Isolation of a Sialogogic Peptide from Bovine Hypothalamic Tissue and Its Characterization as Substance P*

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SUMMARY

A sialogogic peptide has been isolated from bovine hypothalami by gel filtration, ion exchange chromatography, and high voltage paper electrophoresis. The purity of this peptide was established by showing that the molar ratios of the constituent amino acids remained constant after high voltage paper electrophoresis at pH 1.9, 3.5, and 6.5. Its molecular weight (estimated by gel filtration) and its amino acid composition show that the peptide is an undecapeptide composed of Lys, Arg, Glx, Pro, Gly, Leu, Met, and Phe. The NH₂-terminal residue is arginine. Further biological testing showed that the sialogogic peptide isolated is indistinguishable from Substance P; i.e. it stimulates contraction of the guinea pig ileum and the rat duodenum and is a potent vasodepressor. These effects are not inhibited by prior treatment of the test preparation with atropine. This report is believed to present the first complete purification of Substance P.

In 1931 von Euler and Gaddum (1) showed that extracts of various equine tissues, especially brain and intestine, contained a substance that lowered arterial blood pressure when injected intravenously into anesthetized rabbits and stimulated the contraction of intestinal tissue in vitro. Neither of these effects was inhibited by preliminary treatment with atropine. They referred to their partially purified preparation as Powder P and suggested the possible identity between Substance P and the sialogogic peptide. Shortly afterward, Lembeck and Starke (5) reported on the sialogogic activity of their preparations of Substance P and suggested the possible identity between Substance P and the sialogogic peptide.

This report presents a method for obtaining the sialogogic peptide in pure form, its amino acid composition, and further data comparing its biological activity and chemical properties to preparations of Substance P reported by other workers (2, 6-10).

MATERIALS AND METHODS

Analytical Procedures

Protein determinations were done by the method of Lowry et al. (11). Effluents from column chromatographic procedures were monitored by measuring the absorbance of the solution at 260, 280, or 300 nm. Measurements of optical density of solutions of the sialogogic peptide were done with a Cary model 15 recording spectrophotometer with matched sets of quartz cuvettes for test and reference solutions. All amino acid analyses were done on a Beckman-Spinco automatic amino acid analyzer, model 120C, according to the general procedures of Spackman, Stein, and Moore (12). The analyzer was adapted for high sensitivity by the addition of a 4- to 5.1-millivolt range in the recorder. Hydrolyses of the samples were performed for 24 hours at 110° with 0.2 ml of constant boiling 5.7 N HCl in vacuum sealed tubes. 1-Dimethylamino-naphthalene-5-sulfonyl (dansyl) chloride was used to determine the NH₂-terminal residue according to the method of Gray (13). Electrophoresis was carried out at 80 volts per cm on a Michl type electrophoresis apparatus (14). The buffer systems were as follows: (1) pH 1.9: formic acid-acetic acid-water (20:80:000), (2) pH 3.5: pyridine-acetic acid-water (4:40:760), (3) pH 6.5: pyridine-acetic acid-water (80:2.4:720), (4) pH 8.9: 1% NH₄HCO₃ in water. Samples were applied to Whatman No. 1 chromatographic paper in 50 to 200 μl of 0.1 M acetic acid and 10⁻² M mercaptoethanol. A mixture containing 10 to 20 μmoles each of a mixture of standard amino acids was spotted on both sides of the paper. Staining was done by immersing the paper in a solution of ninhydrin-cadmium acetate reagent (15). To recover samples, horizontal strips of paper 2 to 3 cm wide were cut and eluted with 2 to 3 ml of 0.1 M acetic acid-10⁻² M mercaptoethanol. Aliquots of the eluted samples were diluted with 0.9% NaCl before testing for sialogogic activity.

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Biological Procedures

Testing for sialogogic activity was done according to the method of Leeman and Hammerschlag (4). A sialogogic dose was defined as that amount of material that stimulates the secretion of 0.0 ± 0.0 ml of saliva within 2 min following intravenous injection into rats weighing approximately 100 g and anesthetized with pentobarbital, 50 mg per kg. The contractility of freshly dissected sections of guinea pig ilea or rat duodena in response to added samples of peptide was measured in a 40-ml bath of Tyrode's solution (16) at 37°, aerated with a mixture of air-CO₂ (95:5%). The records were made by attaching the muscle to a lever and recording the deflections on a kymograph (Harvard Apparatus Company). Samples of peptide were tested also for their ability to stimulate the contraction of the isolated intestinal tissues after the Tyrode's solution had been made to 0.005 M atropine sulfate, 1.0 × 10⁻⁴ M tryptamine hydrochloride, and 2.0 × 10⁻⁴ M Pyrilamine maleate. These doses of the drugs are known to inhibit the contraction of intestinal tissue in response to effective doses of acetylcholine, serotonin, and histamine (17). The drugs were obtained from Sigma.

Systemic blood pressure was measured with a Grass recorder and histamine (17). The drugs were obtained from Sigma.

The procedure for the isolation of sialogogic peptide from hypothalamic extracts was as follows:

Procedure for Isolation of Sialogogic Peptide

Extraction of Tissue—Fresh frozen bovine hypothalami were obtained from either Swift and Company, South St. Paul, Minnesota, or ERSCO, San Mateo, California. The frozen hypothalami were homogenized to a uniform consistency in a Gilford wood collodion mill in a solution (a volume was used which was 5 times in liters the frozen weight of the tissue in kilograms of acetone-1 N HCl (100:3, v/v)). The mixture was stirred at 4° for 3 hours and then filtered by suction through Whatman No. 1 filter paper. The residue was re-extracted in a solution (a volume was used which was 2 times in liters the original frozen weight of the tissue in kilograms) of acetone-0.01 N HCl (80:20, v/v). The two filtrates were pooled and extracted five times with petroleum ether (b.p. 36-50°C). Each time a volume of petroleum ether was used which was one-fifth of the volume of the combined filtrates. The aqueous phase which contained all of the sialogogic material and some residual acetone was flash-evaporated at room temperature and lyophilized. Extraction and gel filtration of batches of hypothalami larger than 10 kg were performed at the New England Enzyme Center, Tufts University, School of Medicine, Boston, Massachusetts.

Gel Filtration—The lyophilized hypothalamic extract was taken up in 0.1 M acetic acid and centrifuged at 10,000 × g for 20 min before applying the supernatant fluid to a column of Sephadex G-25 (fine) equilibrated with 0.1 M acetic acid at room temperature. Material always was applied in a volume which was less than 5% of the bed volume of the column. The elution profile obtained after the extraction of 2 kg of bovine hypothalami is shown in Fig. 1. For batches of 20 kg of hypothalami, the lyophilized extract first was applied to a large column of Sephadex G-25 (14 × 150 cm), maintained at 4°. Fractions of 250 ml were collected at a flow rate of 40 ml per min. The column was screened for sialogogic activity by taking 0.2- to 1.0-ml aliquots of every third fraction for testing. Fractions from the active region were pooled and lyophilized. The lyophilized residue was rechromatographed on a smaller column of Sephadex G-25. Column size and other chromatographic conditions were identical with those in Fig. 1. Aliquots of 0.1 to 0.5 ml from every fifth tube were tested for sialogogic activity.

Preparative High Voltage Electrophoresis—After chromatography, the lyophilized residue was rechromatographed on a smaller column of Sephadex G-25 maintained at room temperature (Fig. 2). The active fractions were pooled and lyophilized. Cation Exchange Chromatography—The lyophilized residue after the second gel filtration step was taken up in a sufficient volume of 0.4 M pyridine-acetate buffer, pH 5.5, such that the resulting solution had an absorbance at 300 μ of approximately 10.0. This solution was applied to a column of sulfoethyl Sephadex C-25 previously equilibrated with 0.5 M pyridine-acetate, pH 5.5, maintained at room temperature. A stepwise elution with 170 ml of 0.50 M pyridine acetate at pH 5.5 followed by 1000 ml of 0.75 M pyridine-acetate, pH 5.5, was used (Fig. 3). Active fractions from the eluate from the column of sulfoethyl Sephadex were pooled and lyophilized. The residue was then dissolved in a volume of 0.005 M ammonium sufficient to raise the pH of the solution to above 9.0 before applying it to a column of carboxymethyl cellulose-11 (Whatman) that had been equilibrated previously with 0.005 M ammonium. A stepwise elution of 150 ml of 0.005 M ammonium followed by 100 ml of 0.04 M acetic acid was used (Fig. 4).

![Fig. 1. Gel filtration of bovine hypothalamic extract on Sephadex G-25. Column size: 8 × 100 cm. Flow rate: 10 ml per min.](http://www.jbc.org/)

![Fig. 2. Second gel filtration of sialogogic material from a large column of Sephadex G-25.](http://www.jbc.org/)
**FIG. 3.** Chromatography on sulfoethyl Sephadex C-25 of sialogogic material after gel filtration. Applied material was pooled from tubes 140 to 180 of Fig. 2. Column size: 1.8 X 20 cm. Fraction size: 17 ml. Flow rate: 1 ml per min. Aliquots of 20 to 50 μl from every tube were diluted to 1 ml with 0.9% NaCl and tested for sialogogic activity.

**FIG. 4.** Chromatography on Cm-cellulose of sialogogic material after sulfoethyl Sephadex chromatography. Applied material was pooled from tubes 36 to 40 of Fig. 3. Column size: 1.4 X 15 cm. Fraction size: 8 ml. Flow rate: 1 ml per min. Aliquots of 20 to 50 μl from every tube were diluted to 1 ml with 0.9% NaCl and tested for sialogogic activity.

Enzymatic Studies

The enzymes used were obtained from Worthington. Trypsin, two times recrystallized, was treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone according to the method of Carpenter (18) to inactivate any trace of chymotrypsin. Samples of 10 sialogogic doses each of the sialogogic material obtained after chromatography on Cm-cellulose were incubated in 1 ml of buffer for 3 hours at 37° with either 10 μg of an enzyme or 10 μg of an enzyme that previously had been put in a boiling water bath for 10 min. The solution used for the hydrolysis with trypsin was 0.5% NH₄HCO₃ with pepsin, 0.01 M HCl; with chymotrypsin, 0.05 M Tris-HCl (pH 7.15); and with carboxypeptidase A and B, 0.2 M N-ethylmorpholine acetate (pH 8.5). Aliquots of the mixtures were tested for sialogogic activity immediately after the incubation period.

Sixty micrograms of the pure sialogogic peptide were incubated with 15 μg of trypsin in 0.3 ml of 0.5% NH₄HCO₃ for 4 hours at 37°. The reaction was terminated by drying the sample under reduced pressure at room temperature. The residue was applied

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**TABLE I**

Summary of purification of sialogogic peptide

The starting material was 20 kg of frozen hypothalami. The total sialogogic doses reported are those recovered after each purification step.

| Purification step                  | Total protein (mg) | Total sialogogic doses (μg) | Sialogogic doses (μg/μg) |
|------------------------------------|--------------------|----------------------------|--------------------------|
| Initial extraction                 | 100,000            | 30,000                     | 0.3                      |
| First gel filtration on Sephadex G-25 | 2,000              | 25,000                     | 12                       |
| Second gel filtration on Sephadex G-25 | 1,000              | 20,000                     | 20                       |
| Chromatography on sulfoethyl Sephadex | 5                  | 8,000                      | 1,300                    |
| Chromatography on Cm-cellulose     | 1                  | 5,000                      | 5,000                    |
| Paper electrophoresis              | 0.150              | 2,000                      | 13,000                   |

* Protein was determined by the method of Lowry et al. (11).

 Protein was calculated from quantitative amino acid analyses.

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The abbreviation used is: Cm, carboxymethyl.
Twenty micrograms of the pure peptide were mixed with 3 pg of terminal residue according to the method of Amber (19). Above.

Addition of purity were run successively on the same column of Sephadex G-25. Column size: 1.8 X 60 cm. Fraction size: 4 ml. Flow rate: 2 ml per min. Elution solvent: 0.1 M acetic acid-0.2 M NaCl.

Estimation of Size

Samples of oxidized insulin A chain (mol. wt. 2984), arginine vasopressin (mol. wt. 1089), and sialogogic material at all stages of purity were run successively on the same column of Sephadex G-25 at room temperature (Fig. 6). From these data we conclude that the sialogogic peptide has a molecular weight greater than 1000 but less than 2000. This is in agreement with the molecular weight of 1340 calculated from the amino acid composition.

Enzymatic Studies

Trypsin—Incubation of the sialogogic peptide with trypsin did not destroy its activity nor did it cleave the peptide. Electrophoresis at pH 3.5 of material incubated with trypsin again showed only one ninhydrin-positive spot; this spot corresponded to the only region of activity, and it had an electrophoretic mobility identical with that of the untreated peptide. Quantitative amino acid analysis of the material eluted from the region corresponding to the ninhydrin stain gave a composition indistinguishable from the original peptide: Lys (1.1), Arg (0.9), Glx (2.1), Pro (2.3), Gly (1.3), Met (0.6), Leu (0.9), Phe (1.8).

Pepsin and Chymotrypsin—Incubation of the sialogogic peptide with pepsin or chymotrypsin completely destroyed the sialogogic activity. Incubation with inactivated pepsin or chymotrypsin had no effect.

Fig. 6. Estimation of molecular weight of sialogogic peptide by gel filtration on column of Sephadex G-25. Column size: 1.8 X 60 cm. Fraction size: 4 ml. Flow rate: 2 ml per min. Elution solvent: 0.1 M acetic acid-0.2 M NaCl.

Amino Acid Composition

The amino acid composition of the pure peptide is given in Table II. The absorption spectrum of the pure peptide confirmed the lack of tyrosine and also showed the absence of trypto-
The following amounts of the pure sialogogic peptide were added at the arrows: a and b, 0.25 μg; c and d, 0.05 μg; e, 0.25 μg.

**TABLE III**

| Amino acid | Condition A* | Condition B* |
|------------|--------------|--------------|
|            | Composition | Molar ratio | Composition | Molar ratio |
| Lysine     | 3.9 mmoles  | 1.0          | 4.2 mmoles  | 1.0          |
| Arginine   | 4.2 mmoles  | 1.1          | 3.8 mmoles  | 0.0          |
| Glutamic acid | 7.9 mmoles | 2.0          | 9.7 mmoles  | 2.2          |
| Proline    | 7.9 mmoles  | 2.0          | 8.7 mmoles  | 2.0          |
| Glycine    | 5.8 mmoles  | 1.5          | 8.6 mmoles  | 2.0          |
| Methionine | 2.1 mmoles  | 0.5          | 1.3 mmoles  | 0.3*         |
| Leucine    | 3.7 mmoles  | 0.9          | 4.6 mmoles  | 1.1          |
| Phenylalanine | 6.6 mmoles | 1.7          | 8.1 mmoles  | 1.9          |
| Serine     | 1.3 mmoles  | 0.3          | 1.6 mmoles  | 0.4          |
| Alanine    | 0.9 mmoles  | 0.2          | Trace       | Trace        |

* Condition A: 0.65 to 0.75 mobility (mobility: lysine, 1.00), 120 doses recovered; 60 doses per analysis; Condition B: 0.45 to 0.65 mobility (mobility: lysine, 1.00), 150 doses recovered, 75 doses per analysis.

* Elevated glycine values were due to contamination during electrophoresis. Blank strips of paper eluted as controls yielded glycine and smaller amounts of serine and alanine, respectively. The contamination problem was negligible when larger amounts of material were used.

* The low methionine value is partly explained by the fact that the methionine sulfone and methionine sulfoxide peaks were unusually high in this sample. When these peaks were calculated as part of the value for methionine, the molar ratio of methionine increased to at least 0.6.

**Fig. 7.** Effect of pure sialogogic peptide on contraction of isolated intestinal tissue. Bath size: 40 ml. For further details see "Materials and Methods." a, b, and c, Tyrode's solution made to 10⁻² μm atropine, 10⁻⁶ μm tryptamine, and 2 × 10⁻⁶ μm Pyrilamine. The following amounts of the pure sialogogic peptide were added at the arrows: a and b, 0.25 μg; c and d, 0.05 μg; e, 0.25 μg.

**Fig. 8.** Effects of pure sialogogic peptide on the blood pressure of an anesthetized rat. Body weight: 250 g. a, 0.2 μg per kg of peptide injected at arrow; b, rat previously treated with atropine, 50 mg per kg, 30 min before injection of 0.2 μg per kg of peptide at arrow. After electrophoresis for 30 min was spread over 10 cm of paper at pH 6.5 and 15 cm of paper at pH 6.5. That this smear of activity can still be attributed to one peptide is supported by two observations. (a) The homogeneous peptide obtained after electrophoresis at pH 3.5 was rerun at pH 6.5. Sialogogic material was eluted from two broad regions of the paper, but the amino acid compositions of the material from the two regions of paper were essentially the same (Table III). (b) Sialogogic material obtained after chromatography on CM-cellulose was run first at pH 6.5. Samples eluted from the region of paper representing an electrophoretic mobility of 0.55 to 0.75 and of 0.2 to 0.55 (mobility: lysine, 1.00) were reapplied separately to paper and run under identical conditions. The faster moving material had the same broad distribution of activity as the original run, indicating that the smear of activity could be accounted for by one peptide. However, the electrophoretic mobility of the slower moving material was unchanged. This strongly suggests that the sialogogic peptide is modified irreversibly during electrophoresis at 6.5.

At pH 8.9 the sialogogic activity still migrates toward the cathode with an electrophoretic mobility of 0.0 to 1.0 (mobility: lysine, 1.0).

**Characterization of Sialogogic Peptide as Substance P**

**Studies on Isolated Intestinal Tissue**—The pure peptide was found to stimulate the contraction of isolated guinea pig ileum and rat duodenum even in the presence of atropine, tryptamine, and Pyrilamine (Fig. 7). These results show that the activity of the peptide is not mediated via acetylcholine, serotonin, or histamine.

**Blood Pressure Studies**—Intravenous injection of one-tenth of a sialogogic dose of pure peptide lowered the arterial blood pressure of the anesthetized rat (Fig. 8). A nearly maximal fall in blood pressure was seen at one-fifth of a sialogogic dose. Preliminary treatment of the rat with atropine sulfate had no effect on the activity of the sialogogic peptide.

**DISCUSSION**

The sialogogic peptide isolated from bovine hypothalami is an undecapeptide containing Lys₁, Arg₂, Gln₃, Pro₄, Gly₅, Met₆, Leu₇, and Phe₈. The calculated molecular weight of 1340 agrees well with the estimate of its size obtained by gel filtration (Fig. 6). The best evidence for the purity of the peptide reported here is the constant molar ratio of the constituent amino acids ob-
amino acids are found after amino acid analyses of our sialogogic peptide. The amino acid compositions reported for Substance P contain Asp, Ser, Ala, Thr, Val, Ile, and Tyr, in addition to the amino acids found in the sialogogic peptide. These same contaminating amino acids in the sialogogic peptide are contained within the amino acid compositions reported for partially purified Substance P (6, 7, 10). Two lines of evidence suggest that at least two of the three possible carboxyl groups in the peptide are not free: (a) a progressive increase in mobility with respect to lysine going from pH 1.9 to 9.8 electrophoresis, and (b) absorption on Cm-cellulose at pH 10.4.

Since Substance P has never been purified to homogeneity, it only has had a biological definition. There is general agreement that at least one peptide referred to as Substance P has the following properties in the presence of atropine (2): (a) it is a potent hypotensive agent, (b) it stimulates the contraction of various smooth muscle preparations, and (c) it is distinguishable from kinins in that it can stimulate the contraction of rat duodenal tissue. The hypothalamic peptide isolated on the basis of its sialogogic activity meets these criteria.

Comparison of the chemical and physical properties of the sialogogic peptide with those reported for partially purified samples of Substance P from several different tissues and species suggests that these chemical entities are very similar if not identical (Table IV). Table V gives the amino acid compositions that have been reported for Substance P. The constituent amino acids in the sialogogic peptide are contained within the amino acid compositions reported for Substance P, with the exception of methionine. It has been our experience and that of Zuber (7, 22) that the molar ratio of methionine relative to other amino acids increases with further purification. Qualitatively, the amino acid compositions reported for Substance P contain Asp, Ser, Ala, Thr, Val, Ile, and Tyr, in addition to the amino acids found in the sialogogic peptide. These same contaminating amino acids are found after amino acid analyses of our sialogogic preparations after Cm-cellulose chromatography but are separated from the sialogogic peptide by high voltage electrophoresis.

In further support of the close similarity of the sialogogic peptide and Substance P, Haefely and Hürllimann (10) and Vogler et al. (9) noticed that their highly purified samples stimulated copious salivation when injected intravenously into conscious dogs.

The specific activity of the peptide isolated here appears to be at least 6 times greater than that reported for the most highly purified preparations of Substance P (6-10). Lembeck and Starke (5) reported that approximately 2,000 Euler units per Kg stimulated the secretion of 50 mg of saliva in rats. Assuming the sensitivity of their test rats to be the same as ours, one sialogogic dose would be the equivalent of 200 Euler units. Thus the pure peptide has a calculated specific activity of 2,600,000 Euler units per mg of protein. The highest specific activity for a preparation of Substance P previously reported is that of Zuber (7) of 400,000 Euler units per mg of protein.

### Table IV

| Property                  | Sialogogic peptide | Substance P | Authors                  |
|---------------------------|--------------------|-------------|--------------------------|
| Molecular weight          | 1340               | 1,650 ± 250 | Vogler et al. (6)         |
| Electrophoretic mobility relative to lysine at pH 1.9 | 1.1                | 0.8-1.0     | Meinardi and Craig (8)    |
| Isoelectric point         | >pH 8.9            | >pH 8.5     | Vogler et al. (9)         |
| NH₂-terminal residue      | Arginine           | Arginine    | Boissonnas et al. (6)     |
| Ether                     | Insoluble          | Insoluble   | Vogler et al. (9)         |
| Ethanol                   | Soluble            | Soluble     | Geipert et al. (20)       |
| 60% (NH₄)₂SO₄             | Insoluble          | Insoluble   | Boissonnas et al. (6)     |
| Chymotrypsin              | Activity destroyed | Activity destroyed | Lembeck and Zetler (2)     |
| Pepsin                    | Activity destroyed | Activity destroyed | Lembeck and Zetler (2)     |
| Carboxypeptidase A        | Activity not destroyed | Activity not destroyed | Lembeck and Zetler (2)     |
|                          |                    |             | Zuber (7)                 |

### Table V

| Amino acid   | 1st | 2nd | 3rd | 4th |
|--------------|-----|-----|-----|-----|
| Lysine       | +   | +   | 1-2 | 1.2 |
| Histidine    | -   | -   | 0   | 0.5 |
| Arginine     | +   | +   | 1-2 | 1.4 |
| Aspartate    | +   | +   | 1   | 0.8 |
| Threonine    | +   | +   | 1   | 0.5 |
| Serine       | +   | +   | 2   | 0.7 |
| Glutamic acid| +   | +   | 2   | 1.9 |
| Proline      | +   | +   | 2   | 2.1 |
| Glycine      | +   | +   | 2-3 | 1.7 |
| Alanine      | +   | +   | 2-3 | 0.6 |
| Valine       | +   | +   | 1   | 0.8 |
| Methionine   | -   | -   | 1   | 0.63|
| Isoleucine   | +   | +   | 1   | 0.3 |
| Leucine      | +   | +   | 2-3 | 1.6 |
| Tyrosine     | -   | +   | 1   | 0.0 |
| Phenylalanine| +   | +   | 1-2 | 2.0 |

* Boissonnas et al. (6). Starting material: bovine and equine intestine.
* Vogler (9). Starting material: bovine intestine.
* Zuber (7). Starting material: bovine brain.
* The values reported by Meinardi and Craig (8) were divided by 1.6 to make comparison to the sialogogic peptide easier. Starting material: goat hypothalamus.
Whether or not there is a single Substance P-peptide is not yet clear. Some preliminary evidence suggests that there may be more than one (8, 23). Zetter introduced a method for separating crude preparations of Substance P into three biologically active fractions, Fₐ, F₀, and Fₕ, by chromatography on aluminum oxide. Fraction Fₐ is not Substance P as it does not have vasodepressor activity. Both F₀ and Fₕ satisfy the original criteria established by von Euler and Gaddum (1), but Fₐ produces relaxation instead of contraction of rat duodenal tissue. Recently, Baldau and Gebhardt (24) have reported that a relaxing component can be separated from Fₐ upon further purification, unmasking a peptide that can contract the rat duodenum. Whether the active substances in F₀ and Fₕ are peptides with different compositions or altered forms of the same peptide is not known. Lembeck and Starke have shown that F₀ can be converted to Fₐ by shaking with aluminum oxide (25). The results of our electrophoretic experiments at pH 6.5 show that the sialogogic peptide can be changed into a slower moving component with no resultant change in amino acid composition.

Two other undecapeptides—physalaemin, isolated from the skin of a South American amphibian (26), and eledoisin, isolated from the salivary glands of cephalopods (27)—have been shown to have the biological activities of Substance P; i.e., they stimulate the contraction of smooth muscle tissue (5), and are potent vasoconstrictors. Both F₀ and Fₕ contain no detectable amounts of the amino acids in common with physalaemin and eledoisin. Furthermore, all three peptides stimulate the secretion of saliva at doses less than 2 μg per kg when given via tail vein in rats. The chemical and biological similarities of these three peptides make it tempting to speculate that these peptides had a common origin during evolution.

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