rats is that it is merely a compensatory mechanism secondary to the induced hypovolemia.

Finally, to ensure that the effects of central AM loss on blood volume were not due to alterations in kidney function, we examined plasma creatinine levels. Water restriction did not alter plasma creatinine concentrations, and there was no difference between AM and control ribozone-treated groups. PEG treatment, as expected (37), decreased kidney function, which resulted in elevated plasma creatinine levels in AM and control ribozone treatment groups. Again there was no statistical difference between AM and control ribozone treatment groups.

The studies presented here suggest that brain-derived AM is a physiologically relevant regulator of AVP secretion. A true understanding of the role of a peptide like AM comes only when the production or action of endogenous peptide is compromised. The pharmacological data on the AM-induced stimulation of AVP release are matched by a decrease in plasma AVP levels caused by a loss of endogenous AM, thereby supporting our hypothesis that brain-derived AM is a true physiological regulator of AVP secretion. Although the ribozone construct decreases AM as well as PAMP protein levels, central PAMP administration did not alter AVP secretion or thirst (19). It was, therefore, concluded that the effects of the AM ribozone on AVP secretion are most likely due to loss of the positive drive for hormone secretion normally expressed by endogenous AM.

The data presented here, in combination with previously published data (2, 3, 5, 10, 15, 25, 29, 31, 39, 40, 44), indicate that AM is a physiological regulator of fluid and electrolyte homeostasis. It is important to determine whether brain-derived AM exerts long-term control of AVP secretion and production. Certainly, data indicating that ribozone compromise of central AM for 48 h led to moderate increases in water intake and urine output would suggest that AM may be an important long-term regulator of AVP. Data on plasma AVP levels in AM heterozygote (+/−) mice have not been reported, and there are no models of long-term postdifferentiation and/or site-specific compromise of AM. However, understanding the physiological role of brain-derived AM becomes increasingly important, because the peptide is being examined as a potential therapeutic agent for the treatment of septic shock (43) and a variety of cardiovascular disorders, including essential hypertension (7, 11, 38), myocardial infarction (13, 20), atherosclerosis (35), and congestive heart failure (9, 21, 24).

REFERENCES


