Proc. Natl. Acad. Sci. USA
Vol. 78, No. 8, pp. 5221–5224, August 1981
Neurobiology

Identification, characterization, and distribution of secretin immunoreactivity in rat and pig brain

(neuropeptide/peptide/radioimmunoassay/high-pressure liquid chromatography)

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Communicated by Louis B. Flexner, April 17, 1981

ABSTRACT **Secretin immunoreactivity was detected in the central nervous system of the rat and pig with a highly specific radioimmunoassay. The secretin immunoreactivity in the rat and pig brain and duodenum extracts was fractionated by using a reverse-phase high-pressure liquid chromatographic system. The immunoreactive secretin from pig brain and duodenum coeluted precisely with synthetic porcine secretin. However, immunoreactive secretin extracted from rat brain and duodenum eluted slightly before porcine secretin. These data suggest a slight difference in the structure of rat and pig secretin. The detection of secretin in the brain lays the groundwork for studies to determine the role of the peptide in central nervous system function.**

In 1902, Bayliss and Starling identified a compound in acid extracts of the duodenum that caused pancreatic secretion (1). They named this compound secretin and suggested that it belonged to a class of chemical messengers which they called hormones. The structure of the first hormone was elucidated by Jorpes and Mutt (2). Secretin was shown to be a basic peptide containing 27 amino acids and an amide terminus. The peptide has numerous pharmacological actions, but its one defined physiological role is clearly endocrine in nature (cf. refs. 3–6). The stimulus for secretin release is duodenal acidification. The target organ of the hormone is the pancreas, and the response is pancreatic secretion of bicarbonate and water. Thus, secretin belongs to a rapidly growing class of endocrine and paraendocrine peptides.

In recent years, it has become evident that most peptides once thought to be localized solely in the brain or in the gastrointestinal tract actually serve neurotransmitter or hormonal roles in both regions. Secretin is one peptide that has generally been thought to be an exception to this trend, as it was thought to be located only in the gastrointestinal system (cf. ref. 7). This seemed particularly surprising because the existence of central neuronal systems containing the structurally related peptides glucagon and vasoactive intestinal peptide had been described (8–13). In the light of the similarities in structure and, to some extent, function among the members of the secretin/glucagon/VIP family of peptides, it seemed inconsistent that there would be such a drastic difference in localization of these peptides. For this reason, the existence of immunoreactive secretin in the brain was investigated.

The recent demonstration of secretin bioactivity in the brain (14) indicated that secretin, like the other members in its family of peptides, might, in fact, be contained in the brain. The purpose of this study was (*i*) to investigate the presence of immunoreactive secretin in rat and pig brain, (*ii*) to characterize the immunoreactive peptide in brain, and (*iii*) to determine the distribution of immunoreactive secretin in rat brain.

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METHODS

Regional Distribution. Sprague–Dawley male rats weighing 250–325 g (Zivic–Miller, Allison Park, PA) were housed six per cage in a colony room with a 12:12 light/dark 24-hr cycle and were allowed free access to food and water. Seven rats were decapitated; the brains and intestines were removed and dissected over ice. Brain regions were frozen on dry ice and weighed. The proximal 5–6 cm of duodenum was collected and cleaned. Tissue samples were placed in 5–10 vol of 0.5 M acetic acid and boiled for 15 min. Samples were homogenized by sonication or by use of a Polytron homogenizer (Brinkmann). Variously sized aliquots of the supernatant of an 8000 × *g* centrifugation at 4°C were removed, concentrated in a vacuum centrifuge (Savant, Hicksville, NY), and analyzed by radioimmunoassay (RIA).

Porcine brain and cleaned intestine were obtained within 30 min after slaughter and frozen on dry ice. Samples were then boiled in 0.5 M acetic acid and homogenized with a Polytron homogenizer. Tissue was centrifuged as described for the rat, and samples of supernatant were removed, concentrated, and analyzed by RIA.

Characterization. Immunoreactive secretin from rat and pig brain and duodenum was characterized by reverse-phase high-pressure liquid chromatography. Tissue samples were processed as described for the regional distribution studies. Supernatants were concentrated in a vacuum centrifuge, resuspended in triethylammonium phosphate buffer, filtered by a Millipore thick prefilter in series with an HA filter to remove particulate matter and were chromatographed. Alternatively, supernatants were injected through a C_{18} Sep Pak cartridge (Waters Associates). The effluent was recycled through a second Sep Pak cartridge to maximize recovery of secretin. The Sep Pak cartridges were rinsed with 6 ml of water to remove salts, ions, amino acids, small peptides, and other hydrophylic compounds. Secretin and other relatively hydrophobic compounds were eluted from the Sep Pak cartridges with a solution of 0.2 M HCl/absolute ethanol, 1:1 (vol/vol). The acid/ethanol eluate was concentrated in a vacuum centrifuge, resuspended in triethylammonium phosphate, filtered to remove particulates, and chromatographed. To some tissue homogenates, 10 μg of synthetic porcine secretin (Peninsula Laboratories, San Carlos, CA) was added to determine possible effects of the extraction procedure or effects of chromatographing large amounts of tissue homogenate on the elution of secretin.

Abbreviation: RIA, radioimmunoassay.

The high-pressure liquid chromatographic system consisted of a Waters Associates model 204 liquid chromatograph equipped with a model UK6 injector, two model 6000A pumps, a model 660 programmer, a model 440 absorbance detector, a Schoeffel variable wavelength detector, an Omni Scribe recorder (Houston Instruments, Houston, TX), and a 4 mm × 30 cm column of μBondapak/C_{18} (Waters Associates). A mobile phase consisting of acetonitrile and triethylammonium phosphate buffer was used (15). Triethylammonium phosphate buffer was prepared each week with 4 liters of 0.083 M phosphoric acid in water purified by a Millipore reverse-osmosis system in series with a Millipore Milli-Q ion-exchange/charcoal-filtration system, (Millipore). The pH of the buffer was then adjusted to 3.0–3.5 with triethylamine. The triethylamine was purified periodically by distilling over 1% *p*-toluenesulfonyl chloride or 2% (wt/vol) naphthyl isothiocyanate. Distillation was performed slowly, and only the middle 80% was collected; the first and last 10% of the distillate were discarded. On the day of use, both acetonitrile (Waters Associates) and the triethylammonium phosphate buffer were filtered with Millipore FA and HA filters, respectively. The solvents were then degassed by a combination of sonication and reduced pressure.

Extracts or synthetic porcine secretin were injected onto the chromatograph. A 50-min linear gradient of 27–42% (vol/vol) acetonitrile in triethylammonium phosphate was used to elute secretin from the column. One-minute fractions were collected from the column, and aliquots were concentrated in a vacuum centrifuge and measured for secretin immunoreactivity.

RIA. A commercially available secretin antiserum generated in a rabbit toward secretin conjugated to bovine serum albumin (Calbiochem–Behring no. 720094) was used for the RIA. Secretin was iodinated by the chloramine-T method (16) as described (17) and was purified by QAE-Sephadex column chromatography. The secretin RIA method described by Burhol and Waldum (18) was adapted and used. All steps of the RIA were at 4°C. In brief, on the first day of the assay, concentrated samples in duplicate (and often at different dilutions) or standards in triplicate from 1.5 pg–2000 pg were resuspended in the RIA buffer (100 mM sodium acetate, pH 4.0/3.0 mM sodium azide/10 mM EDTA/0.5% egg albumin) to a final volume of 400 μl. Antiserum diluted 1:4000 was added in 50 μl of the sodium acetate buffer (final incubation dilution of 1:40,000 before immunoprecipitation). On the second day of the assay, about 6000 cpm of iodinated secretin (approximately 200 μCi/μg; 1 Ci = 3.7×10^{10} becquerels) were added in 50 μl of the sodium acetate buffer. Prior to addition, iodinated secretin was purified on a Sep Pak cartridge, concentrated, and resuspended in buffer. On day 4, 200 μl of 5–10% sheep anti-rabbit IgG antiserum and 200 μl of 2% (vol/vol) normal rabbit serum, both diluted in phosphate-buffer saline (pH 7.4), were added to immunoprecipitate the rabbit anti-secretin complexes. On day 5, tubes were centrifuged at 5000 × *g* at 4°C, and the supernatants were aspirated and discarded. Bound secretin was counted in a Micromedic γ counter, and picogram equivalents were automatically calculated on a log/logit program. For the distribution studies, samples from all regions were analyzed in the same RIA.

RESULTS

The secretin antiserum used in this study seemed highly specific and was of a reasonably high titer. The antisera did not recognize either glucagon (Eli Lilly) or vasoactive intestinal peptide (Peninsula Laboratories), structurally related peptides, or the nonsulfated form of cholecytokinin (Peninsula Laboratories) in amounts up to 10 μg (less than 0.01% displacement of ^{125}I-labeled secretin). The sensitivity of the assay was 10 pg. Intraassay coefficient of variation was less than 5%.

The radioimmunoassay detected only one immunoreactive peptide in extracts of rat and pig brain and duodenum, which coeluted from the C_{18} column similarly to synthetic porcine secretin (Figs. 1 and 2). The retention time of synthetic secretin was unaffected by the extraction procedure or column loading as performed in this study. However, closer examination of Fig. 2 shows that the peak of rat immunoreactive secretin consistently eluted from the C_{18} column approximately 1–2 min before synthetic porcine secretin. In contrast, immunoreactive material from pig brain and duodenum coeluted precisely with synthetic porcine secretin. In addition to this form of secretin, preliminary results showed a high molecular weight form of secretin in acid extracts of brain.

Secretin had a widespread distribution of immunoreactivity in the rat brain with an approximately 6-fold difference in concentration between the highest concentrations in the thalamus

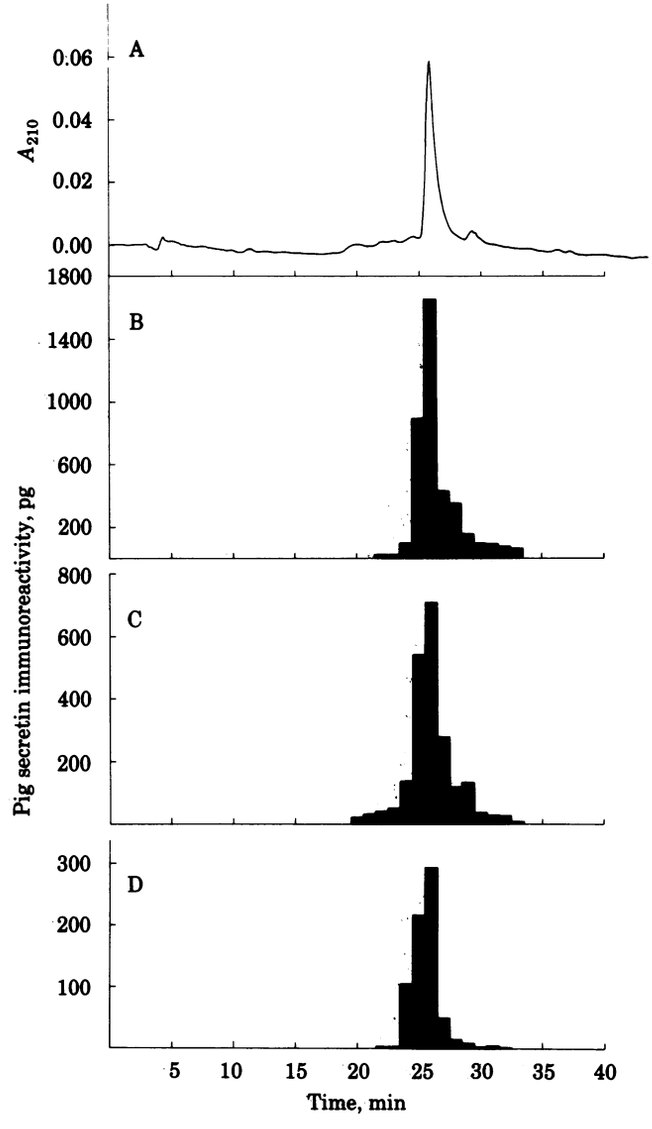

FIG. 1. High-pressure liquid chromatograms of synthetic pig secretin and immunoreactivity in pig brain and duodenum extracts: the retention time of 1.0 μg of synthetic pig secretin at 210 nm (*A*); secretin immunoreactivity (pg) shown by synthetic pig secretin (*B*), pig duodenum extract (*C*), and pig brain extract (*D*). The delay time between detector and fraction collector is corrected for on these chromatograms.

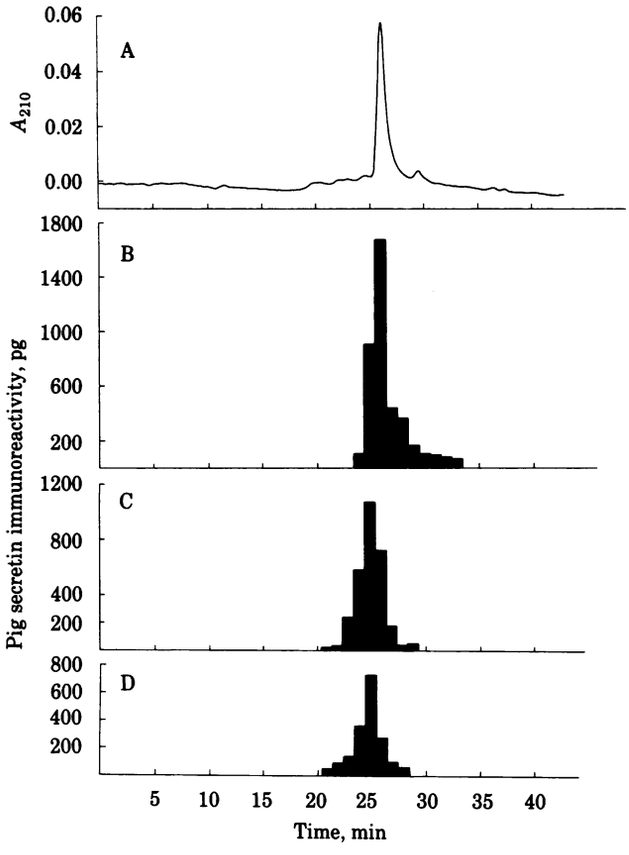

FIG. 2. High-pressure liquid chromatograms of synthetic pig secretin (*A*) and immunoreactivity from rat brain (*D*) and duodenum (*C*) extracts, expressed in picogram equivalents to porcine synthetic secretin (*B*).

and hypothalamus and lowest concentrations in the cerebral cortex (Table 1).

The olfactory bulb also had high secretin concentrations. Moderate concentrations were measured in the striatum, septum, hippocampus, midbrain, and cerebellum, although the cerebral cortex contained the highest overall amount of immunoreactive secretin in the brain. Lowest concentrations were measured in the cerebral cortex and the medulla and pons.

Secretin concentrations were particularly high in the pituitary and pineal gland. In fact, concentrations of immunoreactive secretin in these regions were higher than any brain region and were approximately 17 times higher than the concentration in cerebral cortex. The content of immunoreactive secretin in the duodenum was approximately 10 times higher than that found in the highest brain region. When assayed at various dilutions, brain and duodenum samples showed parallel displacement of ^{125}I-labeled secretin with synthetic secretin.

DISCUSSION

The results of this study describe the occurrence of immunoreactive secretin in rat and pig brain and confirm the findings of Mutt *et al.* (14), who have demonstrated a secretin bioactive material in the porcine brain. Characterization studies show that immunoreactive secretin from pig brain and duodenum coelutes from the high-pressure liquid chromatography precisely with synthetic porcine secretin. Immunoreactive secretin from rat brain and duodenum has a slightly different elution time and, therefore, may be slightly different in structure than porcine secretin. It has been reported that although bovine secretin is probably identical in structure to porcine secretin, chicken secretin has about 50% sequence dissimilarity to pig peptide (19). Therefore, it might be possible that a minor structural alteration in rat secretin might change its retention time in high-pressure liquid chromatography without eliminating its immunoreactivity. It is possible, however, that a minor structural change in rat secretin might slightly alter rat secretin immunoreactive potency and change the apparent concentration as measured by RIA and alter its chromatographic characteristics. The relative concentrations of secretin between regions within a species would not be affected by such a structural alteration.

Table 1. Distribution of secretin in the rat brain and duodenum*

Region	Secretin, pg per mg wet weight	Secretin, pg per region
Forebrain		
Olfactory bulb	33.4 ± 2.3	1,812.6 ± 110.6
Cerebral cortex	7.4 ± 0.4	6,147.9 ± 276.10
Striatum	15.5 ± 1.6	2,193.2 ± 249.2
Septum	16.9 ± 1.6	2,597.8 ± 238.9
Hippocampus	15.4 ± 2.6	2,115.7 ± 322.1
Thalamus	45.0 ± 4.1	1,633.4 ± 99.0
Hypothalamus	39.0 ± 8.0	1,996.7 ± 361.0
Midbrain	17.94 ± 2.1	2,578.8 ± 220.7
Hindbrain		
Medulla and pons	9.8 ± 1.1	2,334.2 ± 267.0
Cerebellum	18.5 ± 1.0	5,335.0 ± 339.0
Pineal gland	129.8 ± 12.8	310.3 ± 49.0
Pituitary gland	127.2 ± 10.5	1,570.4 ± 120.4
Duodenum (proximal 5 cm)	44.6 ± 4.2	56,746.0 ± 6621.0

* Number of rats, seven.

Secretin immunoreactivity in the brain could be synthesized either in neurons within the brain or in endocrine cells of the gastrointestinal tract from where it is circulated to the brain and is present primarily in blood vessels. A number of recent findings indicate the former possibility. First, neurons in rat brain containing immunoreactive secretin have been identified immunocytochemically. Second, neither perfusion of the rat brain with saline prior to sacrifice nor duodenumectomy for 5 days prior to sacrifice affects the concentration of secretin in the brain.

The widespread localization of secretin in the rat brain may indicate that this peptide may be involved in diverse physiological functions. Brain receptors for secretin have been inferred from evidence demonstrating cyclic AMP accumulation after incubation of secretin with cultured brain cells or a neuroblastoma–glioma cell line (20, 21). High concentrations of secretin in the hypothalamus and pituitary and pineal glands may point to a role in neuroendocrine function. Consistently, Fuxe *et al.* (22) have found that central injections of secretin increase dopamine turnover in the median eminence and reduce prolactin secretin. Interestingly, the secretin injections decreased dopamine turnover in the nucleus accumbens and olfactory tubercle. The knowledge of the localization of secretin throughout the brain lays the groundwork for further physiological studies on its role in central nervous system function.

We acknowledge Calbiochem–Behring for providing the antisera and, in particular, S. Khoury and G. Kritchevsky of Calbiochem–Behring for helpful discussion and assistance. The authors also thank Mrs. Dorothy Allison for typing this manuscript. T.L.O. is a Research Associate in the Pharmacology–Toxicology Program of the National Institute of General Medical Sciences, National Institutes of Health. R.L.M. is a Burroughs Wellcome Scholar.

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