Cloning of the cDNA encoding the urotensin II precursor in frog and human reveals intense expression of the urotensin II gene in motoneurons of the spinal cord
(neuropeptides/amphibians/evolution)

YOALINE COULOUARN*, ISABELLE LEHRMANN**, SYLVIE JEGOU*, YOUSSEF ANOuar*, HERVE TOSTIVINT*, JEAN CLAUDE BEAUVILLAIN‡, J. MICHAEL CONLON§, HOWARD A. BERN¶, and HUBERT VAUDRY*

*European Institute for Peptide Research (IFRMP 23), Laboratory of Cellular and Molecular Neuroendocrinology, Institut National de la Santé et de la Recherche Médicale, Unité 413, Centre National de la Recherche Scientifique, University of Rouen, 76821 Mont-Saint-Aignan, France; ‡Institut National de la Sante et de la Recherche Médicale, Uniteé 422, 59045 Lille, France; §Regulatory Peptide Center, Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178; and ¶Department of Integrative Biology and Cancer Research Laboratory, University of California, Berkeley, CA 94720

Contributed by Howard A. Bern, September 23, 1998

ABSTRACT Urotensin II (UII) is a cyclic peptide initially isolated from the caudal neurosecretory system of teleost fish. Subsequently, UII has been characterized from a frog brain extract, indicating that a gene encoding a UII precursor is also present in the genome of a tetrapod. Here, we report the characterization of the cDNAs encoding frog and human UII precursors and the localization of the corresponding mRNAs. In both frog and human, the UII sequence is located at the C-terminal position of the precursor. Human UII is composed of only 11 amino acid residues, while fish and frog UII possess 12 and 13 amino acid residues, respectively. The cyclic region of UII, which is responsible for the biological activity of the peptide, has been fully conserved from fish to human. Northern blot and dot blot analysis revealed that UII precursor mRNAs are found predominantly in the frog and human spinal cord. In situ hybridization studies showed that the UII precursor gene is actively expressed in motoneurons. The present study demonstrates that UII, which has long been regarded as a peptide exclusively produced by the urophysis of teleost fish, is actually present in the brain of amphibians and mammals. The fact that evolutionary pressure has acted to conserve fully the biologically active sequence of UII suggests that the peptide may exert important physiological functions in humans.

The caudal neurosecretory system of teleost fish, which terminates in the urophysis, exhibits morphofunctional similarities to the hypothalamoneurohypophysial complex (1). Two major regulatory peptides have been isolated from urophysial extracts and chemically characterized. Urotensin I (UI) is a 41-amino acid residue peptide that is structurally similar to mammalian corticotropin-releasing factor and to the frog skin peptide sauvagine (2). Urotensin II (UII) is a cyclic 12-amino acid residue peptide that has some sequence similarity, but is not homologous, to somatostatin-14 (3, 4). The characterization of UII from the urophysis of various teleost species has shown that the structure of the C-terminal cyclic hexapeptide has been fully conserved, while several substitutions occur in the N-terminal region of the molecule.

It has long been thought that UII peptides were confined to the caudal neurosecretory system of fish (4). However, immunohistochemical and biochemical studies have shown that this concept is not correct. In particular, UII has been isolated from whole-brain extracts of trout and skate (5) as well as sea lamprey and river lamprey (6). Concurrently, UII has been characterized in the brain of the European green frog Rana ridibunda, demonstrating for the first time that a UII-related peptide is also present in the central nervous system of a tetrapod (7). Moreover, the occurrence of UII-like immunoactivity has been reported in the cerebral ganglia of the gastropod Aplysia californica (8).

The fact that UII-like molecules are present in distant taxa, from molluscs to amphibians, suggests that urotensin-related peptides may also occur in mammals. In support of this hypothesis, a peptide with a high degree of sequence similarity with UII, called urocortin, has been characterized recently in the rat (9) and human brain (10). In addition, specific binding sites for UII have been demonstrated in rat blood vessels (11) and fish UII exerts cardiovascular effects in rat (12) and rabbit (13). It has also been found that the mouse anococcygeus muscle has special sensitivity to UII (14). However, to date, the existence of UII has never been reported in mammals. In the present study, we have characterized the cDNAs encoding the UII precursors in frog and human.

MATERIALS AND METHODS

Isolation of Frog Prepro-UII cDNA. Recombinants (2.5 × 10^4) of an amplified Uni-Zap frog (R. ridibunda) brain cDNA library were screened with a degenerate 23-mer oligonucleotide {5'-AC(AG)CA(AG)TA(TC)TTCCA(AG)AA(AG)CA(TC)TC-3'} (ScienceTec, Les Ulis, France) designed from the C-terminal amino acid sequence ECFWKYCV of frog UII. The oligonucleotide probe was 3'-end labeled with [32P]dCTP by using terminal transferase (Promega). Prehybridization was performed at 37°C for 4 hr in 5× SSPE (0.9 M NaCl/0.05 M sodium phosphate buffer, pH 7.7/0.005 M EDTA)/0.1% SDS/10× Denhardt’s solution (1× Denhardt’s solution = 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% BSA)/50 μg/ml tRNA/50 μg/ml denatured salmon sperm DNA. Overnight hybridization was carried out at 37°C in 5× SSPE/0.1% SDS. Filters were washed three times for 10 min at room temperature in 5× SSPE/0.1% SDS and directly exposed onto Kodak X-Omat films for 16 hr at −70°C with intensifying screens. A single full-length clone was obtained. The rescued PCR-amplified insert was subeloned into pGEM-T (Promega) and sequenced on both strands with SP6 and T7 primers by using the Thermo sequenase fluorescent labeled primer cycle.

Abbreviations: UI, urotensin I; UII, urotensin II; EST, expressed sequence tag; RT-PCR, reverse transcription–PCR.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF104117 (frog prepro-UII) and AF104118 (human prepro-UII)].

To whom reprint requests should be addressed.
sequencing kit (Amersham). The sequence of this clone was confirmed after plaque purification and full characterization of a second clone obtained during the screening of 250,000 new recombinants.

Isolation of Human Prepro-UII cDNA. An expressed sequence tag (EST) potentially encoding a peptide with significant identity to frog UII was registered in GenBank EST division (accession no. AA535545). This sequence derived from an EST analysis of cDNA clones obtained from bulk colon tumors. Two oligonucleotide primers (5' - AACCCAA-GAGGAAATTTGAGAAAGTT-3' and 5' - CCAGGTAA-CAATGAACAGGGTGTAG-3') deduced from the EST sequence were designed to synthesize a 269-bp fragment by reverse transcription–PCR (RT-PCR) from a human colon tumor sample. This PCR product was labeled with [32P]dCTP by random priming and was hybridized to a multiple tissue dot blot containing poly(A) RNA from 50 human tissues along with appropriate positive and negative controls (MasterBlot, CLONTECH). Hybridization and washes were performed according to the manufacturer’s recommendations. The blot was exposed to X-Omat film (Kodak) and the hybridization signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.

RT-PCR Amplification. Total RNA from 14 different frog tissues was prepared as mentioned above. The concentration of total RNA was determined by measuring the optical density signals were quantified by using the DENSYSLAB software (Bio-Probe systems, Montreuil-Sous-Bois, France). The highest hybridization signal was obtained in the spinal cord. Thus, human spinal cord poly(A) RNA (CLONTECH) was used for amplification of the 5'-end of human UII cDNA with a commercially available rapid amplification of cDNA ends (RACE) kit (Marathon cDNA amplification kit, CLONTECH).

Northern Blot Analysis. Total RNA from frog brain, hypothalamus, and spinal cord was extracted by the acid guanidinium-thiocyanate-phenol-chloroform procedure (15) by using Tri reagent (Sigma). Samples (30 μg each) of total RNA from whole frog brain, frog hypothalamus, and spinal cord, or poly(A) RNA from human spinal cord (2 μg) (CLONTECH) were run on a formaldehyde agarose gel, transferred onto a nylon filter and hybridized with a [32P]dCTP-labeled PCR product containing most of the coding sequence of frog or human UII cDNA.
at 260 nm. Total RNA (5 μg) from each frog tissue sample was converted to single-stranded cDNA (SuperScript II, Life Technologies, Eragny, France) with oligo (dT)12–18 primer (25 μg/ml) and was analyzed by PCR with the primers 5′-TCTTTTCCGTTGGCCCATCAG-3′ and 5′-CGTCACTAGATGGAACTGGTTAATCA-3′. All PCR-based procedures were performed in a final volume of 50 μl containing first-strand cDNA (10% of reverse transcription reaction), 1 unit Tfi DNA polymerase (Promega), Tfi polymerase buffer (Promega)/2.5 mM MgSO4/0.4 mM dNTPs/10% dimethyl sulfoxide/50 pmol of each primer. The PCR reactions were performed for 30 cycles (94°C, 30 sec; 50°C, 90 sec; 72°C, 90 sec). The amplified products were analyzed in 1.5% agarose gels, blotted on a nylon membrane, and hybridized with a [32P]ATP-labeled internal oligonucleotide (5′-CAATC-3′) or the sense (F) prepro-UII riboprobe and were exposed for 2 weeks onto x-ray film. The amplified products were analyzed by PCR with the primers 5′-CAATC-3′ and 5′-CTCTTTCGGTGTTGCGCCAACATTACAGATC-3′. Some PCR products were subcloned into pGEM-T and sequenced.

In Situ Hybridization. Frog and human sense and antisense riboprobes were prepared by in vitro transcription of PCR products obtained with prepro-UII specific primers (5′-TCCCTTTCGTTGGCCCATCAG-3′ and 5′-GAACCAGTATTTCCGAAACAT-3′; for frog; 5′-AGGGTTCC-3′ and 5′-CTCTTTCGGTGTTGCGCCAACATTACAGATC-3′ for human) extended at the 5′-end with the SP6 and T7 RNA polymerase promoters, in the presence of [35S]UTP (Amersham) or digoxigenin-11-UTP (Boehringer Mannheim) and T3 or T7 RNA polymerase (Promega).

Adult male frogs were anesthetized and perfused transcardially with 4% paraformaldehyde. The brains were postfixed for 24 hr in 4% paraformaldehyde, embedded in Tissue-Tek (Leica, Rueil Malmaison, France) and frozen in liquid nitrogen. Frontal sections (12-μm thick) were cut in a cryostat and kept at −80°C until use. Sections were pretreated as previously described (16) and covered with prehybridization buffer (50% formal/mide/0.6 M NaCl/10 mM Tris-HCl, pH 7.5/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.1% BSA/1 mM EDTA, pH 8.0/550 μg/ml denatured salmon sperm DNA/50 μg/ml yeast tRNA). Hybridization was performed overnight at 55°C in the same buffer (except for salmon sperm DNA used at 60 μg/ml) supplemented with 10 mM DTT, 10% dextran sulfate, and heat-denatured riboprobes. The [35S]-labeled and digoxigenin-labeled probes were diluted in hybridization buffer to a final concentration of 5 × 10⁶ dpm/ml and 1:100 (vol/vol), respectively. The slices were washed in 2× SSC saline sodium citrate (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) at 60°C and treated with RNase A (50 μg/ml) for 60 min at 37°C. Five final high-stringency washes were performed in 0.1× SSC, 14 mM β-mercaptoethanol, 0.05% sodium pyrophosphate at 60°C. Sections hybridized with the [35S]-labeled riboprobes were then dehydrated in graded ethanol and were exposed onto Hyperfilm-βmax (Amersham) for 2 weeks. Sections hybridized with the digoxigenin-labeled riboprobes were washed in buffer 1 (100 mM Tris-HCl and 150 mM NaCl, pH 7.5), incubated for 30 min in blocking buffer (2% Boehringer blocking agent in buffer 1) and incubated for 2 hr in buffer 1 containing 1:500 alkaline phosphatase-conjugated antidigoxigenin antibodies (Boehringer Mannheim), 1% normal sheep serum, and 0.1% Triton X-100. The sections were rinsed twice for 10 min in buffer 1 and once for 10 min in buffer 2 (100 mM Tris-HCl/100 mM NaCl and 50 mM MgCl2, pH 9.5) and then incubated for 3 hr in a chromagen solution consisting of Fast Red TR/Naphthol AS-MX and 3 mM Levamisole (Sigma). The reaction was stopped by rinsing in TE buffer (10 mM Tris-HCl/1 M EDTA, pH 8.0). The sections were examined under a Leitz Orthoplan microscope.

RESULTS

Characterization of Frog and Human Prepro-UII cDNA.

The ORFs of the frog and human UII precursor cDNAs encode proteins deduced to be 127 and 124 amino acids long, respectively (Fig. 1). The organization of both frog and human UII precursors is similar to that of the carp UII prohormone, i.e., all precursor proteins are composed of a consensus N-terminal signal-peptide sequence, an N-terminal flanking peptide, a conserved proteolytic processing site (Lys/Arg-Lys-Arg), and the UII sequence located at the C-terminal extremity of each precursor. The N-terminal flanking peptides of carp, frog, and human UII precursors do not exhibit appreciable sequence similarities. Human UII is composed of only 11 amino acid residues, whereas frog and carp UII possess 13 and 12 residues, respectively. The C-terminal cyclic heptapeptide of frog and human UII is fully conserved. In contrast, the N-terminal region of the peptide is highly variable. In frog, as in carp, the C-terminal region of the flanking peptide contains a potential dibasic cleavage site (Arg-Lys and Arg-Arg, respectively), which could possibly generate the conserved
dipeptide Gln-Phe. However, in human, the sequence of the corresponding dipeptide appears to be totally different (Pro-Tyr).

**Distribution of Frog Prepro-UII mRNA.** Expression of the prepro-UII gene was studied in the frog brain by Northern blot analysis (Fig. 2A). A strong hybridization signal corresponding to an mRNA of ~1 kb was observed in the spinal cord. In contrast, no signal could be detected in the whole brain or hypothalamus. The distribution of prepro-UII mRNA in various frog tissues was studied by RT-PCR assay and Southern blot analysis (Fig. 2B). A PCR product of the correct size (548 bp) was observed in the spinal cord and posterior brain. A faint band was also found in the pituitary. In contrast, no band could be detected in the diencephalon or in the other tissues examined, including lung, intestine, pancreas, liver, adrenal, ovary, testis, and skin.

The precise localization of prepro-UII mRNA in the central nervous system of the frog was studied by in situ hybridization. All somatic motor nuclei of the brainstem were specifically labeled with the mRNA probe (Fig. 3). In particular, a strong hybridization signal was observed in the trochlear nucleus (Fig. 3A), the facial motor nucleus (Fig. 3B), the abducens nucleus (Fig. 3C), the glossopharyngeal nucleus (Fig. 3C), and the hypoglossal nucleus (Fig. 3D). An intense signal was found also in the ventral horn of the spinal cord (Fig. 3E). The cellular distribution of UII precursor mRNA was studied by using a digoxygenin-labeled mRNA probe (Fig. 4). Prepro-UII mRNA was seen in about one-third of the motoneurons (which were identified by acetylcholinesterase histochemistry on consecutive sections; data not shown) of the motor nuclei of the brainstem (Fig. 4A) and spinal cord (Fig. 4B). In addition, an intense hybridization signal was observed in a few neurons of the nucleus reticularis inferior (Fig. 4C).

**Distribution of Human Prepro-UII mRNA.** The tissue distribution of human prepro-UII mRNA was studied by dot blot analysis (Fig. 5A). Of the 50 different tissues tested, the spinal cord exhibited by far the strongest hybridization signal. Prepro-UII mRNA was observed also in the medulla oblongata, but the signal intensity was much lower than in the spinal cord. In peripheral tissues, the presence of prepro-UII mRNA was detected in kidney, spleen, small intestine, thymus, prostate, pituitary gland, and adrenal gland and, to a lesser extent, in stomach, pancreas, ovary, and liver (Fig. 5A). Northern blot analysis revealed the presence of a single band of ~700 bp (Fig. 5B).

Labeling of sections of the cervical portion of a human spinal cord by in situ hybridization showed that prepro-UII mRNA was located in motoneurons (Fig. 5C).

**DISCUSSION**

The present report describes the cloning of the cDNAs encoding the frog and human homologues of prepro-UII. So far, the sequence of prepro-UII has been determined only in the carp *Cyprinus carpio*, in which two isoforms (prepro-UII-α and -γ) have been cloned (17). We now describe the molecular characterization of prepro-UII in tetrapods and we provide evidence for the occurrence of UII in mammals.

The amino acid sequence of frog and human prepro-UII exhibits little overall similarity to carp prepro-UII-α (12% and 16%, respectively) and carp prepro-UII-γ (22% and 16%, respectively). Likewise, the overall amino acid sequence similarity between frog and human prepro-UII is low (25%). The C-terminal region of the precursors, which includes the UII peptides, is the only domain that exhibits significant amino acid sequence identity. In particular, the cyclic heptapeptide Cys-Phe-Trp-Lys-Tyr-Cys-Val, which is essential for the biological activity of UII (11), has been conserved totally from lamprey to human (Fig. 6), which diverged some 560 million years ago (18). The fact that strong evolutionary pressure has acted to preserve the sequence of the biologically active region of UII, whereas the N-terminal flanking region of the precursor has no significant similarity, indicates that UII almost certainly has important physiological functions. The sequence of a dipeptide (Gln-Phe) flanked by basic amino acid cleavage sites, located upstream of the UII peptide, was found to be identical in carp (17), flounder (19), and frog. However, in human prepro-UII, the structure of this peptide is totally different, suggesting that UII is the only bioactive peptide derived from the precursor, at least in mammals.

Characterization of UII in carp urophysis and frog brain extracts has demonstrated already that the UII precursor is actually processed to generate the mature peptide (7, 20). The fact that the Lys-Arg dibasic site flanking UII at the N terminus has been conserved from fish (17) to mammals suggests that, in human, UII is also generated in vivo. In
support of this hypothesis, it has been reported previously that the Lys-Arg dibasic site is more efficiently cleaved by prohormone-converting enzymes than any other pairs of basic residues (21).

The existence of sequence similarities between fish UII and somatostatin has long been mentioned (1), but the cloning of their respective precursors did not reveal the existence of significant sequence homology (17, 22), suggesting that they were not derived from a common precursor (4, 23). In agreement with this notion, sequence alignment of the cDNA encoding frog and human prepro-UII with those encoding the precursors of the two frog somatostatin variants (16) and human somatostatin (24) or cortistatin (25) showed clearly that the genes encoding UII and somatostatin precursors do not derive from a common ancestor.

In both frog and human, the highest concentration of prepro-UII mRNA was found in the spinal cord. The presence of the mRNA was also detected in the medulla oblongata by RT-PCR and dot blot analysis, respectively. In situ hybridization studies showed that, in frog and human, the prepro-UII gene is specifically expressed in a subpopulation of motoneurons in the spinal cord. In frog, prepro-UII mRNA was found also in several motor nuclei of the medulla oblongata. In agreement with these observations, we have observed previously in the frog the presence of UII-like immunoreactivity in motoneurons distributed along the entire length of the spinal cord and in a subset of neurons located in the motor nuclei of the medulla oblongata (26). We have found also that the frog spinal cord and medulla oblongata both contain high concentrations of a peptide coeluting with synthetic UII (26). Taken together, these data suggest that, in frog and human, UII could be involved in the regulation of locomotor activities. In fish, UII is present not only in the urophysis but also in a midsagittal column of cerebrospinal fluid-contacting neurons located along the entire length of the spinal cord and brainstem (27, 28). In frog, the prepro-UII gene is apparently expressed exclusively in the posterior part of the central nervous system, and possibly in the pituitary. In human, other brain regions and

Fig. 5. Tissue distribution of human prepro-UII mRNA. (A) Dot blot analysis of prepro-UII mRNA expression in various human tissues. The master blot (CLONTECH) consisted of 50 human-tissue poly(A) RNAs (80–448 ng per dot) normalized by using the RNA expression levels of eight housekeeping genes. Positive controls consisted of human genomic DNA. Negative controls included yeast and Escherichia coli RNA or DNA, as well as human repetitive genomic sequences. The blot was probed with the human prepro-UII cDNA probe and exposed for 2 days onto an X-Omat film. (B) Northern blot analysis of prepro-UII mRNA expression in the human spinal cord. Spinal cord poly(A) mRNA (2 μg) was hybridized with the cDNA probe. The molecular weight was determined by using RNA markers. (C) X-ray autoradiographs showing the distribution of prepro-UII mRNA in the human spinal cord. Coronal sections were hybridized with the antisense (Top) or sense (Bottom) prepro-UII riboprobe and exposed for 10 days onto x-ray film.
several peripheral tissues appeared to contain prepro-UII mRNA, suggesting that UII may exert a wider range of biological activities in human than in other vertebrates.

The functional significance of UII in motoneurons, especially in the human spinal cord, is currently a matter of speculation. Several neuropeptides are present in motoneurons of different species, notably during development or after lesioning (29). However, calcitonin gene-related peptide is the only neuropeptide consistently found so far in motoneurons of adult vertebrates (30, 31). The intense expression of the prepro-UII gene in motoneurons of the spinal cord in both frog and adult human suggests that UII, like calcitonin gene-related peptide, may play important functions at the neuromuscular junction, such as regulation of the expression of nicotinic receptor subunits (32). Alternatively, UII may act locally to modulate survival and/or regeneration of motoneurons, as previously proposed for calcitonin gene-related peptide (33).

This work is dedicated to the memory of Professor I. I. Geschwind of the University of California at Davis, who conducted the first meaningful work on the chemistry of active urophysial principles, and to Dr. Tomoyuki Ichikawa of the Tokyo Metropolitan Institute of Neurological Sciences, who helped clone fish prepro-urotensin II. We thank Dr. M. M. Ruchoux (Laboratory of Anatomopathology, Centre Hospitalier et Universitaire de Lille, Lille, France) for providing the human spinal cord sample and Patrice Bizet for skillful technical assistance. This work was supported by the Institut National de la Santé et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Lille-Amiens-Sante et de la Recherche Médicale (U413 and U422), the National Science Foundation, and the Conseil Régional de Haute-Normandie. Y.C. was the recipient of a fellowship from the LARC (Coulouarn et al. 1998)