

SOMATOSTATIN-RELATED PEPTIDES ISOLATED FROM THE EEL GUT: EFFECTS ON ION AND WATER ABSORPTION ACROSS THE INTESTINE OF THE SEAWATER EEL

TOSHIHIRO UESAKA¹, KEIICHI YANO², MOTOO YAMASAKI²,
KEI NAGASHIMA¹ AND MASA AKI ANDO^{1,*}

¹*Laboratory of Physiology, Faculty of Integrated Arts and Sciences,
Hiroshima University, Higashi-Hiroshima, Hiroshima 724, Japan*
and ²*Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd,
3-6-6 Asahimachi, Machidashi, Tokyo 194, Japan*

Accepted 9 November 1993

Summary

Four somatostatin-related peptides were isolated from eel guts. Two of them were the same as eel SS-25II (eSS-25II) and eel SS-25I (eSS-25I) isolated from European eel pancreas. The remaining two peptides were C-terminal tetradecapeptides (eSS-14II and eSS-14I) of eSS25II and eSS-25I, respectively. These four peptides all enhanced the serosa-negative transepithelial potential difference and short-circuit current across the seawater eel intestine after pretreatment with isobutylmethylxanthine, serotonin (5-HT) and methacholine, an agonist of acetylcholine (ACh). Among these peptides, eSS-25II was the most potent enhancer, followed by eSS-25I and eSS-14II. Since the large peptide (eSS-25II) acts at a lower concentration than the small somatostatin (eSS-14II), the 11 N-terminal amino acid residues seem to potentiate somatostatin action in the eel intestine. In contrast, eSS-14II was more potent than mammalian SS-14, indicating that the three amino acid residues (Tyr¹⁸, Gly²¹, Pro²²) in the C-terminal portion also contribute to the potency of somatostatin. Endogenous somatostatin (eSS-25II) activated net Na⁺, Cl⁻ and water fluxes across the seawater eel intestine. This stimulatory action was not inhibited by tetrodotoxin or yohimbine, an adrenergic antagonist, indicating that eSS-25II does not act through neuronal firing or through catecholamine release. Thus, eel somatostatins may act directly on the enterocytes, but on a distinct receptor from that for adrenaline, to antagonize the inhibition of NaCl and water absorption by 5-HT and ACh in the seawater eel intestine.

Introduction

Regulation of ion and water transport across the intestine is essential for the survival in sea water or fresh water of euryhaline teleosts, such as eel and flounder. However, only a

*To whom reprint requests should be addressed.

Key words: somatostatin, HPLC purification, ion transport, water absorption, eel intestine, *Anguilla japonica*.

few regulators have been proposed for the teleost intestine, and their effects are controversial. Mainoya and Bern (1984) have reported that porcine vasoactive intestinal peptide (VIP) inhibits NaCl and water absorption across the intestine of the tilapia. However, in the flounder intestine, VIP does not inhibit NaCl absorption but stimulates only Cl⁻ secretion (O'Grady and Wolters, 1990). NaCl absorption across the flounder intestine is inhibited by rat atrial natriuretic peptide (O'Grady *et al.* 1985). Urotensins are also thought to act as regulators of intestinal ion and water absorption, but their effects are different in freshwater and seawater tilapia (Mainoya and Bern, 1982, 1984).

Our previous study has demonstrated that ion and water absorption across the seawater eel intestine is reduced by acetylcholine (ACh), serotonin (5-HT) and eel atrial natriuretic peptide (Mori and Ando, 1991; Ando *et al.* 1992) and enhanced by catecholamines (Ando and Kondo, 1993; Ando and Omura, 1993). In addition, the existence of other endogenous regulators in the eel intestine has also been proposed by Mori and Ando (1991).

The present study was intended to isolate such endogenous regulators from the eel gut, to characterize their structure and to elucidate their mode of action. Four somatostatin-related peptides were extracted from eel gut, purified, and their primary structures identified. By comparing their structure and potency with those of mammalian somatostatins, we were able to examine their structure-activity relationships. Furthermore, we also demonstrate that eel somatostatin stimulates NaCl and water absorption across the intestine of the seawater eel independently from neuronal firing or catecholamine release.

Materials and methods

Purification and structural analysis

Three hundred cultured Japanese eels, *Anguilla japonica*, were decapitated and the gut excised immediately, frozen on dry ice and stored at -80 °C. The frozen tissues (593 g) were pulverized in a stainless-steel mortar cooled with liquid nitrogen and immediately boiled in three volumes of distilled water for 10 min. After cooling, trifluoroacetic acid (TFA) was added to a final concentration of 0.1%. The mixture was homogenized with a Polytron mixer. The homogenate was centrifuged (10 000g for 30 min) at 4 °C, and the supernatant was treated with acetone (final concentration 66%). After acetone precipitation, the supernatant was evaporated to dryness. The dried material was dissolved in 0.1% TFA (50 ml) and forced through disposable C18 cartridges (Sep-Pak, Millipore, Milford, MA). The retained material was eluted with 50% acetonitrile containing 10% 2-propanol and 0.1% TFA, and the eluate was evaporated. The concentrated material was applied to a column of Toyopearl HW-40F (2.6 cm × 100 cm; Tosoh, Tokyo, Japan) and eluted with 1 mol l⁻¹ acetic acid and 10% 2-propanol at rate of 1.5 ml min⁻¹. A sample of each fraction (18 ml) was assayed for its ability to enhance transepithelial potential difference (PD) across the eel intestine. Bioactive fractions were pooled and subjected to HPLC separation (LC-6AD system, Shimadzu, Kyoto, Japan) with a reverse-phase column (Asahipak C8P-50, Asahi Chemical Industry, Kanagawa, Japan). The retained material was eluted with a 60-min linear gradient of 0% to 60%

acetonitrile containing 10% 2-propanol and 0.1% TFA, and each fraction was bioassayed. Bioactive fractions were applied to a reverse-phase column (TSKgel ODS-80T_M, Tosoh) and eluted with a 100-min linear gradient of 0% to 20% acetonitrile containing 5% 2-propanol and 0.1% TFA. The active fractions were applied to a cation-exchange column (TSKgel CM-5PW, Tosoh) and eluted with a 35-min linear gradient of 0–0.35 mol l⁻¹ NaCl in 20 mmol l⁻¹ phosphate buffer (pH 6.7) containing 10% 2-propanol. Bioactive peaks were rechromatographed on a reverse-phase column (TSKgel ODS-80T_M) with a 50-min linear gradient of 5% to 15% acetonitrile in 5% 2-propanol and 0.1% TFA. Final purification was performed using the same column under isocratic conditions (see Fig. 1).

Amino acid compositions were determined with a PICO-TAG amino acid analysis system (Millipore). Amino acid sequence analyses of the peptides were carried out by automated Edman degradation with a gas protein sequencer (PPSQ-10, Shimadzu). To determine molecular mass, fast atom bombardment mass spectrometry (FAB-MS) was performed with JMS-HX110A (Jeol, Tokyo, Japan).

Measurement of biological activity across the eel intestine

Each fraction was assayed for its ability to enhance the PD across the seawater eel intestine after pretreatment with 10⁻⁵ mol l⁻¹ isobutylmethylxanthine (IBMX), 10⁻⁶ mol l⁻¹ serotonin (5-HT) and 10⁻⁶ mol l⁻¹ methacholine (MCh), an agonist of acetylcholine. The same experimental conditions were used to determine the effects of adrenaline on the seawater eel intestine (Ando and Omura, 1993). Before bioassay, the cultured Japanese eels, weighing about 220 g, were kept in sea water (20 °C) for more than 1 week. After decapitation, the intestine was removed and the outer muscle layers were stripped off according to previous methods (Ando and Kobayashi, 1978). The mucosal segment (posterior section) was opened and mounted as a flat sheet between two Lucite Ussing-type half-chambers with an exposed area of 0.5 cm². Tissue was bathed in Krebs' bicarbonate Ringer's solution consisting of (mmol l⁻¹): 118.5 NaCl, 4.7 KCl, 3.0 CaCl₂, 1.2 MgSO₄, 24.9 NaHCO₃ and 1.2 KH₂PO₄ and containing 5 mmol l⁻¹ glucose and alanine. The bathing solution (2.5 ml each) was kept at 20 °C and circulated continuously and bubbled with a 95% O₂:5% CO₂ gas mixture (pH 7.4).

The PD was recorded through a pair of calomel electrodes with a polyrecorder (EPR-151A, Toa Electronics, Tokyo, Japan) as the potential of the serosa with respect to the mucosa. To determine the tissue resistance (R_t), rectangular pulses, 30 μ A for 500 ms, were applied across the intestinal sheet every 5 min through an isolator (SS-201J, Nihon Kohden, Tokyo, Japan) connected to a stimulator (SEN-3301, Nihon Kohden). The short-circuit current (I_{sc}) was obtained from the ratio of PD to R_t .

Net water flux was calculated directly from the difference between the rates of effluent and perfusate flow in the perfusion system. Details of simultaneous measurements of net water flux and PD have been described elsewhere (Ando *et al.* 1986). Briefly, the serosal fluid was perfused through the everted intestine at a constant rate (around 150 μ l min⁻¹) and the effluent was collected every 10 min. The difference between the two rates (net water flux) was measured every 10 min. In some preparations, net Na⁺, K⁺ and Cl⁻ fluxes

were also calculated simultaneously from the collected fluid and ionic concentrations as described previously (Ando, 1983). Na^+ and K^+ concentrations were measured by flame photometry (FPF-2A, Hiranuma, Mito, Japan) and Cl^- concentration was determined with a chloride counter (CL-5M, Hiranuma).

Acetyl- β -methylcholine bromide, (-)-adrenaline, 5-hydroxytryptamine creatine sulphate, 3-isobutyl-1-methylxanthine (IBMX), tetrodotoxin (TTX) and yohimbine HCl were purchased from Sigma Chemical (St Louis, MO). Somatostatin-14 (SS-14) and SS-25 were obtained from Peninsula Laboratories (Belmont, CA).

Results

Characterization of the purified peptides

The extract from the eel guts was initially forced through C18 cartridges. The retained material was separated by gel filtration chromatography into two parts (fractions 19–25 and 26–47) in the next step. From the high-molecular-mass fractions, two peptides, which were temporarily named EI-6 and EI-7 from their retention time in a reverse-phase

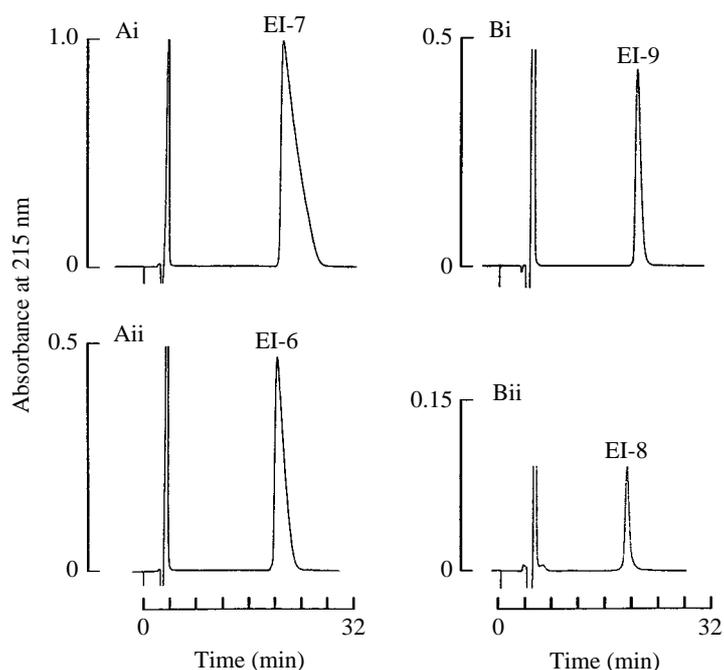


Fig. 1. The final HPLC purification of EI-7, EI-6, EI-9 and EI-8. The active fractions obtained from the preceding chromatographic step were applied to the reverse-phase column. (A) Absorbance profile (at 215 nm) of EI-7 (i) and EI-6 (ii). They were eluted isocratically with 11.5% acetonitrile containing 5% 2-propanol and 0.1% trifluoroacetic acid (TFA) (pH 2.2). Flow rate was 0.8 ml min^{-1} . (B) Absorbance profile (at 215 nm) of EI-9 (i) and EI-8 (ii). They were eluted isocratically with 14.5% acetonitrile containing 5% 2-propanol and 0.1% TFA (pH 2.2). Flow rate was 0.5 ml min^{-1} .

column, were isolated after several steps of HPLC separation. In the same way, from the low-molecular-weight fractions, another two peptides were purified; they were provisionally named EI-8 and EI-9. Each peptide enhanced the serosa-negative PD after pretreatment with IBMX, 5-HT and MCh. Fig. 1 shows chromatograms of the final purification of each peptide. Clearly, EI-6, EI-8 and EI-9 gave a single peak, indicating the homogeneity of these peptides. Although EI-7 demonstrated a large peak width, this fraction was homogeneous, since only a single band was detected on tricine SDS-PAGE (data not shown).

The amino acid sequence of EI-7 was as follows: Ser-Val-Asp-Asn-Gln-Gln-Gly-Arg-Glu-Arg-Lys-Ala-Gly-X-Lys-Asn-Phe-Tyr-Trp-Lys-Gly-Pro-Thr-Ser. Although Cys is not detected in this sequence analysis, quantitative amino acid analysis revealed that this peptide contains two Cys residues (Table 1), and fast atom bombardment mass spectrometry (FAB-MS) gave a molecular ion peak in the spectrum at 2858 m z^{-1} (M+H)⁺. Therefore, the primary structure of EI-7 was regarded as Ser-Val-Asp-Asn-Gln-Gln-Gly-Arg-Glu-Arg-Lys-Ala-Gly-Cys-Lys-Asn-Phe-Tyr-Trp-Lys-Gly-Pro-Thr-Ser-Cys. This sequence is the same as that of SS-25II isolated from European eel pancreas (Conlon *et al.* 1988).

EI-6 had a similar amino acid composition except for a lower Lys content (Table 1), suggesting that Lys may be modified. An amino acid sequence of Ser-Val-Asp-Asn-Gln-

Table 1. Amino acid composition of the eel somatostatin-related peptides

	EI-6	EI-7	EI-8	EI-9
Asx	2.8 (3)	3.2 (3)	1.0 (1)	1.0 (1)
Cys(O ₃ H)*	1.9 (2)	2.0 (2)	1.6 (2)	1.5 (2)
Glx	2.7 (3)	3.1 (3)	—	—
Ser	1.9 (2)	2.1 (2)	1.4 (1)	1.2 (1)
Gly	3.0 (3)	3.2 (3)	2.4 (2)	2.2 (2)
His	—	—	—	—
Arg	2.0 (2)	2.0 (2)	—	—
Thr	1.1 (1)	1.2 (1)	1.1 (1)	1.1 (1)
Ala	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)
Pro	1.1 (1)	1.2 (1)	1.0 (1)	1.1 (1)
Tyr	0.9 (1)	0.9 (1)	0.9 (1)	0.9 (1)
Val	1.0 (1)	1.0 (1)	—	—
Met	—	—	—	—
Ile	—	—	—	—
Leu	—	—	—	—
Phe	1.2 (1)	1.0 (1)	1.1 (1)	1.0 (1)
Trp	ND	ND	ND	ND
Lys	1.5 (2)	2.6 (3)	0.9 (1)	1.6 (2)
Hyl	+	—	+	—

ND, not determined.

Hyl, hydroxylysine.

*Cysteine was detected as cysteic acid.

Parentheses indicate the amount of an amino acid residue relative to Ala, where Ala=1.

Gln-Gly-Arg-Glu-Arg-Lys-Ala-Gly-X-Lys-Asn-Phe-Tyr-Trp-X-Gly was obtained, but the C-terminal sequence after Gly in this peptide could not be determined. Amino acid analysis revealed that EI-6 also contained two Cys residues (Table 1). A molecular ion peak was obtained at $2874 \text{ m z}^{-1} (\text{M}+\text{H})^+$ in the native EI-6. After reducing EI-6, the molecular ion peak shifted to $2876 \text{ m z}^{-1} (\text{M}+\text{H})^+$, indicating the existence of a disulphide bond between two Cys residues. The difference in molecular mass of 16 (2858 versus 2874 m z^{-1}) between EI-7 and EI-6 suggests that the twentieth amino acid residue (Lys) of EI-7 is hydroxylated in EI-6. Hydroxylysine was detected in the amino acid analysis of EI-6 (Table 1). This sequence was the same as that of SS-25I isolated from European eel pancreas (Conlon *et al.* 1988).

The amino acid sequence of EI-9 was Ala-Gly-X-Lys-Asn-Phe-Tyr-Trp-Lys-Gly-Pro-Thr-Ser. Amino acid analysis indicated that EI-9 also contained two Cys residues (Table 1). On the basis of the amino acid sequence and the composition of EI-7 and EI-9, it was suspected that EI-9 was a C-terminal fragment of EI-7. The amino acid composition of EI-8 was similar, except for a lower Lys content (Table 1). By analogy with the case of EI-7 and EI-6, EI-8 was suspected to be a hydroxylated form of EI-9. Hydroxylysine was detected in the amino acid analysis of EI-8 (Table 1). These tetradecapeptides are not isolated from European eel pancreas, and we named them EI-9 and EI-8, eel somatostatin-14II (eSS-14II) and eSS-14I, respectively. On the basis of these results, each isolation yield from 593 g of guts was $34.2 \mu\text{g}$ (12.0 nmol) of eSS-25II (EI-7), $13.2 \mu\text{g}$ (4.6 nmol) of eSS-25I (EI-6), $3.1 \mu\text{g}$ (2.0 nmol) of eSS-14II and $0.5 \mu\text{g}$ (0.3 nmol) of eSS-14I.

Functions of eel somatostatins

Although it is evident that somatostatins isolated from the gut enhanced the serosa-negative PD, it is not clear yet whether somatostatin acts directly on the intestinal epithelium or whether its action is mediated through other factors, such as neurones and endocrine cells. Fig. 2 shows the effects of eSS-25II after pretreatment with TTX or after adrenaline action has been blocked. Even after blocking neuronal firing with $10^{-6} \text{ mol l}^{-1}$ TTX, eSS-25II enhanced the serosa-negative PD and I_{sc} ; this was accompanied by an increase in R_{t} (Fig. 2A). This indicates that eSS-25II acts on the epithelium without firing the enteric nervous system. Although these effects of eSS-25II are similar to those of adrenaline (Ando and Omura, 1993), eSS-25II had the same effects even after the adrenaline receptor had been blocked with yohimbine (Fig. 2B). Table 2 summarizes the effects of eSS-25II on these three electrical variables after pretreatment with IBMX, 5-HT and MCh.

The stimulatory effects of eSS-25II were dose-dependent, with a threshold concentration of $10^{-10} \text{ mol l}^{-1}$ and a maximal effect at $10^{-9} \text{ mol l}^{-1}$. Fig. 3 shows dose-response curves for the effects of the various somatostatin-related peptides on I_{sc} . eSS-25II was the most potent, followed by eSS-25I, mammalian SS-25 isolated from ovine hypothalamus (Bohlen *et al.* 1980), eSS-14II and mammalian SS-14. Although the same SS-14 as in mammals has been isolated from European eel pancreas (Conlon *et al.* 1988), its effect on intestinal I_{sc} was the smallest.

Since the major role of the intestine of the seawater eel is the absorption of water, the

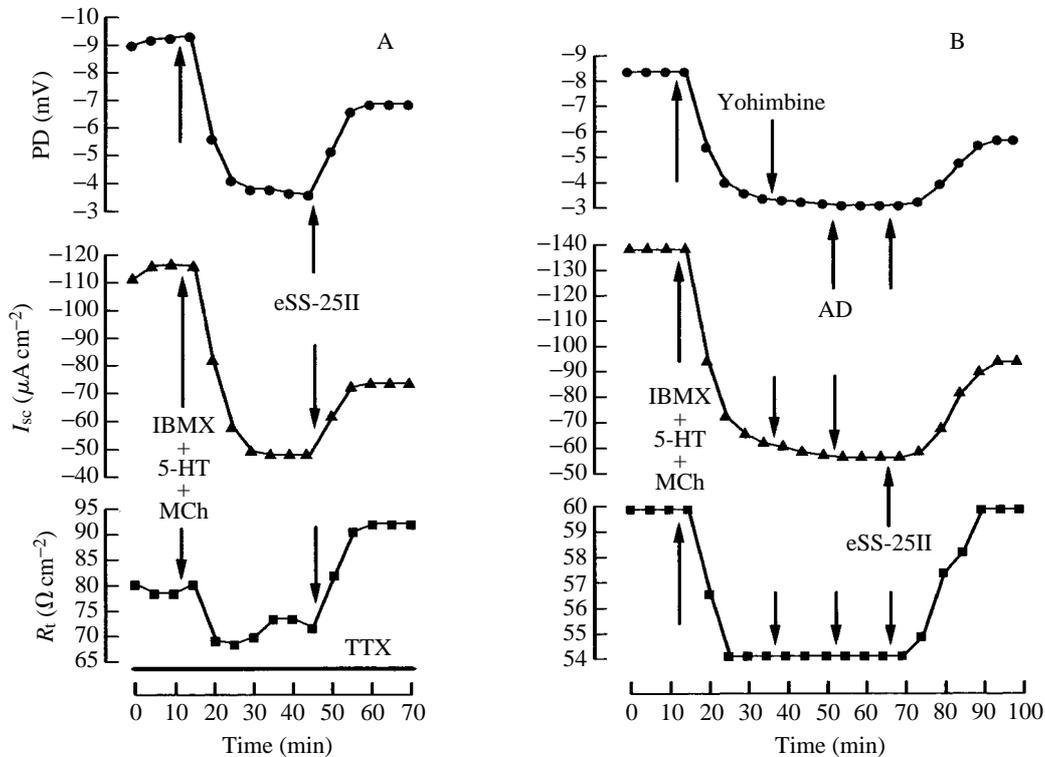


Fig. 2. Independent action of eSS-25II on neural firing and catecholamine release. (A) Effects of eSS-25II on PD (●), I_{sc} (▲) and R_t (■) in the presence of tetrodotoxin (TTX). Eel SS-25II was added to the serosal fluid (second arrows) after pretreatment with $10^{-5} mol l^{-1}$ isobutylmethylxanthine (IBMX), $10^{-6} mol l^{-1}$ serotonin (5-HT) and $10^{-6} mol l^{-1}$ methacholine (MCh) (first arrows). (B) Effects of eSS-25II on PD (●), I_{sc} (▲) and R_t (■) after pretreatment with yohimbine. Yohimbine ($5 \times 10^{-6} mol l^{-1}$) was applied to the serosal fluid (second arrows). At the third arrow adrenaline ($10^{-8} mol l^{-1}$) was added to the serosal bathing medium, and at the fourth arrow eSS-25II ($10^{-9} mol l^{-1}$). The tracing shows the results from one experiment. Two other experiments gave similar results.

effect of eSS-25II on net water flux was examined. When eSS-25II was added to the serosal fluid after pretreatment with IBMX, 5-HT and MCh, the net water flux increased gradually; this was accompanied by an increase in PD (Fig. 4). During the period of enhanced net water flux, net Na^+ and Cl^- fluxes were also increased. However, the effects of eSS-25II ($10^{-9} mol l^{-1}$) on ion and water fluxes were examined in only two preparations, since a large amount of peptide must be perfused continuously in the perfusion system and the amount of purified peptide was small. Therefore, a synthesized mammalian somatostatin (SS-25) was used instead of eSS-25II. The effects of SS-25 were similar to those of eSS-25II, although a higher concentration ($10^{-7} mol l^{-1}$) of SS-25 was required. Table 3 shows effects of SS-25 on PD and net ion and water fluxes.

Table 2. Effects of eel somatostatin (eSS-25II) on the transepithelial potential difference (PD), short-circuit current (I_{sc}) and tissue resistance (R_t) after pretreatment with IBMX, 5-HT and MCh

	PD (mV)	I_{sc} ($\mu\text{A cm}^{-2}$)	R_t (Ωcm^2)
Control	-7.7 ± 0.6	-96.2 ± 9.7	82.0 ± 5.4
IBMX ($10^{-5} \text{ mol l}^{-1}$) +5-HT ($10^{-6} \text{ mol l}^{-1}$) \dagger +MCh ($10^{-6} \text{ mol l}^{-1}$) \dagger	$-2.5 \pm 0.3^{**}$	$-37.9 \pm 6.0^{**}$	$68.0 \pm 4.6^*$
eSS-25II ($10^{-9} \text{ mol l}^{-1}$)	$-6.7 \pm 0.4\ddagger$	$-80.1 \pm 9.2\ddagger$	$86.2 \pm 6.9\ddagger$

Values are mean \pm S.E.M., $N=5$.

* $P < 0.01$, ** $P < 0.001$ compared with the control (paired t -test).

$\dagger P < 0.01$, $\ddagger P < 0.001$ compared with those treated with IBMX, 5-HT and MCh (paired t -test).

IBMX, isobutylmethylxanthine; 5-HT, serotonin; MCh, methacholine.

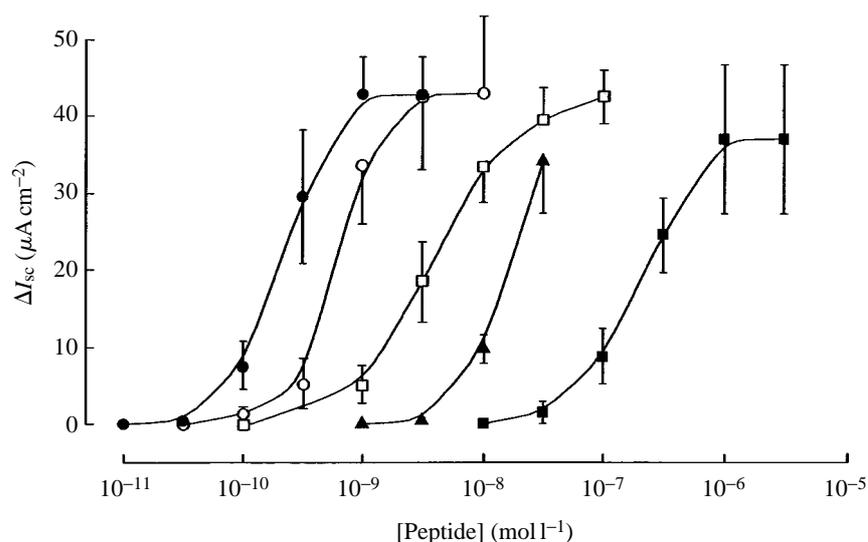


Fig. 3. Dose-response curves of various somatostatin-related peptides (eSS-25II, ●; eSS-25I, ○; SS-25, □; eSS-14II, ▲; SS-14, ■). The change in the I_{sc} after addition of the corresponding somatostatin was plotted against its concentration (on a logarithmic scale). Each point and vertical bar indicate the mean value and S.E.M. ($N=5$).

Discussion

Four somatostatin-related peptides were isolated from eel guts. Two of them were the same as SS-25II (eSS-25II) and SS-25I (eSS-25I) isolated from European eel pancreas (Conlon *et al.* 1988). The remaining two peptides were the C-terminal tetradecapeptides of eSS-25II and eSS-25I. Since such tetradecapeptides have not been isolated from European eel pancreas, we named them eel SS-14II (eSS-14II) and eSS-14I. As shown in

Table 3. Effects of mammalian somatostatin (SS-25) on transepithelial potential difference (PD), net ion fluxes (J_{net}^{Na} , J_{net}^{K} , J_{net}^{Cl}) and net water flux ($J_{net}^{H_2O}$) across the seawater eel intestine

	PD (mV)	J_{net}^{Na} ($\mu\text{equiv cm}^{-2} 10 \text{ min}^{-1}$)	J_{net}^{K} ($\mu\text{equiv cm}^{-2} 10 \text{ min}^{-1}$)	J_{net}^{Cl} ($\mu\text{cm}^{-2} 10 \text{ min}^{-1}$)	$J_{net}^{H_2O}$ ($\mu\text{l cm}^{-2} 10 \text{ min}^{-1}$)
Control	-8.4±0.5	2.24±0.19	-0.06±0.02	2.51±0.12	15.4±1.3
IBMX ($10^{-5} \text{ mol l}^{-1}$) +5-HT ($10^{-6} \text{ mol l}^{-1}$) +MCh ($10^{-6} \text{ mol l}^{-1}$)	-3.6±0.4†	0.99±0.12†	-0.12±0.02†	1.24±0.12†	9.5±1.0†
SS-25 ($10^{-7} \text{ mol l}^{-1}$)	-7.4±0.5**	2.13±0.18**	-0.06±0.02*	2.02±0.17*	12.6±1.5*

Values are means ± s.e.m. (N=7).

† $P < 0.001$ compared with the control (paired t -test).

* $P < 0.01$, ** $P < 0.001$ compared with those treated with IBMX, 5-HT and MCh (paired t -test).
IBMX, isobutylmethylxanthine; 5-HT, serotonin; MCh, methacholine.

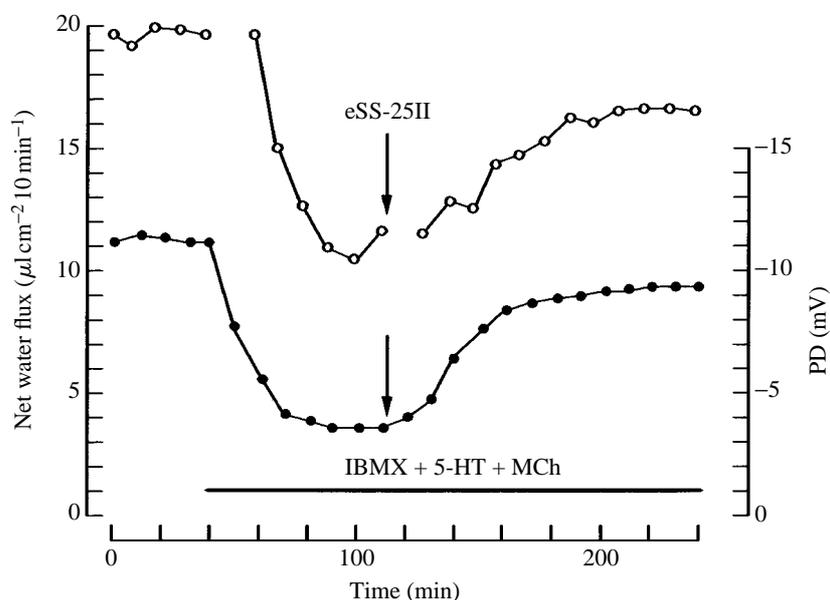


Fig. 4. Effects of eSS-25II on the PD (●) and the net water flux (○) across eel intestine. The horizontal bar marks the period when IBMX (10^{-5} mol l $^{-1}$), 5-HT (10^{-6} mol l $^{-1}$) and MCh (10^{-6} mol l $^{-1}$) were applied to the serosal fluid. At the arrows, 10^{-9} mol l $^{-1}$ eSS-25II was added to the same side. The tracing shows the results from one experiment. One other experiment gave similar results.

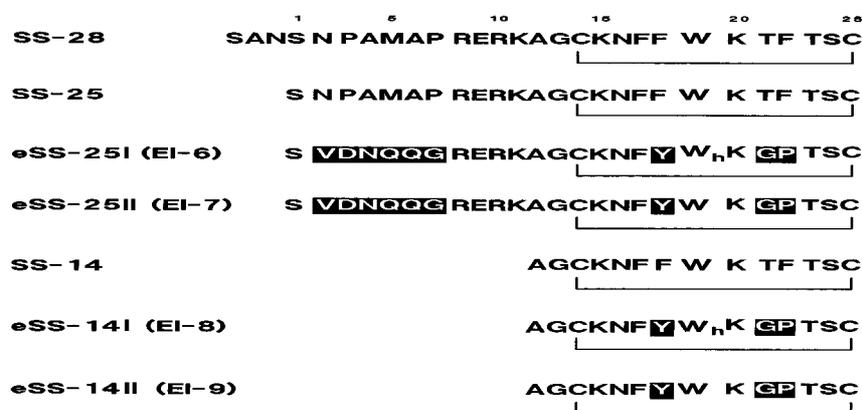


Fig. 5. Primary structures (single letter code) of the various somatostatin-related peptides. SS-28, SS-25 and SS-14 are mammalian somatostatins. The numbering at the top starts from Ser (S) of eel somatostatin. The intramolecular disulphide linkage between Cys residues is represented by a line. Amino acid residues that differ between eel and mammals are indicated by dark boxes.

Fig. 5, both eSS-25II and eSS-25I have a processing site at Arg¹⁰-Lys¹¹. Therefore, it is likely that eSS-14II and eSS-14I are produced in the intestine by processing eSS-25II and eSS-25I, respectively. However, the effect of eSS-14II on the I_{sc} was smaller than that of

eSS-25II (Fig. 3), suggesting that eSS-25II rather than eSS-14II acts physiologically, at least in the intestinal absorption of NaCl. However, it cannot be ruled out that eSS-14II and eSS-14I are involved in other functions of the intestine, such as motility or nutrient absorption. They may contribute to regulation of the functions of other organs, such as the stomach and the pancreas.

The role of hydroxylation of Lys²⁰ in eel somatostatin is not clear yet. The hydroxylated form seems to have a reduced action on intestinal absorption, because a higher concentration of the hydroxylated form (eSS-25I) than of eSS-25II is required to increase I_{sc} .

Fig. 5 shows the primary structure of the various somatostatin-related peptides studied. SS-28 is a large mammalian somatostatin, which is considered to be cleaved to generate SS-14 at the Arg-Lys processing site (Gluschankof *et al.* 1984; Conlon, 1989). In the mammalian intestine, the short SS-14 is thought to be an effective form of somatostatin, since SS-14 stimulates ion transport across the porcine jejunum at lower concentrations than does SS-28 *in vitro* (Brown *et al.* 1990). In contrast, in the eel intestine, the N-terminal 11 amino acid residues potentiate somatostatin action, since SS-25 and eSS-25II have 100 times the potency of SS-14 and eSS-14II, respectively.

Although high concentrations are required, both eSS-14II and SS-14 enhanced I_{sc} (Fig. 3). Since the amino acid sequences of these peptides are included in eSS-25II and SS-25 respectively, the structure essential for somatostatin action must exist in eSS-14II and SS-14. Among these tetradecapeptides, 11 amino acid residues are common, including the disulphide linkage between two Cys residues. Presumably, a tertiary structure formed by these common residues will be necessary for activity. Besides the disulphide linkage, five amino acid residues from position 18 to position 22 (Tyr-Trp-Lys-Gly-Pro) may be important, since hydroxylation of Lys²⁰ or substitution of Phe, Thr and Phe for Tyr¹⁸, Gly²¹ and Pro²² reduced the potency of the somatostatins (Fig. 3). Although six amino acid residues in the N terminus differ between SS-25 and eSS-25II, the role of these amino acid residues is not clear yet.

The present study is the first investigation of the effect of eel somatostatin in a homologous system. Eel somatostatin (eSS-25II) extracted from eel guts stimulated Na⁺, Cl⁻ and water absorption across the seawater eel intestine after pretreatment with IBMX, 5-HT and MCh (Fig. 4; Table 3). This suggests that endogenous somatostatin (eSS-25II) antagonizes the effects of 5-HT and acetylcholine. Although similar antagonism to these inhibitors has been demonstrated by adrenaline, the somatostatin effect is not due to adrenaline release or neuronal firing, because its effects are not blocked by yohimbine, a fairly specific antagonist of adrenaline in the seawater eel intestine (Ando and Omura, 1993), or by TTX, which blocks neuronal firing. Presumably, somatostatin acts directly on the enterocytes. *In vitro*, somatostatin may act on a receptor distinct from the adrenergic one, but in concert with adrenaline, to antagonize the inhibition of NaCl and water absorption caused by 5-HT and acetylcholine in the seawater eel intestine.

We wish to thank Professors Yojiro Muneoka and Makoto Kobayashi, Faculty of Integrated Arts and Sciences, Hiroshima University, for their helpful advice. This research was supported in part by Grants-in-Aid no. 02804062 from the Ministry of

Education, Sciences and Culture, Japan, and no. 9233 from Salt Science Research Foundation, Japan.

References

- ANDO, M. (1983). Potassium-dependent chloride and water transport across the seawater eel intestine. *J. Membr. Biol.* **73**, 125–130.
- ANDO, M. AND KOBAYASHI, M. (1978). Effects of stripping of the outer layers of the eel intestine on salt and water transport. *Comp. Biochem. Physiol.* **61A**, 497–501.
- ANDO, M. AND KONDO, K. (1993). Noradrenalin antagonizes effects of serotonin and acetylcholine in the seawater eel intestine. *J. comp. Physiol. B* **163**, 59–63.
- ANDO, M., KONDO, K. AND TAKEI, Y. (1992). Effects of eel atrial natriuretic peptide on NaCl and water transport across the intestine of the seawater eel. *J. comp. Physiol. B* **162**, 436–439.
- ANDO, M. AND OMURA, E. (1993). Catecholamine receptor in the seawater eel intestine. *J. comp. Physiol. B* **163**, 64–69.
- ANDO, M., SASAKI, H. AND HUANG, K. C. (1986). A new technique for measuring water transport across the seawater eel intestine. *J. exp. Biol.* **122**, 257–268.
- BOHLEN, P., BRAZEAU, P., BENOIT, R., LING, N., ESCH, F. AND GUILLEMIN, R. (1980). Isolation and amino acid composition of two somatostatin-like peptides from ovine hypothalamus: somatostatin-28 and somatostatin-25. *Biochem. biophys. Res. Commun.* **96**, 725–734.
- BROWN, D. B., OVEREND, M. F. AND BRADLEY, G. T. (1990). Neurohormonal regulation of ion transport in the porcine distal jejunum. Actions of somatostatin-14 and its natural and synthetic homologs. *J. Pharmac. exp. Ther.* **252**, 126–134.
- CONLON, J. M. (1989). Biosynthesis of regulatory peptides – evolutionary aspects. In *The Comparative Physiology of Regulatory Peptides* (ed. S. Holmgren), pp. 344–369. London, New York: Chapman and Hall.
- CONLON, J. M., DEACON, C. F., HAZON, N., HENDERSON, I. W. AND THIM, L. (1988). Somatostatin-related and glucagon-related peptides with unusual structural features from the European eel (*Anguilla anguilla*). *Gen. comp. Endocr.* **72**, 181–189.
- GLUSCHANKOF, P., MOREL, A., GOMEZ, S., NICOLAS, P., FAHY, C. AND COHEN, P. (1984). Enzymes processing somatostatin precursors: An Arg-Lys esterpeptidase from the rat brain cortex converting somatostatin-28 into somatostatin-14. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6662–6666.
- MAINOYA, J. R. AND BERN, H. A. (1982). Effects of teleost urotensins on intestinal absorption of water and NaCl in tilapia, *Sarotherodon mossambicus*, adapted to freshwater or seawater. *Gen. comp. Endocr.* **47**, 54–58.
- MAINOYA, J. R. AND BERN, H. A. (1984). Influence of vasoactive intestinal peptide and urotensin II on the absorption of water and NaCl by the anterior intestine of the tilapia, *Sarotherodon mossambicus*. *Zool. Sci.* **1**, 100–105.
- MORI, Y. AND ANDO, M. (1991). Regulation of ion and water transport across the eel intestine: Effects of acetylcholine and serotonin. *J. comp. Physiol. B* **161**, 387–392.
- O'GRADY, S. M. AND WOLTERS, P. J. (1990). Evidence for chloride secretion in the intestine of the winter flounder. *Am. J. Physiol.* **258**, C243–C247.
- O'GRADY, S. M., FIELD, M., NASH, N. T. AND RAO, M. C. (1985). Atrial natriuretic factor inhibits Na–K–Cl cotransport in teleost intestine. *Am. J. Physiol.* **249**, C531–C534.