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Vasotocin Genes of the Teleost Fish *Catostomus commersoni*: Gene Structure, Exon–Intron Boundary, and Hormone Precursor Organization[†,‡]

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Received September 8, 1989

ABSTRACT: cDNA clones encoding two members of the vasotocin hormone precursor gene family have been isolated from the white sucker *Catostomus commersoni*. The hormone is encoded by at least two distinct genes, both of which are expressed, as indicated by Northern blot analysis. Genomic DNA amplified by the polymerase chain reaction has been used to define exon–intron boundaries. Both vasotocin genes contain introns in positions corresponding to those found in the gene of their mammalian counterpart vasopressin. The predicted vasotocin precursors show a surprising degree of sequence divergence, amounting to 45% at the amino acid level, of which only approximately half can be accounted for by conservative amino acid changes. The precursors include a hormone moiety followed by a putative neurophysin sequence that is longer at the C-terminus by a tract of some 30 amino acids by comparison to their mammalian counterpart. Each of these sequences contains a leucine-rich core segment resembling that found in copeptin, a glycopeptide moiety present in mammalian vasopressin precursors.

Neuropeptides were probably among the earliest molecules to play a signaling role in the evolving nervous system. Initially it had been assumed that regulatory peptides, such as vasopressin and oxytocin, would be found exclusively within vertebrates [reviewed in Krieger (1983)], where they can function as endocrine hormones, neurotransmitters, and paracrine factors [reviewed in Hoffman (1987) and Ishikawa et al. (1987)]. A wealth of studies have shown, however, that vasopressin and oxytocin are the predominant mammalian homologues of a larger, structurally conserved, family of neuropeptide hormones (Table I), whose members are distributed throughout the animal kingdom, including groups as diverse as molluscs and insects [reviewed in Acher and Chauvet (1988)]. These peptides all consist of nine amino acids with cysteine residues in positions one and six forming a disulfide bridge. While Asn[5], Pro[7], and the C-terminal glycine amide are also completely conserved, amino acid variations exist at positions 2, 3, and 4, giving rise to the different family members shown in Table I and, most significantly, at position 8 where the change from Leu or a related lipophilic amino acid to Arg or Lys accounts for a vasopressin-type as opposed to an oxytocin-type function (Hruby & Smith, 1987). In all

Table I: Vasopressin–Oxytocin Neuropeptide Hormone Family[a]

OXYTOCIN FAMILY		
CYS TYR ILE GLN ASN CYS PRO LEU GLY·NH2	*OXYTOCIN*	*mammal*
CYS TYR ILE GLN ASN CYS PRO ILE GLY·NH2	*MESOTOCIN*	*non-mammalian tetrapod*
CYS TYR ILE SER ASN CYS PRO ILE GLY·NH2	*ISOTOCIN*	*bony fish*
CYS TYR ILE SER ASN CYS PRO GLN GLY·NH2	*GLUMITOCIN*	*cartilagenous fish*
CYS TYR ILE GLN ASN CYS PRO VAL GLY·NH2	*VALITOCIN*	
CYS TYR ILE ASN ASN CYS PRO LEU GLY·NH2	*ASPARGTOCIN*	
VASOPRESSIN FAMILY		
CYS TYR PHE GLN ASN CYS PRO ARG GLY·NH2	*ARG·VASOPRESSIN*	*mammal*
CYS TYR PHE GLN ASN CYS PRO LYS GLY·NH2	*LYS·VASOPRESSIN*	
CYS PHE PHE GLN ASN CYS PRO ARG GLY·NH2	*PHENYPRESSIN*	
CYS TYR ILE GLN ASN CYS PRO ARG GLY·NH2	*VASOTOCIN*	*non-mammalian vertebrate*
CYS PHE ILE ARG ASN CYS PRO LYS GLY·NH2	*LYS·CONOPRESSIN*	*mollusc (Conus)*
CYS ILE ILE ARG ASN CYS PRO ARG GLY·NH2	*ARG·CONOPRESSIN*	*mollusc (Conus)*
CYS LEU ILE THR ASN CYS PRO ARG GLY·NH2	*DIURETIC HORMONE*	*insect (Locust)*

[a] The cysteine residues are linked by a disulfide bridge. Mammals: in general, oxytocin and arginine vasopressin are found in mammals except in pig which has lysine vasopressin and the Methatherian marsupials which have mesotocin, lysine vasopressin, and phenypressin either in addition or as alternatives to their normal mammalian counterparts. Nonmammalian tetrapods (birds, reptiles, and amphibians): mesotocin and vasotocin. Bony fish (e.g., sucker): vasotocin and isotocin. Cartilaginous fish: vasotocin and glumitocin (rays), vasotocin, valitocin, and aspargtocin (sharks). Mollusc: in fish-hunting snails (*Conus*), two peptides have been identified, namely, lysine conopressin and arginine conopressin. Locust: *Locusta migratoria* contains an arginine vasopressin like diuretic hormone. Compiled from Proux et al. (1987), Acher and Chauvet (1988), and Gray et al. (1988).

[†] This work was supported by grants from the Deutsche Forschungsgemeinschaft to D.R. and the Medical Research Council of Canada to K.L.

[‡] The nucleic acid sequences in this paper have been submitted to GenBank under Accession Numbers M25145 (IT-1), X15821 (IT-2), M25144 (VT-1), and J02889 (VT-2).

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vertebrates other than mammals, vasotocin has been chemically identified in place of vasopressin while in nonmammalian tetrapods and bony fish oxytocin is replaced respectively by mesotocin and isotocin (Acher & Chauvet, 1988).

Cloning studies in mammals have revealed that oxytocin and vasopressin are encoded by distinct, but structurally related genes and synthesized as part of larger precursor molecules [reviewed in Richter (1987)]. These contain, in addition to the hormone moiety, a cysteine-rich protein termed neurophysin, which probably plays a role in transporting the hormone from the site of synthesis, the hypothalamus, to the site of storage, the neurohypophysis. The vasopressin precursor includes an additional entity, a glycopeptide or copeptin (Acher & Chauvet, 1988), which may be the conjectured hypothalamic prolactin-releasing factor (Nagy et al., 1988).

In order to gain further insights into the evolution of the vasopressin–oxytocin neuropeptide family, we have examined the molecular basis for the presence of vasotocin and isotocin precursor proteins in the hypothalamic region of the teleost fish *Catostomus commersoni* (white sucker). We report here the gene and precursor organization for vasotocin as compared to that for its mammalian counterpart vasopressin.

Experimental Procedures

Materials. Klenow enzyme and restriction enzymes were obtained from Boehringer Mannheim; *Taq* DNA polymerase was from Cetus, Offenbach, FRG; deoxyadenosine 5′-[α-^{35}S]triphosphate (500 Ci/mmol; 1 Ci = 37 GBg) and adenosine 5′-[γ-^{32}P]triphosphate (6000 Ci/mmol) were from Du Pont de Nemours GmbH, Dreieich, FRG, and deoxycytidine 5′-[α-^{32}P]triphosphate (3000 Ci/mmol) came from Amersham-Buchler, Braunschweig, FRG.

Screening of a λgt11 Library and Nucleotide Sequence Analysis. A *Catostomus commersoni* hypothalamic region cDNA library, constructed in the expression vector λgt11, was screened as described previously, using either fully degenerate oligonucleotide pools corresponding to the first seven amino acids of vasotocin (Heierhorst et al., 1989) or appropriate cDNA inserts isolated from primary clones. Inserts from positive clones were either sequenced directly (Chen & Seeberg, 1985) or subcloned into M13 vectors (Yanish-Perron et al., 1985) and their identities confirmed by sequence analysis on both strands (Sanger et al., 1977).

Polymerase Chain Reaction (PCR)[1] *and Genomic DNA Sequencing.* Recombinant λgt11 DNA (0.2 μg) or sucker liver genomic DNA (100 ng) was subjected to PCR amplification (Saiki et al., 1988), using specific oligonucleotide primers directed against appropriate regions of the respective cDNA sequences. Reaction mixtures and conditions were essentially as described previously (Heierhorst et al., 1989) except that after initial denaturation at 94 °C for 5 min, templates were subjected to 30 cycles of denaturation for 1 min at 94 °C, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min, with the final extension reaction being prolonged by a further 5 min. Single-stranded DNA templates for direct sequencing of amplified genomic sucker DNA were generated as described previously (Heierhorst et al., 1989) and sequenced (Sanger et al., 1977) by using the same oligonucleotides as sequencing primers. Alternatively, products of the amplification reaction were phosphorylated and cloned into the *Sma*I site of M13mp18 (Yanish-Peron et al., 1985) prior to sequencing as above.

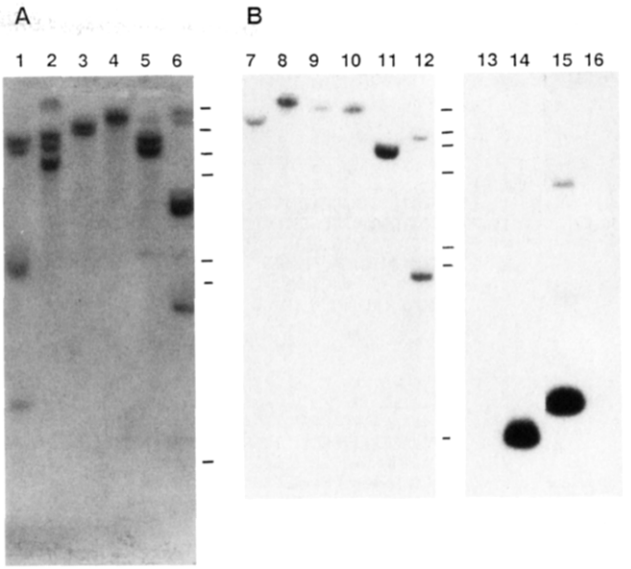

FIGURE 1: Southern blot analysis of sucker genomic DNA. Ten micrograms of DNA from an individual sucker liver was digested with various restriction enzymes, electrophoresed on 0.8% agarose gels, and blotted onto nylon membranes. The following restriction enzymes were used: *Pst*I, lanes 1, 7; *Bam*HI, lanes 2, 8; *Eco*RI, lanes 3, 9; *Sst*I, lanes 4, 10; *Hin*dIII, lanes 5, 11; *Eco*RV, lanes 6, 12. Blots were hybridized with ^{32}P-labeled cDNA probes, washed stringently (last wash 0.1 × SSC/0.1% SDS; 60 min, 65 °C), except for lanes 13–16 (last wash 1.0 × SSC/0.1% SDS; 60 min, 65 °C), and autoradiographed for 30 h. Blot hybridization with (A) ^{32}P-labeled VT-1 cDNA (nr 1–539) and (B) VT-2 cDNA (nr 1–721). For positions of the nucleotide residues (nr), see Figure 2. Lanes 13–16 show controls for the VT cDNAs; lanes 13 and 15 contained VT-2 cDNA and lanes 14 and 16 VT-1 cDNA. Lanes 13 and 14 were hybridized to ^{32}P-labeled VT-1 cDNA [as in (A)] and lanes 15 and 16 to ^{32}P-labeled VT-2 cDNA [as in (B)]. The extra band at ca. 3.8 kb seen in lane 15 is due to incomplete digestion of the plasmid. Markers (*Hin*dII-restricted, ^{32}P-labeled λ-DNA of fragment sizes 23 130, 9416, 6557, 4361, 2322, 2027, and 564 bp) are indicated by horizontal lines.

Northern and Southern Blot Analysis. Poly(A+) RNA (10 μg) was denatured with glyoxal (Thomas, 1983), separated on a 1.2% (w/v) agarose gel, and transferred to Hybond N membranes (Amersham) according to the supplier's instructions. Similarly, 10 μg of sucker genomic liver DNA, prepared by standard methods (Maniatis et al., 1982) from individual fish, was digested to completion with various restriction enzymes, fractionated on a 0.8% (w/v) agarose gel, and transferred as above. In both cases, hybridizations were carried out under standard conditions (Maniatis et al., 1982) using 10^6 cpm/mL labeled cDNA probes (Feinberg & Vogelstein, 1984) specific for the respective cDNAs.

Results

Southern Blot Analysis. Southern blotting and hybridization of genomic DNA isolated from individual fish were carried out by using the vasotocin precursor cDNA isolated previously as hybridization probe (Heierhorst et al., 1989; this precursor being referred to here as VT-1). This revealed either two or four bands in digests probed with VT-1 cDNA (Figure 1A, lanes 1–6), suggesting either the occurrence of endonuclease restriction sites within introns and/or the existence of more than one gene for the VT precursor.

To test whether the vasotocin hormone precursor is indeed encoded by more than one gene, the λgt11 white sucker hypothalamic library was searched for a second VT-encoding cDNA using the fully degenerate oligonucleotide pools for vasotocin (Heierhorst et al., 1989). This yielded cDNA clones

[1] Abbreviations: PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; IT, isotocin; VT, vasotocin; NP, neurophysin; nr, nucleotide residue(s); bp, base pair(s); SSC, 0.15 M NaCl/0.015 M sodium citrate; SSPE, 0.18 M NaCl, 10 mM NaH_2PO_4 (pH 7.4), and 1 mM EDTA (ethylenediaminetetraacetate).

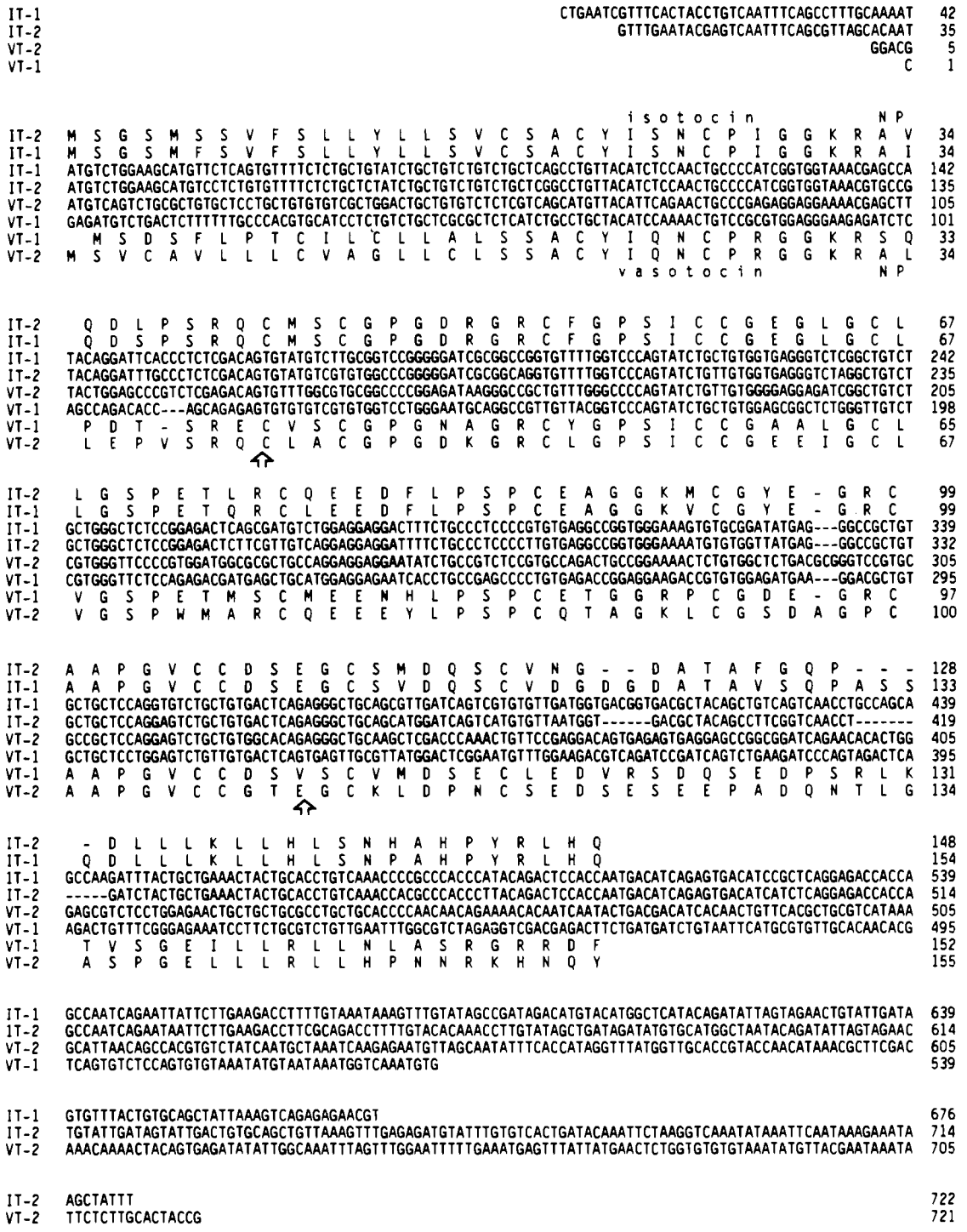

FIGURE 2: Comparison of nucleotide and deduced amino acid sequences of sucker isotocin (Figueroa et al., 1989) and vasotocin precursors. The largest cDNA clone encoding the VT-2 precursor consists of 721 bp excluding the poly(A) tail and exhibits a single open reading frame of 155 amino acids, giving rise to a calculated molecular weight of 16077. For details of IT-1, IT-2, and VT-1 cDNAs, see Heierhorst et al. (1989) and Figueroa et al. (1989). The nucleotide and amino acid residues are numbered at the right. The arrows point to the position of the introns within the two vasotocin genes.

for a second type of vasotocin precursor, termed VT-2, which showed a different nucleotide sequence as compared to VT-1 (Figure 2).

The two VT cDNAs failed to cross-react even under relatively low washing stringencies (Figure 1B, lanes 13–16), and hence the VT-2 cDNA hybridized to a completely different set of DNA restriction fragments (Figure 1B, lanes 7–12) as compared to those recognized in the VT-1 cDNA-probed Southern blot (Figure 1A). These data, together with the identification of distinct mRNAs for VT-1 and VT-2 (see later), argue strongly for the existence of at least two distinct genes for vasotocin, within the genome of *Catostomus commersoni*.

Analysis of the Exon–Intron Boundaries within the Vasotocin Genes. To determine the exon–intron boundaries of the *Catostomus* vasotocin genes, genomic DNA and cloned cDNA were amplified in parallel using the polymerase chain reaction (PCR) technique (Saiki et al., 1988). Specific "forward" and "reverse" primers were designed to flank positions in the VT-1 cDNA sequence corresponding to the known locations of introns in the gene of the mammalian counterpart vasopressin (Schmale et al., 1983). When the VT-1 cDNA was amplified

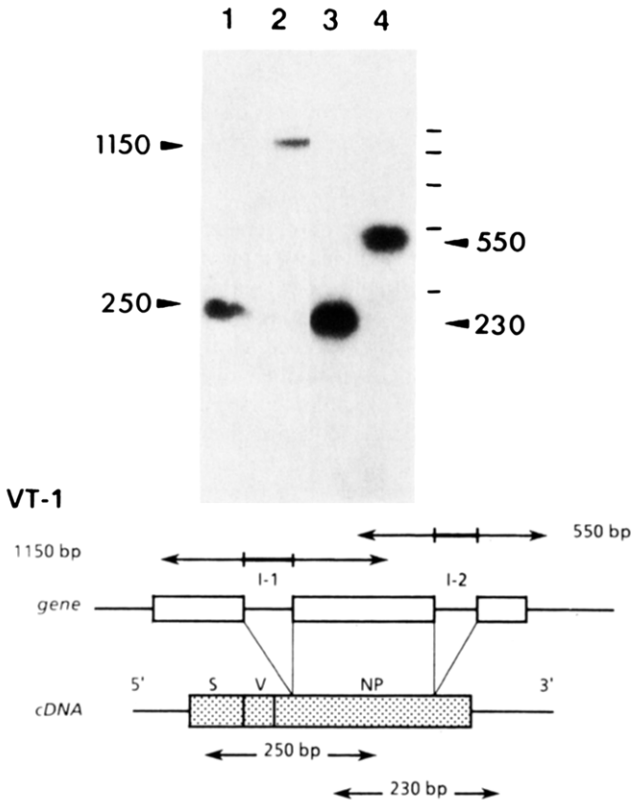

FIGURE 3: Location of the introns within the VT-1 gene. VT-1 cDNA (lanes 1 and 3) and liver genomic DNA from an individual sucker (lanes 2 and 4) were subjected to PCR amplification as described under Experimental Procedures using "forward–reverse" oligonucleotide primer pairs designed to flank positions corresponding to the introns of mammalian vasopressin (Schmale et al., 1983). These are as follows: first intron (lanes 1 and 2, nr 31–50 and 281–262; second intron (lanes 3 and 4), nr 254–277 and 488–465 [see Figure 2 for nucleotide residue (nr) positions]. One-tenth of the reaction products were analyzed on a 1.2% (w/v) agarose gel, blotted onto nylon membranes, and hybridized with a VT-1-specific ^{32}P-labeled oligonucleotide probe (nr 161–144 for lanes 1 and 2 and nr 329–352 for lanes 3 and 4). *Hae*III-digested ϕX174 DNA was used as size marker; horizontal lines indicate fragment sizes of 1353, 1078, 832, 603, and 281/271 bp. Arrows indicate the positions of amplified DNA fragments. The structural organization of the VT-1 gene is shown below the relevant blots, including the locations of the amplified fragments which are indicated by horizontal lines (arrowed). The protein-coding regions are represented by stippled boxes. S, signal peptide; V, vasotocin; NP, neurophysin; I-1, intron 1; I-2, intron 2. The schemes are not drawn to scale.

by using the primers as outlined in the legend to Figure 3, the expected DNA fragments of 250 and 230 bp in size were obtained (Figure 3, lanes 1 and 3). Amplification of genomic DNA using the same primers yielded larger fragments indicating the existence of introns within the protein coding regions. Using the "forward–reverse" primer pair designed to locate the first putative intron, a fragment of 1150 bp was identified, suggesting the presence of an intron of ca. 900 bp (Figure 3A, lane 2) within the VT-1 gene. The other pair of "forward–reverse" primers used to identify the second intron gave rise to a DNA fragment of 550 bp, suggesting an intron size of 320 bp (Figure 3A, lane 4). Sequencing of all the PCR-amplified genomic DNA products shown in Figure 3 confirmed the presence of exon–intron junctions in positions corresponding exactly to those found in the mammalian vasopressin genes.

Amplification of VT-2 cDNA using appropriate primer pairs resulted in the expected fragments of 240 and 340 bp for VT-2 cDNA (Figure 4, lanes 1 and 3) while fragments of 360 and 1400 bp were obtained with sucker genomic DNA as template

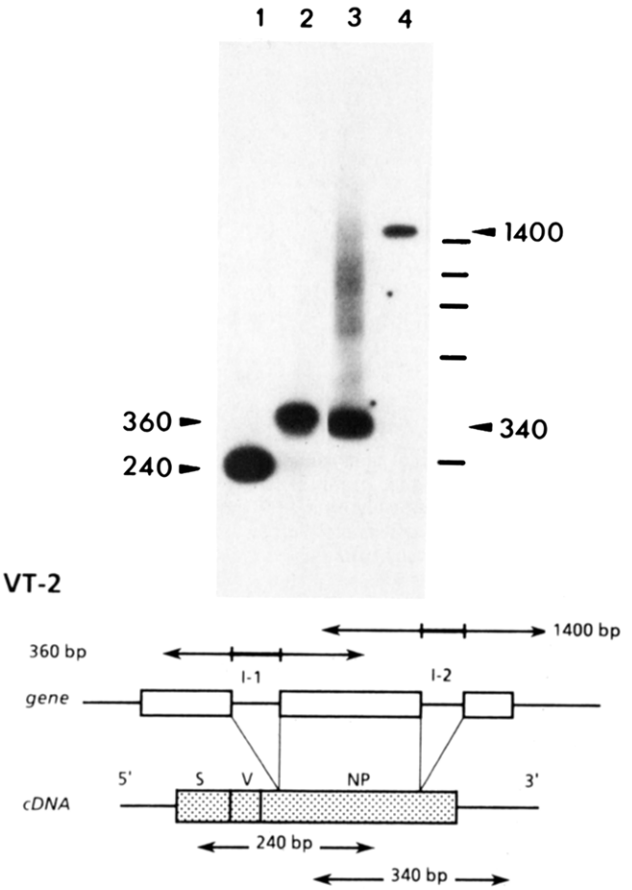

FIGURE 4: Location of the introns within the VT-2 gene. VT-2 cDNA (lanes 1 and 3) and liver genomic DNA from an individual sucker (lanes 2 and 4) were PCR amplified and analyzed as described in the legend to Figure 3 but using PCR amplification primer pairs and specific oligonucleotides deduced from the cDNA sequence of VT-2. "Forward–reverse" primer pairs were the following: first intron (lanes 1 and 2), nr 35–54 and 280–261; second intron (lanes 3 and 4), nr 180–197 and 517–497 [see Figure 2 for nucleotide residue (nr) positions]. Membrane-bound PCR amplification products were hybridized with a VT-2-specific ^{32}P-labeled oligonucleotide probe corresponding to nr 215–248 of the VT-2 cDNA (Figure 2). Size markers and depiction of the structural organization of the VT-2 gene are as described in the legend to Figure 3.

(Figure 4, lanes 2 and 4), suggesting that the VT-2-encoding gene also contains two introns in positions corresponding to those in mammalian vasopressin, one of 120 bp and the other of 1060 bp, a result that was confirmed by sequence analysis of the cloned PCR products.

Organization of cDNAs and Predicted Precursors for Vasotocin. Sequence analysis of the VT-2 precursor cDNA (Figure 2) confirmed that it exhibits a single open reading frame encoding a predicted vasotocin hormone moiety, followed by a putative neurophysin sequence similar to that in the previously described VT-1 counterpart in that it is longer at its C-terminus by a tract of about 30 amino acids as compared to the mammalian vasopressin-associated neurophysin (Heierhorst et al., 1989). Both neurophysin sequences do, however, contain a leucine-rich core segment resembling that found in the copeptin of the mammalian vasopressin precursor.

The degree of nucleic acid and amino acid sequence divergence between the predicted vasotocin precursors is quite remarkable (Figure 2). Thus, they share only 55% amino acid identity, although approximately half of the differences can be accounted for by conservative changes. Major areas of similarity are located in the region corresponding to the hormone moiety and "constant" central domain (precursor amino acid positions VT-1, 40–106; and VT-2, 42–109) of the

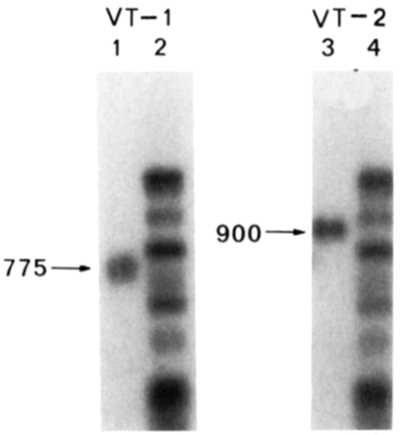

FIGURE 5: Northern blot analysis of vasotocin precursor-encoding poly(A+) RNA. Ten micrograms of sucker hypothalamic poly(A+) RNA (lanes 1 and 3) was glyoxylated, separated electrophoretically on a 1.2% (w/v) agarose gel, and blotted. The following ^{32}P-labeled cDNA fragments (for nucleotide positions, see Figure 2) were used: lane 1, VT-1 cDNA (nr 441–535); lane 3, VT-2 cDNA fragment (nr 556–704); markers (lanes 2 and 4) as shown in Figure 3 (Medac, Hamburg, FRG).

neurophysin. Closer inspection of the nucleotide and predicted amino acid sequences of VT-1 and VT-2 reveals several intriguing differences between the two precursors. Thus, while the longer cDNA length for VT-2 can, for the most part, be ascribed to an absence of a polyadenylation signal in a position corresponding to that in VT-1, the precursor length for VT-2 of 155 amino acid residues is itself 3 amino acids longer than that for VT-1. In comparison to VT-1, the VT-2 precursor contains an extra amino acid in each of the signal peptide, the first neurophysin "variable" domain, and the "constant" central domain (precursor amino acid positions 4, 38, and 97, respectively).

Comparison of sucker vasotocin-associated neurophysins with each other and with the known mammalian, avian, and amphibian counterparts reveals a low overall amino acid conservation, even within the central "constant" domain. Significantly, however, the 14 cysteine residues that play important structural and functional roles by forming a series of disulfide bridges between different parts of the polypeptide backbone (Burman et al., 1989) are conserved in every neurophysin analyzed to date, including all of the sucker neurophysins.

Expression of Vasotocin Precursor-Encoding mRNAs. Northern blot analysis of fish hypothalamic region poly(A+) RNA (Figure 5) using probes specific for each hormone transcript revealed that the mRNA for VT-1 predominates over that for VT-2. These results are consistent with the cDNA clone abundances: when the λgt11 library was rescreened by using the cDNA probes for VT, cDNA clones were obtained in the ratio of 70% VT-1 type to 30% VT-2 type (40 clones analyzed).

The Northern blot data show that VT-1 is encoded by a mRNA of 775 bases while that for VT-2 is longer at 900 bases (Figure 5). The distinct nature of these transcripts coupled with the disparity between the two VT cDNA sequences rules out the possibility that these differences can be explained by polymorphic variation within the population of fish used to prepare the mRNA. Rather, the data suggest that each of the VT transcripts is encoded by distinct genes, both of which are transcribed.

Discussion

The data presented here show that the hypothalamic neurosecretory system of the teleost fish *Catostomus commersoni* contains at least two distinct polyprotein precursors for the neuropeptide vasotocin. These are encoded by discrete genes, both of which are expressed.

The predicted vasotocin precursors display a similar overall organization to each other and to their mammalian counterpart, vasopressin (Schmale et al., 1983), with the exception that they lack a typical glycopeptide. This similarity of organization extends to the level of gene organization, insofar as both vasotocin genes contain two introns in positions exactly homologous to these found in rat vasopressin. Thus, the hormone and neurophysin moieties are encoded on distinct exons, although the latter is encoded jointly by the second and third exons. In comparison to their overall organizational similarities, the two vasotocin precursor cDNAs show a surprising degree of nucleotide sequence divergence. This results in a 45% difference in their predicted amino acid sequences, of which only approximately half can be accounted for by conservative amino acid changes. This is in marked contrast to the two predicted sucker isotocin precursors which are highly homologous, this amounting to nearly 90% similarity at the amino acid level (Figueroa et al., 1989).

A notable feature of the predicted vasotocin precursors is that the putative neurophysins are C-terminally extended by approximately 30 amino acids in comparison to their mammalian counterpart (Schmale et al., 1983). These extensions show some similarity to the sucker isotocin-associated neurophysins (Figueroa et al., 1989) and the mammalian vasopressin-associated copeptin in particular, in containing comparable leucine-rich core segments (Heierhorst et al., 1989), raising the possibility that the mammalian copeptin arose by the introduction of a functional cleavage signal into a larger ancestral neurophysin.

The presence of two genes for the vasotocin precursors in the genome of the teleost fish *Catostomus commersoni* is consistent with the tetraploid hypothesis for the evolution of the *Catostomids* (Uyeno & Smith, 1972). This asserts that the modern *Catostomids* have gained an additional evolutionary "fitness" by duplicating their entire chromosomal complement, thus producing two unlinked copies of every gene within the *Catostomid* genome. This is borne out, to some degree, by the extraordinarily wide environmental range displayed by this group of fish (Uyeno & Smith, 1972). However, such a hypothesis predicts that since all genes in the *Catostomid* genome should have been duplicated simultaneously, each pair of duplicates ought to have accumulated the same degree of genetic divergence in the intervening period. The approximate 10% amino acid difference between the coding regions of the two isotocin precursor genes (Figueroa et al., 1989) is consistent with a chromosomal duplication event occurring some 100 million years ago, assuming a 1% divergence in amino acid sequence per 10 million years (Nathans et al., 1986). In contrast, the 45% amino acid heterogeneity between the two vasotocin precursors is indicative of a much earlier gene duplication occurring perhaps 450 million years ago. Such antiquity leads one to speculate whether this particular gene duplication is unique to the teleosts or if it in fact preceded the separation of the lineages leading to fish and mammals. Assuming that two preexisting vasotocin genes underwent coordinate duplication with the ancestral isotocin gene approximately 100 million years ago, then one should expect to find two related "VT-1 type" and two "VT-2 type" precursor genes. Further, each pair of VT-1 and VT-2 genes should show the same degree of genetic variation as the two isotocin genes. Preliminary data indeed support this notion, suggesting that there are at least two genes for "VT-1 type"

precursors, with different sized introns and ca. 10% amino acid variation in the protein-coding regions.

ACKNOWLEDGMENTS

We thank Helen Wilson of Nanton (Alberta) for help in fish collection; Henk Zwiers, Evelyn and David Ko, Ilona Berbeckar, and Estrella Gonzalez (Calgary) for assistance with the collection of fish hypothalami and preparation of total RNA; Werner Rust, Sylke Engel-Haskiris, and Marion Däumigen-Kullmann (Hamburg) for technical assistance; and Dr. Wilhelm Kullmann (Hamburg) for synthesizing the oligonucleotides.

Registry No. DNA (*Catostomus commersoni* vasotocin VT-1 messenger RNA complementary), 125048-68-2; DNA (*Catostomus commersoni* vasotocin VT-2 messenger RNA complementary), 125048-69-3; vasotocin VT-1 (*Catostomus commersoni* precursor reduced), 125048-71-7; vasotocin VT-2 (*Catostomus commersoni* precursor reduced), 125048-72-8; isotocin (*Catostomus commersoni* reduced), 124920-92-9; vasotocin (*Catostomus commersoni* reduced), 17451-88-6; isotocin, 550-21-0; vasotocin, 9034-50-8.

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Stabilization of the Topoisomerase II–DNA Cleavage Complex by Antineoplastic Drugs: Inhibition of Enzyme-Mediated DNA Religation by 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide†

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Received September 29, 1989; Revised Manuscript Received November 9, 1989

ABSTRACT: In order to elucidate the mechanism by which the intercalative antineoplastic drug 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) stabilizes the covalent topoisomerase II–DNA cleavage complex, the effect of the drug on the DNA cleavage/religation reaction of the type II enzyme from *Drosophila melanogaster* was examined. At a concentration of 60 μM, *m*-AMSA enhanced topoisomerase II mediated double-stranded DNA breakage ~5-fold. Drug-induced stabilization of the enzyme–DNA cleavage complex was readily reversed by the addition of EDTA or salt. When a DNA religation assay was utilized, *m*-AMSA was found to inhibit the topoisomerase II mediated rejoining of cleaved DNA ~3.5-fold. This result is similar to that previously reported for the effects of etoposide on the activity of the *Drosophila* enzyme [Osheroff, N. (1989) *Biochemistry 28*, 6157–6160]. Thus, it appears that structurally disparate classes of topoisomerase II targeted antineoplastic drugs stabilize the enzyme's DNA cleavage complex primarily by interfering with the ability of topoisomerase II to religate DNA.

Topoisomerase II is a ubiquitous enzyme that is essential for the viability of eukaryotic cells (Vosberg, 1985; Wang, 1985; Osheroff, 1989a). Fundamental to the enzyme's physiological DNA strand passage reaction is its ability to create and religate double-stranded breaks in the nucleic acid backbone (Vosberg, 1985; Wang, 1985; Osheroff, 1989a).

In addition to its important cellular functions, topoisomerase

†This work was supported by Grant GM-33944 from the National Institutes of Health. M.J.R. was a trainee under Grant 5 T32 CA-09582 from the National Cancer Institute.

0006-2960/90/0429-2511$02.50/0