The Isolation of a New Hypotensive Peptide, Neurotensin, from Bovine Hypothalami*

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SUMMARY

A hypotensive peptide, designated neurotensin, has been discovered and isolated in pure form from acid-acetone extracts of bovine hypothalami by column chromatography and paper electrophoresis. The results of amino acid analyses of material recovered after paper electrophoresis at pH 3.5, 6.5, and 8.9 and chromatographic analyses of the dansylated material indicate that the peptide isolated by this procedure is homogeneous. Its amino acid composition and apparent molecular weight estimated by chromatography on Sephadex G-25 indicate that neurotensin is a tridecapeptide composed of Lys, Arg, Asx, Glx, Pro, Ileu, Leu, Tyr. Neurotensin lacks a free NH₂-terminus; however, it possesses a free COOH terminus which can be acted upon by carboxypeptidase A. Neurotensin induces hypotension in the rat and can stimulate the contraction of guinea pig ileum and rat uterus; however, it produces relaxation of the rat duodenum. These pharmacological properties classify it as a "kinin," yet its chemical composition distinguishes it from any known peptide.

During the course of purification of substance P from bovine hypothalamic extracts (1), the presence of a different peptide was detected that produces a visible vasodilation in the exposed cutaneous regions of anesthetized rats. This response occurs within seconds after intravenous injection and is associated with an acute hypotension. Higher doses cause a distinctive cyanosis that persists for minutes. This report presents a method for obtaining the peptide, which we have named neurotensin, in pure form, its amino acid composition, and some further characterization of its chemical and biological properties. These data serve to distinguish it from other known mammalian peptides (2).

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MATERIALS

Bovine hypothalami, obtained from Swift & Co., St. Joseph, Mo. or Fort Worth, Texas, were excised within 1 hour of killing and frozen immediately. The tissue fragments, extending from the optic chiasma to the mamillary bodies, weighed 8 to 10 g each. Albino rats of various sizes, hypophysectomized rats, and female guinea pigs weighing about 300 g were obtained from Charles River Breeding Labs. Sephadex media were obtained from Pharmacia Chemical Co., Silica Gel G plate from Schawarts Mann, and chromatography papers from Whatman. Trypsin (25 units per mg), treated with L-(l-tosylamido-2-phenyl)ethyl chloromethyl ketone, and α-chymotrypsin (45 units per mg) were purchased from Worthington Biochemical Corp. Diisopropylphosphorofluoridate-treated carboxypeptidase A (35 units per mg), diisopropylphosphorofluoridate-treated carboxypeptidase B, and leucine aminopeptidase (200 units per mg) were also from Worthington. Thermolysin (A grade) was obtained from Calbiochem Corp. and aminopeptidase M (12 units per mg) from Henley & Co. Five times recrystallized papain was a generous gift from Dr. M. J. Gorbunoff, Biochemistry Department, Brandeis University, and was found by us to have a specific activity of 16 units per mg using benzoyl arginine methyl ester as substrate (3). Pronase (1 unit per mg) was a product of Sigma Chemical Co. Oxidized insulin A chain (glycyl) and the S-amino acid peptide, Phe-Val-Gln-Trp-Met-Asp-Thr, were products of Mann Research Labs. Synthetic oxytocin, synthetic vasopressin, synthetic phylsiamin, and bacitracin were obtained from Sigma Chemical Co.

The following drugs were obtained from the indicated source: Sigma, propranolol-HCl, atropine sulfate, and tryptamine-HCl; Winthrop, phenoxybenzamine and isoproterenol; Nutritional Biochemicals, acetylcholine chloride; P. Falag & Bauer, pyrilamine maleate; Parke-Davis, adrenaline chloride; Abbott Laboratories, sodium pentobarbital (Nembutal) and sodium heparin. All reagents were Fisher certified products.

METHODOLOGY

Analytical Procedures

Gel Chromatography and Ion Exchange Chromatography—The Sephadex G-25 and sulfoethyl-Sephadex C-25 were prepared as recommended by the manufacturer. Desending gel chromatography was carried out at a flow rate of 0.3 to 0.5 ml per min per cm² at room temperature or 4°C. Linear gradient elution of ion
exchange columns was performed as described by Bailey (4). Column eluates were monitored by measuring their absorbance at 280 or 300 nm with a Zeiss spectrophotometer. Protein concentrations of column eluates were expressed as units of absorbance at 280 nm with the assumption that one absorbance unit represented 1 mg of protein per ml.

High Voltage Paper Electrophoresis—High voltage paper electrophoresis was performed on Whatman No. 1 or 3MM paper using a Michie type electrophoresis apparatus (5) at 80 volts per cm with Varsol as an inert cooling medium. The buffer systems consisted of (a) pH 1.9, formic acid-acetic acid-water (150:100:750); (b) pH 3.5, pyridine-acetic acid-water (4:40:760); (c) pH 6.5, pyridine-acetic acid-water (80:2.4:720); and (d) pH 8.9, 1% ammonium carbonate in water. When preparative electrophoresis was to be carried out, the Whatman papers were washed with 20% pyridine and then 20% acetic acid for several days before use. Samples were applied to the paper in 0.2 ml acetic acid, 0.01 M mercaptoethanol so as not to exceed a load of 0.1 mg of protein per cm and 1.0 mg of protein per cm on Whatman No. 1 and 3MM paper, respectively. Appropriate standard amino acids (10 nmoles each) were spotted on both sides of the samples and peptides were located by staining a guide strip with ninhydrin-cadmium acetate reagent (6) or with the chlorine, o-tolidine method (7). Strips (1.5 to 2.0 cm in width) of the unstained section of the paper were eluted with 1 to 2 ml of 0.2 M acetic acid, 0.01 M mercaptoethanol (8). After lyophilization samples were diluted into 0.85% NaCl solution for biological testings. The electrophoretic mobility of neurotensin was calculated relative to lysine according to the method of Offord (9).

Amino Acid Analysis—Amino acid analyses were done according to Spackman et al. (10) with the aid of a Beckman model 120C automatic amino acid analyzer equipped with a high sensitivity adapter (11), precision ±3% on a 2 to 10 n mole range. Samples of peptides (5 to 10 nmoles) were routinely hydrolyzed with 0.25 ml of constant boiling HCl in evacuated, sealed tubes at 105° for 20 to 40 hours. Although tyrosine losses during acid hydrolyses have been reported to be about 10% in 24 hours (12), we have found that at very low concentrations tyrosine can be degraded at 5-fold this rate (Fig. 1). The destruction rate is enhanced when the sample is first eluted from paper and impeded in the presence of phenol. Therefore, to minimize destruction of tyrosine, 25 µl of 0.1 M phenol were added to some tubes (13). For those analyses where phenol was not present tyrosine values were calculated from a time curve of the destruction extrapolated to zero time (Fig. 1). In order to quantitate the amounts of cysteine, cystine, and methionine in the peptide, performic acid oxidation was performed as described by Hirs (14). The dried sample was oxidized for 12 hours at 0° with 0.25 ml of performic acid reagent, diluted with cold water, lyophilized, and acid hydrolyzed for amino acid analysis. Spectral analyses of the pure peptide were performed on a Cary model 15 recording spectrophotometer to determine tyrosine and tryptophan content.

Dansylation Procedure—DNS*-chloride was used to determine the NHz-terminal residue according to the procedure of Gray (15). The DNS peptide was hydrolyzed in constant boiling HCl at 105° for 4 and 20 hours as recommended by Gros et al. (16) and examined by two-dimensional chromatography on 5 x 5-cm sheets of polyamide using the four solvent systems described by Hartley (17).

Enzymatic Procedures—Enzymatic digestions were done by incubating 10 doses of pure peptide with a 1:50 molar ratio of enzyme at 38° for 4 hours in 0.1 ml of the appropriate buffer. The buffer systems were as follows: pronase, 0.05 Tris-HCl buffer, pH 7.6; papain, 0.20 M ammonium acetate, pH 5.6, 0.03 M mercaptoethanol; thermolysin, 0.20 M NH4HCO3, pH 8.2; a-chymotrypsin, trypsin, and carboxypeptidase A and B, 0.10 M NH4HCO3, pH 7.8; leucine aminopeptidase, 0.05 M sodium barbital, pH 8.5, 0.01 M MgCl2; aminopeptidase M, 0.06 M sodium phosphate, pH 7.8. Control solutions consisted of active peptide incubated with enzyme that had been boiled for 5 min. The reactions were terminated by addition of 3 drops of acetic acid; the samples were lyophilized, dissolved in 0.85% saline, and tested in rats for their vasodilatory and hypotensive activity. In the cases where enzymatically released free amino acids were determined, approximately 200 doses of pure neurotensin were used per digest and analyses were performed on the amino acid analyzer.

Biological Procedures—Testings for the sialogogic activity of substance P were done according to the procedure of Leeman and Hammerechlag (1). One sialogogic dose is that amount of material that stimulates the secretion of 50 ± 10 µl of saliva when injected intravenously into an anesthetized 100 g rat. The biological activity of neurotensin was monitored by observing the characteristic vasodilation of the exposed cutaneous regions of anesthetized rats that occurs within seconds following intravenous injection of samples. The intensity and duration of the response was noted and a minimal active dose was determined. Since this vasodilation was found to be associated with a transient fall in blood pressure, a dose of peptide was defined as that amount of material per 100 g body weight which when given intravenously to an anesthetized rat causes a fall in blood pressure of 35 ± 5 mm Hg. Systemic blood pressure was measured with a Hewlett-Packard recorder and pressure transducer (pre-amplifier 8805 B, recorder 7782 A, transducer 267 BC) following cannulation of the carotid artery in rats weighing 250 to 300 g and anesthetized with pentobarbital (50 mg per kg). Test samples were dissolved in 0.85% saline and administered through a cannula in the femoral vein. The effects of pretreatments with various drugs on the response to neurotensin was examined by

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1 The abbreviation used is: DNS, 1-dimethylaminonaphthalene-5-sulfonyl.
the following procedure, each drug being tested in a separate
group of rats. The animal was first shown to be responsive to
an agonist in a dose producing a deviation in blood pressure
of 30 to 40 mm Hg; then, 40 min after administration of the ap-
propriate antagonist, the effect of the agonist was shown to be
completely inhibited. Shortly afterward neurontin was tested in
the same rat. The drugs, atropine sulfate (35 mg per kg),
propranolol (5 mg per kg), and phenoxybenzamine (10 mg per
kg) were administered subcutaneously to prevent the blood pres-
sure responses to the agonists, acetylcholine (0.5 µg per kg),
isoproterenol (0.5 µg per kg), and epinephrine (1.0 µg per kg),
respectively. Acute bilateral adrenalectomy was performed un-
der pentobarbital anesthesia. Hypophysectomized rats were
bathed in Tyrode’s solution (21). All muscles were allowed to
equilibrate in the bath for 30 min before the experiments were
in modified Locke’s solution (20), while all other tissues were
suspended in a 40 ml bath maintained at 37°C and aerated with a
be in proestrous by their vaginal smear (19). All tissues were
examined. Rat uteri were taken from virgin rats determined to
The effect of neurotensin on the contractility of freshly dissected
mixture of O2-CO2 (95:5). Uterine contractility was examined
with an assumed specific gravity of 1) was homogenized to a
filtrate was mixed at 4°C with one-third its volume of petroleum
ether (b.p. 36 to 50.9°C); the ether-acetone phase was discarded
and the process repeated three to four times until the discarded
phase was transparent. The acetone-water phase was then
evaporated under reduced pressure (water bath temperature of
35–45°C) to remove acetone and the aqueous residue was finally
lyophilized. The extraction and initial fractionation steps on a
preparative scale were performed at the New England Enzyme
Center, Tufts University, School of Medicine, Boston, Massa-
Chromatography on G-25 Sephadex—Fractionation of the ex-
tact was performed in two successive steps on a 20-liter and a
5-liter column of Sephadex G-25 (fine). The activity of neuro-
tensin is masked at this stage; however, substance P, a sialogogic
undecapeptide (22) also present in these tissues, occupies the
same region and was used as a marker. The lyophilized ex-
tract was taken up in 0.1 m acetic acid (600 ml/22 kg of hy-
pothalami) and after its pH was adjusted to 4, the suspension
was centrifuged at 10,000 X g for 20 min at 4°C. The super-
natant was resuspended in solvent (400 ml/20 kg of hypothalami)
and recentrifuged. The combined supernatant fluid was then
applied to a 20-liter column of Sephadex G-25 (fine) which was
equilibrated with 0.1 m acetic acid at 4°C (Fig. 2) and the column
eluates were screened for sialogogic activity. Material from the ac-
tive region was pooled, lyophilized, taken up in 0.1 m acetic acid
(100 ml/20 kg of hypothalami), and rechromatographed on a
5-liter column kept at room temperature (Fig. 3). Again the active
region was pooled and lyophilized.

Cation Exchange Chromatography—The material was then sub-
jected to ion exchange chromatography on sulfoethyl-Sephadex
C-25, with volatile buffers. Material representing 22 kg of
tissue (about 800 mg of protein) was taken up in 200 ml of 0.05
M pyridine-acetate buffer, pH 3.1, and was applied at room
temperature to a 50-ml column of sulfoethyl-Sephadex equilibrated
with this buffer. The column was developed with a linear
gradient which was generated by using 750 ml of 0.05 M pyridine-
acetate, pH 3.1, in the mixing chamber and 750 ml of 2.0 M

Whole blood, obtained anaerobically, was assayed for pO2,

\[ pO_2, pCO_2, \text{ and pH at } 37^\circ \text{C in an Instrumentation Laboratory analyzer} \]

(models 213 and 313). The analyses were done by Dr. Earl
Weiss, Department of Respiratory Diseases, The St. Vincent
Hospital of Worcester, Mass. Female albino rats, 90 to 130 g,
were anesthetized with pentobarbital intraperitoneally (45 mg
per kg) 40 min before use. Rats were then injected intraven-
ously via tail vein with 0.85% saline or with neurotensin in
saline, and arterial blood was withdrawn from the abdominal
aorta into heparinized syringes at various times after injection.
Samples were kept in ice water until the analyses were done.

Procedure for Isolation of Neurotensin

Extraction of Tissues—The frozen tissue (usually 20 to 45 kg
with an assumed specific gravity of 1) was homogenized to a
uniform consistency with an equal volume of -20°C acetone-
1 N HCl (100:3 v/v) in a Gilford Wood colloid mill; then 3 more
volumes of this solvent were added and the suspension was
stirred overnight at 4°C. The mixture was suction filtered through
Whatman No. 31 paper on Buchner funnels and the filtrate set
aside. The residue was resuspended in a volume of acetone-
0.01 N HCl (80:20 v/v) that was three times the original volume
of the tissue and filtered as above. The two filtrates were pooled.
Repetitive petroleum ether extraction of the combined filtrates
to remove lipids as well as acetone was performed as follows: the
filtrate was mixed at 4°C with one third its volume of petroleum
ether (b.p. 36 to 50.9°C); the ether-acetone phase was discarded
and the process repeated three to four times until the discarded
phase was transparent. The acetone-water phase was then
evaporated under reduced pressure (water bath temperature of
35–45°C) to remove acetone and the aqueous residue was finally
lyophilized. The extraction and initial fractionation steps on a
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FIG. 2. Gel chromatography of bovine hypothalamic extract on
Sephadex G-25. Sample, acid acetone extract representing 22 kg
wet weight of tissue; column size, 14 X 150 cm (resin volume 20
liters); fraction size, 300 ml; eluent, 0.1 M acetic acid; flow rate,
20 ml per min. Sialogogic activity (O——O) was measured by
injecting aliquots of eluates into test rats.
pyridine-acetate, pH 5.5, in the reservoir. The protein concentration of the eluates was monitored at 280 and 300 nm (Fig. 4), and neurotensin activity (unmasked by this fractionation step) was located by the visual vasodilation method. The active region was pooled, lyophilized, and the doses of neurotensin present were quantitated using the rat blood pressure method. Material pooled from two of these columns of sulfoethyl-Sephadex (representing 45 kg of tissue) was applied in a 10-cm band to Whatman No. 3MM paper and subjected to electrophoresis. Fig. 6a shows the pattern of ninhydrin staining material obtained and the doses of neurotensin recovered from corresponding regions of the paper. Material recovered from Region G (50% of the activity) was found to be a pure peptide. The remainder of the activity can be recovered as a pure peptide by further electrophoresis at either pH 6.5 or pH 8.9. Neurotensin has an electrophoretic mobility relative to lysine of 0.4, 0.2, and 0.3 at pH 3.5, 6.5, and 8.9, respectively.

RESULTS

Purification and Yields

Table I summarizes the results of a typical purification procedure starting with 45 kg of bovine hypothalami. By this procedure the extracted peptide is purified approximately 200,000-fold. Approximately 3 to 5 nmoles of pure neurotensin can be obtained per kg of wet tissue. Assuming a 75% yield through the initial gel chromatography steps, it can be estimated that there are about 20 nmoles of this peptide per kg of tissue in the initial extract. Since re-extraction of the initial precipitate with acetone-0.01 M HCl (60:40 v/v) yields more of a vasodilatory peptide that probably is neurotensin, there may be as much as 35 nmoles of neurotensin present per kg of hypothalami. Thus, on a molar basis the concentration of neurotensin in bovine hypothalami is only about 20% that of substance I (22) and 40% that of luteinizing hormone-releasing factor (23).

Homogeneity and Composition

Several lines of evidence show that the biologically active peptide obtained is homogeneous. The material obtained after electrophoresis at pH 3.5 runs as a single peptide during electrophoresis at pH 6.5 (Fig. 6b), and when the isolated peptide is treated with DNS-chloride and chromatographed on Silica Gel G only one fluorescent peptide spot is visible having an RF = 0.68 using butanol-pyridine-acetic acid-water (15:10:3:12) and an RF = 0.52 using butanol acetic acid water (4:1:1). The molar ratios of the constituent amino acids are integral and remain constant after electrophoresis at pH 3.5, 6.5, and 8.9 (Table II). Furthermore, the biological specific activity also remains constant after re-electrophoresis. Spectrophotometric analyses of the peptide (Fig. 7) confirmed the presence of 2 moles of eluates into test rats.

Fig. 3. Rechromatography of sialogogic region on Sephadex G-25. Sample, material representing 22 kg of tissue, Region 38 to 50 in Fig. 2; column size, 8 x 100 cm (resin volume 5 liters); fraction size, 20 ml; eluent, 0.1 m acetic acid; flow rate, 8 ml per min. Sialogogic activity (O—O) was measured by injecting aliquots of eluates into test rats.

Fig. 4. Ion exchange chromatography on sulfoethyl-Sephadex C-25. Sample, material representing 22 kg of tissue, Region 160 to 180 in Fig. 3; column size, 2.2 x 15 cm (resin volume 50 ml); fraction size, 20 ml; column buffer, 0.05 m pyridine-acetate, pH 3.1. Neurotensin and sialogogic activities, determined by injecting lyophilized aliquots dissolved in 0.85% saline into test rats, were eluted in Fractions 50 to 60 and 65 to 75, respectively (see inset). ---, an estimate of the pH gradient.

Fig. 5. Rechromatography of neurotensin region on sulfoethyl-Sephadex C-25. Sample, material representing 45 kg of tissue, Region 50 to 60 in Fig. 4; column size, 1.1 x 10 cm (resin volume 10 ml); fraction size, 10 ml; column buffer, 0.01 m pyridine-acetate, pH 5.5. ---, an estimation of the pyridine concentration. Neurotensin activity, measured after injection of lyophilized aliquots of every third fraction into test rats, was eluted from the column in Fractions 80 to 100 (see upper inset). To remove pyridine, which itself absorbs at 280 nm, each of these fractions was lyophilized, then each residue was dissolved in 2.0 ml of 0.10 m acetic acid and the A280 nm determined (see lower inset).
Fig. 6. High voltage paper electrophoresis of highly purified neurotensin. a, stained guide strip and distribution of neurotensin activity recovered after preparative electrophoresis at pH 3.5. Neurotensin, 7000 doses, was applied across 10 cm at the origin; 150 doses were spotted on the guide strip. Leu indicates leucine spotted at origin. Conditions: 60 min at 80 volts per cm; ninhydrin was established by amino acid analysis of 4 nmoles of pure peptide that had been subjected to performic acid oxidation. The following molar ratios were obtained: cysteic acid, 0; methionine sulfoxide, 0; aspartic acid, 1.05; glutamic acid, 2.0; proline, 2.0; isoleucine, 0.8; and leucine, 1.8. Tyrosine, lysine, and arginine were not determined.

Estimation of Molecular Weight of Neurotensin

Fig. 8 shows the elution position of neurotensin as measured by vasoconstrictor activity relative to a number of other peptides of known molecular weight. The apparent molecular weight of neurotensin obtained from this plot is 1600. The minimum molecular weight of the tridecapeptide calculated from its amino acid composition is 1673.

End Group Determination

Neurotensin was found to lack a free NH₂ terminus. Chromatography of the acid hydrolysates of neurotensin treated with DNS-chloride yielded only O-DNS-tyrosine and N⁶-DNS-lysine and no α-DNS-amino acid derivatives, indicating that the α-amino group of the NH₂-terminal residue is blocked. Additional support for this conclusion is that enzymatic digests of 10 nmoles of peptide with leucine aminopeptidase and 4 nmoles of peptide with carboxypeptidase A and I released stoichiometric amounts of leucine, isoleucine, and tyrosine and destroyed its biological activity, indicating that the COOH terminus is free.

TABLE I
Summary of purification of neurotensin 45 kg of bovine hypothalami

| Purification step | Total protein | Total neurotensin doses recovered | Neurotensin yield | Neurotensin specific activity |
|------------------|--------------|----------------------------------|------------------|-----------------------------|
| 80% Acetone extraction | 270,000⁺ | 40,000 | 15,000⁺ | 100 | 0.06 |
| First gel chromatography on Sephadex G-25 | 10,000⁺ | 33,000 | | | |
| Second gel chromatography on Sephadex G-25 | 1,500⁺ | 30,000 | 11,000 | 75 | 7.0 |
| First chromatography on SE-Sephadex | 250⁺ | 20,000 | 7,500 | 50 | 30.0 |
| Second chromatography on SE-Sephadex | 10⁺ | 6,000 | 40 | 600.0 |
| Paper electrophoresis pH 3.5 | 0.3⁺ | 3,100 | 21 | 10,300.0 |

⁺ Protein is expressed as absorbance units at 280 nm.

Enzymatic Studies

Incubation of biologically active material with the relatively nonspecific endopeptidases, pronase and papain, as well as enzymes with a high substrate specificity such as trypsin, chymotrypsin, and thermolysin destroyed its ability to produce vasoconstriction and to lower blood pressure in rats. These re-
**Biological Studies**

**Vasodilation and Cyanosis**—Intravenous injection of neurotensin (>200 pmoles per kg) produces within 30 s a visible dilatation of the small vessels in the exposed cutaneous regions of anesthetized rats, particularly noticeable in the ears, the feet, and around the mouth. Larger doses (>1.0 nmoles per kg) evoke this response and then cause a visible cyanosis within minutes after injection. The strength and duration of these effects is directly related to the dose of neurotensin injected. The cyanosis is not associated with a change in the partial pressure of oxygen (pO₂) or carbon dioxide (pCO₂) in arterial blood. When arterial blood was drawn from a control group of anesthetized rats (nine animals) 2 to 3 min after intravenous injection with 0.85% saline, the following values (mean ± standard error) were obtained: pO₂, 84 ± 4 mm Hg; pCO₂, 36 ± 4 mm Hg; pH 7.36 ± 0.03.

**Rat Blood Pressure**—Neurotensin is a potent hypotensive agent in the anesthetized rat, the threshold intravenous dose for a measurable response being about 100 pmoles per kg. The magnitude of the hypotensive effect depends upon the starting level of blood pressure, being diminished in rats with lower basal levels (Table III). The hypotensive effect of neurotensin exhibits acute tachyphylaxis, i.e. a second equal dose administered 1, 10, or 60 min after an active first dose produces no effect (Fig. 9); however, a second dose given several hours later is effective. Table III also shows that the hypotensive effect of neurotensin is not significantly altered by adrenalectomy, hypophysectomy, or prior administration of atropine sulfate, phenoxybenzamine, or propranolol.

**Vascular Permeability**—Neurotensin can cause a marked increase in vascular permeability which is easily demonstrated. Within minutes after intravenous injection of >500 pmoles per kg of peptide into an anesthetized rat, the animal’s limbs appear swollen and edematous. Intravenous injection of this amount of neurotensin into animals previously injected with Evans blue dye causes a greater leakage of the albumin-bound dye into the extravascular space than is visible in the control animals. If

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**Table III**

| Group               | No. of animals | Basal pressure* | Response to fall in pressure | Tachyphylaxis* |
|---------------------|----------------|-----------------|-----------------------------|----------------|
| Intact (high)       | 23             | 102 ± 3         | 36 ± 2                      | Yes            |
| Intact (low)        | 4              | 52 ± 6          | 15 ± 2                      | Yes            |
| Adrenalectomized    | 7              | 99 ± 7          | 32 ± 3                      | Yes            |
| Hypophysectomized   | 11             | 77 ± 6          | 35 ± 3                      | Yes            |
| Atropine (35 mg/kg) | 7              | 80 ± 9          | 30 ± 4                      | Yes            |
| Phenoxybenzamine (10 mg/kg) | 6 | 91 ± 5 | 27 ± 3                      | Yes            |
| Propranolol (5 mg/kg) | 5         | 76 ± 6          | 24 ± 4                      | Yes            |

* Starting blood pressure, mean ± S.E. Experimental procedure is described under “Methods.”

* Response observed 30 s after intravenous injection of 0.6 nmoles per kg of neurotensin, mean ± S.E.

* Tachyphylaxis was defined as the lack of response to a second equal dose given 5 min after the first.

* Refers to initial basal blood pressure level.

* One hour postoperative.

* Postoperative, 1 to 4 days.
neurotensin is given intradermally a greater amount of extractable dye accumulates at its site of the injection than that of saline (Fig. 10). Another indication of this increased permeability is the finding that the hematocrit of arterial blood increased from a control value (mean ± range) of 46 ± 2% in saline-injected animals (five rats) to 79 ± 9% in the experimental group (seven rats) 15 min after intravenous injection of 0.2 nmoles per kg of neurotensin.

Studies on Isolated Intestinal Tissue—Neurotensin stimulates the contraction of guinea pig ileum and rat uterus and the relaxation of rat duodenum in an organ bath (Fig. 11). Neurotensin was found to be as potent as bradykinin in its effect on guinea pig ileum and rat duodenum, but only one-fifth as potent as bradykinin on rat uterus. These responses still occur in the presence of atropine, tryptoamine, pyrilamine, phenoxybenzamine, and propranolol, showing that these effects are not mediated by acetylcholine, serotonin, or histamine nor do they involve α- or β-adrenergic receptors.

**DISCUSSION**

A new hypotensive peptide, designated neurotensin, has been isolated from bovine hypothalami and found to be a tridecapeptide composed of Lys, Arg, Asx, Glx, Pro, Ileu, Leuz, Tyrz. At the time of our initial report (24) the results indicated the presence of only one tyrosine moiety in this peptide. This is now known to have been an error caused by the degradation of tyrosine during acid hydrolysis of the minute amounts of peptide obtained. The apparent molecular weight of the extracted biologically active material measured by gel chromatography is 1600; this is in good agreement with the minimum molecular weight of the isolated peptide as calculated from its amino acid composition. However, since polypeptides can be generated from proteins during harsh stages of extraction and purification, it is conceivable that this tridecapeptide exists in hypothalamic tissue as part of a larger molecule. The evidence indicates that although the COOH terminus of isolated neurotensin is free, the NH2 terminus is blocked. It is possible that one of the two glutamic acid residues occupies the NH2-terminal position in the cyclized pyrrolidone carboxylic acid form as is true of two other hypothalamic peptides, thyrotropin releasing factor and gonadotropin releasing factor (25).
A reasonable suggestion is that it may play a neural role within this peptide. Since neurotensin is present in the hypothalamus, its property resembles that reported for the undecapeptide, rana-tensin, of the blood pressure of the anesthetized dog (27). The potency of neurotensin in producing a change in vascular permeability is comparable to that reported for bradykinin (18).

One can only speculate as to the physiological function of this peptide. Since neurotensin is present in the hypothalamus, a reasonable suggestion is that it may play a neural role within this tissue perhaps as a transmitter or as a modulator of nervous activity. It has been suggested that substance P, another peptide neural constituent, may be a sensory transmitter (28) and, indeed, recent evidence indicates that this peptide at a concentration of $10^{-4}$ M can cause depolarization of motor neurons in the spinal cord of the frog (29). However, the hypothalamus is also a major site of neurosecretion in mammals; therefore, an alternate suggestion is that neurotensin may function as a neurohormone. If this peptide does not prove to be a posterior pituitary hormone, nor a releasing factor for intermediate or anterior pituitary hormones, there is still an additional possibility. There is anatomical evidence to suggest that there are neurosecretory cells with short neurosecretory processes that synapse on capillaries within the hypothalamus (30). A neurosecretory substance could reach peripheral targets by this neurovascular pathway. The biological properties of neurotensin that have been investigated are manifest following intravenous injection and are not eliminated by hypophysectomy of the test animal. If any of these responses operate physiologically it could represent an instance where a hypothalamic peptide plays a neurohormonal role affecting visceral function independent of the pituitary. However, neurotensin may not be confined to the central nervous system; it may be a constituent of peripheral nerve or even other cells. The fact that neurotensin is a vaso- dilatory peptide which can induce changes in vascular permeability suggests that it may be one of the as yet unidentified peptides that play a role in neurogenic vasodilation (31) or the inflammatory response (32).

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