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A Potent Dipsogenic Action of Homologous Angiotensin II Infused at Physiological Doses in Eels

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ABSTRACT—Angiotensin II at pharmacological doses induces drinking in various vertebrate species. However, its role in physiological regulation of drinking remains to be determined.Eel angiotensin II was infused slowly into freshwater eels to examine whether sustained, physiological increases in its plasma level, as observed after transfer to seawater, stimulate drinking. Hourly infusion was initiated with 0.9% NaCl, followed by increasing concentrations of angiotensin II (0.1–10 pmol/kg/min), and ended with only saline again for 2 hr.

Angiotensin II infusion at a rate of 0.1 pmol/kg/min significantly induced drinking; neither plasma angiotensin II concentration nor plasma atrial natriuretic peptide (ANP), Na concentrations or arterial pressure were significantly altered. The dipsogenic effect of angiotensin II was dose-dependent, and enhanced drinking disappeared quickly after infusate was switched back to saline. Blood pressure and plasma ANP concentration increased dose-dependently at more than 1 pmol/kg/min, and both parameters declined to their initial levels after return to normal saline infusion. By contrast, plasma Na concentration was decreased by more than 1 pmol/kg/min of angiotensin II.

These results show that drinking is induced by angiotensin II within physiological range in water-replete freshwater eels. At higher doses, enhanced drinking occurs despite hypertension, hyponatremia and increased plasma ANP, all of which are inhibitory to elicitation of drinking. Thus, it is likely that angiotensin II could be a physiological stimulus for drinking in the eel.

INTRODUCTION

Angiotensin II is established as a potent dipsogen in all vertebrate species from fishes to mammals (Kobayashi *et al*., 1979; 1983; Hazon *et al*., 1989; see Kobayashi and Takei, 1996). In teleost fishes, bolus injections of angiotensin II into the circulation induced drinking in euryhaline species such as eels (Takei *et al*., 1979), flounder (Carrick and Balment, 1983) and killifish (Malvin *et al*., 1980). In contrast to angiotensin II, atrial natriuretic peptide (ANP) is recognized as a major antidipsogenic hormone in mammals and eel (Antunes-Rodrigues *et al*., 1985; Tsuchida and Takei, 1998). These hormones seem to regulate drinking in various vertebrate species (Fitzsimons, 1998).

After water deprivation in tetrapods or after transfer to seawater of euryhaline fishes, plasma renin activity and plasma angiotensin II concentration increased continuously for hours (Blair-West *et al*., 1972; Henderson *et al*., 1976; Okawara *et al*., 1987; Takei *et al*., 1988a; Tierney *et al*., 1995). The dipsogenic action of angiotensin II has been demonstrated by bolus injections after which its plasma concentration increases only transiently to pharmacological levels because of its short life in plasma (Naden *et al*., 1985). Therefore, infusion is a better route of hormone delivery to mimic the physiological changes that occur in plasma after natural dipsogenic stimuli. In mammals, the involvement of angiotensin II in physiological drinking has been extensively studied by slow infusion of the hormone (Abraham *et al*., 1975; Fitzsimons *et al*., 1978). However, the dipsogenic action of angiotensin II has not yet been examined by infusion in fishes (see Kobayashi and Takei, 1996).

In the present study, low doses of eel angiotensin II were delivered into the circulation by slow infusion, and its effect on drinking was examined in water-replete freshwater eels that drink scarcely *ad libitum*. Plasma angiotensin II concentration was measured during the infusion to monitor its plasma level. Factors that influence drinking such as blood pressure, plasma ANP concentration and osmolality (Hirano and Hasegawa, 1984; Takei *et al*., 1988b; Tsuchida and Takei, 1998) were also measured during the infusion. Care was taken to minimize changes in blood volume during infusion because its change sensitively alters drinking rate in eels (Hirano, 1974).

MATERIALS AND METHODS

Animals

Cultured Japanese eels, *Anguilla japonica*, were purchased from a local dealer. They were kept in a 1-ton, freshwater tank for more

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than 1 week before use. Water in the tank was continuously filtered, aerated and thermo-regulated at 18±0.5°C. Eels were not fed after aquisition. They weighed 198.7±2.6 g (mean±SEM, n=7) at the time of experiment.

Surgical procedures

Eels were anesthetized in 0.1% (w/v) tricaine methanesulfonate (Sigma, St. Louis, USA) for 10 min. Vinyl tubes (o.d. : 1.5 mm) were inserted into the esophagus and the stomach of eels as described previously (Takei *et al.*, 1998). In addition, polyethylene tubes (o.d. : 0.8 mm) were inserted into the dorsal aorta for infusion of angiotensin II and blood pressure measurement, and into the ventral aorta for blood sampling. The eels that bled more than 0.1 ml during surgery were not used for the experiment because they showed elevated drinking and plasma angiotensin II level.

After surgery, eels were transferred to plastic troughs through which aerated and thermo-regulated water constantly circulated. The vinyl tube placed in the esophagus collected the water taken by drinking and was connected to a drop counter for continuous measurement of drinking rate. The water drunk was reintroduced into the stomach through the second vinyl tube by means of a pulse injector synchronized with the drop counter (Takei *et al.*, 1998). The catheter in the dorsal aorta was connected via 3-way stopcock to a plastic syringe for infusion of angiotensin II, and to a pressure transducer (DX-300, Nihon Kohden, Tokyo) and a polygraph (366 system, NEC-Sanei, Tokyo) for continuous measurement of blood pressure. Eels were allowed to recover for more than 18 hr post-operatively.

Experimental protocol

The eels received hourly infusion of a vehicle solution, followed by increasing doses of homologous [Asn^1,Val^5] angiotensin II (Peptide Institute, Inc., Osaka) at 0.1, 1 and 10 pmol/kg/min, and ended with a vehicle solution infusion for 2 hr. At the exchange of infusates, 50 μl of new infusate was injected as a prime to obtain stable plasma angiotensin II level quickly. As a vehicle, 0.9% NaCl solution was used which contained 0.01% Triton X-100 (Nakarai Chemicals, Kyoto) to prevent adsorption of angiotensin II to the walls of tube and syringe. Since the infusion rate was 0.3 ml/hr, the same volume of blood was withdrawn every hour to nullify the change in blood volume. Blood was collected into a chilled syringe containing 10% 2K-EDTA (10 μl/ml blood collected). The blood was centrifuged, and plasma was used for measurements of plasma Na concentration and osmolality, and eel ANP (Kaiya and Takei, 1996) and angiotensin II (Tsuchida and Takei, 1998) by radioimmunoassay. The intraassay and interassay coefficients of variation were 5.1% and 11.5% for ANP and 5.2% and 12.6% for angiotensin II, respectively. Na concentration was determined in an atomic absorption spectrophotometer (Z5300, Hitachi, Tokyo) and plasma osmolality in a vapor pressure osmometer (Wescor 5500, Logan, USA). All determinations were made in duplicate or triplicate.

Analyses of data

The time-course data were analyzed statistically by ANOVA followed by Tukey's test at each time point. Significance was determined at $p<0.05$. All results were expressed as means±SE of the mean.

RESULTS

Angiotensin II infusion in freshwater eels induced dose-dependent increases in drinking rate with parallel increases in plasma angiotensin II concentration (Fig. 1). The increase in drinking rate was significant at a dose as low as 0.1 pmol/kg/min of angiotensin II, whereas significant increases in plasma angiotensin II concentration occurred at doses of 1

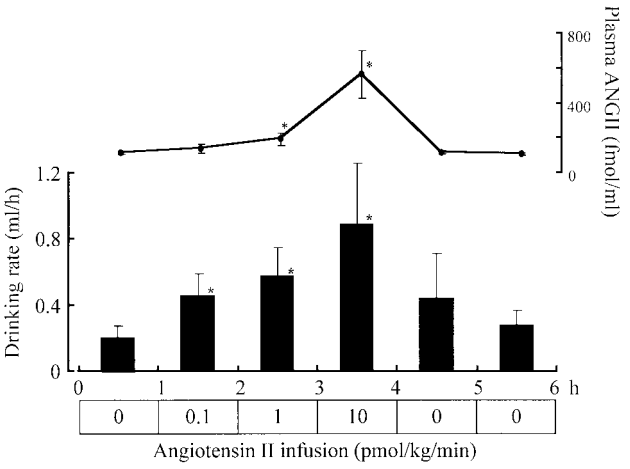

Fig. 1. Time course of changes in drinking rate and plasma angiotensin II (ANGII) concentration in freshwater eels (n=7) during hourly infusion of different doses of angiotensin II as indicated below the abscissa. Vehicle (0.9% NaCl with 0.01% Triton X-100) infusions occurred before and after angiotensin II infusion. Values are shown as means±SEM. *p<0.05 compared with the value during the initial vehicle infusion.

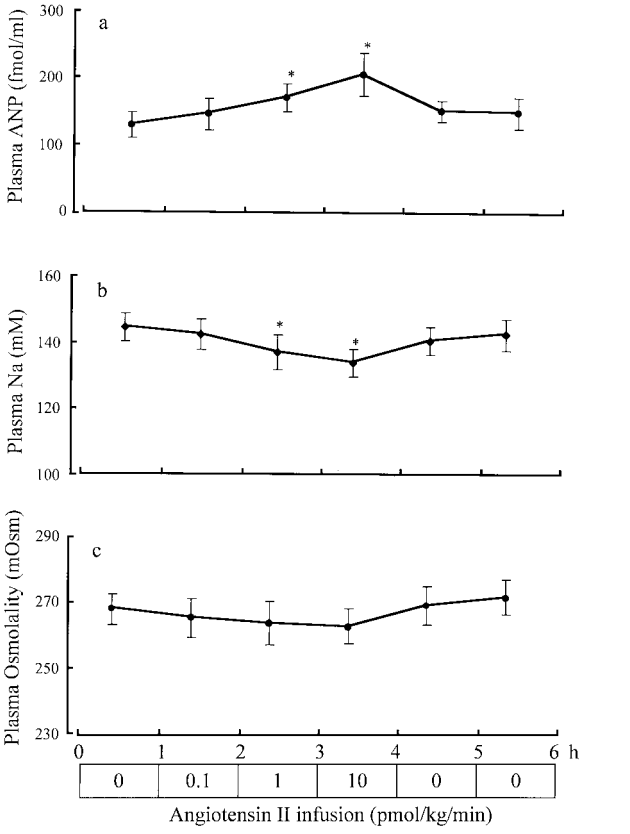

Fig. 2. Time course of changes in (a) plasma atrial natriuretic peptide (ANP) concentration, (b) plasma Na concentration, and (c) plasma osmolality in freshwater eels (n=7) during hourly infusion of different doses of angiotensin II. Values are shown as means±SEM. *p<0.05 compared with the value during the initial vehicle infusion.

and 10 pmol/kg/min. Even at 1 pmol/kg/min, plasma angiotensin II level (ca. 150 fmol/ml) was well within the range of normal plasma levels of eels. The increase in drinking rate was approximately 4.2-fold and that of angiotensin II was 9-fold at 10 pmol/kg/min. Plasma angiotensin II concentration decreased abruptly to the initial level within 1 hr after infusate was returned to saline.

Plasma ANP concentration increased dose-dependently during angiotensin II infusion (Fig. 2a). The increase was significant at 1 and 10 pmol/kg/min, and the level returned slowly to the initial level after return to saline infusion. In contrast to the increases in plasma hormone levels, plasma Na concentration decreased during angiotensin II infusion (Fig. 2b). Significant decreases occurred at 1 and 10 pmol/kg/min. Plasma osmolality exhibited a similar trend as plasma Na concentration, but there were no significant changes (Fig. 2c).

After a priming injection of angiotensin II before the start of infusion, arterial blood pressure increased transiently. However, the level reached equilibrium within 10 min after the start of infusion, indicating stable plasma angiotensin II level thereafter. Both arterial blood pressure and pulse pressure increased dose-dependently during angiotensin II infusion with significant increases at doses of 1 and 10 pmol/kg/min (Fig. 3). Blood pressure returned to the initial level abruptly, but pulse pressure was still higher than the initial level for 1 hr after saline infusion. Tachycardia was noted at doses greater than 1 pmol/kg/min (data not shown).

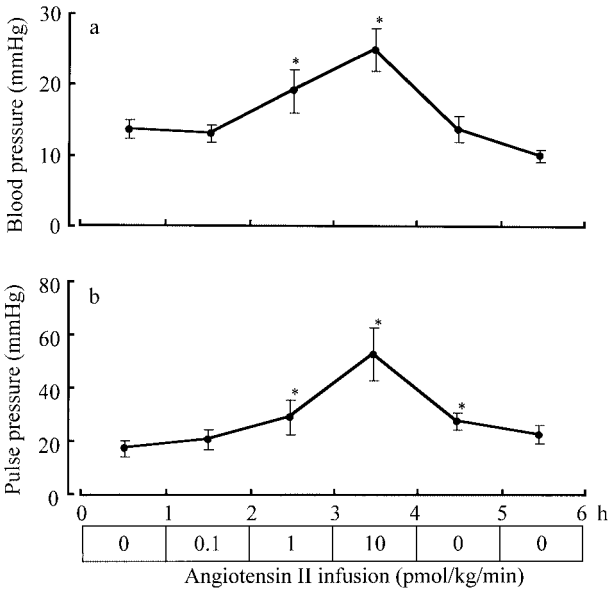

Fig. 3. Time-course changes in (a) mean arterial blood pressure, and (b) pulse pressure in freshwater eels (n=7) during hourly infusion of different doses of angiotensin II. Values are shown as means±SEM. *p<0.05 compared with the value during the initial vehicle infusion.

DISCUSSION

Role of the renin-angiotensin system in physiological thirst

The dipsogenic effect of angiotensin II has been demonstrated in diverse vertebrate species with variations in its potency relating to ecological demand for water (Kobayashi *et al.*, 1979; 1983). The involvement of angiotensin II in the physiological regulation of thirst has been a target of intensive research in mammals, and two approaches have been used to evaluate this possibility. One approach is made by the use of inhibitors of the renin-angiotensin system such as angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists. The results typically indicate the important role of the system in the drinking response to various thirst stimuli (Malvin *et al.*, 1977; Barney *et al.*, 1983; Blair-West *et al.*, 1997). In seawater teleost fish, ACE inhibitors potently inhibit drinking (Balment and Carrick, 1985; Hazon *et al.*, 1989; Tierney *et al.*, 1995; Fuentes and Eddy, 1997). However, our recent data suggest that in the eel the effect of an ACE inhibitor (SQ14225) is due not to the inhibition of the renin-angiotensin system but to the activation of the kallikrein-kinin system (Takei, Y., Tsuchida, T., Li, Z. and Conlon JM, unpublished data). Angiotensin II analogs such as salarasin ([Sar^1, Ala^8] angiotensin II) and nonpeptide antagonists such as losartan, which are routinely used in mammals, are not effective in fishes (Nishimura *et al.*, 1978; Tierney *et al.*, 1997). Therefore, inhibitors of the renin-angiotensin system identified in mammals are not reliable *a priori* to assess the role of angiotensin II in the physiological regulation of drinking in fishes.

Another experimental approach can be made by infusing angiotensin II to maintain its plasma level within the physiological range observed after natural dipsogenic stimuli. In mammals, the results appear to be variable depending on the species. In the sheep, the plasma angiotensin II concentration that induced reliable drinking was beyond the level observed after water restriction (Abraham *et al.*, 1978). By contrast, significant drinking was induced by ca.10 pmol/kg/min of angiotensin II infusion in the dog which yielded plasma levels within physiological limits (Fitzsimons *et al.*, 1978). This dose was mildly vasopressor as observed in this study. In the rat, drinking was induced at 100 pmol/kg/min of angiotensin II infusion, where the plasma level rose to 458 fmol/ml; a concentration almost equal to the level seen after 48-h water deprivation (Anke *et al.*, 1988).

In the current study, eels were extremely sensitive to the dipsogenic effect of angiotensin II. Drinking was induced at doses as low as 0.1 pmol/kg/min which produced an average plasma level of 79.4 fmol/ml, a value even lower than that reported previously for seawater eels (94.1 fmol/ml, Okawara *et al.*, 1987). Significantly, no vasopressor effect was noted at this dose of infusion. Therefore, eels are more sensitive to the dipsogenic action than to vascular action of angiotensin II. The dipsogenic effect also appears to be stronger than the renal effect in eels since doses greater than 10 pmol/kg/min

were required to induce natriuretic and diuretic effects in American eels (Nishimura and Sawyer, 1976).

It is generally accepted that vasopressor drugs are antidipsogenic and vasodepressor drugs are dipsogenic in mammals (Fitzsimons, 1998). Accordingly, the dipsogenic potency of angiotensin II is enhanced when its vasopressor effect is suppressed by simultaneous administration of vasodepressor drugs in the rat (Robinson and Evered, 1987). Since vasopressor drugs are antidipsogenic also in the eel (Hirano and Hasegawa, 1984), the absence of vasopressor effect at 0.1 pmol/kg/min of angiotensin II may explain in part why angiotensin II exhibited such high dipsogenic potency in eels in the present study.

Effects of angiotensin II on plasma ANP and Na concentrations

It is generally believed that the renin-angiotensin system and the natriuretic peptide system act as functional antagonists in the regulation of fluid and electrolyte homeostasis and blood pressure (Schiffrin *et al.*, 1993). With regard to secretion, ANP inhibits renin release both *in vivo* and *in vitro* (see Ruskoaho, 1992), but angiotensin II stimulates ANP secretion at 1–10 pmol/kg/min of infusion in dogs (Volpe *et al.*, 1990). Similarly, ANP infusion depressed plasma angiotensin II concentration in the eel (Tsuchida and Takei, 1998) whereas angiotensin II infusion at 1–10 pmol/kg/min increased plasma ANP level in this study. The increase in ANP concentration observed here at higher infusion doses may reflect elevated ANP secretion in response to concomitantly increased arterial pressure. However, increases in pulse pressure and heart rate as observed in the present study may also be involved because positive inotropic and chronotropic changes to the heart are known to stimulate ANP secretion (see Ruskoaho, 1992).

Angiotensin II decreased plasma Na concentration in freshwater eels in this study. It has been proposed that angiotensin II modulates the activity of ion transporters such as Na^+,K^+-ATPase and Na^+/H^+ antiporter in various tissues of mammals (Sacommani *et al.*, 1990; Aperia *et al.*, 1994). Similar effects are also suggested in the gill and intestine of European eels (Vilella *et al.*, 1996; Marsigliante *et al.*, 1997). We also found that angiotensin II alters Na^+,K^+-ATPase activity in a biphasic manner in isolated eel gill cells from freshwater eels; stimulation at low doses and inhibition at high doses (Takei *et al.*, 1999). Thus, suppression of brancheal Na^+,K^+-ATPase activity may result from the increased plasma levels of angiotensin II following infusion in freshwater eels.

Altogether, the data from this study support a direct dipsogenic effect of angiotensin II in the eel at physiological concentrations.

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