Effects of homologous atrial natriuretic peptide on drinking and plasma ANG II level in eels


Takamasa Tsuchida and Yoshio Takei. Effects of homologous atrial natriuretic peptide on drinking and plasma ANG II level in eels. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1605–R1610, 1998.—The effects of eel atrial natriuretic peptide (ANP) on drinking were investigated in eels adapted to freshwater (FW) or seawater (SW) or in FW eels whose drinking was stimulated by a 2-ml hemorrhage. An intra-arterial infusion of ANP (0.3–3.0 pmol · kg⁻¹ · min⁻¹), which increased plasma ANP level 1.5–20-fold, inhibited drinking dose dependently in all groups of eels. The drinking rate recovered to the level before ANP infusion within 2 h after infusate was replaced by saline. The inhibition at 3.0 pmol · kg⁻¹ · min⁻¹ was profound in FW eels and hemorrhaged FW eels, whereas significant drinking still remained after inhibition in SW eels. Plasma ANG II concentration also decreased dose dependently during ANP infusion and recovered to the initial level after saline infusion in all groups of eels. The decrease at 3.0 pmol · kg⁻¹ · min⁻¹ was large in FW eels and hemorrhaged FW eels compared with that of SW eels. Thus the changes in drinking rate and plasma ANG II level were parallel during ANP infusion. Plasma sodium concentration and osmolality decreased during ANP infusion in SW and FW eels, and they were restored after saline infusion. In hemorrhaged FW eels, however, ANP infusion did not alter plasma sodium concentration and osmolality. Hematocrit did not change during ANP infusion in any group of eels. Collectively, ANP infusion at physiological doses decreased drinking rate and plasma ANG II concentration in parallel in both FW and SW eels. It remains undetermined whether the inhibition of drinking is caused by direct action of ANP or through inhibition of ANG II, which is known as a potent dipsogen in all vertebrate species, including eels.

Water intake; Anguilla japonica

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MATERIALS AND METHODS

Animals. Cultured Japanese eels, Anguilla japonica, were purchased from a local dealer. They were kept in a 1-ton FW tank for >1 wk before use (FW-adapted eels). Some eels were transferred to a 0.5-ton SW tank and acclimated there for >2 wk before use (SW-adapted eels). Water in the tank was continuously filtered, aerated, and thermoregulated at 18 ± 0.5°C. Eels were not fed after purchase. They weighed 181.5 ± 2.8 g (mean ± SE, n = 24) at the time of experiment.

Surgical procedures. Eels were anesthetized on 0.1% (wt/vol) tricaine methanesulfonate (Sigma, St. Louis, MO) neutralized with sodium bicarbonate for 10 min. Then vinyl tubes (1.5 mm OD) were inserted into the esophagus and stomach of eels as described previously (39). The esophageal catheter was used for measurements of drinking rate, and the stomach catheter was used for reintroduction of drunk water. In addition, polyethylene tubes (0.8 mm OD) were inserted into the dorsal and ventral aorta for infusion of drugs and blood collection, respectively. The eels that bled >0.05 ml (0.7% of blood volume) during surgery were excluded from the experiment.

After surgery, eels were transferred to a plastic trough through which aerated and thermoregulated (18°C) water constantly circulated (Fig. 1). The catheter placed in the esophagus was connected to a drop counter for continuous
measurement of drinking rate. The drunk water that dropped from the esophageal catheter (0.03 ml/drop) was reintroduced into the stomach with FW (for FW-adapted fish) or 80% SW (for SW-adapted fish) by means of a pulse injector synchronized with the drop counter (39). Eighty-percent SW was used for SW fish because drunk SW that dropped from the esophageal catheter was diluted to 78.7 ± 0.02% (n = 4) because of desalting by the esophagus (13). The catheters in the aortas were connected to plastic syringes filled with 0.9% NaCl solution. The syringe connected to the dorsal aorta was set in the infusion pump for ANP infusion (Fig. 1). Eels were allowed to recover for 18 h postoperatively.

Experimental protocol. Three groups of eels were used in this experiment: 1) FW-adapted eels, 2) FW eels subjected to 2 ml of hemorrhage (28.6% blood volume) on the previous day, and 3) SW-adapted eels. The second group was prepared because drinking rate of FW eels was too low to demonstrate clear inhibition, and because hypovolemia is a potent stimulus for drinking in the eel (11). The eels received hourly infusions of isotonic saline followed by increasing doses of eel ANP (Peptide Institute, Osaka, Japan) at 0.3, 1.0, and 3.0 pmol kg⁻¹ min⁻¹ and ended with saline infusion for 2 h. Infusion rate was 0.3 ml/h, whereas 0.6 ml of blood was sampled every hour from the ventral aorta into the chilled plastic syringe containing 10% 2K-EDTA (10 µl/ml blood). Fifty microliters of collected blood were transferred into a capillary for measurements of hematocrit and plasma sodium concentration and osmolality. After centrifugation of the blood sample, plasma was used for measurements of eel ANP and ANG II by radioimmunoassay. The blood cells were washed two times with 1 ml 0.9% saline, reconstituted with 0.3 ml of saline, and injected into the circulation within 5 min after blood sampling. Each drop from the esophageal catheter was monitored as a spike in the recorder, and water intake every 10 min was stored in the memory of the drop counter (Fig. 1). The details of the drop counter and pulse injector system have been described elsewhere (39).

Eel ANP concentration was determined by homologous radioimmunoassay (32). Eel ANG II concentration was measured by radioimmunoassay for mammalian [Asp¹, Ile⁵] ANG II (42). The antibody used in this assay exhibited 75% cross-reactivity with eel [Asn¹, Val⁵] ANG II (Fig. 2). ¹²⁵I-labeled [Asn¹, Val⁵] ANG II was prepared by a lactoperoxidase method and isolated by reverse-phase HPLC (ODS-120T column, 0.46 × 25 cm; Tosoh, Tokyo, Japan) with a linear gradient of CH₃CN from 15 to 60% for 60 min as used for iodination of eel ANP (32). The HPLC completely separated monoiodinated ANG II from unlabeled and diiodinated ANG II. The dilution curve of immunoreactive ANG II in eel plasma was parallel to the standard curve of eel ANG II (Fig. 2). ANG II concentration was measured directly with 40 µl of eel plasma, because recovery of synthetic eel ANG II added to the plasma was 93.2 ± 7.8% (n = 4) and because the values of direct measurement and after extraction of acidic acetone (27) do not differ (95.5 ± 4.7%, n = 4). Furthermore, reverse-phase HPLC of extracted eel plasma revealed that the immunoreactive ANG II was detected only at the elution position identical to [Asn¹, Val⁵] ANG II (data not shown). The intra-assay and interassay coefficients of variation were 5.8 and 14.0%, respectively.

The sodium concentration in plasma was determined in an atomic absorption spectrophotometer (Hitachi 180-50, Tokyo, Japan), and plasma osmolality was determined in a vapor pressure osmometer (Wescor 5500, Logan, UT). All determinations were made in duplicate or triplicate.

Analysis of data. Student's t-test was applied for comparison of different groups of eels. A paired t-test or Fisher's exact probability test was used for analysis of time course data. Significance was set at P < 0.05. All results are expressed as means ± SE.

Fig. 1. Experimental setup for continuous measurement of drinking rate in an esophagus- and stomach-cannulated eel. Each drop of drunk water was reintroduced into the stomach by a pulse injector synchronized with a drop counter. Catheters in dorsal and ventral aortas were used for ANP infusion and blood sampling, respectively.

Fig. 2. Standard curve for radioimmunossay of eel [Asn¹, Val⁵] ANG II (A) using ¹²⁵I-labeled eel ANG II. Displacement curve of human [Asp¹, Ile⁵] ANG II (●), against which antiserum was originally raised, was also given. Serial dilution curve of eel plasma (●) was parallel with standard curve of eel ANG II.
RESULTS

Plasma ANP concentration. Plasma ANP concentration increased dose dependently during ANP infusion in all groups of eels (Table 1). After infusate was changed back to saline, plasma ANP level was still high for 1 h but fell to the initial level in 2 h.

Changes in drinking rate. Drinking rate was very low in FW eels; however, ANP infusion decreased it to zero (Fig. 3A). The drinking rate recovered gradually during saline infusion, and it rebounded above the initial level after 2 h. The dose-dependent inhibition was more apparent in hemorrhaged FW eels, which exhibited greater initial drinking, and eels drank practically no water at 3.0 pmol·kg⁻¹·min⁻¹ (Fig. 3B). ANP also inhibited copious drinking of SW eels in a dose-dependent manner (Fig. 3C). However, the inhibition was small, so that SW eels drank more water than FW eels and hemorrhaged FW eels at 3.0 pmol·kg⁻¹·min⁻¹.

The inhibition disappeared after saline infusion also in SW eels.

Changes in plasma ANG II level. Plasma ANG II concentration decreased dose dependently during ANP infusion, and it was restored to the initial level after infusate was replaced by saline in all groups of eels (Fig. 4). However, the percentage of decrease was much smaller in SW eels compared with that of FW eels and hemorrhaged FW eels.

Plasma sodium concentration, osmolality, and hematocrit. Plasma sodium concentration and osmolality were higher in SW eels than in FW eels and hemorrhaged FW eels (Fig. 5). Plasma sodium concentration and osmolality decreased dose dependently in both FW and SW eels during the ANP infusion, and the levels were restored gradually after saline infusion. However, there were no changes in plasma sodium concentration and osmolality in hemorrhaged FW eels. Hematocrit did not change during ANP infusion in all groups.

Table 1. Changes in plasma ANP concentration (fmol/ml) during eel ANP infusion in FW, HFW, and SW eels

<table>
<thead>
<tr>
<th>Eels</th>
<th>n</th>
<th>Saline (0)</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>Saline (0)</th>
<th>Saline (0)</th>
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<tr>
<td>FW</td>
<td>8</td>
<td>67.0 ± 5.6</td>
<td>112.4 ± 45.0</td>
<td>396.2 ± 80.1</td>
<td>1,532.7 ± 294.5</td>
<td>327.8 ± 107.9</td>
<td>101.6 ± 30.5</td>
</tr>
<tr>
<td>HFW</td>
<td>8</td>
<td>68.9 ± 16.1</td>
<td>100.7 ± 20.0</td>
<td>302.5 ± 55.8</td>
<td>1,616.5 ± 271.0</td>
<td>510.7 ± 159.4</td>
<td>67.8 ± 31.6</td>
</tr>
<tr>
<td>SW</td>
<td>8</td>
<td>73.3 ± 14.9</td>
<td>193.7 ± 45.4</td>
<td>468.1 ± 58.0</td>
<td>1,713.2 ± 400.8</td>
<td>766.1 ± 265.8</td>
<td>109.2 ± 43.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANP, atrial natriuretic peptide; FW, freshwater; HFW, hemorrhaged FW; SW, seawater.

Fig. 3. Effects of eel atrial natriuretic peptide (ANP) infusion (0.3–3.0 pmol·kg⁻¹·min⁻¹) on drinking rate in freshwater (FW) eels (n = 8; A), hemorrhaged FW eels (n = 8; B), and seawater (SW) eels (n = 8; C). Values are shown as means ± SE. *P < 0.05 from initial value by paired t-test.

Fig. 4. Effects of eel ANP infusion (0.3–3.0 pmol·kg⁻¹·min⁻¹) on plasma ANG II concentration in FW eels (n = 8; A), hemorrhaged FW eels (n = 8; B), and SW eels (n = 8; C). Values are shown as means ± SE. *P < 0.05 from initial value by paired t-test.
The ANP level after infusion of 3.0 pmol·kg⁻¹·min⁻¹ of individual and seasonal variation of intact fishes was observed even in intact eels in the cerebral ventricles (2, 41). However, the inhibition was observed even in intact eels in the present study. These results support the notion that eels are highly sensitive to the antidipsogenic effect of ANP. Furthermore, the antidipsogenic effect of ANP is more potent than the dipsogenic effect of ANG II in the eel (33), although the reverse is true in mammals (43).

Cellular dehydration, extracellular dehydration, and ANG II are major dipsogenic stimuli in birds and mammals (7, 20, 37). Hypovolemia and ANG II also stimulate drinking in the eel (11), but cellular dehydration caused by injection of hypertonic saline suppresses drinking in both FW and SW eels despite a concomitant increase in plasma ANG II level (38). Because ANP is a powerful antidipsogen in the eel, as shown in this study, and because hypernatremia profoundly augments ANP secretion in the eel (18), the unexpected inhibition of drinking after injection of hypertonic saline in the eel may be due to increased plasma ANP. An unexpected hypotension observed after injection of hypertonic saline in the eel may also be due to increased plasma ANP (30).

ANP is secreted in response to an increase in blood volume, and the increased ANP decreases blood volume by inhibiting the intake and stimulating the excretion of water and sodium (3, 28). In contrast, renin is released in response to hypovolemia and ANG II stimulates the intake and inhibits the excretion of water and sodium (20). Therefore, the renin-angiotensin system antagonizes the natriuretic peptide system in every aspect of osmoregulation. ANP inhibits renin secretion both in vivo and in vitro in selected species of mammals (4, 10), but the effect is still controversial, especially in vivo, because it decreases blood pressure (19, 29). In the present study, low doses of ANP decreased plasma ANG II concentration in vivo without changing blood pressure and blood volume. In the eel, therefore, ANP appears to be highly potent for inhibiting renin secretion by direct actions.

The present study showed that the inhibition of drinking by ANP is accompanied by a parallel decrease in plasma ANG II concentration in both FW and SW eels. Furthermore, the percentage inhibition of drinking and plasma ANG II level was similar in both FW and SW eels. Therefore, it is possible that the inhibition of drinking by ANP is mediated by a decrease in plasma ANG II. In fact, ANG II is a potent dipsogen in the eel (11), but the effect is still controversial, especially in vivo, because it decreases blood pressure (19, 29). In the present study, low doses of ANP decreased plasma ANG II concentration in vivo without changing blood pressure and blood volume. In the eel, therefore, ANP appears to be highly potent for inhibiting renin secretion by direct actions.

In mammals, ANP has been shown to inhibit drinking and sodium appetite when administered into the cerebral ventricles (2, 41). However, the inhibition was observed only in animals whose appetite was stimulated by dehydration or ANG II injection. In contrast, the inhibition was observed even in intact eels in the present study. These results support the notion that eels are highly sensitive to the antidipsogenic effect of ANP. Furthermore, the antidipsogenic effect of ANP is more potent than the dipsogenic effect of ANG II in the eel (33), although the reverse is true in mammals (43).
and increases again gradually thereafter (38). The plasma ANP level increases for 1–2 h after exposure to SW and gradually decreases to the FW level (17). Therefore, plasma ANP level does not differ between FW and SW eels, as shown in this study. Comparing the changes in drinking rate and plasma ANP level, it seems that ANP is involved in the temporary inhibition of drinking for 1–2 h after exposure to SW. Because active desalting of drunk SW occurs by the esophagus, the eels most likely suffer from severe hypertenatemia if the initial burst of drinking continues after SW exposure. It seems, therefore, that the transient depression of drinking for 1–2 h after SW exposure may be beneficial to counteract the excess hypertenatemia during the initial phase of SW adaptation.

Perspectives

The present study shows that ANP decreases drinking rate and plasma ANG II level (probably renin secretion) in the eel at doses that do not affect blood pressure. This is rather surprising because, in mammals, vascular effect usually overrides the other effects and ANP knockout mice exhibit normal drinking and plasma ANG II but lower blood pressure compared with normal mice (15). Therefore, the eel may serve as a good model to analyze how ANP is involved in the mechanisms regulating drinking and renin secretion.

Another important aspect for future studies is whether ANP acts directly on the brain to inhibit drinking or the inhibition is mediated by depressed plasma ANG II. ANP is known to act on the brain to inhibit drinking in mammals (2, 43), but aquatic fish can stop and start drinking only by constriction and relaxation of the esophageal sphincter. Because direct application of ANG II into the cerebral ventricles of eels shows its action on the brain (31), a similar technique can be used for ANP to clarify its site of action. The mediation of ANG II in ANP action can be examined by infusing ANP with ANG II to maintain normal plasma ANG II level during ANP infusion or by using various inhibitors of the renin-angiotensin system. In A. anguilla, a bolus injection of ANG I-converting enzyme inhibitor depresses drinking in SW fish but not in FW fish (40). Thus ANG II does not seem to be involved in normal drinking in FW eels.

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