Salmon cardiac natriuretic peptide is a volume-regulating hormone

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Tervonen, Virpi, Heikki Ruskoaho, Tiina Lecklin, Mika Ilves, and Olli Vuolteenaho. Salmon cardiac natriuretic peptide is a volume-regulating hormone. Am J Physiol Endocrinol Metab 283: E353–E361, 2002.—The present study tested the hypothesis that salmon cardiac peptide (sCP), a new member of the family of natriuretic peptides, has an important role in the regulation of fluid balance and cardiovascular function. Intra-arterial administration of sCP increased urine output in salmon. It had a diuretic effect in rat as well, but the potency was lower. sCP increased the sodium excretion in proportion to the increased urine flow. Blood pressure was not affected by sCP in either species. Acute volume expansion elevated the plasma level of sCP in salmon, and an acute transfer of salmon from fresh to sea water decreased the circulating sCP level. Cardiac immunoreactive sCP or sCP mRNA levels were not affected by transfer to sea water. These results indicate that sCP has an important physiological role in defending salmon against volume overload but that it does not appear to contribute to the short-term regulation of blood pressure. sCP provides an excellent model of the general mechanisms of regulation of the A-type (atrial) natriuretic peptide system.

osmoregulation; diuresis; natriuresis; volume overload; hypoperosmotic environment

CONTROL OF WATER AND SALT BALANCE involves a complex interplay of regulatory mechanisms. The cardiac natriuretic peptides play an important part in this regulation (18, 21). The first member of the peptide family, atrial (A-type) natriuretic peptide (ANP) (7) has been shown to be a multifunctional hormone protecting volume and pressure homeostasis (18, 25). In mammals, the release of ANP from the heart is enhanced by stretch of cardiac myocytes caused, for example, by an increase in blood volume. ANP reduces the cardiac load by decreasing the systemic blood pressure and intravascular volume. The volume-depleting action is a result of increased excretion of water and electrolytes in the kidney and inhibition of the renin-angiotensin-aldosterone system (18, 21).

Although the biology of the natriuretic peptides has been studied extensively, the cellular and molecular elements important for the physiological actions are not yet well understood. We used salmon cardiac peptide (sCP), a recently discovered teleost cardiac hormone related to the natriuretic peptides (32, 34) as a model in our attempt to clarify these mechanisms. As judged by the cDNA and gene sequence, sCP represents a new subgroup of the natriuretic peptide family (20, 32). However, the storage, processing, and molecular sizes of the stored and circulating forms resemble those of ANP (13, 35). In addition, the release from isolated ventricle is similarly sensitive to mechanical load and utilizes the regulated pathway, as does ANP secretion from the mammalian cardiac atria (13). Finally, the basal gene expression of ANP and sCP appears to be regulated by similar elements (20). Thus sCP could be used to elucidate the conserved features important for the actions and regulation of the ANP-like peptide hormones. We have now examined the biological profile of sCP, with special emphasis on its potential role as a volume regulating hormone.

MATERIALS AND METHODS

Experimental animals. Salmon (Salmo salar) of both sexes, aged 4–5 yr and weighing 640 ± 120 g, were obtained from the Finnish Game and Fisheries Research Institute (Taivalkoski, Finland, 65°6’ lat.), where they were reared in fresh water under natural temperature and photoperiod. The fish were transported to the Department of Physiology, University of Oulu, in closed plastic packages filled with oxygenated water and were released into water tanks containing unchlorinated, recirculating tap water. The water quality was maintained by biological filtration and biweekly water changes of ~20%. The salmon were stocked in a 1,500-l tank at a density of ~16 fish per tank. The experiments were performed in November and January, when the water temperature was 0.2°C and the seasonal photoperiod was 4 h light and 20 h dark. In the laboratory, the maintenance and experimental ambient temperature was 6°C and the light-dark cycle was 8:16 h. Dissolved oxygen levels in the experimental tanks were >80% oxygen saturation.

The male Sprague-Dawley rats used (318 ± 26 g, mean ± SD) were from the colony of the Center of Experimental Animals at the University of Oulu. The rats were housed in
plastic cages and had free access to rat maintenance chow and water. Room temperature of 22°C, humidity of 40%, and a 12:12-h light-dark environmental light cycle were maintained. The experimental design was approved by the Animal Experimentation Review Board of the University of Oulu.

**Bolus injection of synthetic sCP into salmon.** For the administration of sCP, measurement of arterial blood pressure, and collection of blood samples, the salmon were cannulated via the dorsal aorta as described previously (29). The fish were anesthetized in well aerated tricine solution (100 mg/l, MS-222, Sigma Chemical, St. Louis, MO), the pH of which was adjusted to 7.0 with sodium carbonate. The anesthesia took place at the temperature of the water in the tank. When the animals became unresponsive to handling, they were weighed, placed on the operating table, and covered with a cold wet towel. The gills were not irrigated during the operation. The dorsal aorta was cannulated near its origin via the buccal cavity with PE-50 tubing (0.96 mm OD, 0.58 mm ID; Portex, Hythe, UK) with the help of a stainless steel wire. The cannula was pretreated with a small amount of heparinized saline (100 IU/ml). The cannula was exteriorized through the snout via a short PE-10 tube (1.57 mm OD, 1.14 mm ID, Portex), which pierced the upper jaw through the snout. The cannula was filled with heparinized saline (100 IU/ml) and plugged with a stainless steel pin. The urinary bladder was catheterized through the urinary pore with PE-50 tubing filled with saline and worked ~4 cm into the animal. This cannula was anchored with a ligation (3–0 coated polyester fiber; Deknalon, Hamburg, Germany) to the anal fin. The whole procedure, from immersing the fish into the tricine solution to reviving the fish, took 15 min or less. The fish were placed in individual black plastic tubes floating in the holding tank and were allowed to recover overnight. On the next day, the dorsal aortic cannula was flushed with heparinized saline and attached to a three-way stopcock (Codan, Esbjerg, Denmark), one arm of which was connected to a pressure transducer (Micron Instruments, Simi Valley, CA) and a Grass polygraph (Grass Instruments, Quincy, MA). Zero pressure was adjusted to the level of the water surface, and the calibration was carried out using a tube filled with water. The experiment was started by monitoring the baseline dorsal aortic pressure for 20 min, before which a blood sample (1 ml) was withdrawn from the arterial cannula for the measurement of basal plasma immunoreactive (ir)-sCP and the amino-terminal (NT) fragment of pro-sCP (NT-pro-sCP). The blood sample was immediately centrifuged in a microfuge (1 min, 13,000 rpm; Heraeus Instruments, Osterode, Germany). The plasma was removed into a chilled Eppendorff tube on ice, and the blood cells were gently resuspended in 0.5 ml of cold salmon Ringer solution (in mmol/l: 140 NaCl, 10 NaHCO3, 2 NaH2PO4, 1 MgSO4, 1 CaCl2, 4 KCl, pH 7.8) and injected back into the fish. This injection lasted 1 min and was followed by a 0.2-ml flush of heparinized saline (100 IU/ml). Synthetic sCP (32) or vehicle (salmon Ringer solution) was injected into the dorsal aorta as a single bolus. An sCP dose of 60 pmol/kg was delivered in volumes ranging from 0.3 to 0.5 ml, and the peptide injection was followed by a 0.1-ml saline flush. Arterial blood samples of 0.5 ml were taken at 30, 60, 90, 120, and 150 min after the injections. The plasma, obtained from centrifugation in a microfuge (2 min, 13,000 rpm), was placed on ice. These blood samples were replaced with an equal volume of salmon Ringer solution. The urine catheter emptied into polyethylene tubes that were placed below the water surface outside the holding tank to ensure constant urine flow from the bladder. Fractions of 30 min were collected using a fraction collector for the measurement of volume, sodium, and potassium concentrations. The ion concentrations were measured from urine with an ion selective analyzer (Kone Instruments, Espoo, Finland) and the osmolality with an osmometer (Advanced Wide-Range Osmometer, Advanced Instruments, Needham Heights, MA). After the experiments, the fish were killed with a sharp blow on the head. All plasma and urine samples were stored at −70°C.

**Infusion of synthetic sCP into rats.** The physiological actions of mammalian ANP and B-type, or brain, natriuretic peptide (BNP) are mediated via the ANP receptors (NPR-A). Because mature sCP structurally resembles mammalian cardiac natriuretic peptides, we were interested in whether characteristics important for ligand recognition by the mammalian natriuretic peptide receptors, specifically NPR-A, are present in sCP. Thus the effectiveness of synthetic sCP was studied in rat. The surgical preparation and experimental setup have been described previously (16). Briefly, under anesthesia (1 part Hypnorm (Janssen Pharmaceutical, Beerse, Belgium), 1 part Dormicurum (Roche, Espoo, Finland), 2 parts sterile water (3.3 ml/kg ip), a PE-60 catheter (0.76 mm ID, 1.22 mm OD; Intramedic, Becton-Dickinson, Sparks, MD) was placed into the abdominal aorta through the left femoral artery, and a PE-50 catheter (0.58 mm ID, 0.965 mm OD, Intramedic) was inserted into the femoral vein. All of the catheters were exteriorized behind the neck, filled with heparinized saline (500 IU/ml) solution, and plugged with a stainless steel pin. After the operation, the rats were housed individually in cages and had free access to food and water. A day after the operation, the rats were placed individually in metabolic cages without food and water. The arterial catheter was attached to a pressure transducer (Micron Instruments, Los Angeles, CA), and the mean arterial pressure (MAP) and heart rate (HR) were recorded with Ponemah Physiology Platform software (Gould Instrument Systems, Valley View, OH). The venous catheter was connected to a syringe infusion pump (Braun Perfusor ED, Braun Melsungen, Melsungen, Germany) for administration of peptide or vehicle (0.9% NaCl). Rats were left undisturbed for 30 min before the recording of hemodynamic variables and collecting of urine.

The experiment was started by recording the MAP and HR for 25 min before 1.0 ml blood was drawn from the arterial catheter for the measurement of basal plasma ir-NT-pro-ANP and ir-BNP levels. Because the plasma sample size did not allow measurement of both ir-ANP and ir-BNP, the NT-pro-ANP analysis was chosen for the estimation of secretion of pro-ANP-derived peptides. When blood pressure, HR, and right atrial pressure were stabilized near to control values (in ~5 min), synthetic sCP was administered intravenously at a dose of 660 pmol·kg−1·min−1 for 150 min by means of an infusion pump; the infusion rate was 20 µl/min. In control experiments, only the vehicle was infused. Arterial blood samples of 1.0 ml were taken into K2-EDTA tubes at 30, 60, 90, 120, and 150 min. Samples were centrifuged immediately (13,000 rpm, 5 min), and the separated plasma was stored at −20°C. The drawn blood was replaced with an equal volume of blood from a donor rat. Donor blood was obtained from conscious rats that were chronically cannulated into the femoral artery for this purpose. The drawnblood volume in donor rats was replaced by 0.9% NaCl. Urine was collected every 30 min over 2.5 consecutive hours for volume, sodium, potassium concentration and osmolality measurements. The urine ion concentrations were measured with an ion selector and the osmotic concentration with an osmometer. After the experiments, the rats were anesthetized with a bolus injection of anesthetic solution via the

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arterial cannula and decapitated. All of the plasma and urine samples were stored at −70°C.

**Acute volume overload in salmon.** Salmon were given an intra-arterial infusion of saline to find out whether acute blood volume expansion affects the release of sCP. Cannulation was performed as described for bolus injection of synthetic sCP into salmon. The acute volume overload was caused by intra-arterial administration of precooled 0.9% NaCl (10 ml/kg body wt during 2–3 min). For the measurement of the plasma levels of ir-sCP and NT-pro-sCP, arterial blood samples of 0.5 ml were drawn in chilled tubes containing 1.5 mg K₂-EDTA/ml blood at 10, 20, and 30 min and at 1, 2, and 3 h and centrifuged at +4°C to separate the plasma. The plasma samples were stored at −70°C until assayed. All of the blood samples were replaced with an equal volume of saline. After the experiments, the fish were killed with a sharp blow on the head.

**Transfer of salmon from fresh water to sea water.** We used an acute transfer of cannulated salmon from fresh water to sea water to study the effect of exposure to a hyperosmotic environment on the regulation of the sCP system. The dorsal aorta and the urinary bladder were cannulated as described, except that the urinary pore was sealed with cyanoacrylate adhesive and a small piece of vinyl. The fish were placed in individual plastic tubes floating in a 300-l experimental tank. Two to three tubes were placed in each tank containing well aerated, filtered, recirculating tap water. The water quality was followed daily with measurements of dissolved oxygen saturation (OxyGuard International, Birkerød, Denmark) and weekly with measurement of nitrite (Aqua Vital Multi-sticks; Aquarium Münster, Telgte, Germany). The next day, a 0.7-ml blood sample was drawn via the arterial cannula for the measurement of basal plasma ir-sCP, ir-NT-pro-sCP, Na⁺, Cl⁻, K⁺, and osmolality. Urine was collected via the catheter as two 60-min fractions by use of an LKB Redirac fraction collector (LKB, Bromma, Sweden) to determine the volume and NT-pro-sCP, Na⁺, and K⁺ concentrations. The fish were randomly divided into two groups, which were acutely transferred into 300-l tanks containing either fresh water or artificial sea water. The transfer was not associated with any mortalities, but two fish showed slight restlessness for a few minutes after the transfer. Blood samples of 0.7 ml were drawn at 30, 60, and 120 min and at 24, 48, and 72 h after the transfer. The plasma was separated by centrifugation at +4°C and stored at −70°C. The blood cells were gently resuspended in 0.5 ml of chilled salmon Ringer solution and injected back into the fish. In the fish kept in fresh water, the urine was collected in two 60-min fractions starting from the transfer, as described. In the fish transferred to sea water, the urine flow had decreased to very low levels within 2 h after the transfer. To obtain a volume large enough for determination of the electrolytes, osmolality, and ir-NT-pro-sCP, the urine was collected in 24-h fractions. After the experiments, the fish were killed with a sharp blow on the head. The heart was removed and the atrium and ventricle separated, weighed, and frozen in liquid N₂. The plasma and tissue samples were stored at −70°C.

**Determination of sCP mRNA by quantitative RT-PCR.** Total RNA was extracted from salmon cardiac tissue by means of the acid-pheno1 method (6). Aliquots of the guanidine isothiocyanate homogenates were stored at −70°C for use in radioimmunooayassay. The cDNA first strand was synthesized using Moloney murine leukemia virus reverse transcriptase. The quantitative PCR reactions were performed with the ABI 7700 Sequence Detection System using the TaqMan chemistry and the primers and the bifunctional probes for sCP and 18S RNA described previously (20).

**Radioimmunoassays.** The assays for rat BNP (19), rat NT-pro-ANP (39), sCP (35), and NT-pro-sCP (38) were performed as described previously. For the direct NT-pro-sCP assay, salmon plasma samples were diluted 1:50. The dilution was 1:2.5 for rat plasma samples in the direct NT-pro-ANP assay. The salmon and rat plasma samples and salmon urine samples were extracted with SepPak C₁₈ cartridges (Waters, Milford, MA) before the sCP and BNP radioimmunoassays. Aliquots of the homogenates obtained during RNA extraction (see **Determination of sCP mRNA by quantitative RT-PCR**) were used to measure ir-sCP in the tissue samples. No more than 1 µl of the guanidine isothiocyanate extract was used directly in the radioimmunoassay because of the interference caused by the strongly chaotropic buffer. The urine samples were extracted as described for plasma samples for use in the NT-pro-sCP radioimmunoassay.

**Statistical analyses.** Results are expressed as means ± SE unless otherwise indicated. An unpaired Student’s t-test was used for comparison between treated and control groups. The results from repeated measures were analyzed using one-way analysis of variance followed by the Newman-Keuls test. The level P < 0.05 was considered statistically significant.

**RESULTS**

To find out the acute effects of sCP, a bolus injection (60 pmol/kg) was given into the dorsal aorta of chronically cannulated salmon, and a constant infusion of sCP (660 pmol·kg⁻¹·min⁻¹ for 150 min) was given intravenously to conscious Sprague-Dawley rats. We first determined the circulating levels of sCP. The bolus injection elevated the salmon plasma level of ir-sCP from 125 ± 8 to 285 ± 21 pmol/l within 30 min (P < 0.001, n = 11; Fig. 1). Plasma ir-sCP returned to a level indistinguishable from the control level by 60 min after the injection. In control fish, the circulating ir-sCP concentration did not change significantly during the study period from the basal level of 126 ± 4 pmol/l (n = 9). Circulating NT-pro-sCP was not affected by the bolus injection of sCP (Fig. 1). As expected from the cross-reactivity profile of our sCP antiserum (34), ir-sCP was undetectable in rat plasma in the basal conditions. The infusion of synthetic sCP resulted in the very high plasma ir-sCP concentration of ~25 nmol/l (Fig. 2A). sCP infusion did not have any

![Fig. 1. Plasma concentrations of immunoreactive (ir) salmon cardiac peptide (sCP) and amino-terminal (NT)-pro-sCP in salmon before and after a bolus injection of synthetic sCP (60 pmol/kg).](http://www.ajpendo.org)
significant effect on the endogenous levels of rat plasma NT-pro-ANP or BNP (Fig. 2, B and C).

The renal effects of sCP were studied by collection of urine and analysis of the excreted volume and electrolyte concentrations. The bolus injection of synthetic sCP to salmon resulted in a fourfold increase in the urine flow ($P < 0.01$, $n = 7$; Fig. 3A). The maximal increase from 0.4 ± 0.1 to 1.7 ± 0.3 ml·kg$^{-1}$·h$^{-1}$ was detected during the first 30 min after the sCP bolus, and the significant diuretic effect lasted for ≥90 min. The urine flow remained constant in control fish injected with salmon Ringer solution (0.5 ml·kg$^{-1}$·h$^{-1}$). The administration of sCP did not cause any significant changes in the urine Na$^+$ concentration or osmolality, but the total amount of Na$^+$ and osmolytes excreted was increased in proportion to the diuretic effect (Fig. 4, B and C).

In contrast to the remarkable effects on renal function, synthetic sCP did not appear to have any significant influence on the cardiovascular parameters measured. The mean blood pressure was not affected by the sCP administration in salmon or rats. In the control group of salmon, the resting baseline dorsal aortic pressure ($P_{DA}$) was 26.5 ± 2 mmHg and in the experimental group 25.5 ± 1.3 mmHg. At the end of the experimental period, $P_{DA}$ in the control group was 21.5 ± 1.9 mmHg ($n = 5$) and in the experimental group 21.4 ± 1.5 mmHg ($n = 8$). In the rats, the MAP varied between 119 ± 4 and 121 ± 5 mmHg during the infusion in the control and experimental groups, without any significant differences between the groups or time points. In the rats, the HR did not show any significant changes as a result of infusion of sCP, averaging 386 ± 7 beats/min in the control group and

Fig. 3. Effect of synthetic sCP (60 pmol/kg bolus; ●) or vehicle (○) on urine flow rate (A) and renally excreted Na$^+$ (B) and osmolytes (C) in fresh-water salmon. Results are expressed as means ± SE; $n = 7$ for sCP injections, $n = 8$ for controls. **$P < 0.01$ and *$P < 0.05$ compared with baseline value.
Mechanical load enhances the secretion of sCP from isolated perfused salmon ventricle (13, 32). To study whether loading the heart by acute volume expansion influences the plasma levels of sCP in vivo, salmon were given an intra-arterial bolus of saline. Theoretically, the bolus of 10 ml/kg body wt saline increases the blood volume by ~25%. The circulating level of ir-sCP increased on average 1.7-fold (from 153 ± 37 to 256 ± 79 pmol/l; n = 7) within 10 min after the administration of the saline bolus (P < 0.05; Fig. 5A). The sCP plasma level had returned to the baseline value within 60 min after the volume expansion. Plasma NT-pro-sCP concentration did not change significantly, varying between 840 ± 99 and 1,030 ± 115 pmol/l (n = 7; Fig. 5B).

Increased osmolality has been shown to stimulate the cardiac release of ANP from isolated rat atria (3). Teleosts dehydrate after transfer to sea water (8), and their plasma ionic concentrations are higher in sea water than in fresh water (10). To study whether the increased plasma osmolality and ionic concentration have any effects on the circulating sCP or the cardiac peptide and sCP mRNA levels, fresh-water salmon were acutely transferred to sea water. Cannulated salmon were used to study the effect of dehydration and increased plasma osmolality, resulting from the hyperosmotic environment, on the levels of circulating and cardiac sCP as well as those of cardiac sCP mRNA. The plasma osmolality was significantly increased to 330 ± 1 mosmol/kgH2O (n = 5) 2 h after the transfer to sea water (P < 0.05) compared with that found in the fresh-water controls (313 ± 6 mosmol/kgH2O; n = 5) and continued to increase throughout the experiment (Fig. 6A). The baseline plasma concentrations of Na⁺, Cl⁻, and K⁺ were 159.6 ± 1.7, 132.6 ± 1.8, and 2.2 ± 0.06 mmol/l, respectively (n = 10), in accord with previous studies (27, 31). The plasma Na⁺ and Cl⁻ concentrations were significantly elevated, starting at 24 h

Fig. 4. Effect of synthetic sCP (330 pmol·kg⁻¹·min⁻¹ infusion; filled bars) or vehicle (open bars) on the total amount of urine (A), sodium (B), and osmolytes (C) excreted during the study period in rats. Results are expressed as means ± SE; n = 8.

Fig. 5. Effect of acute plasma expansion on the plasma concentration of ir-sCP and NT-pro-sCP in salmon. The plasma volume was increased at 0 min by a bolus injection of saline (10 ml/kg body wt). Results (means ± SE; n = 7) were calculated by dividing the peptide level at each time point by the mean level at 0 min. *P < 0.05 compared with baseline value.
In the present study, the urine flow, urine osmolality, and Na⁺ concentration were examined to evaluate the amount of renally removed volume and sodium in salmon in hypo- and hyperosmotic environments. In addition, the presence of NT-pro-sCP in urine was studied by radioimmunoassay. The urine flow rate in cannulated adult salmon kept in fresh water was 2.7 ± 0.6 ml·kg⁻¹·h⁻¹ during the experiment. The transfer of salmon from fresh to sea water decreased the urine flow almost immediately to very low levels (Fig. 8A; P < 0.05, n = 3). The minimum urine flow rate of 0.2 ± 0.1 ml·kg⁻¹·h⁻¹, which was maintained to the end of the experiment, was reached within 2 h after the transfer to sea water. The baseline (fresh water) urine osmolality was 54 ± 10 mosmol/kg H₂O and the Na⁺ concentration 22.3 ± 3.4 mmol/l (Fig. 8, B and C; n = 6). During the 3-day experiment, the urine osmotic concentration remained practically unchanged in salmon kept in fresh water. On the other hand, the urine osmolality increased significantly within 24 h after the transfer in the salmon transferred to sea water (Fig. 8B; P < 0.05, n = 3). Because of the very small urine volume, it was not possible to analyze the urine osmolality at 2 h after the transfer to sea water. The urine Na⁺ concentration was significantly higher 24 h after the transfer to sea water (Fig. 8C; P < 0.001, n = 3) compared with the salmon staying in fresh water. ir-NT-pro-sCP was detectable in solid-phase extracted urine samples. In fresh-water salmon, the levels were 9.3 ± 2 pmol/l (n = 7). No statistically significant differences were detected in the urine ir-NT-pro-sCP levels between the experimental groups or among the time points.

**DISCUSSION**

We recently discovered a novel cardiac peptide hormone, sCP, a teleost representative of the family of
natriuretic peptides (20, 32, 34). The natriuretic peptides have previously been found to have direct and indirect diuretic, natriuretic, and hypotensive effects (21, 22, 41). Now, we wanted to test whether the biological profile of sCP is consistent with the presumed central role of the natriuretic peptides in the regulation of teleost fluid balance and cardiovascular function.

A potent diuretic effect is one of the most characteristic features of the natriuretic peptides (18, 21). In teleosts, homologous heart extract and heterologous ANP have been found to cause diuresis in toadfish (15), and eel and rat ANP has been reported to exert diuretic effects in trout (23). In the present study, intravenous administration of synthetic sCP increased urine output in both salmon and rats. In salmon, a single dose of 60 pmol/kg caused an impressive diuretic response. The urine flow was still significantly elevated at 90 min after the bolus, even though the plasma sCP concentration had returned to the baseline level within 60 min. In rats, infusion of a fairly large amount of sCP (330 pmol·kg⁻¹·min⁻¹) was required to induce the diuretic effect. It does, however, show that the structural features of sCP and those of ANP and BNP are sufficiently conserved, so that rat NPR-A receptor can bind sCP. The relatively prolonged diuretic action of sCP in salmon is consistent with previous results on ANP in mammals (2, 5) showing that the cellular response, and thereby the biological effect, outlasts the changes in plasma peptide concentrations. On the other hand, sCP infusion did not affect the endogenous levels of rat plasma NT-pro-ANP, despite our previous findings that ANP can directly inhibit its own release via NPR-A receptors in rats (17). Nor did sCP have any significant effect on the circulating levels of BNP, as might have been expected on the basis of earlier findings in a genetic model of ANP deficiency that increased synthesis of BNP can compensate for the decreased concentration of ANP (30).

In mammals, besides diuresis, ANP causes an increased excretion of electrolytes, especially Na⁺ and Cl⁻ (18, 21). In toadfish, homologous heart extracts and mammalian ANP have been found to increase the excretion of Na⁺ but not of Mg²⁺, Ca²⁺, or K⁺ (15). In fresh-water trout, eel ANP has been reported to increase the urine osmolarity (23). In those studies, a statistically significant natriuretic response exceeding the diuresis was, however, obtained only with very high doses of natriuretic peptides (20 µg of mammalian ANP per toad or 10 µg/kg body wt of eel ANP per trout). In the present study, sCP caused a significant natriuretic response in both salmon and rat, but the response was proportionate to the diuretic effect. Accordingly, the urine osmolality did not change significantly in response to sCP administration. Thus our present results indicate that sCP participates in teleost volume regulation by its potent capability to increase urine output and sodium excretion. It should be remembered, however, that, in contrast to the domination of the kidney in terrestrial vertebrates, both the kidneys and the gills are important sites of water and electrolyte excretion in fishes (10). The potential role of the gill as a target organ to sCP should be carefully examined in future studies.

Another key characteristic of the natriuretic peptide is their hypotensive action (18, 25). However, the decrease of blood pressure in normotensive experimental animals has usually been achieved only with high doses of peptides. It appears that the decrease of the blood pressure and the concomitant increase of the heart rate require doses of ANP that exceed those causing diuresis and natriuresis (2, 15, 23, 28, 40). In fresh-water eels, administration of homologous ANP with a dose of ~50 pmol/kg has been found to induce a decrease in the mean arterial pressure (24). In the present study, a bolus intra-arterial injection of sCP into salmon, causing brisk and prolonged diuresis, did not affect the mean arterial pressure. Moreover, the mean arterial pressure as well as the heart rate were unchanged in rats in which sCP was infused at doses resulting in very high plasma levels and significant diuresis. Thus, in the biological profile of sCP, the effects on the fluid and electrolyte balance appear to predominate over those on the cardiovascular system.

Teleosts are hyposmotic to fresh water but hypersmotic to sea water. Thus they have chronic problems with the salt and water balance because of the ionic and osmotic gradients between the epithelia and the environment. Fresh-water fish face a threat of volume...
overload and salt loss, whereas marine teleosts tend to lose water and gain salt (4, 10). Euryhaline fish such as migrating salmon face both kinds of challenges and must therefore be able to modulate the level and type of osmoregulation. Thus they provide an ideal model for studies on osmo- and ionoregulatory mechanisms. The cardiac natriuretic peptides have been shown to be important for the regulation of the water and salt balance in both physiological and pathophysiological conditions in mammals; therefore, peptide hormones belonging to this family have been proposed to have an essential role in fish osmoregulation as well (9, 10). In mammals, volemic stimuli are potent regulators of the cardiac natriuretic peptide release (25, 36), whereas in the eel an increase in plasma osmolality rather than blood volume has been proposed to be a major regulator of eel atrial and ventricular natriuretic peptides (12). In the present study, we examined the effect of increased plasma volume and dehydration and enhanced osmolality on sCP in salmon.

In mammals, atrial stretch is the most widely recognized stimulus for ANP secretion (25, 36). The increase of plasma volume, resulting in heart wall stretch, has been shown to elevate the circulating ANP concentration in a large number of animal species (1, 12, 14, 26, 37). In the present study, acute volume overload of fresh-water salmon with saline (25% of estimated blood volume) induced a 1.7-fold increase of the circulating sCP concentration. In recent studies (13, 32), we have shown that sCP secretion from isolated salmon ventricle is rapidly and dose-dependently increased by mechanical load. It may be argued that the response to volume expansion in intact salmon was modest, considering the brisk increase of sCP secretion that loading causes in perfused salmon ventricle. However, the exact magnitude of the atrial and ventricular pressure increase caused by the volume expansion is not known. Moreover, isotonic saline injected into the dorsal aorta is prone to diffuse rapidly into the interstitial compartment. On the other hand, fresh-water salmon are facing the threat of volume overload to begin with, and the response to plasma volume expansion probably would have been more prominent in salmon with reduced circulating volume. Nevertheless, considering the biological effects of sCP (see RESULTS), we propose that the volume expansion-induced elevation of circulating sCP levels is an important homeostatic defense mechanism.

In contrast to the stimulating effect of plasma volume expansion on circulating sCP, increased plasma osmolality appears to be associated with a decreased level of sCP. Plasma ir-sCP was significantly lower in salmon kept in sea water for 3 days compared with the control fish remaining in fresh water. On the other hand, the exposure to sea water did not have any significant effect on the levels of ir-sCP or sCP mRNA in cardiac tissue, apparently the sole site of sCP production (20, 32, 35).

The plasma concentration of NT-pro-sCP remained unchanged, or even tended to increase, on transfer of salmon from fresh water to sea water. This is surprising, because NT-pro-sCP is formed by cardiomycocytes from the same precursor as sCP and released into the bloodstream in equimolar concentrations with sCP (35). The elimination pathways of the two peptides are, however, completely different. In analogy with mammalian ANP and BNP, sCP is rapidly eliminated, presumably by binding to receptors both with and without guanyl cyclase activity and by neutral endopeptidase-mediated degradation. On the other hand, NT-pro-sCP, a biologically inert peptide, probably lacks specific elimination pathways and is slowly excreted to the urine. In the present study, we demonstrated by homologous radioimmunoassay the presence of NT-pro-sCP in salmon urine. Previously NT-pro-ANP has been found in the urine in mammals (11). Thus the divergent responses of circulating levels of sCP and NT-pro-sCP may be explained by two mechanisms. Transfer to sea water could enhance the elimination of sCP, most probably by increasing the amounts of clearance receptors or by enhancing neutral peptidase activity. This would result in decreased plasma sCP levels and stable NT-pro-sCP levels without any change in the cardiac secretion rate of pro-sCP-derived peptides. In a recent study (33), we found strong evidence that modulation of the elimination rate explains the alterations of sCP plasma levels caused by varying ambient temperature. On the other hand, the presumably slower elimination, due to the greatly decreased urine output, would counteract the reduced production of NT-pro-sCP and could thus mask the decrease of plasma NT-pro-sCP levels. Further studies are required to find out their relative importance.

In mammals, the physiological function of ANP is thought to be the protection of the heart against volume and pressure overload (25). The secretion of ANP is stimulated by cardiac myocyte stretch. It causes diuresis, natriuresis, vasorelaxation, fluid shift from the intravascular to the interstitial compartment, and the inhibition of volume-conserving hormonal systems. Our present results suggest that sCP has an equivalent homeostatic role in teleosts to ANP in mammals in the regulation of volume and electrolyte balance. We demonstrated in the present study that sCP possesses potent diuretic and natriuretic activity. Its release from the heart is stimulated by increased extracellular volume in vivo, as found in the present study, and by increased mechanical load in vitro, as reported in our previous studies (13, 32). The similar physiological actions of mammalian and salmon cardiac natriuretic peptides, as well as the ability of rat natriuretic peptide receptors to recognize sCP, indicate high functional conservation of the natriuretic peptide system, spanning the large phylogenetic distance between teleosts and mammals. Thus sCP provides an excellent model for studies on the basic mechanisms of regulation of the A-type natriuretic peptides.

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