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THE STIMULATORY EFFECTS OF NEUROTENSIN AND RELATED PEPTIDES IN RAT STOMACH STRIPS AND GUINEA-PIG ATRIA

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1 The stimulatory effects of neurotensin (NT) and several NT fragments were evaluated in two pharmacological preparations: rat stomach strips and isolated spontaneously beating atria of guinea-pigs.

2 In rat stomach strips, NT elicited a dose-dependent contractile effect in concentrations varying between 1.3×10^{-9} and 5.4×10^{-7} M.

3 The contractile effect of NT (1.3 and 5.4×10^{-8} M) in this tissue was not modified by atropine (3.4×10^{-7} M), methysergide (2.0×10^{-6} M), a mixture of cimetidine (8.0×10^{-6} M) and diphenhydramine (7.8×10^{-6} M), indomethacin (1.4×10^{-5} M), 8-Leu-angiotensin II (1.0×10^{-6} M), glucagon (2.0×10^{-6} M) or somatostatin (3.0×10^{-7} M).

4 Rat stomach strips desensitized by bradykinin (6.1×10^{-6} M) or substance P (7.4×10^{-6} M) maintained their sensitivities to NT (1.3 and 5.4×10^{-8} M).

5 In guinea-pig atria, NT produced a dose-dependent positive inotropic action in concentrations varying between 5.4×10^{-10} and 2.7×10^{-7} M.

6 The inotropic effect of NT (2.7×10^{-9} M) was not influenced by methysergide (2.8×10^{-6} M), atropine (3.4×10^{-7} M), practolol (1.5×10^{-5} M), 8-Leu-angiotensin II (1.0×10^{-6} M), or indomethacin (1.4×10^{-5} M), but it was reduced by 37% by cimetidine (4.0×10^{-5} and 2.0×10^{-4} M). A combination of cimetidine (4.0×10^{-5} M) and diphenhydramine (3.9×10^{-6} M) did not produce a greater inhibition of NT than cimetidine alone.

7 Atria desensitized by bradykinin (6.1×10^{-6} M) or glucagon (2.0×10^{-6} M) maintained their sensitivities to NT (2.7×10^{-9} M). Substance P was inactive both as an agonist or antagonist of NT.

8 These results suggest the existence of specific NT receptors in rat stomach strips and guinea-pig atria.

9 The data derived from our structure-activity study suggest that the minimum structure required for the full stimulation of NT receptors in these two preparations is H-Arg^{9}-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH. The sequence PyroGlu^{1}-Leu^{2}-Tyr^{3}-Glu^{4}-Asn^{5}-Lys^{6}-Pro^{7}-Arg^{8}- and the amino acids Ile^{12} and Leu^{13} appear to contribute mainly to the affinity or binding of NT to its receptor. The chemical groups responsible for the full activation (intrinsic activity) of NT receptors seem to be located in the sequence -Arg^{9}-Pro^{10}-Tyr^{11}.

Introduction

Neurotensin (NT) is a newly discovered peptide (Carraway & Leeman, 1973) which is distributed in the central nervous system (Carraway & Leeman, 1976; Uhl, Kuhar & Snyder, 1977; Kobayashi, Brown & Vale, 1977) and in some regions of the digestive tract (Carraway & Leeman, 1976; Orci, Baetens, Rufner, Brown, Vale & Guillemin, 1976; Polack, Sullivan, Bloom, Buchan, Facer, Brown & Pearse, 1977). The peptide exhibits a large spectrum of biological activities (for reviews, see Leeman, Mroz & Carraway, 1977; Bissette, Manberg, Nemeroff & Prange Jr., 1978). However, its physiological role remains unknown. The recent demonstration of the presence of NT-like immunoreactivity in the blood of man (Blackburn, Bloom & Polack, 1978) as well as of its elevated blood concentration following the ingestion of food (Mashford, Nilsson, Rokaeus & Rosell, 1978; Besterman, Bloom, Sarson, Blackburn, Johnston,

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Patel, Stewart, Modigliani, Guerin & Mallinson, 1978) raised the possibility that NT behaves as a circulating hormone.

Specific NT antagonists should prove to be very useful for evaluating the possible pathophysiological role of NT. Such compounds are not yet available despite the fact that structure-activity studies oriented toward the identification of the chemical groups responsible for the biological activity of NT have already been published (Carraway & Leeman, 1975; Segawa, Hosokawa, Kitagawa & Yajima, 1977; Rivier, Lazarus, Perrin & Brown, 1977; Loosen, Nemeroff, Bissette, Burnett, Prange Jr., & Lipton, 1978). The results of these studies provide convincing evidence that the major determinants of the biological activity (e.g. hypothermia, hypotension, hyperglycaemia, gut contraction) of NT reside primarily in its COOH-terminal region (Leeman *et al.*, 1977; Loosen *et al.*, 1978). Whether the receptors mediating the various biological effects of NT are all of the same type remains to be determined.

Preliminary reports have been published on the positive chronotropic and inotropic effect of NT (Quirion, Rioux & Regoli, 1978) and its contractile action in rat stomach strips (Rokaeus, Burcher, Chang, Folkers & Rosell, 1977). As an extension of these studies, we decided to evaluate further the interaction between NT and its cardiac and smooth muscle receptors using various antagonists and desensitizing drugs. This paper also describes our attempts to identify the chemical groups responsible for binding NT to its receptors and those required for their activation.

Methods

General procedures

Albino Wistar rats of either sex (Can. Breeding Lab., St-Constant, Quebec) weighing between 250 and 350 g were killed by a blow on the neck and exsanguinated by cutting the carotid arteries. The stomach was taken out. The fundus was separated from the whole organ, emptied of its content, washed with a cold (4°C) Krebs solution and cut longitudinally into strips averaging 3.0 cm long and 1.5 mm wide (Vane, 1957). The tissues were mounted under a resting tension of 2.0 g in 15 ml organ baths containing an oxygenated (95% O_2 and 5% CO_2) Krebs solution maintained at 37°C with a thermostated circulator (Haake model FJ). The composition of the Krebs solution was as follows (mM): NaCl 118.0, $MgSO_4.7H_2O$ 1.18, KH_2PO_4 1.18, glucose 5.55, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and KCl 4.7. The tissues were equilibrated for 45 to 60 min before being challenged with drugs. The contractions of the tissues were recorded by means of force displacement transducers (Grass, FT03) coupled to a Grass polygraph (Model 79).

Male or female guinea-pigs (450 to 550 g) were purchased from a local breeder. The animals were killed by a blow on the neck and the heart rapidly removed and placed in an oxygenated Krebs solution as above. Both atria were dissected out from the ventricles and cleaned of fat and blood. The tissues were mounted under a tension of 0.5 g in 15 ml organ baths in the presence of an oxygenated Krebs solution maintained at 30°C with a thermostated circulator. Under these conditions, the atria started beating spontaneously. The force of contraction was recorded as described above for the stomach strip. The period of equilibration before the injections of drugs was 45 to 60 min.

Construction of dose-response curves

In preliminary experiments, we found that reproducible responses to NT were obtained in rat stomach strips when the intervals between the injections were 15 to 30 min for low to medium concentrations and 55 to 60 min for higher ones. In guinea-pig atria, it was 20 to 30 min and 55 to 60 min respectively. The volume of drug injections varied between 0.05 and 0.2 ml.

The dose-response curves to NT or one of its fragments were obtained by consecutive applications of increasing concentrations of the peptide to the tissues. The usual schedule of injections in rat stomach strips was as follows: NT (ED_{100}) → NT (ED_{10} to ED_{40}) → NT fragment (ED_{10} to ED_{40}) → NT (ED_{50} to ED_{80}) → NT fragment (ED_{50} to ED_{80}) → NT (ED_{80} to ED_{100}) → NT fragment (ED_{80} to ED_{100}). A similar schedule of injections was used for the guinea-pig atria except that the maximally effective concentration of NT (ED_{100}) was given only at the end of the experiment. No more than two dose-response curves (one for NT and one for NT fragment) were obtained on each tissue.

Specificity of action of neurotensin in rat stomach strips and guinea-pig atria

We investigated the possibility that the stimulatory action of NT in these preparations was due to the release of endogenous substances (e.g. acetylcholine, noradrenaline, histamine, 5-hydroxytryptamine, prostaglandins), was influenced by one of them, or was provoked by the interaction of NT with other receptors such as those for angiotensin, bradykinin or others. In these experiments, NT and control agonists were tested in the absence and presence of relatively high concentrations of various drug antagonists. The stimulant action of NT was also measured

on tissues desensitized with various drugs for which no antagonist was available.

Drugs and solutions

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (5-HT), atropine sulphate (Sigma), histamine dihydrochloride (Fisher), diphenhydramine hydrochloride (Parke-Davis), cimetidine (Smith Kline & French), indomethacin lactose (Merck), acetylcholine chloride (ACh, Sigma), 1-Asp, 8-Leu-angiotensin II (8-Leu-AT_{II}) (synthesized in our laboratory by the late Dr W.K. Park), somatostatin (Peninsula), glucagon (Eli Lilly), neurotensin (Peninsula) and bradykinin triacetate (synthesized in our laboratory by Dr Serge St-Pierre). Neurotensin (NT) and its fragments were also synthesized in our laboratory (St-Pierre, Quirion, Regoli & Rioux, unpublished). The details of the synthesis and purification procedures will be published elsewhere at a later date. The primary structure of the various NT fragments used in these experiments is described in Table 1. Indomethacin was dissolved in trizma base (Sigma) (0.2 M). All other drugs were dissolved in saline (NaCl, 0.9% w/v). Stock solutions of histamine, adrenaline and acetylcholine were acidified to pH 4.0 with HCl. Ascorbic acid (10^{-4} M) was added to each daily dilution of adrenaline. Concentrations of all drugs are expressed in moles per litre of the salt.

The results are expressed as means ± the standard error of the mean (s.e. mean). The statistical significances were evaluated by Student's *t* test for paired samples and *P* values of 0.05 or less were considered significant.

Results

Effects of various drugs on the stimulant action of neurotensin in rat stomach strips and guinea-pig atria

The contractile effects of NT (1.3 and 5.4 × 10^{-8} M) in rat stomach strips were not modified by methysergide, atropine, a mixture of cimetidine and diphenhydramine, and 8-Leu-AT_{II} while those produced by their respective agonists (5-HT, ACh, histamine, angiotensin II (AT_{II}) were abolished. Indomethacin, a potent inhibitor of prostaglandin synthesis (Vane, 1971) also failed to inhibit the effect of NT in this preparation. These results are summarized in Table 2.

Rat stomach strips exposed to high concentrations of bradykinin (6.1 × 10^{-6} M) for 60 min or substance P (7.4 × 10^{-6} M) for 40 min became desensitized to bradykinin and substance P, respectively. These tissues remained fully responsive to medium concentrations (1.5 and 6.2 × 10^{-8} M) of NT (6 experiments). High concentrations of glucagon (2.0 × 10^{-6} M) or somatostatin (3.0 × 10^{-7} M) did not influence the contractile effects of NT in rat stomach strips (6 experiments).

The inotropic action of NT (2.7 × 10^{-9} M) in isolated spontaneously beating atria of guinea-pigs was not inhibited by methysergide, atropine, practolol and 8-Leu-AT_{II} while the effects produced by their respective agonists were depressed or abolished. The responses of the tissues to NT were not modified by indomethacin. These results are presented in Table 3. On the other hand, as shown in Table 4, cimetidine (4.0 × 10^{-5} or 2.0 × 10^{-4} M) was found to inhibit partially the inotropic effects of NT and histamine, but not those elicited by an equipotent concentration

Table 1 Primary structure of neurotensin and various fragments

Name	1	2	3	4	5	6	7	8	9	10	11	12	13
Neurotensin (NT)	<Glu-	Leu-	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{2-13}	—	Leu-	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{3-13}	—	—	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{4-13}	—	—	—	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{6-13}	—	—	—	—	—	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{7-13}	—	—	—	—	—	—	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{8-13}	—	—	—	—	—	—	—	Arg-	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{9-13}	—	—	—	—	—	—	—	—	Arg-	Pro-	Tyr-	Ile-	Leu.OH
NT_{10-13}	—	—	—	—	—	—	—	—	—	Pro-	Tyr-	Ile-	Leu.OH
NT_{1-12}	<Glu-	Leu-	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr-	Ile.OH	
NT_{1-11}	<Glu-	Leu-	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro-	Tyr.OH		
NT_{1-10}	<Glu-	Leu-	Tyr-	Glu-	Asn-	Lys-	Pro-	Arg-	Arg-	Pro.OH			

Table 2 Effects of various inhibitors on the contractile effects of neurotensin (NT) and other agonists in the rat stomach strip

Antagonist (M)	*Agonist*	*Dose* (M)	*Contraction (g)* *Before*	*After*
Methysergide	5-HT	2.8×10^{-9}	1.7 ± 0.1	0*
(2.0×10^{-6})		2.8×10^{-8}	2.1 ± 0.2	0*
	NT	1.3×10^{-8}	1.7 ± 0.2	1.7 ± 0.2
		5.4×10^{-8}	2.8 ± 0.2	2.7 ± 0.1
Atropine	ACh	3.4×10^{-9}	1.7 ± 0.1	0*
(3.4×10^{-7})		3.4×10^{-8}	2.3 ± 0.2	0*
	NT	1.3×10^{-8}	1.6 ± 0.2	1.7 ± 0.2
		5.4×10^{-8}	2.4 ± 0.2	2.4 ± 0.2
Cimetidine	Hist	2.2×10^{-5}	2.6 ± 0.3	0*
(8.0×10^{-6})		8.7×10^{-5}	3.4 ± 0.3	0*
+ diphenhydramine	NT	1.3×10^{-8}	1.9 ± 0.2	1.8 ± 0.2
(7.8×10^{-6})		5.4×10^{-8}	3.0×0.3	2.6 ± 0.2
Indomethacin	NT	1.3×10^{-8}	1.8 ± 0.2	1.6 ± 0.2
(1.4×10^{-5})		5.4×10^{-8}	2.9 ± 0.2	3.0 ± 0.2
8-Leu-AT_{II}	AT_{II}	1.0×10^{-9}	1.3 ± 0.1	0*
(1.0×10^{-6})		5.0×10^{-9}	1.7 ± 0.1	0*
	NT	1.3×10^{-8}	1.8 ± 0.2	1.9 ± 0.2
		5.4×10^{-8}	2.9 ± 0.2	2.8 ± 0.2

The results are expressed as means ± s.e. mean of 6 to 8 experiments. The antagonists were left in contact with the tissues for 20 min before repeating the injections of agonists. 5-HT = 5-hydroxytryptamine; ACh = acetylcholine; Hist = histamine; AT_{II} = angiotensin II; M = Molar concentration; g = g of tension developed.
* $P < 0.001$.

Table 3 Effects of various inhibitors on the inotropic action of neurotensin (NT) and other agonists in spontaneously beating atria of guinea pigs

Antagonist (M)	*Agonist*	*Dose* (M)	*Developed tension (% increase)* *Before*	*After*
Methysergide	5-HT	2.8×10^{-7}	$+61.9 \pm 1.8$	$+17.6 \pm 0.2$*
(2.8×10^{-6})	NT	2.7×10^{-9}	$+67.8 \pm 1.0$	$+67.6 \pm 1.0$
Atropine	ACh	6.8×10^{-9}	-45.2 ± 1.0	0*
(3.4×10^{-7})	NT	2.7×10^{-9}	$+67.2 \pm 1.2$	$+67.5 \pm 1.1$
Practolol	Ad	6.2×10^{-8}	$+63.4 \pm 0.9$	0*
(1.5×10^{-5})	NT	2.7×10^{-9}	$+67.2 \pm 0.8$	$+65.3 \pm 1.0$
8-Leu-AT_{II}	AT_{II}	5.0×10^{-8}	$+61.5 \pm 3.4$	0*
(1.0×10^{-6})	NT	2.7×10^{-9}	$+68.0 \pm 2.9$	$+66.8 \pm 2.1$
Indomethacin	NT	2.7×10^{-9}	$+67.5 \pm 1.1$	68.1 ± 0.9
(1.4×10^{-5})				

The results are expressed as means ± s.e. mean of 7 to 8 experiments. The antagonists were left in contact with the tissues for 20 min before repeating the injections of agonists. Ad = adrenaline; 5-HT = 5-hydroxytryptamine; ACh = acetylcholine; AT_{II} = angiotensin II.
* $P < 0.001$.

of adrenaline. The inotropic action of NT and histamine could not be abolished by cimetidine. A combination of cimetidine (4.0×10^{-5} M) and diphenhydramine (3.9×10^{-6} M) inhibited by more than 90% the inotropic effect of histamine and by 30 to 35% that produced by NT.

The inotropic action of NT (2.7×10^{-9} M) was fully maintained in isolated atria previously desensitized by exposure to high concentrations of bradykinin (6.1×10^{-6} M) or glucagon (2.0×10^{-6} M) (6 experiments). Substance P was inactive in this preparation both as an agonist or as an antagonist of NT (6 experiments). The selective blockade of NT-induced inotropic effects by somatostatin in guinea-pig atria has been described before (Quirion, Regoli, Rioux and St-Pierre, 1979).

Dose-response curves of neurotensin and neurotensin fragments in rat stomach strips and cimetidine-treated guinea-pig atria

The guinea-pig atria were exposed to cimetidine (4.0×10^{-5} M) throughout the experiments in order to eliminate the histaminergic component of the stimulant effect of NT and eventually, of NT fragments (see above). Without this precaution, it would have been difficult to compare on the same basis the potency of the various peptides since we did not know whether all the peptides had the ability to release tissue histamine or, alternatively, to interact with histamine receptor.

The dose-response curves obtained in rat stomach strips and guinea-pig atria are illustrated in Figures 1 and 2, respectively. A more complete description of the results is presented in Table 5. For the sake of clarity, we did not include in the figures the dose-response curves of NT fragments exhibiting approximately the same potency as NT. NT_{10-13} is inactive in both tissues. The dose-response curves to NT_{2-13}, NT_{3-13}, NT_{4-13}, NT_{6-13}, NT_{7-13}, and NT_{8-13} remained parallel to that for NT and exhibited the same maximum responses. Only minor losses (10 to 48%) of potency (when compared to NT) were observed with these compounds. NT_{9-13} exhibited 11% and 1.3% of the potency of NT in rat stomach strips and guinea-pig atria, respectively. The ability of NT_{9-13} to activate the receptors was fully maintained. On the other hand, NT_{1-12} and NT_{1-11} showed large decreases in potency in both preparations and none (NT_{1-12}) or slight reduction (NT_{1-11}) in their maximum effects (Table 5). NT_{1-10} was completely inactive as an agonist or antagonist in the two preparations (4 experiments).

Discussion

The ability of NT to stimulate or to inhibit the contractions of intestinal smooth muscles is well established (Carraway & Leeman, 1973; Segawa *et al.*, 1977; Rokaeus *et al.*, 1977; Kitabgi & Freychet, 1978).

Table 4 Effect of histamine receptor blocking drugs on the positive inotropic action of neurotensin (NT) and histamine (Hist) in spontaneously beating atria of guinea-pigs

Antagonist (M)	*Agonist*	*Dose* (M)	*Developed tension (% increase)* *Before*	*After*
Cimetidine	Hist	8.7×10^{-8}	56.8 ± 2.6	34.3 ± 1.8***
(8.0×10^{-6})	NT	2.7×10^{-9}	64.0 ± 2.9	63.9 ± 2.9
Cimetidine	Hist	8.7×10^{-8}	58.4 ± 3.2	23.7 ± 1.1***
(4.0×10^{-5})	Ad	6.2×10^{-8}	63.1 ± 2.5	65.5 ± 3.5
	NT	2.7×10^{-9}	65.9 ± 3.3	42.3 ± 3.2***
Cimetidine	Hist	8.7×10^{-8}	57.6 ± 3.0	22.9 ± 2.7***
(2.0×10^{-4})	Ad	6.2×10^{-8}	64.9 ± 2.6	66.2 ± 3.1
	NT	2.7×10^{-9}	66.1 ± 2.7	40.7 ± 3.3**
Cimetidine	Hist	4.3×10^{-8}	25.0 ± 1.1	0*
(4.0×10^{-5})	Hist	8.7×10^{-8}	63.7 ± 1.1	0*
+ diphenhydramine	Hist	4.3×10^{-6}	86.5 ± 3.2	5.2 ± 1.4****
(3.9×10^{-6})	NT	5.4×10^{-10}	20.6 ± 1.5	14.1 ± 2.0*
	NT	2.7×10^{-9}	65.2 ± 2.5	43.1 ± 4.0***
	NT	5.4×10^{-8}	104.4 ± 4.5	76.7 ± 3.2***

The results are expressed as means ± s.e. mean of 6 to 8 experiments. The antagonists were left in contact with the tissues for 20 min before repeating the injections of Hist or NT.
* $P < 0.02$; ** $P < 0.005$; *** $P < 0.001$.

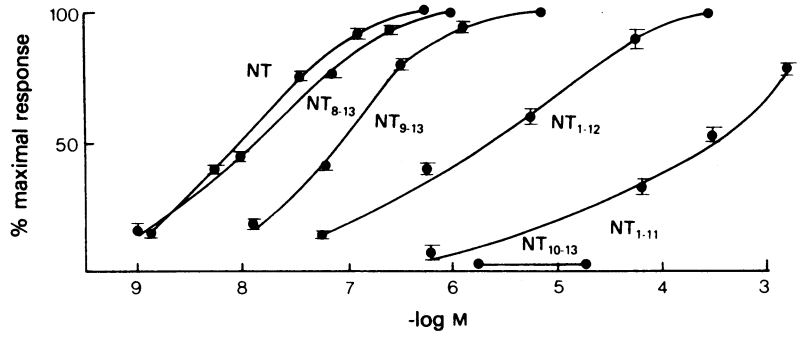

Figure 1 Dose-response curves illustrating the contractile effects of increasing concentrations of neurotensin (NT) and various NT fragments as measured in rat stomach strips. Each point is the mean value; vertical lines show s.e. mean. The number of individual determinations is given in Table 5.

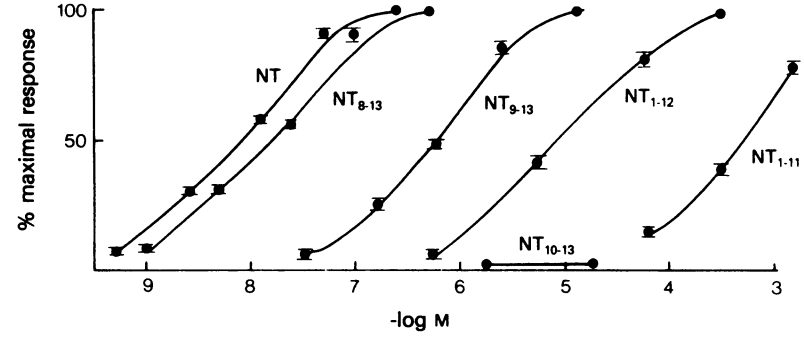

Figure 2 Dose-response curves illustrating the positive inotropic effects of increasing concentrations of neurotensin (NT) and various NT fragments as measured in isolated spontaneously beating atria of guinea-pigs. Each point is the mean value; vertical lines show s.e. mean. The number of individual determinations is given in Table 5. The guinea-pig atria were exposed to cimetidine (4.0×10^{-5} M) throughout the experiments.

The guinea-pig isolated ileum has been used for structure-activity studies of NT (Carraway & Leeman, 1975; Segawa *et al.*, 1977). However, its usefulness for such studies may be questioned since the action of NT in this preparation was shown recently to be biphasic (relaxation followed by a contraction) and to involve at least two components: a direct action on the muscle fibre causing relaxation and an indirect action possibly due to the release of acetylcholine from intramural cholinergic nerves and associated with a contraction (Kitabgi & Freychet, 1978). On the other hand, the rat fundus strip seems to be a suitable assay organ for NT, its fragments or derivatives. The contractile effect of NT in this preparation was shown to be unmodified by atropine, hexamethonium, morphine, methysergide and 7-OH-tetrahydrocannabinol (Rokaeus *et al.*, 1977). These results led to the suggestion that NT does not stimulate this tissue by releasing acetylcholine or 5-hydroxytryptamine. Our results support this interpretation and further suggest that the contractile effect of NT in rat stomach strips is not mediated by the intramural release of histamine or prostaglandins. The myotropic effect of NT was also not influenced by 8-Leu-AT_{II}, a well characterized angiotensin antagonist (Regoli, Park & Rioux, 1974) and by somatostatin and glucagon, two peptides known to be present in gastric tissues (Unger, Ketterer & Eisentraut, 1966; Arimura, Sato, Dupont, Nishi & Schally, 1975). Rat stomach strips desensitized with bradykinin or substance P

were found to maintain their sensitivity to NT. These results strongly suggested the existence of specific NT receptors in smooth muscle cells of rat stomach.

The positive inotropic action of NT in isolated spontaneously beating atria of guinea-pigs as well as its inhibition by somatostatin were described previously (Quirion *et al.*, 1978; Quirion *et al.*, 1979). In this paper, we have provided evidence that part of the action of NT in this organ seems to be mediated by the release of tissue histamine or by the interaction of NT with cardiac histamine receptors. This hypothesis is based on the observation that cimetidine, a specific H_2-receptor antagonist (Brimblecombe, Duncan, Durant, Emmet, Ganellin & Parsons, 1975), inhibited partially (30 to 35%) the inotropic action of NT. A maximally effective concentration of cimetidine used in combination with diphenhydramine produced a greater inhibition of histamine-induced inotropic effects than cimetidine alone. This observation is consistent with previous reports which suggested the existence of H_1- and H_2-receptors in guinea-pig atria (Reinhart, Wagner & Schumann, 1974). However, the drug combination did not produce a larger inhibition of the action of NT than cimetidine alone. Other antagonists such as methysergide, atropine, practolol, 8-Leu-AT_{II} and indomethacin did not influence the cardiostimulant effect of NT. The desensitization of the atria by bradykinin or glucagon did not modify NT-induced inotropic effects. These results suggest that NT produced its inotropic action by interacting with specific NT receptors presumably located in atrial cell membranes. Part of this action appears to be mediated either by the release of tissue histamine or a direct interaction with histamine receptor, or by both mechanisms. Further studies are needed to clarify this issue.

Both rat stomach strips and cimetidine-treated atria of guinea-pigs were found suitable for studying the relationship between the chemical structure of NT and its myotropic or inotropic activity. Besides their relatively high sensitivity to NT, the two preparations allowed the measurement of at least two complete dose-response curves to NT or its fragments. Using both assays to evaluate the biological activities of NT and NT fragments, we came to the following conclusions: (a) The minimum structure required to produce the maximum response in the two preparations is H-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH; (b) The sequence 1–8 and the amino acids Ile^{12} and Leu^{13} contribute mainly to the affinity or binding of NT to its receptors; this is supported by the fact that NT_{2-13}, NT_{3-13}, NT_{4-13}, NT_{6-13}, NT_{7-13}, NT_{8-13}, NT_{9-13}, NT_{1-12} and NT_{1-11}, albeit less potent than NT, were able to elicit more or less the same maximum response as NT; (c) The sequence 9–11 (Arg^9-Pro^{10}-Tyr^{11}) appears to contain the chemical groups responsible for the intrinsic activity or ability of NT to stimulate its receptors; this is consistent with the absence of effect of NT_{10-13} and NT_{1-10}. Further studies with NT derivatives substituted in position 9, 10 and 11 are needed before definite conclusions could be drawn on the relative contribution of Arg^9,

Table 5 Maximum effects (ME), effective concentrations producing 50% of ME (ED_{50}) and relative potencies of neurotensin and several of its fragments as measured in rat stomach strips and guinea-pig atria

	Rat stomach strips			*Guinea-pig atria*		
	ME (%)	*ED_{50}* ($\times 10^{-8}$ M)	*Relat. potency*	*ME* (%)	*ED_{50}* ($\times 10^{-8}$ M)	*Relat. potency*
Neurotensin (NT)	100	1.1 ± 0.1(68)	100	100	0.9 ± 0.1(65)	100
$NT_{(2-13)}$	100	1.2 ± 0.2(7)	90	100	1.0 ± 0.1(8)	94
$NT_{(3-13)}$	100	1.2 ± 0.2(7)	90	100	1.1 ± 0.1(7)	84
$NT_{(4-13)}$	100	1.3 ± 0.1(7)	85	100	0.94 ± 0.1(7)	99
$NT_{(6-13)}$	100	1.4 ± 0.2(7)	78	100	1.3 ± 0.1(7)	73
$NT_{(7-13)}$	100	1.7 ± 0.1(7)	65	100	1.9 ± 0.1(6)	49
$NT_{(8-13)}$	100	1.6 ± 0.2(7)	69	100	1.8 ± 0.2(6)	52
$NT_{(9-13)}$	100	10.0 ± 1.0(7)	11	100	69.0 ± 6.0(7)	1.3
$NT_{(10-13)}$	0	—(7)	—	0	—(7)	—
$NT_{(1-12)}$	100	170.0 ± 20.0(8)	0.65	100	1000 ± 100(6)	0.09
$NT_{(1-11)}$	80–100	25,000 – 35,000(3)	<0.01	80–100	40,000 – 50,000(3)	<0.01
$NT_{(1-10)}$	0	—(4)	—	0	—(4)	—

ED_{50} values are expressed as means ± s.e. means. In parentheses, the number of individual determinations. ED_{50} values of NT_{1-11} were approximate. The guinea-pig atria were exposed to cimetidine (4.0×10^{-5} M) throughout the experiments.

Pro^{10} and Tyr^{11} to the intrinsic activity of NT. Our conclusions give further support to the hypothesis put forward by various authors concerning the critical role of the C-terminal region of NT for its biological activity (see the introduction). The structural requirements of NT receptors in cardiac and smooth muscle tissue appear to be similar to those observed in other organs but further studies with specific NT antagonists are needed to support this conclusion.

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