Potent cardiovascular effects of homologous urotensin II (UII)-related peptide and UII in unanesthetized eels after peripheral and central injections

Shigenori Nobata,1 John A. Donald,2 Richard J. Balment,3 and Yoshio Takei1

1Laboratory of Physiology, Atmosphere and Ocean Research Institute, University of Tokyo, Chiba, Japan; 2School of Life and Environmental Sciences, Deakin University, Victoria, Australia; and 3Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Submitted 17 September 2010; accepted in final form 28 November 2010

Nobata S, Donald JA, Balment RJ, Takei Y. Potent cardiovascular effects of homologous urotensin II (UII)-related peptide and UII in unanesthetized eels after peripheral and central injections. Am J Physiol Regul Integr Comp Physiol 300: R437–R446, 2011. First published December 1, 2010; doi:10.1152/ajpregu.00629.2010.—We cloned cDNAs encoding urotensin II (UII)-related peptide (URP) and UII in Japanese eel, Anguilla japonica, the former being the first such cloning in teleost fishes. Unlike the exclusive expression of UII in the urophysis, the URP gene was expressed most abundantly in the brain (medulla oblongata) followed by the urophysis. Peripheral injections of URP into eels increased blood pressure by 16.1 ± 0.8 mmHg at 0.1 nmol/kg in central arterial blood pressure (PVA) and with similar potency and efficacy to that of UII (relative potency of URP to UII = 0.83). URP/UII and ANG II preferentially acted on the branchial and systemic circulations, respectively, and the duration of effect was distinct among the three peptides in the order of UII (60 min) > URP (30 min) > ANG II (14 min) in PVA. Urandite, a mammalian UII receptor antagonist, inhibited the URP effect (−63.6 ± 5.2%) to a greater extent than for UII (−39.9 ± 5.0%). URP and UII constricted isolated eel branchial and systemic arteries, showing their direct actions on the vascular smooth muscle. Central injection of URP increased blood pressure by 12.3 ± 0.8 mmHg at 50 pmol/eel in PVA and with similar efficacy but less potency (relative potency = 0.47) and shorter duration, compared with UII. The central actions of URP/UII were more potent on the branchial circulation than on the systemic circulation, again opposite the effects of ANG II. The similar responses to peripheral and central injections suggest that peripheral hormones may act on the brain. Taken together, in eels, URP and UII are potent cardiovascular hormones like ANG II, acting directly on the peripheral vasculature, as well as a central vasomotor site, and their actions are mediated to different degrees by the UII receptor.

teleost; urotensins; caudal neurosecretory system; urandite; vasopressor; tachycardia

UROTENSIN II (UII) IS A CYCLIC neuropeptide initially isolated from the caudal neurosecretory system (CNSS) of the goby, Gillichthys mirabilis (26), on the basis of its smooth muscle-contracting activity. Thereafter, UII has been identified not only in fishes but also in the brain and spinal cord of the bullfrog, Rana catesbeiana, chicken, Gallus gallus, and many species of mammal (6). The mature form of UII has a cyclic structure formed by a disulfide bond at the COOH terminus, in which the CFWKYCV/I sequence is fully conserved in the sequences observed to date, while the NH2 terminus is rather variable. Recently, UII-related peptide (URP), a UII paralog generated by a whole genome duplication (32), was identified as a UII-immunoreactive molecule in the rat brain (29). The putative mature form of URP is an octapeptide consisting of ACFWKYCV/I in amphibians, birds, and mammals, and it shares the cyclic sequence with UII, although precursor sequences are quite different. As inferred by the completely conserved sequence in the cyclic structure, the cyclic hexapeptide is important for the biological activities of URP and UII (12, 18, 19) and has sequence similarities with the cyclic bioactive region of somatostatin (26).

There is accumulating evidence that UII plays an important role in cardiovascular regulation in vertebrates. However, the data are variable and are dependent on the species, vascular beds, and experimental design. UII is a vasoconstrictor in isolated arteries of various species and is generally more potent than endothelin-1, but it also acts as a vasodilator in some arteries through the release of nitric oxide (1). In vertebrates except for mammals, homologous UII constricted bullfrog arterial rings (34), and central and peripheral injection of trout UII elevated the dorsal aortic pressure in the trout (17). However, in the coronary arteries of chinook salmon, trout UII and rat UII caused vasodilation and vasoconstriction, respectively (27). Finally, in dogfish, Scyllorhinus canicula, homologous UII elevated arterial blood pressure, in part, by catecholamine release and constricted isolated arteries (10). Thus, the role of UII in cardiovascular regulation is complex in vertebrates examined thus far. It is also suggested that UII is involved in osmoregulation and renal function (21), and it is implicated in human disease, as circulating UII levels are elevated in patients with hypertension, congestive heart failure, and renal failure (1).

In contrast to the extensive studies of UII, the function of URP remains less clear because of its relatively recent discovery. Rat URP induced hypotension in anesthetized rats (29) and dilated the coronary arteries of chinook salmon and rat (27). On the other hand, URP exerted a potent contractile effect on endothelium-denuded rat aortic rings (4). URP is probably a vasoconstrictor in the trout as peripheral injection of human URP at a high dose (1.0 nmol/kg) elevated dorsal aortic pressure (17).

The effects of UII and URP are mediated through activation of a G protein-coupled receptor called the UT receptor (30). In UT receptor-transfected cells, UII stimulates the PLC pathway (35), and URP causes an increase in cytosolic Ca2+ concentration at an EC50 value comparable to UII (19, 29). It is likely that the slow internalization of UII-bound UT receptor causes the long-lasting vasoconstriction of UII (9, 28). A peptidic antagonist of the UT receptor, urantide, inhibited the binding of UII to the human and rat UT receptor and intracellular signaling (25). Palosuran was synthesized as a highly specific non-
peptidic antagonist to human UT receptor (7), and it inhibited the cell-proliferative effect of UII through the somatostatin receptor but not that of URP (22). Although it has been recently suggested that URP and UII regulate cell proliferation through common and distinct mechanisms, it has been incompletely confirmed (13, 22).

In our preliminary experiments, we found that the vasopressor effect of human URP was more potent than human UII when injected peripherally into unanesthetized eels, and urantide diminished the effects of both peptides (Supplemental Fig. 1A). In parallel with the in vivo study, we performed a database search for URP of teleosts and found that the putative mature sequences were C-terminally extended compared with URPs in tetrapods. Therefore, it was evidently necessary to examine the cardiovascular effects of URP in teleosts using homologous peptides. In this study, we evaluated the cardiovascular effects of URP in eels and compared the effects with UII and ANG II, a potent vasopressor hormone in teleosts, using homologous peptides. We initially isolated cDNAs from the eel brain and caudal neurosecretory system (CNSS) and synthesized the putative mature peptides. These peptides were injected peripherally and centrally into unanesthetized cannulated eels, and the effects on ventral and dorsal aortic blood pressure and heart rate were determined. We also examined the effect of the mammalian UT receptor antagonist, urantide, on the cardiovascular effects of URP and UII. Finally, we examined the in vitro vasoconstrictor effects of URP and UII using isolated eel arteries in vitro.

MATERIALS AND METHODS

Animals. Cultured eels, Anguilla japonica, were purchased from a local dealer. They were acclimated in seawater (SW) without feeding for more than 2 wk before use. Water in the tank was filtered, aerated, and maintained at 18°C. All animal experiments were approved by the Animal Experiment Committee of the University of Tokyo and were performed in accordance with the Manual for Animal Experiments prepared by the committee.

RNA extraction. After anesthesia in 0.1% (wt/vol) tricaine methanesulfonate (Sigma, St. Louis, MO, USA), the brain, CNSS (spinal cord corresponding to the terminal 10 vertebral segments), pituitary, gill, heart, interrenal, head kidney, kidney, esophagus, stomach, intestine, liver, pancreas, spleen, mesentery, red body, skin, and skeletal muscle were isolated from SW eels and immediately frozen in liquid nitrogen. Total RNA was extracted using ISOGEN (Nippongene, Toyama, Japan) for subsequent experiments.

cDNA cloning of eel URP and UII. A double-stranded cDNA pool was prepared from 1.0 μg of total RNA (the brain and CNSS) using a SMART CDNA library construction kit (Clontech Laboratories, Palo Alto, CA, USA). The 5′ region of the cDNAs was amplified by 5′ rapid amplification of cDNA ends (RACE) using the 5′ PCR primer in the kit and degenerate primers designed, according to the known nucleotide sequences of URP and UII in teleosts (Table 1). Gene-specific primers were designed on the basis of the partial sequences, and then the 3′ region of the cDNAs was amplified by the 3′ RACE method using the CDS III/3′ primer in the kit and the gene-specific primers (Table 1). Finally, cDNAs that encompassed the whole coding region of URP and UII were amplified using gene-specific primers designed on the basis of the partial sequences determined using the RACE method. Amplified products were subcloned into pT7blue vector (Novagen, Madison, WI) and then sequenced on a 3130 DNA sequencer (Applied Biosystems, Foster City, CA).

Phylogenetic analyses of eel URP and UII. The putative amino acid sequences of eel URP and UII were aligned with those of other species using the ClustalX version 1.83 (ftp://ftp.igbmc.u-strasbg.fr/pub/ClustalX/), which was followed by manual adjustments for inconsistencies. A phylogenetic tree was constructed by the Bayesian method using MrBayes version 3.1.2 software (http://mrbayes.csit.fsu.edu).

Tissue distribution of URP and UII mRNAs. The tissue distribution of each URP and UII transcript was examined by RT-PCR (n = 3). Single-stranded cDNAs for various tissues were prepared from 0.2 μg of total RNA using SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA), and PCR was performed using Ex Taq DNA polymerase (TaKaRa, Tokyo, Japan). β-Actin cDNA (accession no. AB074846) was used for an internal standard. The PCR was performed as follows: 25 cycles for β-actin and 30 cycles for URP and UII of denaturation (94°C, 30 s), annealing (60°C, 30 s), and extension (72°C, 45 s). The amplified DNA fragments were electrophoresed on a 1.2% agarose gel and detected by ethidium bromide staining.

Peptide synthesis. Putative mature peptides of eel URP and UII were chemically synthesized by Dr. Akiyoshi Takahashi of Kitasato University and Sigma Aldrich Japan (Tokyo, Japan, respectively). The ring structure of these peptides was achieved by hydrogen peroxide oxidation of the two Cys residues with subsequent purification by reverse-phase HPLC. Human URP and UII, eel ANG II, and urantide, an antagonist of mammalian UT receptor, were purchased from Peptide Institute (Osaka, Japan). Each peptide was dissolved in distilled water at a concentration of 10−3 or 10−4 M, aliquoted and kept at −20°C until use. The stock solution was diluted with 0.9% NaCl containing 0.01% Triton-100 for injection.

Physiological study: effects of URP and UII injected peripherally on cardiovascular function. Eight SW-adapted eels (177.2 ± 6.9 g) were used to examine the cardiovascular effects of URP and UII injected peripherally. Eels were anesthetized in 0.1% (wt/vol) tricaine methanesulfonate (Sigma) for 15 min and placed on an operation board for surgery. The ventral and dorsal aortas were cannulated with polystyrene tubes (0.8 mm OD) for bolus injection of hormones and measurement of blood pressure. After surgery, eels were placed in a plastic trough through which aerated water circulated at 18°C. On the 2nd day after surgery, the blood vessel cannulas were connected via a three-way stopcock to disposable pressure transducers (DX-300, Nihon Koden, Tokyo, Japan) for continuous monitoring of ventral aortic (PVA) and dorsal aortic (PDA) blood pressure, respectively. The signal was amplified by a carrier amplifier (7903, NEC San-Ei, Tokyo, Japan). Blood pressure was recorded by water balance monitoring system MTS00658 (Medical Try System, Tokyo, Japan).

Table 1. Primers used for cloning and RT-PCR analyses

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5′-3′)</th>
<th>Name</th>
<th>Sequence (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fishURP-R1</td>
<td>AGTTEBDVVRMRCARTAYTTTCGAARA</td>
<td>eelURP-R1</td>
<td>TGATGACTGAGATTCGCTCTGGAG</td>
</tr>
<tr>
<td>fishURP-R2</td>
<td>RMRGCAHTYTTCCAARACACHT</td>
<td>eelUII-F1</td>
<td>GGAGAGGAGAGCAGAAAGAG</td>
</tr>
<tr>
<td>fishUII-F1</td>
<td>ARAGAKYHYHTDYTRGARAARC</td>
<td>eelUII-F2</td>
<td>GCAGAGGAGGAGCAGAAAGAG</td>
</tr>
<tr>
<td>fishUII-R1</td>
<td>TTYVYARCGATYTTTTCCAGAA</td>
<td>eelUII-F3</td>
<td>TGAGCTTTCGACCTCAGAGAG</td>
</tr>
<tr>
<td>fishUII-R2</td>
<td>YAVRCAGATYTTTTCCAGARACTC</td>
<td>eelUII-F5</td>
<td>GGAGACATACACTAACAGGAGG</td>
</tr>
<tr>
<td>eelURP-F1</td>
<td>AATGCOAAGCGACAGGACTGCT</td>
<td>eelUII-R1</td>
<td>TAGATGACCTGAGATTTTGAAAC</td>
</tr>
<tr>
<td>eelURP-F2</td>
<td>ACGGTCGCTGAGGACTGCT</td>
<td>eelUII-R2</td>
<td>GCCTAGACGCTACTTCCAGAGAG</td>
</tr>
</tbody>
</table>

AJP-Regul Integr Comp Physiol • VOL 300 • FEBRUARY 2011 • www.ajpregu.org
Eels were injected with 0.01, 0.03, and 0.1 nmol/kg of URP and UII in 0.05 ml of 0.9% NaCl containing 0.01% Triton-100 into the dorsal aorta. For comparison with URP and UII, ANG II was injected at doses of 0.01 and 0.1 nmol/kg. To elucidate the involvement of the UT receptor in the effects of URP and UII, 2 nmol/eel of urantide, an antagonist of the mammalian UT receptor (25), in 0.9% NaCl was injected 10 min before injection of URP and UII (0.01 and 0.1 nmol/kg). Each injection was immediately followed by an injection of 0.05 ml of saline to flush the dead volume of the cannulas. Injection intervals were more than 1 h apart to ensure reproducible responses.

Physiological study: effects of URP and UII injected centrally on cardiovascular function. Eight SW-adapted eels (192.0 ± 2.1g) were used for this experiment. To inject peptides into the brain, a stainless-steel cannula was surgically placed into the third ventricle, according to published protocols (23). Briefly, the optic tectum of the brain was exteriorized by making a hole in the skull and removing the dura mater. A stainless-steel guide cannula (0.35 mm ID, 0.6 mm OD) was inserted to a depth of 0.7 mm from the surface of the brain and fixed with dental cement. The guide cannula was closed with a stainless-steel tube until injection of peptide, and eels were prepared for the measurement of ventral and dorsal aortic blood pressure, as described above. A stainless-steel injection cannula (0.3 mm OD) was connected to the polyethylene tube (0.28 mm ID, 0.61 mm OD) and they were filled with each peptide solution (0.05 μl) and liquid paraffin (remaining space). When the injection cannula was inserted into the guide cannula to inject peptides, P<sub>A</sub> and P<sub>G</sub> temporarily changed and returned to the level of the preinjection within 20 min. Then, the blood pressure was monitored to record the preinjection levels for 10 min. Therefore, injections of peptides were delayed for 30 min after insertion of the injection cannula to obviate any effects of cannula insertion on measured parameters and then given at doses of 5, 15, 30, and 50 pmol/eel in 0.05 μl of 0.9% NaCl.

Physiological study: effects of URP and UII on vascular rings isolated from eels. Eels were anesthetized in 0.1% (wt/vol) tricaine methanesulfonate (Sigma) and the first branchial artery (300–500 μm) and the uterine artery (300–500 μm) were dissected free and placed in HEPES-buffered physiological saline (138 mM NaCl, 5 mM KCl, 1.4 mM MgCl<sub>2</sub>, 10 mM HEPES, 7 mM NaOH, 5.6 mM D-glucose, and 2.5 mM CaCl<sub>2</sub>), pH 7.6; the saline was bubbled with air. The blood vessels were cut into individual rings of ~2 mm and were mounted horizontally between two pieces of 40-μm wire attached to separate jaws of a dual-wire myograph (model no. 410A; Danish Myo Technology, Aarhus, Denmark). The blood vessels were bathed in 5 ml of physiological saline and were maintained at 10°C and gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> to equilibrate the saline. After reaching a stable basal tone, the rings were precontracted with 10 μM phenylephrine and washed with fresh saline. The precontracted rings were then expanded with 10 μM aliciferol (a nonselective <I>Ca<sup>2+</I></sup>-mobilizing agonist), and 10 μM of each peptide solution (0.05 μl) was added to test the effect of the peptide on tone. The concentration of the peptide was doubled every 5 min to a maximum concentration of 100 μM, and the experiments were repeated until the rings were maximally relaxed. The cumulative concentrations of each peptide were 5, 15, 30, and 50 pmol/ring.
20–22°C and bubbled with air. Tension was placed on the arteries by increasing the distance between the internal wires until they were flush against the vessel wall, and they were left to equilibrate for at least 30 min. The myograph was linked to a Myo-Interface system, which was attached to a PowerLab data collection system and a laptop computer. The extent of vasoconstriction of an increasing concentration of URP and UII (1, 10, and 50 nM) was determined as a percentage increase from the starting basal tension.

Statistical analyses. Relative changes in PVA and PDA were compared by Wilcoxon’s signed rank test. Changes in PVA, PDA, and heart rate after each treatment were compared with controls at each dose by the Dunnet test. Changes in PVA, PDA, and heart rate were compared among treatments at each dose by the Tukey test or paired t-test. Relative potency and the 95% confidence interval was calculated by parallel line test. Constriction of the vascular ring was evaluated by Friedman test. Significance was determined at \( P < 0.05 \). All results were expressed as means ± SE.

RESULTS

cDNA cloning of eel URP and UII. The cDNAs encoding eel URP and UII were amplified from the eel brain and CNSS cDNA pool, respectively. These genes were identified as orthologs of URP and UII based on the similarity of the putative mature sequences to those of other vertebrates (Fig. 1) and phylogenetic analysis of the precursor protein (Fig. 2). Eel URP and UII consisted of 135 and 127 amino acid residues, respectively. The putative mature sequence at the COOH terminus had structural characteristics, such as a predicted intramolecular ring formed by two cysteine residues, in which the amino acid sequence (CFWKYC) was identical between URP and UII. Unlike URP of tetrapods, eel URP was characterized by a unique COOH-terminal extension (Thr-Asn) (Fig. 1B). Eel UII and URP were registered in the DDBJ/EMBL/GenBank nucleotide sequence databases (accession nos. AB569617 and AB569618).

Tissue distribution of URP and UII mRNA. Expression of the URP and UII genes was detected by RT-PCR (Fig. 3). The URP gene was highly expressed in the medulla oblongata (MO) but not in other parts of the brain and was only moderately expressed in the CNSS. In one of three eels, URP mRNA was faintly detected in the cerebellum, pituitary, and heart. In contrast, UII mRNA was abundantly expressed in the CNSS.

Effects of URP and UII injected peripherally on cardiovascular function. After peripheral injection of URP and UII, PVA and PDA were increased in a dose-dependent manner (Figs. 4 and 5). The vasopressor effect of URP was statistically significant compared with controls at 0.03 nmol/kg and 0.1 nmol/kg for PVA (increases by 7.6 ± 1.2 and 16.1 ± 0.8 mmHg, respectively) and 0.1 nmol/kg for PDA (5.6 ± 0.6 mmHg). A
significant effect of UII was observed at 0.03 nmol/kg and 0.1 nmol/kg for both PVA (increases by 9.8 ± 1.2 and 15.9 ± 0.7 mmHg, respectively) and PDA (5.5 ± 0.5 and 7.0 ± 0.9 mmHg, respectively). The effect of UII was clearly longer-lasting than that of URP (Fig. 4). The vasopressor effect of URP was significantly lower than that of UII at 0.03 nmol/kg in PVA (Fig. 5, A and B), and the relative potency of URP to UII was 0.83 with the 95% confidence interval of 0.6–1.1 by $2\times 3$ parallel line test. Concomitant with the vasopressor effects, heart rate increased in a dose-dependent manner after injection of URP and UII (Fig. 5 C). The tachycardia caused by URP was statistically significant at 0.1 nmol/kg (increase by 8.3 ± 0.8 beats/min) and that by UII at 0.03 nmol/kg and 0.1 nmol/kg (6.9 ± 1.2 and 8.0 ± 1.9 beats/min, respectively). Peripheral injection of ANG II significantly elevated both PVA and PDA at 0.1 nmol/kg (increases by 9.9 ± 1.6 and 10.4 ± 1.7 mmHg, respectively), but the potency was lower for PVA and higher for PDA than those of URP and UII (Figs. 5, A, B, D) as shown by $2\times 3$ parallel line test, in which the parallelisms of the effect to those of URP and UII were rejected, and the vasopressor effect was significantly lower than those of URP/UII at 0.1 nmol/kg in PVA and the relative potencies to URP/UII in PDA were more than 1.0, even in the 95% confidence interval. ANG II slightly elevated heart rate but not significantly (Fig. 5 C). As shown in Fig. 4, the duration of the vasopressor effect was in order of UII > URP > ANG II, and their significant increases of blood pressure (by paired t-test compared with the value before the injection) were lasting for 60, 30, and 14 min in PVA, and 40, 19, and 16 min in PDA at 0.1 nmol/kg, respectively.

In preliminary experiments, peripheral injections of urantide had no effect on blood pressure at doses of 0.002, 0.02, 0.2, and 2 nmol/eel (Supplemental Fig. 1B). As shown in Fig. 6, urantide consistently inhibited the vasopressor effects of URP and UII. The inhibition was significantly greater for URP ($63.6 ± 5.2\%$) than UII ($39.9 ± 5.0\%$) for PVA at 0.1 nmol/kg.

**In vitro vasoconstrictor effect of URP and UII.** The effect of URP and UII were examined on the contraction of ring preparations isolated from the first branchial artery and the gonadal artery using dual-wire myography. Both peptides significantly constricted the branchial and gonadal artery in a dose-dependent manner at the range of 1.0 to 50 nM ($P < 0.05$ in both arteries treated with URP and UII by Friedman test) (Fig. 7). Compared with URP and UII, ANG II had no effect in the branchial artery and only a slight constriction was observation on one preparation of the gonadal artery ($n = 3$; data not shown). Judging from this and our previous studies (J. A. Donald, unpublished data), it is likely that ANG II has little contractile activity in eel arteries.

**Effects of URP and UII injected centrally on cardiovascular function.** After central injection of URP and UII, PVA and PDA were increased as observed following peripheral injections (Fig. 8). The vasopressor effect of URP was dose dependent

![Fig. 3. Expression of URP and UII genes in the central nervous system (CNS) (A) and other organs of eels (B). β-actin was used as an internal control. As shown in the drawing (A), the CNS was divided to six parts. FB, forebrain; OB, olfactory bulb; Tel, telencephalon; MB, midbrain; OT, optic tectum; Hypo, hypothalamus; HB, hindbrain; Ce, cerebellum; MO, medulla oblongata; Mo, medulla oblongata; SC, spinal cord; Pit, pituitary; CNSS, caudal neurosecretory system.](http://ajpregu.physiology.org/)

![Fig. 4. Mean heart rate, blood pressure of the ventral aorta, and dorsal aorta in eels after peripheral injection of URP, UII, and ANG II at 0.1 nmol/kg ($n = 8$).](http://ajpregu.physiology.org/)
and was significant at 30 pmol/eel and 50 pmol/eel for $P_{VA}$ (increases by 8.8 \pm 2.6 and 12.3 \pm 2.6 mmHg, respectively) but not at any dose for $P_{DA}$ (Fig. 9, A and B). On the other hand, the effect of UUI was significant at doses higher than 15 pmol/eel in $P_{VA}$ (increases by 13.1 \pm 2.3, 12.3 \pm 2.8, and 16.0 \pm 0.9 mmHg, respectively) and at 50 pmol/eel in $P_{DA}$ (5.8 \pm 1.1 mmHg), and almost saturated at doses higher than 15 pmol/eel both in $P_{VA}$ and $P_{DA}$ (Fig. 9, A and B). The vasopressor effect of URP was similar in the efficacy to that of UUI, but less potent than that of UUI as shown by the fact that the relative potency (0.47 and 0.29 in $P_{VA}$ and $P_{DA}$, respectively) of URP to UUI was less than 1.0, even in the 95% confidence interval (0.21~0.84 and 0.06~0.68, respectively) and the effect of URP was significantly lower than that of UUI at 15 pmol/eel both in $P_{VA}$ and $P_{DA}$. As observed in the peripheral injections, the effect of UUI was clearly longer lasting than that of URP (Fig. 8). Heart rate increased in parallel with the vasopressor effect after URP and UUI injections (Fig. 9C). The tachycardia caused by URP was statistically significant at 30 pmol/eel and 50 pmol/eel (increases by 6.4 \pm 1.7 and 6.9 \pm 1.6 beats/min, respectively) and that by UUI at doses higher than 15 pmol/eel (8.9 \pm 2.2, 7.5 \pm 2.1, and 6.9 \pm 1.2 beats/min, respectively). Central injection of ANG II significantly elevated $P_{VA}$ and $P_{DA}$ at 50 pmol/eel (increases by 9.3 \pm 1.8 and 9.4 \pm 1.7 mmHg, respectively). The effect was greater for $P_{DA}$ than for $P_{VA}$ in contrast to those of URP and UUI (Fig. 9D). In contrast to peripheral injection, central injection of ANG II significantly increased heart rate at 50 pmol/eel (increase by 11.4 \pm 2.3 beats/min), which tended to be more potent than URP and UUI (Fig. 9C). As shown in Fig. 8, the duration of the vasopressor effect was in order of UUI > URP > ANG II, and their significant increases of blood pressure were lasting for 120, 120, and 35 min in $P_{VA}$, and 120, 45, and 30 min in $P_{DA}$ at 50 pmol/eel, respectively. The central effects of the three peptides were similar to the peripheral effects.

**DISCUSSION**

Although the putative mature sequence of URP is almost identical among tetrapods, URP has the unique feature of additional Thr-Asn residues at the COOH terminus in teleosts such as the eel, zebrafish, and brook trout (Fig. 1B). In addition, another type of URP, in which the Ala residue at the NH$_2$ terminus was substituted to a Val residue and there is a C-terminal flanking sequence of Ser-Gln-Asn, was found in the genome database of zebrafish and pufferfish (*Tetraodon*).
Thus, we named the two types of teleost URP as URP1 (ACFWKYCVTN) and URP2 (VCFWKYCSQN), respectively. Degenerate primers were designed on the basis of the nucleotide sequences of teleost URPs, and a URP cDNA was identified in the eel. The mature form deduced from consensus cleavage sites was ACFWKYCVTN or URP1, which was confirmed by the phylogenetic analysis of the precursor. Eel URP had high similarity with brook trout URP (55.8%) and zebrafish URP1 (50%) but very low similarity with zebrafish URP2 (9.3%). Although primers were designed to amplify both URP1 and URP2, only URP1 cDNA was identified in eels. Therefore, the URP2 gene may be silenced or lost in eels. On the other hand, the putative mature form of eel UII had high similarity within the ring structure but low similarity at the NH2 terminus with that of other UIIs, respectively, which is a common feature of UII in vertebrates examined thus far. As shown in Fig. 3, the URP gene was expressed in the MO rather than in the urophysis, and expression of the UII gene was exclusively detected in the urophysis, and very weakly but consistently in the midbrain and MO, when cycle number of PCR was increased to 40 cycles. This finding indicates that URP and UII play separate roles in the brain.

In mammals, UII is a vasoconstrictor and/or a vasodilator, depending on the vascular bed being investigated (1) and constricted the vascular smooth muscles in the trout (17). An endothelial nitric oxide system is absent in the eel, Anguilla australis (14), thus eliminating the possibility that UII and URP could cause a nitric oxide-mediated vasodilation. Furthermore, applied URP and UII constricted the branchial and systemic arteries in eels, suggesting that the vasopressor effects of peripherally injected URP and UII are, at least in part, attributable to direct actions on the vascular smooth muscle. In previous experiments in eels using adrenomedullin 2, the cardiovascular effects were different when peptides were injected peripherally or centrally (23). However, the vasopressor effects of URP and UII were quite similar following peripheral and central injections with respect to their preferential action on branchial circulation and duration of effect. These suggest that URP and UII injected peripherally may act on the central target sites in addition to their direct actions on the vasculature. Le Mével et al. (18) proposed that UII stimulates arginine vasotocin (AVT) release from the preoptic nucleus, which then act as vasopressors. Interestingly, the vasopressor effects of URP and UII injected peripherally and centrally are very similar to those of AVT injected peripherally in eels (11).

Compared to ANG II, the vasopressor effects of URP and UII were more potent on the branchial circulation and less...
potent on the systemic circulation, regardless of whether the peptides were injected peripherally or centrally. This difference may be attributable to the preference of each peptide type for the branchial and systemic circulation, respectively. In teleosts, 10 to 70% of the vasopressor effects of angiotensins is mediated by \( \alpha \)-adrenoceptor, thus implicating activation of neural and/or endocrine catecholamine release (24). In contrast, ANG II was a vasodilator in isolated trout arteries (5), suggesting that the direct vasoconstrictory effect of ANG II is dependent on teleost species. In eels, homologous ANG II elevated dorsal aortic blood pressure through activation of adrenergic nerves but not the release of catecholamine (3) and had little contractile activity in eel arteries in vitro in this and our previous studies (J. A. Donald, unpublished data). Thus, it is likely that in eels, both peripheral and central injection of ANG II preferentially acts on the systemic circulation mainly by activation of adrenergic nerves and \( \alpha \)-adrenoceptors. On the other hand, it seems that URP and UII directly and indirectly constricted the branchial and systemic arteries in eels, as described in the preceding paragraph. The shorter-lasting effect of ANG II can probably be attributed to the intracellular kinetics of the ANG II-bound AT\(_1\) receptor that is internalized much faster than the UT receptor and carried to the late endosome (9, 28). Taken together, URP/UII and ANG II elevate blood pressure through different mechanisms.

The most distinctive difference between URP and UII was the duration of the vasopressor effect following either peripheral or central injection of the peptides, with the effect being much shorter for URP than UII. In case of the peripheral injection, this finding is consistent with those in the trout using heterologous URP (18). To elucidate the involvement of the UT receptor in the vasopressor effects of URP and UII, a peptidic antagonist of UT receptor, urantide, which is not exclusive to human UT receptor, was used. Urantide inhibited the vasopressor effects of both peptides, and the antagonistic effect was significantly greater on the effect of URP (63.6%) than that of UII (39.9%), indicating that such effects may be mediated to different degrees for the two peptides by the UT receptor. Palosuran, a nonpeptidic antagonist of human UT receptor, inhibited the cell-proliferative effects of UII through...
the somatostatin receptor but not that of URP (22), and URP and UII regulate cell proliferation of astrocytes through distinct mechanisms (13), suggesting that URP and UII can signal through different pathways. Although the involvement of somatostatin receptors in URP/UII-induced vasopressor effects has not been reported, even in mammals, the different duration of the two peptides actions may be due to ligand preferences for different receptors. In addition, it is possible that the duration of the vasopressor effect is influenced by intracellular kinetics of the UT receptor or alternative signal pathways that is determined by the stabilized conformation of URP- or UII-bound UT receptor in eels. Most seven-transmembrane receptors are thought to signal through two pathways (G protein- and/or β-arrestin-mediated pathways) (15). In fact, two parathyroid hormone analogs selectively activated G protein or β-arrestin signaling pathway (8). Alternatively, the plasma half-life of URP and UII may also affect the duration of the vasopressor effect, but this has not been examined even in mammals.

The expression of URP mRNA was detected in the CNSS as was that of UII, suggesting that URP is secreted into the circulation from the urophysis. Therefore, immunoreactive UII measured previously in plasma may be both URP and UII (16, 20). Plasma UII concentrations are maintained at ~40 and 16 pM in the sucker and goldfish, respectively (16), and 70 pM in the flounder, which was increased 4 times after environmental salinity changes (20). If the injected peptide is distributed rapidly and evenly in the extracellular space of ~155 ml/kg in eels (31), then 0.01 nmol/kg of injected peptide will increase the plasma concentration to 140 pM. Judging from the variation of UII concentration in the flounder, this increase will be within the physiological range, but elevations of blood pressure at this dose were not significant compared with controls. Although the plasma concentrations of URP and UII have not been determined in eels, circulating URP and UII may play a minor role in maintaining resting blood pressure. Consistent with this idea, the peripheral injection of urantide at a dose of 2 nmol/ee1 did not change blood pressure in eels despite the fact that it diminished the vasopressor effects of injected URP and UII at 0.01 nmol/kg. Since the blood from the urophysis flows directly to organs, such as the kidney and intestine, URP released from the urophysis may act on these organs at much higher concentrations. Bernard et al. (2) suggested that UII from the urophysis may act directly on the kidney and intestine.

Concomitant with the vasopressor effects, heart rate did not decrease as expected from the baroreflex-induced bradycardia but increased after injections of URP and UII in eels. In the trout, however, peripheral injection of UII depressed heart rate, which was due to the cardioinhibitory baroreflex by activation of α-adrenoceptor (17). Different from trout, in eels, URP/UII-induced tachycardia probably contributed to the further elevation of blood pressure and masked baroreflexogenic bradycardia.

Perspectives and Significance

UII is a hormone that was initially identified in the urophysis of the goby (26). However, the identification of UII in mammals initiated a burst of studies that demonstrated a variety of new biological actions of UII and its possible involvement in human disease (33). Recent identification of URP affords us a new insight into this intriguing hormone group. In this study, we showed that URP was as potent and efficacious as UII in the eel cardiovascular system when injected into the circulation. The data will give a fresh impetus to reassess the role of URP and UII in teleosts. URP was first identified as a sole UII-immunoreactive substance in the rat brain (29). Consistently, the URP gene was abundantly expressed in the eel brain. Judging from that the vasopressor effects of URP and UII may be mediated by the activation of the vasomotor neuron in the brain, central URP may be involved in maintaining resting blood pressure rather than circulating URP and UII. Although the site of action of URP in the brain is yet uncertain, the commissural nucleus of Cajal in the MO, which is homologous to the nucleus of the solitary tract (NTS) of mammals, is a possible candidate for the site of action, because the NTS is involved in autonomic regulation of blood pressure and expression of the URP gene is strongly detected in the MO. On the other hand, main function of UII may be specialized to osmoregulatory and renal effects in the kidney and intestine as a circulating hormone. In addition, as the UII gene was faintly expressed in restricted area and the peptide may be released in the brain from the neuron originated from the CNSS, UII may have physiological significance in the extremely restricted loci of brain. The localization of URP and UII gene transcripts in the brain and the plasma concentration may provide important clues to assess the central and peripheral roles of these peptides. To this end, in situ hybridization of URP/UII mRNA expression and measurement for the plasma concentration are currently being carried out.

ACKNOWLEDGMENTS

We thank Dr. Akiyoshi Takahashi of Kitasato University for synthesis of eel URP. We thank Dr. Susumu Hyodo, Dr. Makoto Kusakabe and Sanae Hasegawa of this laboratory for valuable comments and technical assistance. This study was financially supported by the Nippon Foundation-Hadal Environmental Science Education Program.

GRANTS

This research was supported by Grant-in-Aid for Basic Research (A) from the Japan Society for the Promotion of Science to Y. Takei (Grant 16207004).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


