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Polygenic Expression of Somatostatin in Lamprey

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SOWER, S. A., Y.-C. CHIANG AND J. M. CONLON. *Polygenic expression of somatostatin in lamprey.* PEPTIDES **15**(1) 151–154, 1994.—Previous studies have led to the identification of three biosynthetically related molecular forms of somatostatin (somatostatin-14, -34 and -37) from the pancreas of the sea lamprey (*Petromyzon marinus*). We have now isolated from the brain of the same species a second form of somatostatin-14 that is identical to mammalian somatostatin-14 and differs from lamprey pancreatic somatostatin-14 by the substitution Ser^{12} to Thr. Larger forms of somatostatin were not identified in lamprey brain in this study. These data suggest that the two molecular forms of lamprey somatostatin-14 are the products of different genes that are expressed in a tissue-specific manner.

Somatostatin Agnathan Lamprey brain Posttranslational processing

THE neurohormone, somatostatin-14 (SS-14), was first isolated from the ovine hypothalamus and was shown to inhibit release of pituitary growth hormone (2). Subsequent studies have shown that somatostatin is widely distributed in vertebrate neuroendocrine tissues [reviewed in (3)]. Only one gene encoding the precursor of SS has been identified in the human (11) and rat (14) whose gene product is posttranslationally processed to SS-14 and SS-28 in a tissue-specific manner (4). The principal islets or Brockman bodies of the anglerfish, *Lophius americanus* (8), and other teleost fish [reviewed in (6)] express a second somatostatin gene encoding a biosynthetic precursor (preprosomatostatin-II) that contains the sequence of [Tyr^7Gly^{10}]SS-14 at its COOH-terminus. In contrast, preprosomatostatin-II is processed to SS-28 or related forms (SS-22 and SS-25) in all species of teleosts studied to date (3,6). More recently, isolation of SS-14 and [Pro^2,Met^{13}]SS-14 from the brain of the frog, *Rana ridibunda*, provides evidence of polygenic expression in amphibian (15).

Lampreys are one of only two extant representatives of the vertebrate class, the agnathans. Agnathans are of particular importance in understanding the evolution of neurohormones because they represent the oldest lineage of vertebrates that diverged over 550 million years ago. In a previous study, Andrews et al. (1) isolated from the pancreas of the sea lamprey, *Petromyzon marinus*, three molecular forms of somatostatin with 14, 34, and 37 amino acid residues that have arisen from alternative pathways of posttranslational processing of prosomatostatin-I. However, lamprey pancreatic SS-14 has Ser in the 12th position compared to Thr in mammalian SS-14. The original aim of the present study was to isolate novel forms of GnRH from lamprey brain using an antiserum raised against lamprey GnRH-I (12) to facilitate identification. Lamprey GnRH-I, lamprey GnRH-III, and SS-14 contain the sequence Trp-Lys in the central region of the peptides, with the result that a peptide with apparent GnRH-like immunoreactivity that was isolated in pure form from an extract of lamprey brain was in fact shown to be a second form of SS-14.

METHOD

Tissue Extraction

Whole brains from 4210 mature male and female sea lampreys (*Petromyzon marinus*) were collected in June and July, 1990, at Hammond Bay Biological Station (Millersburg, MI). Brains were rapidly removed and immediately frozen on dry ice and stored at −80°C until extracted as previously described (13).

Frozen brains were weighed (348 g) and divided into two batches for extraction. Each batch was boiled for 10 min in 2 *M* acetic acid (1 l) and homogenized using a Polytron blender. After centrifugation (18,000 × *g* for 60 min at 4°C), the supernatant (950 ml), containing 0.1% (by vol.) trifluoroacetic acid, was passed through 10 tandemly connected Sep-Pak C18 cartridges (Waters Associates, Milford, MA) at a flow rate of 2 ml/min. Bound material was eluted from the Sep-Paks with 80% (v/v) acetonitrile/water containing 0.1% trifluoroacetic acid. The volume of the effluent from the cartridges was reduced to 10 ml

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(Savant Speed-Vac, Farmingdale, NY) and this material was chromatographed on a 2.5 × 100-cm volume of Sephadex G-25 (Sigma, St. Louis, MO) equilibrated with 2 *M* acetic acid at a flow rate of 2 ml/min. Fractions (10 ml) were collected and the concentration of GnRH-like immunoreactivity was determined by radioimmunoassay at appropriate dilution.

Additional extracts, after partial purification on Sep-Pak cartridges, were chromatographed on a (90 × 5 cm) Biogel P-10 column (Bio-Rad, Richmond, CA) equilibrated with 1 *M* acetic acid at a flow rate of 72 ml/h. Fractions (12 ml) were collected and the presence of SS-like immunoreactivity was determined by radioimmunoassay at appropriate dilution.

The fractions containing maximum immunoreactivity (Fig. 1) were pooled and pumped at a flow rate of 2.0 ml/min onto a 1 × 10-cm Aquapore C8 column (Applied Biosystems, Foster, CA) equilibrated with 0.1% trifluoroacetic acid as previously described (13). The concentration of acetonitrile in the eluting solvent was raised to 21% (v/v) over 10 min, held at this concentration for 30 min, and raised to 50% (v/v) for another 60 min using linear gradients. Absorbance was measured at 214 nm and 2-ml fractions were collected. Fractions from the two extraction batches containing GnRH-like immunoreactivity were rechromatographed on a 1 × 25-cm Vydac 214 TP510 C4 column (Separations Group, Hesperia, CA) equilibrated with 0.1% trifluoroacetic acid at a flow rate of 1.5 ml/min, and individual peaks were collected by hand. The concentration of acetonitrile was raised to 35% (v/v) over 60 min using a linear gradient. Somatostatin was purified to apparent homogeneity by chromatography on a 1 × 25-cm Vydac 218TP510 (C18) column under the same conditions used for the C4 column.

Radioimmunoassay

The GnRH-like immunoreactivity was measured by radioimmunoassay using antiserum (1467) raised against lamprey GnRH-I as previously described (7,13). The antiserum shows 8 × 10^{-7}% reactivity with synthetic SS-14.

The SS-like immunoreactivity was measured using antiserum Go26, raised against SS-14 as described (5,6). The antiserum shows full reactivity with mammalian SS-28 but < 0.1% reactivity with anglerfish SS-28 (1).

Structural Analysis

Amino acid compositions were determined by precolumn derivatization with phenylisothiocyanate using an Applied Biosystems model 420A derivatizer, followed by separation of the phenylthiocarbamyl amino acids by reversed-phase HPLC (13). Hydrolysis in 5.7 *M* hydrochloric acid (24 h at 110°C) of approximately 500 pmol of peptide was performed. Tryptophan and cysteine residues were not determined. The primary structure of somatostatin was determined by automated Edman degradation of approximately 500 pmol peptide using an Applied Biosystems model 471A sequenator. The detection limit for phenylthiohydantoin amino acids was 0.5 pmol.

RESULTS

The initial extract of lamprey brain contained GnRH-like immunoreactivity equivalent to 280 pmol/g of GnRH-I/g wet tissue weight. As previously described (13), the immunoreactivity in the extract, after partial purification on Sep-Pak cartridges, was eluted from a Sepahdex G-25 gel permeation column as three incompletely resolved peaks. Maximum immunoreactivity was detected at the elution volume of synthetic lamprey GnRH-I and the fractions with K_{av} between 0.32 and 0.71 were pooled

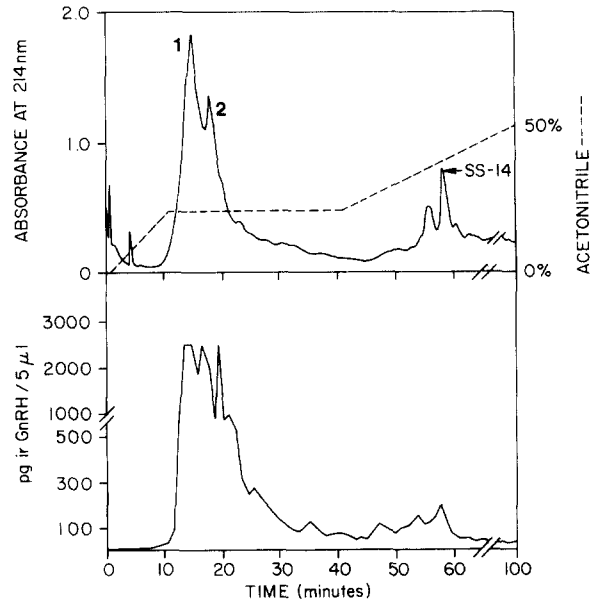

FIG. 1. Elution profile on a reverse-phase Aquapore C8 column of an extract of lamprey brain after partial purification by gel permeation chromatography. Fractions were assayed for GnRH-like immunoreactivity with an antiserum raised against lamprey GnRH-I that cross-reacts with SS-14. Peak I contains lamprey GnRH-III, peak 2 contains lamprey GnRH-I, and peak 3 contains SS-14. Absorbance at 214 nm (upper graph).

and subjected to reversed-phase HPLC on a C8 column (Fig. 1). The early eluting fractions with GnRH-like immunoreactivity, denoted peak 1 and peak 2, were shown to contain lamprey GnRH-I and GnRH-III. The immunoreactive peak denoted peak 3 was rechromatographed on Vydac C4 column (Fig. 2). The apparent GnRH-like immunoreactivity was eluted between 59 and 62 min and purified to apparent homogeneity on a Vydac C18 column, and the final yield of pure peptide was approximately 11 nmol.

Peak 3 was determined to be somatostatin-14 as determinined by amino acid and sequence analysis. The amino acid composition of lamprey brain SS-14 (found: Asx 0.8, Ser 0.7, Gly 1.1, Thr 2.1, Ala 1.1, Phe 3.2, Lys 2.0 residues/mol peptide) indicated that the component was probably identical to mammalian SS-14. Cysteine was present in the peptide but not quantitated. The sequence of the peptide was established by automated Edman degradation as Ala (3701)-Gly (3524)-Xaa-Lys (2298)-Asn (1168)-Phe (2370) -Phe (2363) -Trp (345) -Lys (1547) -Thr (656)-Phe (1168)-Thr (564) -Ser (66)-Xaa, which is the same as mammalian SS-14. The values in parentheses show the yields of phenylthiohydantoin amino acids in picomoles. No amino acid residues were detected during cycles 3 and 14, consistent with the presence of a cystine bridge in the peptide. A mixture of lamprey brain SS-14 and synthetic SS-14 coeluted from a Vydac C18 column as a single sharp peak (data not shown).

After it was demonstrated that peak 3 GnRH was SS-14, the original extract of lamprey brain was chromatographed on a Biogel P-10 column and fractions were analyzed for SS-like immunoreactivity using an antiserum directed against the central region of SS-14 that will detect larger molecular mass forms in extracts of mammalian tissues. As shown in Fig. 3, the SS-like immunoreactivity was eluted as a single peak with the same elution volume as synthetic SS-14.

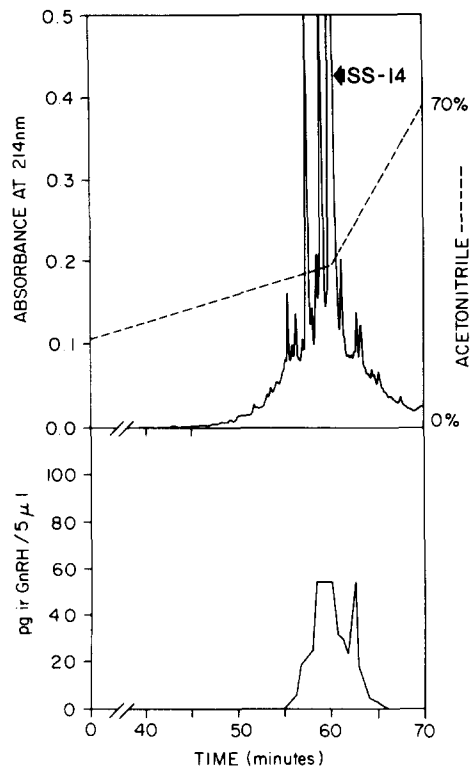

FIG. 2. Peak 3 fractions from C8 column were then eluted on a C4 column. Maximum immunoreactivity was detected between 59 and 62 min. Absorbance at 214 nm (upper graph).

DISCUSSION

The data in the present study support the hypothesis that the complete primary structure of SS-14 has been very highly conserved during vertebrate evolution (3). The amino acid sequence of the peptide is the same in species from all classes of vertebrates that have been studied to date with the exception of the holocephalan fish, *Hydrolagus colliei* (Pacific ratfish) (4). In this fish, [Ser^5]SS-14 was isolated from the pancreas. A previous study resulted in the isolation of another molecular variant of SS-14 ([Ser^{12}]SS-14) from lamprey pancreas together with its *N*-terminally extended forms SS-34 and SS-37 (1). The isolation of SS-14 from lamprey brain, which is identical in amino acid sequence to the peptide from other vertebrates, provides good evidence for the expression of more than one somatostatin gene in an agnathan. Somatostatin-14 was not detected in the lamprey pancreas, suggesting that expression of the different somatostatin genes may be tissue specific. The antiserum to SS-14 used in this study does not detect peptides with [Tyr^7,Gly^{10}]SS-14 at their COOH-termini, leaving the question open to whether the prosomatostatin-II gene is expressed in Agnatha. At the present time, however, there is no evidence for the expression of the preprosomatostatin II gene in species other than teleost fish.

In mammalian tissues, prosomatostatin-I is cleaved either at the Arg^{77}-Lys^{78} dibasic processing site to generate SS-14 or at the Arg^{64} monobasic site to generate SS-28 (10). Both pathways operate in brain tissue. In the pancreas of the agnathans, the sea lamprey (1) and the Atlantic hagfish, *Myxine glutinosa* (5), prosomatostatin is processed primarily to SS-34 by cleavage at a single arginyl residue only. In contrast, SS-14 was the only component with SS-like immunoreactivity detected in the extract of lamprey brain, suggesting that prosomatostatin-I was processed exclusively at the dibasic residue site.

Earlier immunocytochemical studies of lamprey brain revealed that somatostatin perikarya were present in various regions including the ventral hypothalamus, dorsal thalamus, interpeduncular nucleus, and gray area (9). In addition, somatostatin fibers were also found in nearly every part of the brain with particular abundance in the preoptic nucleus and ventral hypothalamus. However, in these same studies, there were no somatostatin fibers in the neurohypophysis. This information would suggest that somatostatin may not act as a neurohormone involved in the regulation of pituitary function, but rather as a brain neurotransmitter and/or neuromodulator.

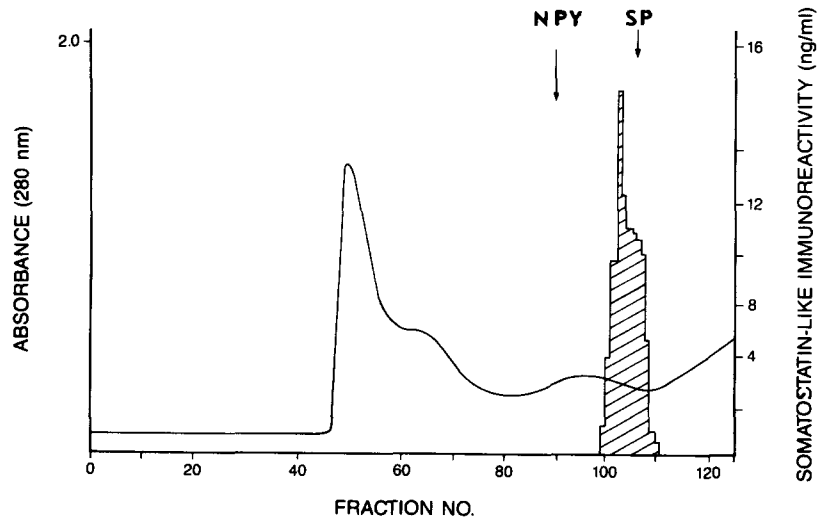

FIG. 3. Elution profile on a Biogel P-10 gel permeation column of an extract of lamprey brain, after partial purification on Sep-Pak cartridges. Somatostatin-like immunoreactivity was measured with an antiserum directed against the central region of somatostatin-14. The arrows show the retention times of porcine NPY (NPY) and substance P (SP).

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REFERENCES

1. Andrews, P. C.; Pollock , H. G.; Elliott, W. M.; Youson, J. H.; Plisetskaya, E. M. Isolation and characterization of a variant somatostatin-14 and two related somatostatins of 34 and 37 residues from lamprey (*Petromyzon marinus*). J. Biol. Chem. 263:15809–15814; 1988.
2. Brazeau, P.; Vale, W.; Burgus, R.; et al. A hypothalamic peptide that inhibits the secretion of pituitary growth hormone. Science 179: 77–79; 1973.
3. Conlon, J. M. Somatostatin: Aspects of molecular evolution. In: Epple, A.; Scanes, C. G.; Stetson, M. H., eds. Progress in comparative endocrinology, vol. 342. New York: Wiley–Liss; 1990:10–15.
4. Conlon, J. M. [Ser^5]somatostatin: Isolation from the pancreas of a holocephalan fish, *Hydrolagus colliei*. Gen. Comp. Endocrinol. 80: 314–320; 1990.
5. Conlon, J. M.; Askensten, U.; Falkmer, S.; Thim, L. Primary structures of somatostatins from the islet organ of the hagfish suggests an anomalous pathway of posttranslational processing of prosomatostatin-1. Endocrinology 122:1855–1859; 1988.
6. Conlon, J. M.; Deacon, C. F.; Hazon, N.; Henderson, I. W.; Thim, L.Somatostatin-related and glucagon-related peptides with unusual structural features from the European eel (*Anguilla anguilla*) Gen. Comp. Endocrinol. 72:181–189; 1988.
7. Fahien, C. M.; Sower, S. A. Relationship between brain gonadotropin-releasing hormone and final reproductive period of the adult male sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. 80:427–437; 1990.
8. Hobart, P.; Crawford, R.; Shen, L. P.; Pictet, R.; Rutter, W. J. Cloning and sequence analysis of cDNAs encoding two distinct somatostatin precursors found in the endocrine pancreas of anglerfish. Nature 288:137–141; 1980.
9. Nozaki, M.; Tsutakara, T.; Kobayashi, H. An immunocytochemical study on the distribution of neuropeptides in the brain of certain species of fish. Biomed. Res. Suppl. 36:135–184; 1984.
10. Patel, Y. C.; Reichlin, S. Somatostatin in hypothalamus, extra-hypothalamic brain and peripheral tissues. Endocrinology 102:523–528; 1978.
11. Shen, L. P.; Rutter, W. J. Sequence of the human somatostatin-1 gene. Science 224:168–141; 1984.
12. Sherwood, N. M.; Sower, S. A.; Marshak, D. R.; Fraser, B. A.; Brownstein, M. J. Primary structure of gonadotropin-releasing hormone from lamprey brain. J. Biol. Chem. 261:4812–4819; 1986.
13. Sower, S. A.; Chiang, Y. C.; Lovas, S.; Conlon, J. M. Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. Endocrinology 132:1125–1131; 1993.
14. Tavianini, M. A.; Hayes, T. E.; Magazin, M. D.; Minth, C. D.; Dixon, J. E. Isolation, characterization, and DNA sequence of the rat somatostatin gene. J. Biol. Chem. 259:11798–11803; 1984.
15. Vaudry, H.; Chartrel, N.; Conlon, J .M. Isolation of [Pro^2,Met^{13}]somatostatin-14 and somatostatin-14 from the frog brain reveals the existence of a somatostatin gene family in a tetrapod. Biochem. Biophys. Res. Commun. 188:477–482; 1992.