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Authors: Ando, Masaaki, Sakaguchi, Kimiyasu, Yasuda, Masanobu, Uesaka, Toshihiro, Kim, Hung Tae, et al.

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## Atrium Contributes to Osmoregulation in Eels Acclimated to Sea Water

Masaaki Ando\*, Kimiyasu Sakaguchi, Masanobu Yasuda, Toshihiro Uesaka, Hung Tae Kim and Akira Kawahara

*Laboratory of Physiology, Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8521, Japan*

**ABSTRACT**—Since highly concentrated NaCl is suspected to enter into the heart of the seawater eels, effects of high NaCl concentration on the atrial beating was examined, and plasma ion concentrations and osmolality were measured simultaneously in the blood collected from the bulbus arteriosus and from the caudal vessels. When 100 mmole l<sup>-1</sup> NaCl was added to the incubation medium, atrial contraction was enhanced significantly. Similar enhancement in the atrial contractility was also observed after addition of NaCH<sub>3</sub>SO<sub>4</sub> (100 mmole l<sup>-1</sup>) or Tris HCl (100 mmole l<sup>-1</sup>), indicating that Na<sup>+</sup> and Cl<sup>-</sup> are not indispensable for the positive inotropic effect. Furthermore, an addition of sucrose (200 mmole l<sup>-1</sup>) also enhanced the contraction. Inversely, hypoosmotic solution reduced the atrial contraction. These results indicate that the eel atrium is sensitive to environmental osmolality. The eel atrium responds even at 20 mmole l<sup>-1</sup> sucrose. Such an inotropic effect of sucrose was not depressed after blocking adrenoceptor with betaxolol, a β<sub>1</sub>-adrenoceptor antagonist, indicating that the effect is not due to adrenaline release from nerve endings. Plasma osmolality and Na<sup>+</sup> concentration were higher in bulbus arteriosus than in caudal vessels, indicating that the eel heart is really exposed to hyperosmotic blood in sea water. The osmotically enhanced atrial contraction may increase the cardiac outflow into the gill. Such property of the atrium would have clear advantages for seawater teleosts, since the concentrated NaCl from the esophagus can be excreted immediately through the gill, without circulating their body, and blood homeostasis can be maintained efficiently.

### INTRODUCTION

Eels adapted to sea water drink the sea water to compensate for osmotic water loss, the ingested sea water is desalted during passing through the esophagus (Hirano and Mayer-Gostan, 1976; Nagashima and Ando, 1994). During desalination, however, extremely concentrated NaCl must be transferred across the esophagus then into the blood circulation. This situation will increase the plasma NaCl concentration. However, plasma Na<sup>+</sup> and Cl<sup>-</sup> concentrations are kept far below the sea water levels (Hirano *et al.*, 1981; Hasegawa *et al.*, 1986), suggesting the existence of some mechanisms to avoid increasing plasma ionic concentrations in the eel.

The fish heart consists of one atrium and one ventricle. Because the eel heart adheres to the esophagus, it is expected that the absorbed NaCl across the esophagus comes back to the heart through a short loop, and is immediately sent to the gill, where NaCl is excreted powerfully. This means that the heart of seawater eel is exposed to a highly concentrated NaCl. However, there are no previous works which have examined the cardiac activity under high NaCl.

The present study was aimed to examine the effects of high NaCl concentration on the cardiac activity of the seawater eel. An isolated atrium was used in this work, since we have established previously that the eel atrium can beat spontaneously for more than 10 hr in normal Ringer solution (Uesaka, 1996; Yasuda *et al.*, 1996). In addition, plasma ion concentrations and osmolality were determined in the blood collected from the bulbus arteriosus and from the caudal vessels to confirm that highly concentrated NaCl really enters into the atrium in the seawater eel.

### MATERIALS AND METHODS

#### Biological activity in the eel atrium

Japanese eels *Anguilla japonica*, from farmed stock and weighing approximately 200 g, were kept in sea water (20°C) for more than 1 week. After decapitation, the heart was rapidly excised and the atrium isolated on ice. It was then tied with two cotton threads, one being connected to the bottom of an experimental chamber and the other to a force transducer (type 45196A, Sanei, Tokyo). The atrium was bathed in Krebs bicarbonate Ringer solution consisting of (in mmol l<sup>-1</sup>): 118.5 NaCl, 4.7 KCl, 3.0 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 24.9 NaHCO<sub>3</sub>. The hyperosmotic Ringer solutions were made by adding 100 mmol l<sup>-1</sup> NaCl, Tris HCl or NaCH<sub>3</sub>SO<sub>4</sub>, or by adding various concentrations of sucrose to the normal Ringer solution. The hypoosmotic 1/2 NaCl Ringer solution was made by halving NaCl concentration in

\* Corresponding author: Tel. +81-824-24-6569;  
FAX. +81-824-24-0759.  
E-mail: mando@ipc.hiroshima-u.ac.jp

the normal Ringer solution. The isoosmotic 1/2 NaCl Ringer solution was made by adding  $118.5 \text{ mmol l}^{-1}$  sucrose to the hypoosmotic 1/2 NaCl Ringer solution. All these solutions contain  $10 \text{ mmol l}^{-1}$  sodium lactate for a metabolic substrate (Milligan and Farrell, 1991).

The bathing solution (2.0 ml) was bubbled with a 95%  $\text{O}_2$ : 5%  $\text{CO}_2$  gas mixture (pH 7.4) at room temperature ( $23\text{--}26^\circ\text{C}$ ). After preloading by 150 mg, spontaneous isometric contraction were converted into electrical signals by a transducer connected to a strain amplifier (6M82, Sanei) and these were recorded using an electronic polyrecorder (EPR-10B, Toa, Tokyo). The rate of contraction (beats  $\text{min}^{-1}$ ; b.p.m.) was measured simultaneously using a tachometer (type 1321, Sanei).

#### Atrial water content

The isolated atrium was incubated in normal Ringer solution for more than 30 min, then transferred to various test solutions (hyper- or hypo-osmotic solution). Under these conditions, wet weight of the atrium was measured every 5 min after blotting on a filter paper. The steady state value was considered as the wet weight in these conditions, usually 15–20 min after treatment. After experiments, the atrium was dried at  $110^\circ\text{C}$  for 24 hr and dry weight was determined. The water content was obtained as the difference between the wet and dry weights and expressed as a ratio to the wet weight.

#### Plasma osmolality and ion concentrations

After anesthetized with 0.1% MS-222 (Sigma Chem., St. Louis, MO, USA), blood (0.9 ml) was collected from the caudal vessels with syringe needle, and then the abdomen was opened. Another blood (0.9 ml) was also collected from the bulbus arteriosus after removing the pericardial membrane. These two blood samples were collected

from the same preparation. Plasma was separated by centrifuging at 4000 rpm for 20 min at  $4^\circ\text{C}$ . Plasma osmolality was determined with an osmometer (Osmette 2007, Precision Systems Inc., Sudbury, Mass, USA).  $\text{Na}^+$  and  $\text{K}^+$  concentrations were measured by flame photometry (FPF-2A, Hiranuma, Mito, Japan), and  $\text{Cl}^-$  concentration with a chloride counter (CL-5M, Hiranuma).

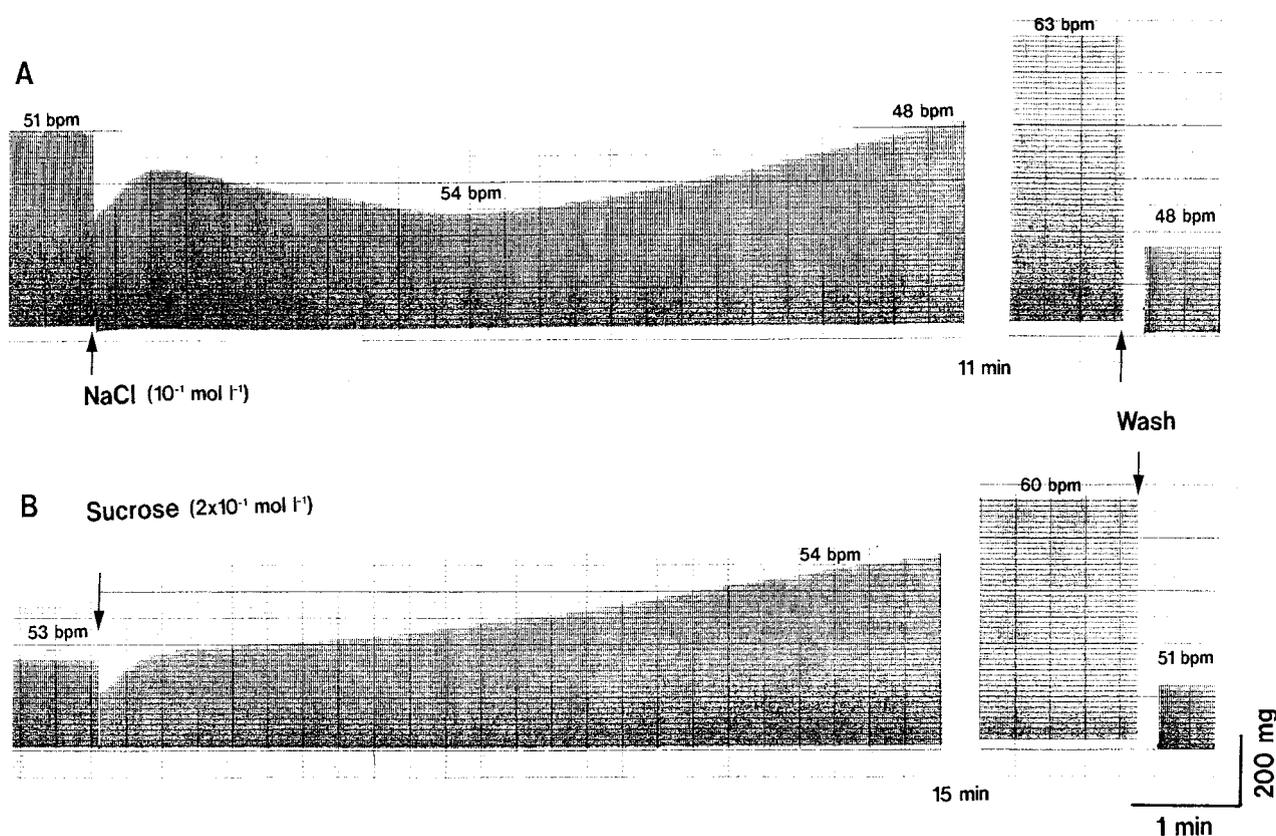
Reagents used were ( $\pm$ ) betaxolol HCl (Mitsubishi Kasei, Tokyo, Japan), (–) adrenaline and veratrine (Sigma). Data are reported as mean  $\pm$  S.E.M.; *N* represents the number of preparations. The statistical significance of differences between means was examined using paired *t*-test. The null hypothesis was rejected for  $P < 0.05$ .

## RESULTS

### Effects of hyperosmolarity

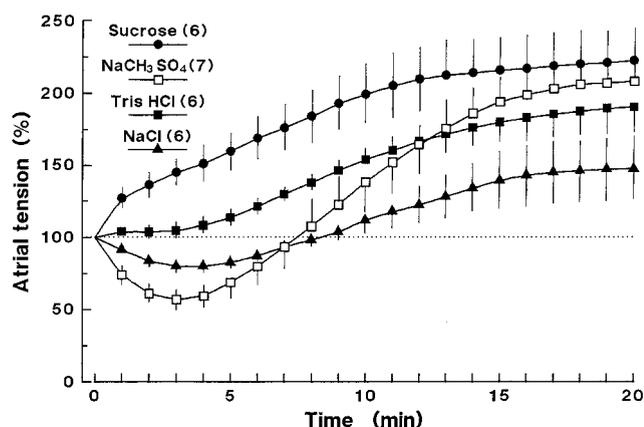
The isolated atrium of the eel contracted spontaneously at a relatively constant rate (50–70 bpm) in normal Ringer solution for more than 10 hr. When the bathing solution was replaced with the hyperosmotic NaCl Ringer solution, the contractility was transiently depressed and then increased gradually, accompanied by an increase in beating rate (Fig. 1A). However, in some preparations only the contractility was increased, without enhancement in the beating rate.

To separate the effect of  $\text{Na}^+$  and  $\text{Cl}^-$ ,  $\text{NaCH}_3\text{SO}_4$  and Tris HCl was used instead of NaCl. However, both salts exhibited qualitatively similar results as in NaCl, finally (after



**Fig. 1.** Effects of hyperosmotic Ringer solution on the atrial beating. A. Effects of NaCl. At the first arrow, high NaCl Ringer solution ( $[\text{NaCl}] = 228.5 \text{ mmol l}^{-1}$ ) was substituted for normal Ringer solution. After transient depression, the tension and beating rate (b.p.m.) were increased gradually. B. Effects of sucrose. At the first arrow, the normal Ringer solution was replaced with hyperosmotic sucrose ( $200 \text{ mmol l}^{-1}$ ) Ringer solution. The tension and beating rate (b.p.m.) were increased gradually.

10 min) enhancement in contractility, though the effect was slightly greater in case of  $\text{NaCH}_3\text{SO}_4$  and Tris HCl treatments (Fig. 2). This indicates that  $\text{Na}^+$  and  $\text{Cl}^-$  are not indispensable



**Fig. 2.** Time courses of the effect of hyperosmolarity. Hyperosmolarity was accomplished by adding  $100 \text{ mmol l}^{-1}$  NaCl ( $\blacktriangle$ ),  $\text{NaCH}_3\text{SO}_4$  ( $\square$ ) or Tris HCl ( $\blacksquare$ ), or by adding  $200 \text{ mmol l}^{-1}$  sucrose ( $\bullet$ ) into the normal Ringer solution. These hyperosmotic solutions were substituted for normal Ringer solution (control) at time zero. The atrial tension is expressed as a relative ratio (%) to the control tension in normal Ringer solution. Each point and vertical bar indicate mean  $\pm$  S.E.M.. Where no S.E.M. are shown, error bars are smaller than symbols. Number of experiments is shown in parenthesis.

**Table 1.** Concentration-dependent effects of sucrose on the atrial beating of the seawater eels

Sucrose concentration ( $\text{mmol l}^{-1}$ )	Increase in atrial beating force (mg)	beating rate ( $\text{beats min}^{-1}$ )
20	$33.4 \pm 7.2^*$	$3.0 \pm 1.2$
50	$63.9 \pm 9.7^*$	$5.0 \pm 1.8$
100	$127.6 \pm 17.5^*$	$1.0 \pm 0.9$
200	$156.6 \pm 14.3^{**}$	$2.5 \pm 1.7$

The effects of sucrose were determined 10 min after application, and expressed as a difference.

Values are Mean  $\pm$  S.E.M.,  $N=6$

\* $P < 0.05$ , \*\* $P < 0.005$  compared to normal Ringer solution (paired  $t$ -test)

for the positive inotropic effect. The transient inhibition was observed only in NaCl and  $\text{NaCH}_3\text{SO}_4$  treatments, but not after Tris HCl.

When sucrose was added, similar enhancement in the atrial contraction was also observed. However, in case of sucrose, the transient inhibition was not observed (Figs. 1B and 2). The inotropic effect of sucrose was concentration-dependent (Table 1). Since the inotropic effect of high NaCl appears to be due to hyperosmolarity, the following experiments were all performed with sucrose.

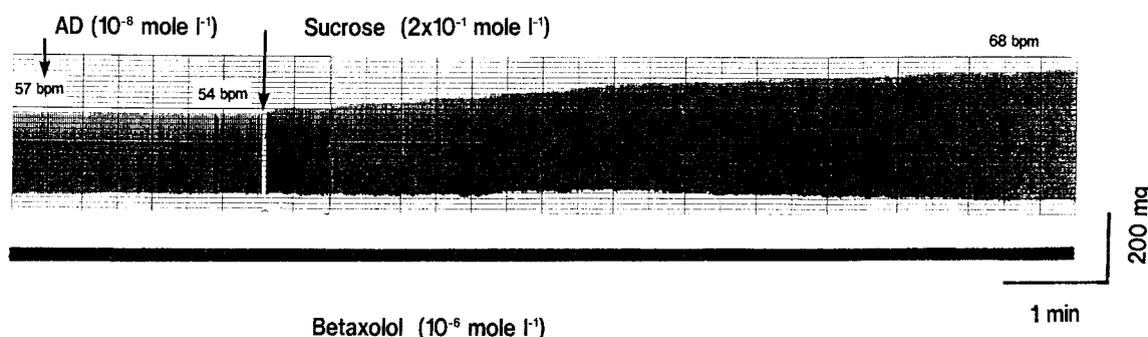
To determine whether hyperosmolarity directly enhance the atrial beating or indirectly through a release of some regulators, such as adrenaline which is a potent stimulator in the eel atrium (Yasuda *et al.*, 1996), effects of hyperosmotic sucrose Ringer solution were examined in the presence of betaxolol, a  $\beta_1$ -adrenoceptor antagonist. As shown in Fig. 3, however, the enhancement in tension and beating rate by sucrose were not blocked by betaxolol, which had been shown to block adrenoceptor completely in the eel atrium (Uesaka, 1996). Even after 30 min treatment with  $0.3 \text{ g l}^{-1}$  veratrine, a neuronal depolarizing agent, similar enhancements were induced by  $200 \text{ mmol l}^{-1}$  sucrose (data not shown). Although hyperosmolarity is expected to secrete atrial natriuretic peptide (ANP) from the eel atrium (Kaiya and Takei, 1996), eel ANP had no effect on the atrial beating (data not shown).

#### Effects of hypoosmolarity

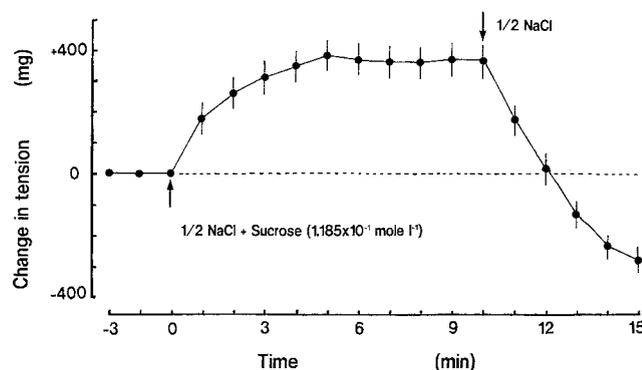
When NaCl concentration was reduced by halving NaCl concentration ( $59.3 \text{ mmol l}^{-1}$  NaCl plus  $118.5 \text{ mmol l}^{-1}$  sucrose; isoosmotic  $1/2$  NaCl Ringer solution), the contractility and beating rate were increased gradually (Fig. 4). In some preparations, the beating rate returned to the initial level after 10 min. After reaching a steady state in isoosmotic  $1/2$  NaCl Ringer solution,  $118.5 \text{ mmol l}^{-1}$  sucrose was omitted, *i.e.* hypoosmotic  $1/2$  NaCl Ringer solution. Under this condition, both tension and beating rate decreased gradually (Fig. 4).

#### Effects on water content

As expected from osmotic water flow, the water content of the eel atrium decreased in hyperosmotic Ringer solution ( $200 \text{ mmol l}^{-1}$  sucrose), and increased in hypoosmotic  $1/2$  NaCl



**Fig. 3.** Effects of hyperosmolarity after blocking adrenoceptor with betaxolol. In the presence of betaxolol ( $10^{-6} \text{ mole l}^{-1}$ ), hyperosmotic sucrose ( $200 \text{ mmol l}^{-1}$ ) Ringer solution was applied (second arrow). The blocking effects of betaxolol is confirmed by a lack of adrenaline (AD) effects (first arrow).



**Fig. 4.** Effect of hypoosmolarity on the atrial tension. Reducing NaCl concentration to half of the normal Ringer solution (1/2 NaCl, [NaCl]=59.8 mmole l<sup>-1</sup>), hypoosmotic 1/2 NaCl Ringer solution was made. After steady beating, the bathing medium (normal Ringer solution) was replaced with isoosmotic 1/2 NaCl Ringer solution, containing 59.8 mmole l<sup>-1</sup> NaCl and 118.5 mmole l<sup>-1</sup> sucrose (first arrow at time zero). At the second arrow (time 10 min), the isoosmotic 1/2 NaCl Ringer solution was replaced with the hypoosmotic 1/2 NaCl Ringer solution. The atrial tension was expressed as changes from control tension in the normal Ringer solution. Each point and vertical bar indicate mean±S.E.M. (N=9). Where no S.E.M. are shown, error bars are smaller than symbols.

**Table 2.** Atrial water content in normal Ringer solution and in hyper- or hypo-osmotic solution

Solution	Water content	Difference
	(%)	(%)
Normal Ringer	84.3±1.7	
200 mmol l <sup>-1</sup> Sucrose	81.6±2.0**	-2.7±0.5
Normal Ringer	84.4±0.9	
Isoosmotic 1/2 NaCl	84.6±1.0	+0.2±0.3
Hypoosmotic 1/2 NaCl	86.1±0.7*	+1.6±0.6

Water content is obtained under steady state condition (15–20 min after treatment). Negative or positive sign in the difference indicates net water loss or water gain, respectively.

Values are Mean±S.E.M., N=6

\*P<0.05, \*\*P<0.005 (paired *t*-test)

Ringer solution (Table 2).

### Plasma Na<sup>+</sup> and Cl<sup>-</sup> concentrations

To confirm that the concentrated NaCl really enters into the heart from the esophagus, blood samples were collected from caudal vessels and bulbus arteriosus in the same eels. As shown in Table 3, plasma osmolality and Na<sup>+</sup> concentra-

tion were significantly higher in the bulbus arteriosus than in the caudal vessels. Cl<sup>-</sup> and K<sup>+</sup> concentrations were not significantly different in these two plasma.

## DISCUSSION

The present study directly demonstrates that hyperosmotic NaCl Ringer solution enhances atrial contraction (Figs. 1A). In some preparations, the beating rate was also increased simultaneously after application of the high NaCl. The enhancement by NaCl does not seem to be ion-specific, because both NaCH<sub>3</sub>SO<sub>4</sub> and Tris HCl finally enhanced the contraction, similarly as in case of NaCl (Fig. 2). Although the effects of NaCH<sub>3</sub>SO<sub>4</sub> or Tris HCl appear to be greater than that of NaCl, this can be explained by a greater reflection coefficient of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup> or Tris<sup>+</sup>, compared to that of Cl<sup>-</sup> or Na<sup>+</sup>, respectively. In other words, NaCH<sub>3</sub>SO<sub>4</sub> and Tris HCl solutions are more effective osmotically than NaCl to dehydrate the myocardium. CH<sub>3</sub>SO<sub>4</sub><sup>-</sup> and Tris<sup>+</sup> have been used as membrane impermeable anion and cation in the eel intestine (Ando, 1980, 1983). These results suggest hyperosmolarity enhances atrial contraction finally. In fact, addition of sucrose (hyperosmolarity) also enhanced the contraction (Figs. 1B and 2). Inversely, the contractility was reduced in hypoosmotic Ringer solution (Fig. 4). All together, eel atrium appears to be sensitive to environmental osmolality.

Although it is well known that hyperosmolarity releases neurotransmitters from nerve endings (Fatt and Katz, 1952; Kita and Van der Kloot, 1977; Kita *et al.*, 1982), the enhanced contraction by hyperosmolarity is not due to catecholamine release, because similar enhancement is obtained even after blocking the adrenoceptor with betaxolol (Fig. 3) or after exhaustion of neurotransmitters with veratrine. However, other possibility, such as neuropeptide release, still remains.

Although Gesser and Mangor-Jensen (1984) describe that hypoosmolarity potentiates ventricular twitch tension in the flounder, our result is against their conclusion. Probably, this discrepancy can be explained by a difference in experimental condition. We made hypoosmotic condition without altering ionic composition (Fig 4), while they lowered both osmolality and Na<sup>+</sup> concentration simultaneously. When Na<sup>+</sup> concentration alone was lowered, while isoosmotic with addition of sucrose, the contractility was enhanced in the eel atrium (Fig. 4). Similar enhancement by isoosmotic low Na<sup>+</sup> solution is also observed in guinea pig heart (Befroy *et al.*, 1999). Even

**Table 3.** Comparison of plasma osmolality and ionic concentrations between bulbus arteriosus and caudal vessels

	Osmolality (mosm kg <sup>-1</sup> )	[Na <sup>+</sup> ] (meq l <sup>-1</sup> )	[Cl <sup>-</sup> ]	[K <sup>+</sup> ]
Bulbus arteriosus	373.6±7.3	163.1±3.2	149.4±4.0	4.6±0.2
Caudal vessels	353.8±8.2**	155.6±3.0*	146.3±3.5	5.2±0.5
Difference	+19.8±3.3	+7.4±3.1	+3.1±3.1	-0.7±0.5

Difference: osmolality or concentration difference between bulbus arteriosus and caudal vessels

Values are Mean±S.E.M., N=14

\*P<0.005, \*\*P<0.001 (paired *t*-test)

in guinea pig heart, hypoosmotic low  $\text{Na}^+$  solutions decreases the contraction (Befroy *et al.*, 1999). Enhancement in the atrial beating by hyperosmolarity may be common in vertebrates, since potentiated contraction is observed after hyperosmolarity in cat and frog atria (Koch-Weser, 1963; Kawata *et al.*, 1983).

When the eel atrium was treated with hyperosmotic NaCl Ringer solution, the contraction was initially reduced and increased finally (Figs. 1A and 2). The transient reduction was observed only after addition of NaCl and  $\text{NaCH}_3\text{SO}_4$  (Fig. 2). Since the common ion between these salts is  $\text{Na}^+$ , the reduction may be due to an increase in the extracellular  $\text{Na}^+$  concentration ( $[\text{Na}^+]_o$ ). As in mammalian and amphibian hearts (Chapman, 1983), fish myocardium also possesses  $\text{Na}^+/\text{Ca}^{2+}$  exchanger which lowers intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) (Tibbits, Moyes and Hove-Madsen, 1992; Tibbits, Philipson and Kashiwara, 1992). Therefore, it is likely that the increase in  $[\text{Na}^+]_o$  stimulates the  $\text{Na}^+/\text{Ca}^{2+}$  exchange, thus lowers  $[\text{Ca}^{2+}]_i$ , which relaxes the myocardium and inhibits contraction initially. The enhancement in contraction after isoosmotic low  $\text{Na}^+$  Ringer solution is explained by an inhibition of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger, and by an increase of  $[\text{Ca}^{2+}]_i$ . However, when water is lost by osmosis (Table 2), myocardium will shrink and intracellular ion concentrations ( $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{H}^+$  etc.) will alter, and the contractility may be enhanced by still unknown mechanisms. However, correlation between cell swelling or shrinkage and intracellular pH have been shown in mammalian heart (Whalley *et al.*, 1991, 1994; Befroy *et al.*, 1999). Whally *et al.* (1991) further report that hyperosmolarity stimulates  $\text{Na}^+/\text{H}^+$  exchanger and induces intracellular alkalinization. On the other hand, Sata *et al.* (1995) demonstrate in *in vitro* system that acidification decreases  $\text{Ca}^{2+}$  sensitivity of cardiac thin filament reconstituted from actin and tropomyosin-troponin complex which are purified from rat myocardium. In relation to their observations, we also observed in the eel atrium that dimethyl-amiloride, an inhibitor of  $\text{Na}^+/\text{H}^+$  exchanger, partially inhibited the sucrose-induced enhancement in contractility (unpublished observation). Therefore, stimulated  $\text{Na}^+/\text{H}^+$  exchanger might be involved in the osmotically enhanced contraction of the eel heart.

Plasma osmolality in the bulbus arteriosus was 20 mosm  $\text{kg}^{-1}$  higher than that in caudal vessels (Table 3). This implicates an entry of hyperosmotic blood into the heart, presumably from the esophagus, since highly concentrated NaCl is absorbed through the esophagus of the seawater eel (Nagashima and Ando, 1994). Because 20  $\text{mmol l}^{-1}$  sucrose enhanced atrial contractility significantly (Table 1), the higher plasma osmolality in the heart might increase the cardiac outflow effectively under *in vivo* condition. Although  $\text{Cl}^-$  concentration was not significantly different between bulbus arteriosus and caudal vessels, this phenomenon may be explained by a  $\text{Cl}^-/\text{HCO}_3^-$  exchange (chloride shift) in erythrocytes.

On the other hand, it is reported that hyperosmolarity induces secretion of ANP in the eel *in vivo* (Kaiya and Takei, 1996) and that ANP stimulates  $\text{Cl}^-$  secretion in the opercular epithelia, a model epithelium for gills, of killifish (Scheide and Zadunaisky, 1988). Thus, it is likely that plasma hyperos-

molarity enhances cardiac outflow by increasing contractility (present study) and stimulates ANP secretion from the myocardium. The enhanced cardiac outflow and ANP level will stimulate NaCl secretion through the gills more efficiently in eels. These responses of the atrium to hyperosmolarity would have clear advantages for seawater teleosts, since the concentrated NaCl from the esophagus can be excreted immediately through the gill, without circulating their body, and blood homeostasis can be maintained efficiently.

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