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Complete Amino Acid Sequence of Urotensin I, a Hypotensive and Corticotropin-Releasing Neuropeptide from *Catostomus*

Abstract. *Urotensin I, purified from extracts of the urophysis of a teleost fish (*Catostomus commersoni*), exhibits potent hypotensive activity (mammals and birds) and corticotropin-releasing activity (both fish and mammals). The primary structure of this 41-residue peptide was determined to be H-Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg-Ile-Glu-Asn-Glu-Arg-Glu-Gln-Ala-Gly-Leu-Asn-Arg-Lys-Tyr-Leu-Asp-Glu-Val-NH_2. Extraction with 0.1*N *HCl at 100°C cleaves the amino-terminal tripeptide, yielding a fully active analog, urotensin I(4–41). The amino acid sequence was confirmed by measuring the biological activity of synthetic urotensin I(4–41). Urotensin I exhibits a striking sequence homology with ovine corticotropin-releasing factor and with frog sauvagine. These three peptides exhibit similar activities in biological test systems.*

The name urotensin and the definitions of the activities of urotensin I to urotensin IV were proposed (*1*) after the purification of a number of fish urophyseal peptides that have potent effects on smooth muscle (*2*). Urotensin I (U_I), a neuropeptide isolated from the urophysis of the teleost *Catostomus commersoni* (white sucker), has mild vasopressor activity in fish and other cold-blooded vertebrates. In contrast, the action of U_I in mammals is of particular interest because of its uniquely selective vasodilatory, hypotensive activity (*3*). During the course of our work on the structure of U_I, Vale *et al.* (*4*) described the amino acid sequence and biological properties of ovine corticotropin-releasing factor (CRF). It then became apparent to us, even at a preliminary stage of our sequence studies, that U_I is structurally similar to ovine CRF and to the related frog skin peptide, sauvagine (*5*). We have now determined the complete amino acid sequence of intact U_I (a 41-residue peptide) and of a fully active analog, U_I(4–41), both of which can be obtained from acid extracts of *C. commersoni* urophyses under appropriate conditions. Further, we have compared the biological activities of naturally occurring U_I with the activities of synthetic U_I, ovine CRF, and sauvagine.

Urotensin I was extracted in 0.1*N* HCl from acetone-dried *C. commersoni* urophyses, either with or without a heating step. Heating at 100°C for 15 minutes (*6*) led to the extraction of active peptide material, which was readily purified by the chromatographic procedures but proved to be a partially hydrolyzed product [U_I(4–41); see below] of the intact peptide present in unheated acid extracts. Because of the comparatively higher yield of peptide from the heated extracts, heat treatment was used for the preparation of peptide used for most of the sequence studies.

Throughout the purification, U_I activity was monitored by measuring the decrease in perfusion pressure in the isolated rat hind limb assay (*7*); the U_I activity was expressed in units defined earlier (*1*). Polyacrylamide gel electrophoresis (PAGE) (7.5 percent acrylamide; tris-HCl, *p*H 9.5; staining by Coomassie blue R; trichloroacetic acid) was also used as a guide for pooling active fractions (*8*).

Peptides were isolated from batches (500 mg) of acetone-dried urophyses (~ 2000 urophyses per batch) and purified by sequential gel filtration, ion-exchange chromatography, and reversed-phase high-performance liquid chromatography (HPLC) (*9*). The initial 0.1*N* HCl extracts contained 12 to 23 units of U_I activity per milligram of protein. Protein content in extracts and fractions was determined with ninhydrin after alkaline hydrolysis or by amino acid analysis, respectively.

Urotensin I was isolated as outlined in the legend of Table 1. Amino acid analyses, after SP-Sephadex chromatography or HPLC, showed close to integral molar ratios for both peptides (Table 1) and, together with sodium dodecyl sulfate–PAGE (*10*), suggested 38 residues for the peptide extracted with the heating step and 41 residues for the peptide extracted without the heating step.

Edman degradation of U_I(4–41) (*11*, *12*) and of fragment mixtures derived from trypsin, *Staphylococcus aureus* protease (SAP), and cyanogen bromide (CNBr) cleavage (*11*), provided a sequence identification for residues 4 to 38 (Fig. 1). Carboxypeptidases A, B, or Y did not release amino acids from the carboxyl terminus of U_I(4–41), suggesting a blocked or conformationally restricted carboxyl terminus. Tryptic, SAP, and CNBr fragments were therefore isolated by HPLC (conditions as given in Table 1 but with various acetonitrile gradients) and characterized by amino acid analysis; the resultant fragments were used to complete the sequence (*13*). Tryptic digests subjected to HPLC yielded two tyrosine-containing peptides, a pentapeptide and a hexapeptide containing an additional lysine. Edman degradation of the hexapeptide gave the sequence Lys-Tyr-Leu-Asp-Glu- (*14*). Only valine remained unaccounted for from the amino acid analysis on the hexapeptide. Valine amide was liberated (35 percent yield after HPLC) by SAP digestion of U_I(4–41) in 0.05*M* ammonium acetate, *p*H 4.0, and identified (*15*) by (i) thin-layer chromatography under conditions that separate valine and valine amide [silica-gel 60 F-254 (Merck); butanol, pyridine, acetic acid, and water (15:10:3:6); and ninhydrin] and (ii) hydrolysis (6*N* HCl for 20 hours at reduced pressure) to valine, identified by amino acid analysis (there was no valine peak in

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the unhydrolyzed sample). Thus, the carboxyl-terminal sequence was confirmed to be Lys-Tyr-Leu-Asp-Glu-Val-NH_2, with further confirmation of the overlap at positions 35 to 36 being provided by Edman degradation on 12 nmole of HPLC-isolated SAP fragment 30 to 40. Intact U_I gave a clear amino-terminal sequence Asn-Asp-Asp-Pro-Pro- by Edman degradation with 10 nmole of peptide (*13*). The sequence could be followed to residue 35 (Fig. 1), in complete agreement with the sequence of $U_I(4–41)$.

The complete amino acid sequence of the naturally occurring 41-residue U_I peptide (molecular weight, 4864) is therefore H-Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg-Ile-Glu-Asn-Glu-Arg-Glu-Gln-Ala-Gly-Leu-Asn-Arg-Lys-Tyr-Leu-Asp-Glu-Val-NH_2. Treatment of U_I with 0.1*N* HCl at 100°C for 15 minutes cleaves the acid-labile (*16*) Asp-Pro bond (between positions 3 and 4) to give the fully active $U_I(4–41)$ with a molecular weight of 4520. The sequence of U_I has been confirmed by the synthesis (*17*) of $U_I(4–41)$, which has the following properties. (i) Its vasodilatory activity in vitro is 400.2 ± 60.9 U/mg as compared with 317.6 ± 66.7 U/mg for the native peptide. (ii) Its behavior is identical with that of the native 38-residue peptide on both PAGE and HPLC [C_{18} μBondapak (10 μm; Waters), 30 by 0.39 cm (inner diameter); 20 minute gradient of 35 to 55 percent CH_3CN (containing 0.1 percent trifluoroacetic acid) in 0.1 percent aqueous trifluoroacetic acid; 1.5 ml/min; 214-nm detector; retention time 12.35 minutes]. (iii) It is indistinguishable from urophysis-derived $U_I(4–41)$ in a highly specific radioimmunoassay for U_I (*18*). (iv) The adrenocorticotropic hormone (ACTH)–releasing activity is not significantly different from that of the natural U_I peptide or from the ovine hypothalamic CRF (*18*). The sequence of *Catostomus* U_I also supports the simultaneously elucidated primary structure of a nearly identical U_I peptide from the urophysis of the carp (*19*).

In mammals, both CRF and sauvagine, like U_I (*3*), exhibit a site-selective (only the superior mesenteric vascular bed is markedly affected), vasodilatory, hypotensive action (*18*). Although U_I and sauvagine exhibit comparable vasodilatory activity on the rat helical mesenteric artery strip (*18*) and in the anesthetized dog (*20*), CRF has only 4 to 6 percent of the vasodilatory activity of U_I. However, like CRF and sauvagine, U_I shows potent ACTH-releasing activi-

```
                          UI(4-41)  5                                  10
UI   H-Asn - Asp - Asp -|Pro - Pro - Ile - Ser - Ile - Asp - Leu -
                        |
CRF  H-Ser - Gln - Glu                          - Leu -
SVG          pGlu - Gly

                                  15                                  20
UI   Thr   - Phe - His - Leu - Leu - Arg - Asn - Met - Ile - Glu -
CRF                                    - Glu - Val - Leu -
SVG  Ser   - Leu - Glu -               - Lys -

                                  25                                  30
UI   Met   - Ala - Arg - Ile - Glu - Asn - Glu - Arg - Glu - Gln -
CRF        - Thr - Lys - Ala - Asp - Gln - Leu - Ala - Gln -
SVG  Ile   - Glu - Lys - Gln -     - Lys       - Lys - Gln -

                                  35                                  40
UI   Ala   - Gly - Leu - Asn - Arg - Lys - Tyr - Leu - Asp - Glu - Val - NH2
CRF          His - Ser -               - Leu -           - Ile - Ala - NH2
SVG          Ala - Asn -           - Leu - Leu -         - Thr - Ile - NH2
```

Fig. 1. Comparison of amino acid sequences of urotensin I (U_I), ovine corticotropin-releasing factor (*CRF*), and sauvagine (*SVG*). Blank spaces for CRF and SVG under U_I amino acid residues denote sequence identity.

Table 1. Amino acid composition of urotensin I peptides. Peptide (5 to 10 μg) was hydrolyzed by 6*N* HCl (200 μl) in a vacuum at 110°C for 20 hours. Tryptophan (not found) was analyzed by hydrolysis in 4*N* methanesulfonic acid containing 0.2 percent 3-(2-aminoethyl)indole, at reduced pressure at 110°C for 20 hours. Cysteine was not detected in any hydrolysis samples. In addition, cysteic acid was not detected in samples that were oxidized with performic acid before hydrolysis. After Biogel P-6 chromatography [column, 85 by 2.6 cm (inner diameter)], with 0.1*N* HCl eluent at 4°C for U_I and 20°C for $U_I(4–41)$, U_I activity was recovered in 30 to 70 percent yields (60 to 220 units per milligram of protein) at an elution constant (K_{av}) of 0.9 for U_I and 0.6 to 0.7 for $U_I(4–41)$. After ion-exchange chromatography on SP-Sephadex [column, 50 by 1.6 cm (inner diameter); pyridine acetate gradient, from 0.2*M* pyridine, *p*H 4.5, to 2.0*M* pyridine, *p*H 6.5, at 4°C], U_I was recovered (elution at *p*H 4.6) in 20 percent yield (overall yield about 10 percent), with a relative mobility (R_F) of 0.72 (PAGE) and specific activity of 350 U/mg; $U_I(4–41)$ was recovered (elution at *p*H 5.1) in 25 to 40 percent yield (overall yield about 16 percent), with an R_F of 0.40 and specific activity of 360 U/mg. After HPLC [column, 25 by 0.46 cm (inner diameter), Lichrosorb C_8, 10 μm, Brownlee; acetonitrile (CH_3CN) gradient, in 0.2*M* ammonium acetate, *p*H 4.0 (1.5 ml/min; 0 to 1 minute in 0 to 10 percent CH_3CN; 1 to 20 minutes in 10 to 55 percent CH_3CN; 280-nm detector)], U_I was eluted (retention time, 17.9 minutes; 60 percent yield; about 6 percent overall yield) with an R_F of 0.72 on PAGE and specific activity of 435 U/mg; $U_I(4–41)$ was eluted (retention time, 19.4 minutes; approximately 100 percent yield; 16 percent overall); with an R_F of 0.4 and specific activity of 317.6 ± 66.7 U/mg. S.I., suggested integral, is the expected number of appearances of the residue in the peptide.

Amino acid residue	Molar ratio					
	Heated			Unheated		
	SP-Sephadex	HPLC	S.I.	SP-Sephadex	HPLC	S.I.
Asx	4.81	5.23	5	8.20	7.77	8
Thr	1.11	1.04	1	1.12	1.00	1
Ser	1.22	0.96	1	1.40	1.47	1
Glx	5.89	5.63	6	5.72	5.80	6
Pro	2.00	2.04	2	1.96	1.82	2
Gly	1.59	1.06	1	1.44	1.48	1
Ala	2.04	2.02	2	1.84	2.03	2
Val	1.22	1.00	1	1.16	0.99	1
Met	1.59	2.04	2	1.64	1.32	2
Ile*	3.22	4.04	4	3.20	2.87	4
Leu	4.91	5.23	5	4.48	4.57	5
Tyr	0.89	0.96	1	1.20	0.99	1
Phe	0.96	1.00	1	1.16	0.99	1
His	0.96	1.04	1	0.76	0.81	1
Lys	1.26	1.00	1	1.00	0.87	1
Arg	3.70	4.15	4	3.70	3.68	4

*Ile values were low in many cases (possibly due to incomplete hydrolysis), but the presence of four Ile residues was confirmed by partial sequence data (*11, 12*).

ty; in fish pituitary preparations, U_I appears to be more potent than the other two peptides in ACTH release (*21*). Thus, our discovery of the sequence homology between U_I and CRF has brought to light a new potential role for CRF as a modulator of cardiovascular dynamics in mammals, as well as revealing a potential role for U_I as a pituitary releasing factor in fish.

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13. Isolation of tryptic, SAP, and CNBr fragments and their analysis were carried out by D. McMaster. In the Edman degradation, a Beckman 890C spinning cup sequencer with a Sequemat P-6 Autoconverter was used. Phenylthiohydantoin amino acids were identified by HPLC [C. L. Zimmerman, E. Appella, J. J. Pisano, *Anal. Biochem.* **77**, 569 (1977)] and by back hydrolysis [E. Mendez and C. Y. Lai, *ibid.* **68**, 47 (1975)].
14. The symbols for the amino acid residues in urotensin I are Asn, asparagine; Asp, aspartic acid; Pro, proline; Ile, isoleucine; Ser, serine; Leu, leucine; Thr, threonine; Phe, phenylalanine; His, histidine; Arg, arginine; Met, methionine; Glu, glutamic acid; Ala, alanine; Gln, glutamine; Gly, glycine; Lys, lysine; Tyr, tyrosine; and Val, valine.
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21. U_I is at least three times as potent as either the ovine CRF or sauvagine in the stimulation of ACTH release from perfused gold fish pituitaries (J. Fryer, K. Lederis, J. Rivier, unpublished observations).
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Synergistic Action of Potassium Chloride and Magnesium Sulfate on Parasitoid Wasp Oviposition

Abstract. *A dilute solution of potassium chloride and magnesium sulfate induces oviposition in artificial eggs by the egg parasitoids* Trichogramma pretiosum *and* T. minutum. *The ability to obtain large numbers of eggs through the use of this inexpensive solution is a major advance toward the development of diets and the large-scale production of* Trichogramma *spp. in vitro.*

Insecticides are not always effective or desirable for the control of many species of insects which cause enormous losses of food and fiber. Egg parasitoids of the genus *Trichogramma* are especially promising biocontrol agents because (i) they parasitize a wide range of host insects (*1*), (ii) they destroy the host in the egg so that no larval feeding damage occurs, (iii) they are readily manipulated and mass-reared in the laboratory, and (iv) host-seeking chemicals are available to enhance the effectiveness of released and indigenous parasitoids in the field (*2*). *Trichogramma* spp. are the most widely studied entomophagous insects and are used for biocontrol on a global basis both in developed and developing countries. Augmentative releases of these parasitoids are restricted by the expense associated with the production of host eggs. Economical mass production of parasitoids on artificial diets (*3*) in quantities suitable for augmentative releases requires techniques for the collection of large numbers of *Trichogramma* spp. eggs. We report the development of an artificial ovipositional stimulant as active as the host egg, which induces *Trichogramma* spp. to deposit eggs in quantities suitable for mass production.

Previous work (*4*) with solutions encapsulated in artificial wax eggs demonstrated that Neisenheimer's solution stimulated oviposition by *T. californicum* Nagaraja and Nagarkatti and "no particular salt, amino acid, or vitamin tested was seen to be essential for eliciting oviposition" (*5*). However, comparisons of the ovipositional activity of *Trichogramma* spp. in lepidopteran hemolymph (*6*) to that in Neisenheimer's solution indicated that the latter solution likely is a weak ovipositional stimulant for *T. californicum*; we observed that it was a poor ovipositional stimulant for *T. pretiosum* Riley. We found that a solution of KCl and $MgSO_4$ is several hundred times more active than Neisenheimer's solution for both *T. pretiosum* and *T. minutum* Riley.

Trichogramma pretiosum and *T. minutum* were reared on eggs of *Sitotroga cerealella* (Olivier), the Angoumois grain moth (*7, 8*). The *p*H of the aqueous test solutions was adjusted to 7.00 with either NaOH (for Neisenheimer's solution) or KOH (for all other solutions). Test solutions were encapsulated inside wax spheres (*4, 5, 9*).

Lepidopteran larval hemolymph generally is rich in potassium and magnesium and is low in sodium (*10*). Because the composition of Neisenheimer's solution (rich in Na^+, low in K^+, and devoid of Mg^{2+}) is different from that of lepidopteran hemolymph and the host's food, we hypothesized that a mixture of K^+ and Mg^{2+} is a better ovipositional stimulant.

Although elemental analyses of *He-*

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