Rainbow trout (Oncorhynchus mykiss) possess two somatostatin mRNAs that are differentially expressed

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Moore, Craig A., Jeffrey D. Kittelson, Melissa M. Ehrman, and Mark A. Sheridan. Rainbow trout (Oncorhynchus mykiss) possess two somatostatin mRNAs that are differentially expressed. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1553–R1561, 1999.—Previously, we isolated a 624-bp cDNA encoding for a 115-amino acid preprosomatostatin containing [Tyr, Gly]-somatostatin (SS)-14 (now designated PPSS-II) obtained from the endocrine pancreas (Brockmann bodies) of rainbow trout. In this study we have characterized a second cDNA obtained from trout pancreas that is 600-bp in length and encodes for a 111-amino acid precursor containing [Tyr, Gly]-SS-14 (PPSS-II'). The nucleotide and amino acid identity between the two cDNAs is 82.3 and 80.5%, respectively. Both PPSS-II' and PPSS-II mRNA were present in esophagus, pyloric ceca, stomach, upper and lower intestine, and pancreas, whereas only SS-II' mRNA was present in brain. PPSS-II' mRNA was more abundant than PPSS-II mRNA in pancreas, whereas PPSS-II' mRNA was more abundant than PPSS-II mRNA in stomach. Fasting increased pancreatic PPSS-II' mRNA levels but had no effect on the levels of PPSS-II' mRNA. These results indicate the existence of two nonallelic pancreatic SS-II genes that are differentially expressed, both in terms of distribution among tissues and in terms of relative abundance within the tissues.

pancreatic somatostatin-II gene; preprosomatostatin-I; preprosomatostatin-II

SOMATOSTATIN (SS) was first isolated as a 14-amino acid peptide from ovine hypothalamus and found to inhibit the release of growth hormone from the pituitary gland (5). Since this initial discovery, SSs have been isolated from numerous tissues in a variety of chemical forms and found to possess a vast array of physiological roles, including neurmodulation, osmoregulation, and the coordination of growth, development, and metabolism (31). The different forms of SSs observed in mammals (e.g., SS-25, SS-28) are NH2-terminal extensions of SS-14 and result from differential processing of the same precursor, preprosomatostatin-I (PPSS-I) (7). A survey of vertebrates reveals the widespread distribution of PPSS-I as SS-14 has been isolated from representative ciclostomes, elasmobranchs, teleost fish, amphibians, reptiles, and birds (10). Teleost fish, in addition to expressing PPSS-I, also possess a second somatostatin precursor, PPSS-II, a molecule that contains [Tyr, Gly]-SS-14 at its COOH terminus. The amino acid sequences of PPSS-II products obtained directly from islet extracts are known for coho salmon (33), eel (9), goldfish (40), sculpin and flounder (8), and tilapia (28). Evidence that teleost PPSSs derive from different mRNAs was first reported in angelfish (16–18); two cDNAs were obtained from pancreatic islets of this species, one encoding for PPSS-I and the other encoding for PPSS-II. Despite the existence of multiple cDNAs encoding for PPSSs in angelfish (16–18) and catfish (21, 24), definitive information regarding the potential differential expression of somatostatin genes has not been reported.

In this study, we used rainbow trout to characterize further the polygenic origin of SS in vertebrates and to evaluate the expression of SS gene products. Rainbow trout are particularly well-suited for this investigation because of the organization of their pancreas, in which the endocrine component (Brockmann body) is anatomically separate from the exocrine component and because the Brockmann body contains comparatively large amounts of SS peptide isoforms localized in discrete cell populations (29).

MATERIALS AND METHODS

Animals. Juvenile rainbow trout, Oncorhynchus mykiss, were obtained from the Garrison National Fish Hatchery near Riverdale, ND. Fish were maintained at North Dakota State University in well-aerated, dechlorinated municipal freshwater (14°C) under 12:12-h light-dark photoperiod and fed to satiety twice daily with Supersweet Feeds (Glenco, MN) trout grower, except 24 h before experiments. In the nutritional state experiment, fish were either fed as usual or fasted for 2 wk before sample collection.

RNA extraction. Tissues were removed from rainbow trout of both sexes after the animals had been anesthetized with 0.01% (wt/vol) 3-aminobenzoic acid ethyl ester (MS-222, Sigma) buffered with 0.2% (wt/vol) sodium bicarbonate. Tissue samples (~25 mg) were placed in 2-ml microfuge tubes and immediately frozen on dry ice. Total RNA was extracted by a modification of the RNAzol method (Cinna/Biotecx Laboratories, Friendswood, TX) described previously (25). Total RNA was quantified by ultraviolet (UV) A260 spectrophotometry and diluted to 15 µg/ml. RNA samples were stored at –70°C until used.

Isolation and sequence analysis of PPSS cDNA. A two-phase rapid amplification of cDNA ends (RACE) PCR-based approach was used for the isolation and characterization of selected cDNA sequences as described previously (25). Briefly, in phase I (Fig. 1A), endogenous poly-A RNA was reverse transcribed from 15 µg of trout pancreatic total RNA with Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD) and a 37-nucleotide antisense adapter primer (GIBCO BRL). Five microliters of the reverse transcription reaction were used as a template for 3'-RACE PCR with an...
**Fig. 1.** Rapid amplification of cDNA ends (RACE) of preprosomatostatin-II’ (PPSS-II’) and PPSS-II’’ mRNA in pancreas and stomach was quantitated by slot-blot analysis (6), a technique similar to RNase protection assay in that it relies on reference to in vitro-synthesized RNA standards and has a sensitivity of ~10^6 molecules but lends itself more readily to the analysis of numerous samples. RNA standards were made by first cloning full-length SS-II’ and SS-II’’ cDNAs in the sense orientation into the PCR 2000 doning vector (Invitrogen). After linearization with Eco R I; Promega; or BamH I (Promega; for SS-II’’ inserts), in vitro RNA synthesis was performed using T7 RNA polymerase (40 units; Promega), according to the manufacturer’s protocol. Full-length cRNA was separated from unincorporated NTPs by ultracentrifugation (100,000 × g) for 7 h at 20°C. The cRNA preparations was assessed by electrophoresis on a 6% polyacrylamide/8.0 M urea gel and verified by sequence analysis. Northern analysis (19) was performed to evaluate the number and size of transcripts as well as to verify that the specific oligonucleotide probes hybridized only with SS-II’ and SS-II’’ transcripts in the total RNA extracted from the Brockmann bodies of trout. Four hundred-fifty microliter transcripts in the total RNA extracted from the Brockmann bodies of trout. Four hundred-fifty microliter transcripts in the total RNA extracted from the Brockmann bodies of trout.

21-base somatostatin gene-specific primer (GSP-1; 5’-GGCT-GCAAGAATTCTTTCTCG-3’) and the universal amplification primer (GIBCO BRL). After an initial denaturation cycle of 94°C for 2 min, 39 PCR cycles were performed, each consisting of 1 min denaturation (94°C), 1 min annealing (42°C), and 1 min extension (72°C). In the last cycle, the extension time was increased to 10 min to ensure complete extension. The resulting PCR product was identified by electrophoresis on an agarose gel containing 1% (wt/vol) agarose (GIBCO BRL) and 2% (wt/vol) NuSeive GTG agarose (FMC Bioproducts, Rockland, ME) in 1× Tris-borate-ethyleneglycol tetraacetic acid followed by ethidium bromide staining and UV transillumination. Amplified fragments were directly cloned into the TA cloning vector PCR 2000 (Invitrogen, San Diego, CA). Positive colonies were identified by agarose gel electrophoresis, as described above, of restriction enzyme digests (Eco R I; Promega, Madison, WI) of purified plasmid preparations (12). One to two micrograms of plasmid DNA was denatured and sequenced by the dideoxy chain-termination method (Sequenase Kit; US Biochemicals, Cleveland, OH) according to the manufacturer’s protocol. All sequences were confirmed by sequencing multiple colonies from at least three independent PCR reactions and with two or more different primers in both directions. In phase II (Fig. 1B), isolation of the 5’ cDNA sequence was accomplished by 5’-RACE PCR (Invitrogen). SS mRNA was exclusively reverse transcribed from pancreatic total RNA using a 20-base antisense oligonucleotide primer complementary to a region of the 3’ fragment isolated in phase I (GSP-2; 5’-GTTGGCCGTGACGTGATTG-3’). The resulting cDNA was purified twice over Glass Max spin columns (GIBCO BRL) to remove unincorporated dNTPs and primer and then “tailed” at the 3’ end with dCTP using terminal deoxynucleotidyl transferase (GIBCO BRL). Five microliters of the tailing reaction were used as a template for 5’-RACE PCR with GSP-2 and anchor primer (GIBCO BRL). Thirty-nine PCR cycles were performed as in 3’-RACE PCR, except Taq polymerase (Perkin-Elmer, Norwalk, CT) was pipetted beneath the layer of mineral oil after the initial 5 min denaturation cycle (26). The amplified product was identified by agarose gel electrophoresis, cloned, and sequenced as described above.

**Phase I**

- **mRNA**
  - Reverse Transcription
  - **cDNA**
    - PCR cycle 1
      - **PCR cycles 2-35**
        - **Tail 3’ end with dCTP**
      - **UAP**
    - **Anchor Primer**

**Phase II**

- **mRNA**
  - Reverse Transcription
  - **cDNA**
    - PCR cycle 1
      - **PCR cycles 2-35**
        - **Tail 3’ end with dCTP**
      - **UAP**
    - **Anchor Primer**
Differential expression of somatostatin genes

RAINSS1 and RAINSS2 mRNAs in various tissues because of its high specificity (amplification of false positives derived from contaminating genomic DNA is excluded) and high sensitivity (36). A dT30 primer (5'-CATGACCTGTACCACTGGCAG-3') containing 17 bases at its 3' end complementary to both SS-II' and SS-II''' (d30) and 30 bases of nonspecific tagging sequence at its 5' end (t30), was used to coreverse transcribe RAINSS-II and RAINSS-II''' mRNA in total RNA isolated from tissues. Five-microliter (15 µg) duplicate aliquots of total RNA were placed in 0.5-ml microfuge tubes and either stored at 4°C or incubated with 5 units of RNase-A (Sigma) for 30 min at 37°C. After RNase-A pretreatment, the remaining reaction components were added to both tube sets (20 µl total volume) so that the final composition was 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl2, 100 µg/ml BSA, 10 mM dithiothreitol, 0.5 µM primer, 2 mM dNTPs, and 5 units of AMV reverse transcriptase (Promega). The reactions were incubated at 37°C for 1 h and stored on ice until used as a template for PCR. Five microfilters of the reverse transcription reaction were used as a template for PCR in a final reaction containing 50 mM KCl, 10 mM Tris·HCl (pH 83 at 25°C), 1.5 mM MgCl2, 0.01 mg/ml gelatin, 200 µM of each dNTP, 0.5 µM upstream SS u30 primer (5'-ATTTGGCA GCCAAGGAGCCGCCTCGCAGCC-3'), 0.5 µM downstream t30 primer (identical to the t30 region of the dT30 primer; 5'-CATGACCTGTACCACTGGCAG-3'), and 0.04 units of Taq DNA polymerase (Perkin Elmer) overlaid with 50 µl of sterile mineral oil. To increase specificity, the annealing temperature was raised to 65°C, and 39 PCR cycles were performed as described previously.

The resulting RS-PCR products were subjected to Southern blot analysis. The amplified cDNAs were separated by agarose gel electrophoresis as described above, and the gel was blotted by capillary transfer to 0.45-µm nitrocellulose membrane (Schleicher and Schuell) overnight (34). The membrane was baked in a vacuum oven (80°C) for 2 h and prehybridized in hybridization solution [5×SSPE (200 mM NaH2PO4, 0.02 M EDTA-Na2), 0.2 M NaH2PO4, 0.02 M EDTA-Na2], 5×Denhardt’s solution (100× solution: 10 g polyvinylpyrrolidone, 10 g BSA, 10 g Ficoll 400, H2O to 500 ml), and 0.5% (vol/vol) SDS] containing 0.1 mg/ml denatured salmon sperm DNA for 2 h at 37°C. The prehybridization mixture was removed, and the membrane was hybridized at 37°C overnight in hybridization solution containing 35S-base SS-II cDNA radiolabeled (1 × 106 cpm/ml) probe. The blot was washed twice with 2×SSPE containing 0.2% (vol/vol) SDS for 20 min at 65°C, and autoradiography was performed (30 h exposure at -90°C using Fuji RX film).

To determine which of the two mRNA species (RAINSS-II' and RAINSS-II'''') were expressed within various tissues, RS-PCR products were subjected to slot-blot analysis. Briefly, 10 µl of RS-PCR product were boiled for 5 min in a 1.5-ml microfuge tube and then immediately placed on ice and diluted with 1,000 µl ice-cold 5× SSPE. Four-hundred-fifty microfilters were then slotted in duplicate directly to 0.2 µm Nytran membrane (Schleicher and Schuell) using a Minifold II slot-blot apparatus (Schleicher and Schuell) under weak vacuum. The wells were washed twice with 500 µl of 5× SSPE, and the membrane was allowed to air dry. The duplicate blots were baked, prehybridized, and hybridized with either SS-II'’-specific or SS-II''''-specific radiolabeled (1 × 106 cpm/ml) oligonucleotide probes. The blots were then washed and autoradiographed as described above.

Primers and probes: Oligonucleotides were either custom synthesized by National Biosciences (Plymouth, MN) or supplied with GibCO BRL 3' and 5'-RACE kits. Oligonucleotides used as probes were 5'-end labeled with γ-[32P]ATP (Amersham) using T4-poly nucleotide kinase (Promega) (34). The full-length SS-II cDNA probe was radiolabeled with α-[32P]CTP by random priming (Prime-a-Gene; Promega) according to the manufacturer’s protocol. All radiolabeled probes were purified over Elutip-D columns (Schleicher and Schuell) according to the manufacturer’s protocol.

Data analysis. Nucleotide and deduced amino acid sequences (coding regions only) were aligned and analyzed with the DOS-based Psi9ine DNA/protein analysis program (North Dakota State University, Department of Biochemistry) and OMEGA 1.0 for Windows 95/NT (Oxford Molecular Group, Campbell, CA). Quantitative data are expressed as means ± SE. The two-tailed Student’s t-test was used to estimate differences between treatment groups. A probability level of 0.05 was used to indicate significance. All statistics were performed using SigmaStat (Jandel Scientific, Palo Alto, CA).

RESULTS

Rainbow trout possess two cDNAs encoding PPSSs that contain [Tyr7,Gly10]-SS-14. An ~260-bp fragment was amplified by 5' RACE PCR from reverse-transcribed total RNA isolated from trout pancreas using an SS-specific upstream primer (GSP-1, Fig. 1). Sequence analysis of this 3' fragment (actual length = 243 bp) revealed six codons followed by a stop codon with 100% identity to the last six codons (+9 to +14) of a trout PPSS containing [Tyr7,Gly10]-SS-14 recently identified and reported by our laboratory (25); the remainder of the fragment consisted of 3' untranslated region, including a polyadenylated tail at the most 3' end. Reverse transcription and 5' RACE PCR with our previously designed GSP-2 primer resulted in the amplification of a 561-bp fragment identical in sequence to that which we reported previously (25).

Reverse transcription and 5' RACE PCR with a newly designed antisense primer unique to the new 3' fragment resulted in the amplification of a 544-bp fragment (Fig. 1B). Overlapping sequence of the 243-bp 3' RACE and 544-bp 5' RACE fragments identified a novel 600-bp cDNA encoding for a second PPSS containing [Tyr7,Gly10]-SS-14, which we have designated PPSS-II', with a single putative initiation site 101 bases downstream from the most 5' end and two putative polyadenylation signal sites. Exhaustive screening of 18–23 colonies from each of three independent 3' RACE and 5' RACE PCRs confirmed the existence of only two cDNAs, one encoding PPSS-II'' and one identical to our previously reported sequence (25) that encodes for the precursor we now designate PPSS-II'.

A comparison between PPSS-II' and our previously reported sequence (25) is shown in Fig. 2. Although PPSS-II' is a 115-amino acid protein containing numerous putative recognition sites for posttranslational modification by converting enzymes, potentially yielding a 28-amino acid SS peptide with [Tyr7,Gly10]-SS-14 at its COOH terminus, PPSS-II'' is a 111-amino acid protein potentially processed to a 25-amino acid SS...
peptide containing [Tyr\textsuperscript{7},Gly\textsuperscript{10}]-SS-14 at its COOH terminus. SS-II\textsuperscript{8} and SS-II\textsuperscript{88} share 82.3% nucleotide and 80.5% amino acid identity.

Despite the similarity of sequence between SS-II\textsuperscript{8} and SS-II\textsuperscript{88}, we took advantage of a 50-base region immediately upstream from the COOH termini of the SS coding regions to design three 20-base oligonucleotides that would specifically bind to SS-II\textsuperscript{8} mRNA, SS-II\textsuperscript{88} mRNA, or to both SS-II\textsuperscript{8} and SS-II\textsuperscript{88} mRNAs (the specificity of these probes was verified by hybridization to in vitro synthesized RNA; see Fig. 6A). Northern analysis using these probes revealed that there was a single transcript encoding PPSS-II\textsuperscript{8} and a single transcript encoding PPSS-II\textsuperscript{88} (Fig. 3).

Two PPSS-II mRNAs are differentially expressed in various tissues. RNA from various tissues was extracted and reverse transcribed. The resulting cDNAs encoding for PPSS-II\textsuperscript{8} and PPSS-II\textsuperscript{88} were coamplified by RS-PCR, electrophoresed on agarose, and subjected to Southern blot analysis using a full-length SS-II cDNA probe (which does not distinguish between SS-II\textsuperscript{8} and SS-II\textsuperscript{88}). With this approach, PPSS-II mRNA was detected in brain, esophagus, pyloric ceca, stomach, upper and lower intestine, and Brockmann bodies (Fig. 4). Duplicate samples pretreated with RNase demonstrated that amplified products were exclusively derived from RNA templates and not false positives derived from contaminating genomic DNA.

Abundance of PPSS-II mRNAs is different in various tissues. Hybridization of the gene-specific oligonucleotide probes to replicate slot-blots containing known quantities of in vitro-synthesized PPSS-II\textsuperscript{8} and PPSS-II\textsuperscript{88} cRNA standards, in the range of 6.5 × 10\textsuperscript{8} to 5.0 × 10\textsuperscript{9} molecules, and RNA extracted from selected tissues allowed for the accurate evaluation of the amounts of PPSS-II\textsuperscript{8} and PPSS-II\textsuperscript{88} mRNAs (Fig. 6). We used this approach to examine the expression of PPSS-II\textsuperscript{8} and of PPSS-II\textsuperscript{88} mRNAs in Brockmann bodies (endocrine pancreas) and stomachs removed from animals under...
normal (fed to satiety twice per day except 24 h before sampling) physiological conditions. Under these conditions, pancreatic SS-II mRNA levels were nearly threefold higher than those of SS-II, estimated to be $8.7 \times 10^8$ molecules/µg total RNA and $3.2 \times 10^8$ molecules/µg total RNA, respectively (Fig. 7A). The concentrations of PPSS-II mRNAs were lower in stomach than in pancreas. In addition, the relative abundance PPSS-II mRNA species in the stomach was opposite that in the pancreas, such that the levels of PPSS-II mRNA were ~10-fold higher than those of PPSS-II mRNA (Fig. 7B).

Abundance of PPSS-II mRNA is modulated by nutritional state. Nutritional state modulated the pattern of pancreatic PPSS-II mRNA expression. Fish that were fasted for 2 wk displayed levels of PPSS-II mRNA that were twofold higher than their continuously fed counterparts (Fig. 8). The levels of PPSS-II mRNA, however, were not affected by food deprivation.

**DISCUSSION**

In the present study, we characterized two cDNAs that encode PPSS containing [Tyr7, Gly10]-SS-14 at their COOH terminus (designated PPSS-II and PPSS-II”) and demonstrated that the two PPSS-II mRNAs are differentially expressed. This is the first report of the coexistence of two different PPSS-IIs. The nucleotide identity between the two cDNAs is 82.3%; the position and extent of the differences suggests the existence of two nonallelic PPSS-II genes. The two PPSS-IIs in rainbow trout are in addition to a single PPSS-I encoding SS-14, which also presumably arise from a separate gene (19).

The deduced PPSS-II and PPSS-II” proteins in rainbow trout Brockmann bodies contain 115 and 111 amino acids, respectively, both slightly shorter than the precursors of anglerfish (16–18) and goldfish (20), the only other known PPSS-IIs containing [Tyr7, Gly10]-SS-14. Rainbow trout PPSS-II” shared 43.5% amino acid identity with anglerfish PPSS-II and 51.3% amino acid identity with goldfish PPSS-II. The amino acid identity...
between rainbow trout PPSS-II’’ and anglerfish PPSS-II was 38.7%, whereas the identity between trout PPSS-II’’ and goldfish PPSS-II was 41.4%. Amino acid identities between rainbow trout PPSS-IIs and precursors derived from gene 1 were lower, between 37.9 and 22.5%. Rainbow trout PPSS-IIs were least similar to the PPSS, giving rise to catfish SS-22. Although the evidence is limited, it appears that evolutionary selection has acted to conserve the biologically active COOH-terminal domain of PPSSs (Fig. 9).

A comparison of nucleotide and predicted amino acid sequences between SS-II’ and SS-II’’ mRNAs. A: serial dilutions of in vitro-synthesized cRNA standards for SS-II’ and SS-II’’; B: total RNA from selected tissues (representative samples of pancreatic RNA are shown) were subjected to slot-blot analysis using 32P-labeled oligonucleotide probes. C: autoradiograms of blots in A were quantitated by scanning laser densitometry and after correction for background were plotted as a function of the number of SS molecules/µg total RNA.

A comparison of nucleotide and predicted amino acid sequences between SS-II’ and SS-II’’ of rainbow trout also helps to resolve questions surrounding the heterogeneity of the SS gene 2 family of peptides among teleosts. For example, 25-amino acid peptides with [Tyr7,Gly10]-SS-14 at their COOH terminus were isolated from eel (9) and coho salmon (33), whereas 28-amino acid peptides with [Tyr7,Gly10]-SS-14 have been isolated from anglerfish (18), flounder (8), goldfish (40), sculpin (8), and tilapia (28). The present findings in trout, in which PPSS-II’ possesses a putative Arg processing site that would give rise to a 25-amino acid peptide containing [Tyr7,Gly10]-SS-14 and in which PPSS-II’’ possesses a putative Arg processing site that would give rise to a 28-amino acid peptide containing [Tyr7,Gly10]-SS-14, suggest that the difference between the 28- and 25-amino acid forms results from a nine nucleotide deletion in the SS coding region.

SS emerged early during the course of evolution. Immunocytochemical evidence places SS in several invertebrate groups, including invertebrate chordates (32), arthropod insects (14), and gastropod mollusks (22). Among vertebrates, a multigenic origin of SS is supported by both cDNA and peptide sequence data. The widespread distribution of SS-14 among vertebrates, from lamprey to mammals (10), suggests strong conservation of the gene (SS gene 1) encoding it. This notion is supported by available cDNA information (2).

SS-II’’ and SS-II’ mRNAs. A: serial dilutions of in vitro-synthesized cRNA standards for SS-II’ and SS-II’’; B: total RNA from selected tissues (representative samples of pancreatic RNA are shown) were subjected to slot-blot analysis using 32P-labeled oligonucleotide probes. C: autoradiograms of blots in A were quantitated by scanning laser densitometry and after correction for background were plotted as a function of the number of SS molecules/µg total RNA.

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Structural data also point to the emergence of additional SS genes. Lamprey, for example, possess variant forms of SS (three successively longer peptides extended at their NH₂ terminus) in addition to SS-14 (1). A variety of peptide sequence data as well as limited cDNA information suggests that teleost fish also possess multiple SS genes. A majority of the reports suggest the existence of two genes: one encoding [Tyr⁷,Gly¹⁰]-SS-14 and one encoding SS-14 (8). Recent reports suggest that some teleosts possess more than two SS genes. For example, the present study shows that rainbow trout possess two distinct cDNAs that give rise to two different PPSSs containing [Tyr⁷,Gly¹⁰]-SS-14 at their COOH termini as well as a third cDNA encoding SS-14 (19). In addition, a recent report also showed that goldfish possess three distinct cDNAs: one encoding SS-14, a second encoding [Glu¹,Tyr⁷,Gly¹⁰]-SS-14, and a third encoding [Pro²]-SS-14 (20). The presence of multiple SS genes also extends to the tetrapods.

Fig. 8. Effects of nutritional state on pancreatic levels of PPSS-IIs. RNA extracted from tissues was subjected to slot-blot quantitation as shown in Fig. 6. Data are presented as means ± SE (n = 8); *significant difference compared with continuously fed group (P < 0.05).

Fig. 9. Comparison of amino acid sequences of PPSS-II⁺ and PPSS-II⁻ with those of PPSSs from 9 other species of vertebrates. Sequence identity was maximized by inserting gaps (dashed lines). Conserved amino acids are shaded. Amino acid sequences were deduced from cDNA sequences from H, human (35); M, monkey (39); B, bovine (37); R, rat (15); C, chicken (27); FR I and FR II, frog I and frog II (38); AF I, anglerfish I (18); AF II, anglerfish II (16–18); CI, catfish I (13); CF I, catfish II (14); GI I-III, goldfish I-III (20); TRI, trout I (19), and ‘TRI++’, trout II (25).
Frogs possess one cDNA that encodes for a PPSS containing [Pro\textsubscript{2},Met\textsubscript{13}]-SS-14 and a second cDNA that encodes a PPSS that contains SS-14 (38). In addition, mammalian cortistatin, a peptide sharing considerable identity to SS-14 that was isolated from the brain of rats (11) may be derived from an alternate SS gene form that emerged in early tetrapod evolution. Whether or not the various SS genes in vertebrates arose through several independent gene duplication events or through a single duplication event predating or concomitant with the appearance of Agnatha, as suggested by Conlon et al. (10), is not known.

Because SS-II\textsuperscript{1} and SS-II\textsuperscript{1′} of rainbow trout are more closely related to each other than to either SS-II or SS-I cDNAs, the duplication event leading to their emergence, probably the tetraploidyization event that appears common to salmonids (30), likely occurred after the duplication, giving rise to the two teleost SS genes, an event estimated to have occurred some 160 million years ago (37). Tetraploidy may also help to explain the presence of multiple SSs in goldfish (20).

The two PPSS-II mRNAs of rainbow trout are differentially expressed. This conclusion is based on several observations. First, the pattern of PPSS-II mRNA and PPSS-II\textsuperscript{1′} mRNA is tissue specific. For example, only PPSS-II\textsuperscript{1′} mRNA was detected in the brain of rainbow trout, whereas both PPSS-II and PPSS-II\textsuperscript{1′} mRNA were detected in pancreas and various regions of the gut. Brain-specific expression of the mRNA encoding the alternate form of SS in frogs (denoted PSS2) (38) and cortistatin (11) also has been reported. Previous immunocytochemical studies support a similar distribution of [Tyr\textsuperscript{7},Gly\textsuperscript{10}]-SS-14-containing peptides in the intestine (4) and stomach (3) of rainbow trout. Second, the abundance of PPSS-II mRNAs was different with specific tissues. Within the Brockmann body of rainbow trout, the predominant message form was that encoding for PPSS-II\textsuperscript{1′}, whereas in the stomach the predominant form was that encoding PPSS-II\textsuperscript{1}. Finally, the pattern of PPSS-II expression within the endocrine pancreas of rainbow trout was modulated by nutritional state. Together, these results suggest that rainbow trout produce two forms of gene 2 SS peptides and that there exist mechanisms to independently regulate the expression of each.

The alternate forms of SS (containing [Tyr\textsuperscript{7},Gly\textsuperscript{10}]-SS-14) in rainbow trout are in addition to SS-14 (19). The functions of the various SS peptides remain to be fully elucidated; however, previous research has suggested that distinctive roles for the gene 1 and gene 2 forms exist. For example, peptides derived from gene 1 (e.g., SS-14, SS-28) were equipotent in their ability to inhibit the release of growth hormone from goldfish pituitary fragments in vitro, whereas peptides derived from alternate genes (e.g., sSS-25, catfish SS-22) had no effect on growth hormone release (23). Similarly, salmon SS-25 (from gene 2) inhibited insulin in rainbow trout, but SS-14 (from gene 1) did not (13).

In summary, the present report describes the characterization of two cDNAs encoding for PPSSs that contain [Tyr\textsuperscript{7},Gly\textsuperscript{10}]-SS-14, consistent with the existence of two nonallelic SS genes and that the mRNAs for the two PPSSs (PPSS-II\textsuperscript{1} and PPSS-II\textsuperscript{1′}) are differentially expressed. These results support the notion of a polygenic origin of somatostatins and suggest the existence of mechanisms to control the differential expression of the multiple SS genes. The regulation of differential gene expression may underlie aspects of the multifunctional nature of the SS family of peptides. Future studies will be conducted to evaluate how differential expression of PPSS-II\textsuperscript{1} and PPSS-II\textsuperscript{1′} is regulated.

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