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Neuromedin N: presence and chromatographic characterization in the rat

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The distribution of neuromedin N and its structurally related peptide, neurotensin, was investigated in the rat and found to be remarkably similar with highest concentrations in the ileum. However, neuromedin N but not neurotensin was found in the kidney. Chromatographic analysis of immunoreactive neuromedin N demonstrated a single peak of immunoreactivity which was distinguishable from the single peak of immunoreactive neurotensin. Neuromedin N is likely to be a naturally occurring peptide and is distinct from neurotensin in rat peripheral tissues.

Neuromedin N; Neurotensin; Chromatography; Neuropeptide distribution; (Rat peripheral tissue)

1. INTRODUCTION

Neuromedin N, a six amino acid peptide, was recently isolated and purified from porcine spinal cord [1]. It has close sequence homology with neurotensin, a thirteen amino acid peptide widely distributed in the central nervous system and gastrointestinal tract of numerous mammalian species [2,3]. Neuromedin N enhances the contractility of guinea pig ileum [1], and has recently been reported to mimic the biological action of neurotensin in the rat ventral tegmental area [4]. The distribution of neuromedin N is unknown at present and because of its structural homology with neurotensin, we have investigated and compared the distribution of immunoreactive neuromedin N with that of neurotensin. Immunoreactive neuromedin N has been analysed by gel filtration and reverse high-pressure liquid chromatography (HPLC).

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2. MATERIALS AND METHODS

2.1. *Immunisation and iodination*

Neuromedin N (Peninsula Lab) was coupled to bovine serum albumin in a molar ratio of 4:1 by the glutaraldehyde method. Six rabbits received 100 nmol neuromedin N initially. Booster injections of 50 nmol conjugated neuromedin N were given at 5-8 weekly intervals. ^{125}I-neuromedin N was prepared by the chloramine T method [5]. The iodination product was purified by HPLC using a Techsil C18 column eluted with a stepwise gradient of acetonitrile from 20 to 80% containing 0.1% aqueous trifluoroacetic acid.

2.2. *Tissue extraction and radioimmunoassay*

Adult Wistar rats were killed by decapitation. Tissues were dissected, weighed, and extracted immediately in boiling 0.5 M acetic acid for 10 min (10:1, v/w). Tissue extracts were centrifuged for 15 min at 4°C, and supernatants were lyophilised. The samples were reconstituted in assay buffer (0.06 M phosphate buffer, pH 7.4, 10 mM EDTA, 0.3% gelatin). Aliquots were taken for assays for neuromedin N and neurotensin.

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Radioimmunoassays were set up in polystyrene tubes in a final volume of 0.8 ml with assay buffer. The final titre of the neuromedin N antiserum used was 1:4000, and the sensitivity of the assay was 75 fmol/tube. Measurements of immunoreactive neurotensin were as previously described [6], with both the C-terminally directed antiserum (NT58), and an N-terminally directed antiserum (a gift from Dr T. Moody).

2.3. *Chromatographic analysis*

Tissue extracts were pooled, and semi-purified on Sep-pak C18 sample preparation cartridges (Waters Associates, USA) prior to chromatography. Gel filtration and HPLC were performed as in [6]. For gel filtration, fractions of 2.0 ml were collected and evaporated to dryness. The fractions were reconstituted in 0.7 ml assay buffer, 0.6 ml of each fraction was assayed for neuromedin N, and 0.5 ml of each fraction was assayed for neurotensin with the C-terminally directed antiserum. For HPLC, fractions of 1 ml were collected and 100 μl of each fraction was assayed for neuromedin N, and neurotensin with both the C- and N-terminally directed antisera.

3. RESULTS AND DISCUSSION

This study demonstrates the discrete localization of neuromedin N in the peripheral tissues of the rat by radioimmunoassay. The cross-reactivity of neuromedin N antiserum with neurotensin 1–13 was 4.2% and with neurotensin 7–13, 9–13, 10–13 (gifts from Dr St Pierre) and lant-6, 6.6%. There was no cross-reactivity with tufsin, neuromedin B, C, and U, or other regulatory peptides including substance P, neurokinins, peptide YY, neuropeptide Y, cholecystokinin, or gastrin. Table 1 shows the distribution of neuromedin N, and neurotensin as measured using both the C- and N-terminally directed antisera. The highest concentrations of

Table 1

Regional distribution of immunoreactive neuromedin N and neurotensin in the rat (pmol peptide/g wet wt, mean ± SE, $n = 8$)

Region	NNLI	NTLI[a]	NTLI[b]
Stomach	1.0 ± 0.6	< 0.4	< 0.6
Jejunum	2.8 ± 1.5	5.7 ± 0.9	5.3 ± 0.8
Ileum	40.8 ± 5.9	89.6 ± 6.4	94.1 ± 8.6
Caecum	7.0 ± 1.4	15.5 ± 1.9	26.3 ± 3.6
Asc. colon	4.5 ± 2.1	15.3 ± 2.2	9.9 ± 2.7
Trans. colon	< 0.8	1.9 ± 0.6	1.6 ± 1.2
Desc. colon	< 0.8	< 0.4	< 0.6
Liver	3.3 ± 1.1	< 0.4	< 0.6
Kidney	17.0 ± 0.6	< 0.4	< 0.6
Heart	6.1 ± 1.6	< 0.4	< 0.6
Lung	3.3 ± 1.3	< 0.4	< 0.6

[a] Immunoreactive neurotensin measured with C-terminal antiserum

[b] Immunoreactive neurotensin measured with N-terminal antiserum

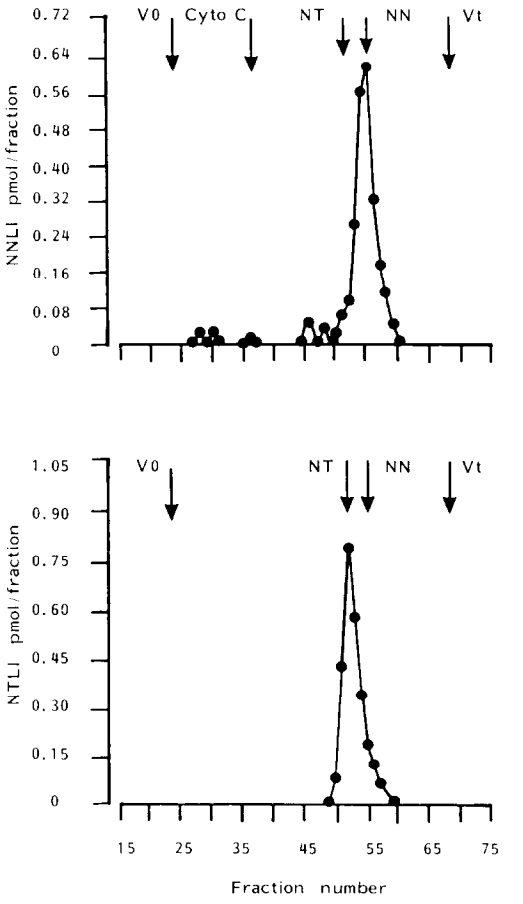

Fig.1. Gel filtration profiles of neuromedin N (NNLI) (upper) and neurotensin (NTLI) (lower) of pooled rat ileal extracts. The Sephadex G-50 superfine column (1.5 × 90 cm) was eluted with 0.06 M phosphate buffer, pH 7.4, containing 0.3% BSA and 0.2 M sodium chloride at 4°C. The elution positions of void volume (V_0) and total volume (V_t), synthetic neuromedin N and neurotensin are indicated.

neuromedin N, 40.8 ± 5.9 pmol/g (mean ± SE), were found in the terminal ileum. Considerable amounts were also found in the rat whole kidney, 17.0 ± 0.6 pmol/g (mean ± SE). Neurotensin had a similar pattern of distribution in the gut. However, 2-3-fold higher concentrations of neurotensin were detected in the same tissue extracts. Chromatographic characterization with both gel filtration and HPLC of the rat ileum and kidney extracts demonstrated that immunoreactivity co-eluted with synthetic neuromedin N (figs 1,2). When the same chromatographic fractions from the ileal extract were assayed with both the C- and N-terminally directed antisera, a single peak of immunoreactivity was detected co-eluting with synthetic neurotensin (fig.3). The elution position of neuromedin N is clearly distinguishable from that of neurotensin. This suggests that the immunoreactivity co-eluting with neuromedin N was not a breakdown product of tissue neurotensin. One of the striking findings in this study is that a considerable amount of immunoreactive neuromedin N is found in the rat kidney, whereas no immunoreactive neurotensin was detected with either neurotensin antisera. The absence of neurotensin in the rat kidney is in agreement with the finding of Godert and Emson [7] in the cat. However, as neurotensin-like receptors are found in the kidney [8], the exact nature of these receptors in relation

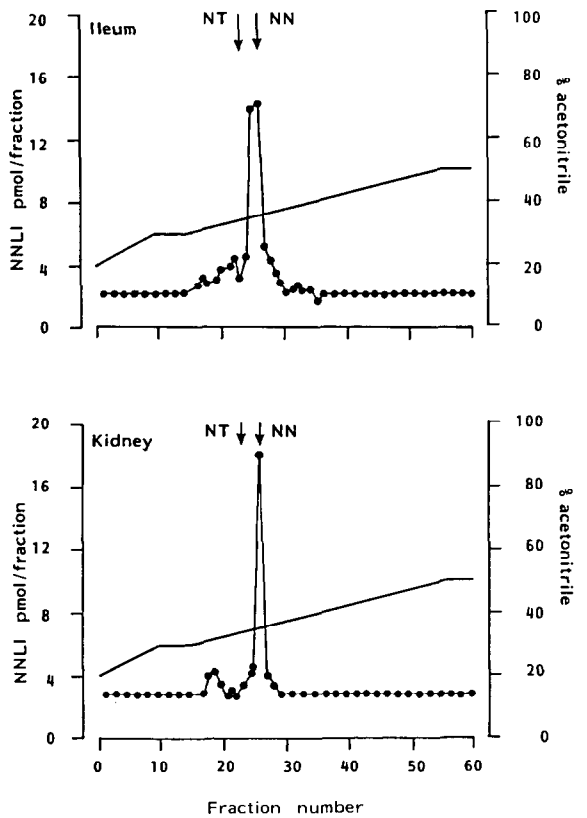

Fig.2. HPLC profiles of neuromedin N (NNLI) of pooled ileal extracts (upper) and pooled kidney extracts (lower). The Techsil C18 column (0.39 × 30 cm) was eluted with a gradient from 20 to 50% (0.5%/min) acetonitrile in distilled water containing 0.1% trifluoroacetic acid. Fractions of 1 ml were collected, and aliquots (0.1 ml) of each fraction were assayed for neuromedin N. The elution positions of neuromedin N and neurotensin are indicated.

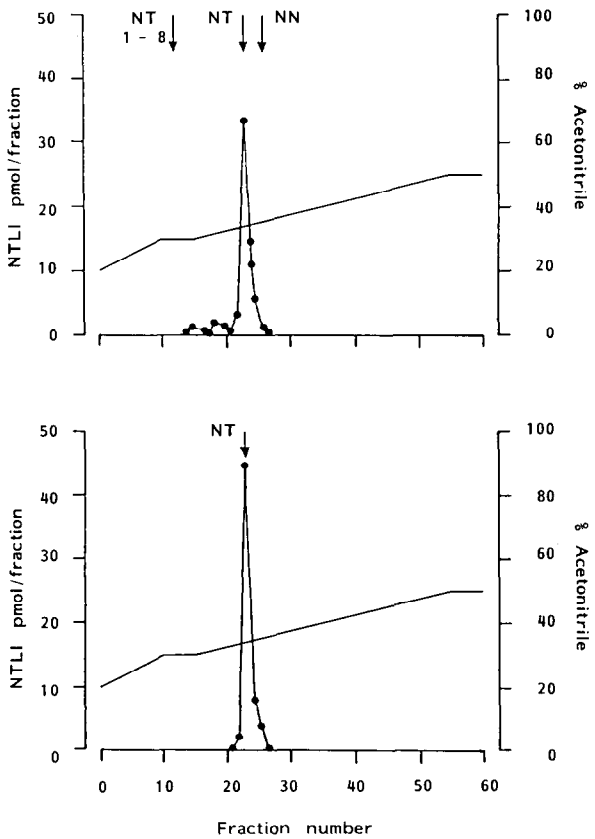

Fig.3. HPLC profiles of neurotensin (NTLI). The same chromatographic fractions of pooled ileal extracts as in fig.2 were measured for neurotensin with the C-terminally directed antiserum (upper) and N-terminally directed antiserum (lower).

to neuromedin N requires elucidation. Checler et al. [9] have most recently reported that neuromedin N binds to neurotensin receptors in the rat hypothalamus. Structurally related bioactive peptides may be derived from the same peptide precursor, a duplication of genetic material having occurred earlier in evolution. Post-translational processing and organ-specific differentiation of the peptide precursor may occur, allowing diversification of biological function. The question that neuromedin N and neurotensin may be derived from the same mRNA remains open but if so it is clear that post-translational enzymic processing must differ from tissue to tissue.

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