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STRUCTURE-ACTIVITY STUDIES WITH NEUROTENSIN: ANALYSIS OF POSITIONS 9, 10 and 11

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1 The stimulant effects of neurotensin (NT) and NT analogues modified in positions 9, 10 or 11 were evaluated and compared in two pharmacological preparations: the rat stomach strip and the isolated spontaneously beating atria of guinea-pig.

2 The data derived from our structure-activity study suggest that Arg^9 and Pro^{10} mainly contribute to the affinity of neurotensin for its cardiac and smooth muscle receptors. Tyr^{11} seems to be more closely involved in the process of receptor activation by NT.

3 The order of potency of some NT analogues modified in position 11 (e.g. [D-Phe^{11}]-NT, [D-Tyr^{11}]-NT) was strikingly different from that described in other systems (e.g. hypothermia test and specific mast cell binding). The importance of this observation is discussed.

Introduction

In a previous study, we have described the use of the rat stomach strip and of the guinea-pig atria to investigate the relationships between the chemical structure and myotropic or inotropic activities of neurotensin (NT) (Quirion, Regoli, Rioux & St-Pierre, 1980), a peptide of nervous and intestinal origin (Carraway & Leeman, 1973; 1976a; 1976b) which is known to exert a large variety of biological effects in mammals (Leeman, Mroz & Carraway, 1977; Bissette, Manberg, Nemeroff & Prange Jr., 1978). The data derived from our structure-activity studies suggested that the N-terminal sequence < Glu^1-Leu^2-Tyr^3-Glu^4-Asn^5-Lys^6-Pro^7-Arg^8 and the amino acids Ile^{12} and Leu^{13} contribute mainly to the affinity or ability of the peptide to bind to its cardiac and smooth muscle receptors while the sequence -Arg^9-Pro^{10}-Tyr^{11} appears to contain the chemical groups responsible for the intrinsic activity or the ability of NT to stimulate its receptors (Quirion *et al.*, 1980). The relative contribution of Arg^9, Pro^{10} and Tyr^{11} to the myotropic or inotropic activities of NT is still unknown.

As an extension of this work, several NT analogues substituted in position 9, 10 or 11 were designed and evaluated for their biological activities. The results described in this paper suggest that Tyr^{11} is more closely involved in the process of receptor activation by NT. Our results are discussed in the light of current knowledge of the relationships between chemical structure and biological actions of NT in other systems.

Methods

General procedures

Albino Wistar rats of either sex (Can. Breeding Lab., St-Constant, Que.) weighing between 250 and 350 g, were used. The animals were killed by a blow on the neck and bled by cutting the carotid arteries. The stomach was taken out. Fundus strips were prepared according to Vane (1957). The tissues were mounted under a resting tension of 2 g in 15 ml organ baths containing an oxygenated, warmed (37°C) Krebs solution.

Hearts were excised from guinea-pigs (450 to 550 g) of either sex purchased from a local breeder. Both atria were separated from the rest of the heart, cleaned of fat and blood and mounted under a tension of 0.5 g in 15 ml organ baths in the presence of oxygenated, warmed (30°C) Krebs solution. Other technical details have been described previously (Quirion *et al.*, 1980). The experimental protocols used to measure the dose-response curves of NT and of its analogues have also been given in detail in the latter paper.

Drugs and solutions

The primary structure of the peptides used in this study are presented in Table 1. These peptides were synthesized in our laboratory by Dr Serge St-Pierre. The details of the synthesis and purification procedures will be published elsewhere. Concentrated solutions of the various peptides were prepared in

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saline (0.9% w/v NaCl solution) and kept frozen until used. Daily dilutions of the compounds were made in saline. Concentration of peptides are expressed in moles/litre of the salt.

The results are expressed as mean ± s.e. of the mean (s.e. mean).

Results

The dose-response curves obtained with NT and its analogues in rat stomach strips and guinea-pig atria are shown in Figures 1 and 2, respectively. Maximum effects, ED_{50} values and relative potencies of the various compounds are presented in Table 2. In rat stomach strips the dose-response curves to [D-Arg^9]-NT and [Ala^9]-NT were displaced to the right of that to NT but remained parallel and exhibited the same maximum. Similar results were obtained in guinea-pig atria (Figure 2). The replacement of Arg^9 with D-Arg decreased the potency of NT by a factor of 150 and 25 in rat stomach strips and guinea-pig atria, respectively. On the other hand, the substitution of Arg^9 with Ala reduced the potency of NT by a factor of 67 in rat stomach strips and 38 in guinea-pig atria.

The dose-response curves to [D-Pro^{10}]-NT (Figures 1 and 2) remained parallel to that to NT and exhibited the same maximum response. However, [D-Pro^{10}]-NT was 1000 and 250 times less potent than NT in the rat stomach strips and guinea-pig atria, respectively. The replacement of Pro^{10} with Gly drastically reduced the potency of NT on the two assay organs. The dose-response curves to [Gly^{10}]-NT exhibited a depressed slope which indicates a possible loss of intrinsic activity (Ariens, 1964). Unfortunately, we could not measure precisely the maximum effect of this compound because of its low potency.

The substitution of Tyr^{11} with D-Tyr, D-Phe, Leu or Ala gave compounds with very low potency in both preparations. The dose-response curves to [D-Tyr^{11}]-NT, [D-Phe^{11}]-NT, [Leu^{11}]-NT and [Ala^{11}]-NT were shifted to the right of that to NT by more than four logarithmic units. The slopes of the various curves appear slightly depressed. The maximum responses could not be assessed accurately, except for [Ala^{11}]-NT in rat stomach strips because of the low potency of these NT derivatives. The replacement of Tyr^{11} with Phe reduced the potency of NT by approximately the same extent (factor of 6 to 7) in rat stomach strips and guinea-pig atria. The dose-response curves to [Phe^{11}]-NT are parallel to that of the reference compound and exhibited the same maximum. On the other hand, [Trp^{11}]-NT was found to be slightly more potent than NT in the rat stomach strips but less active than NT by a factor of 33 in guinea-pig atria (Figures 1 and 2, table 2). None of the NT derivatives described above behave as an antagonist of NT in either rat stomach strips or guinea-pig atria.

Discussion

The results obtained with analogues of NT in which Arg^9 was replaced with D-Arg or Ala suggest that Arg^9 mainly contributes to the affinity of NT for its cardiac and smooth muscle receptors. They also suggest that NT receptors in rat stomach strips are more sensitive to the alteration of Arg^9 than cardiac NT receptors. [D-Arg^9]-NT was reported to be only two times less potent than NT in the test of hypothermia and 6.4 times more potent than the parent compound in the 'mast cells binding' assay (Rivier, Lazarus, Perrin & Brown, 1977). The existence of such a discrepancy between the latter results and ours, raises the possibility that different NT receptors are involved in

Table 1 Primary structure of neurotensin and various analogues

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
[D-Arg^9-NT]	—	—	—	—	—	—	—	—	D-Arg	—	—	—	—
[Ala^9-NT]	—	—	—	—	—	—	—	—	Ala	—	—	—	—
[D-Pro^{10}-NT]	—	—	—	—	—	—	—	—	—	D-Pro	—	—	—
[Gly^{10}-NT]	—	—	—	—	—	—	—	—	—	Gly	—	—	—
[D-Tyr^{11}-NT]	—	—	—	—	—	—	—	—	—	—	D-Tyr	—	—
[Phe^{11}-NT]	—	—	—	—	—	—	—	—	—	—	Phe	—	—
[D-Phe^{11}-NT]	—	—	—	—	—	—	—	—	—	—	D-Phe	—	—
[Ala^{11}-NT]	—	—	—	—	—	—	—	—	—	—	Ala	—	—
[Leu^{11}-NT]	—	—	—	—	—	—	—	—	—	—	Leu	—	—
[Trp^{11}-NT]	—	—	—	—	—	—	—	—	—	—	Trp	—	—

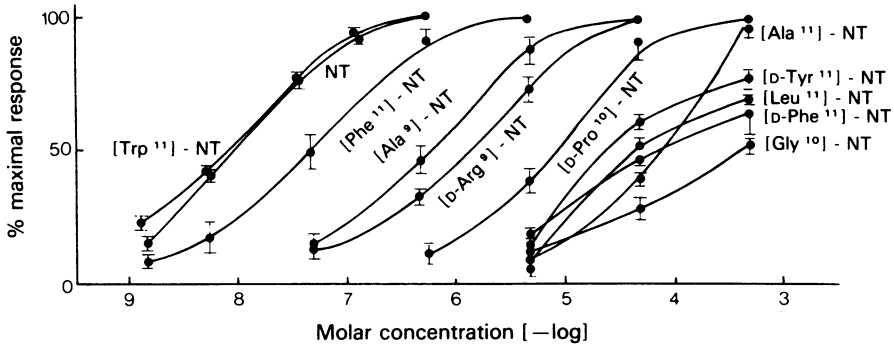

Figure 1 Dose-response curves illustrating the contractile effect of increasing concentrations of neurotensin (NT) and its analogues as measured in rat stomach strips. Each point is the mean value; vertical lines show s.e. mean. The number of individual determinations is given in Table 2.

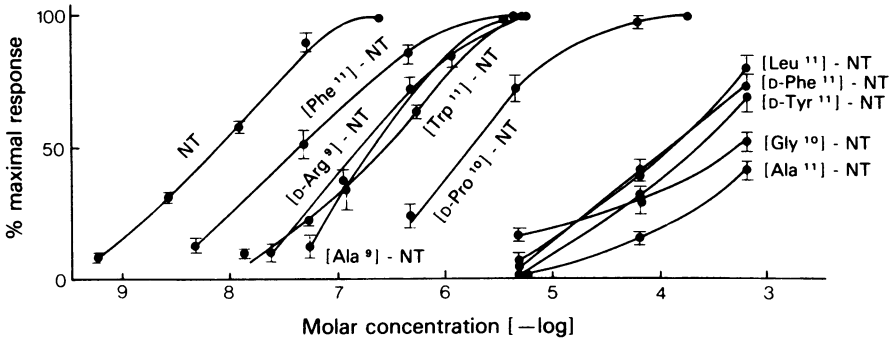

Figure 2 Dose-response curves illustrating the positive inotropic effect of increasing concentrations of neurotensin (NT) and its analogues as measured in isolated spontaneously beating atria of guinea-pig. Each point is the mean value; vertical lines show s.e. mean. The number of individual determinations is given in Table 2.

Table 2 Maximum effects (ME), effective concentrations producing 50% (ED_{50}) of ME and relative potencies of neurotensin and several analogues as measured in rat stomach strip and guinea-pig atria

	Rat stomach strip			*Guinea-pig atria*		
	ME (%)	*ED_{50}* ($\times 10^{-8}$ M)	*Rel. potency*	*ME* (%)	*ED_{50}* ($\times 10^{-8}$ M)	*Rel. potency*
Neurotensin (NT)	100	1.1 ± 0.1 (70)	100	100	0.9 ± 0.1 (64)	100
[D-Arg^9]-NT	100	170.0 ± 50.0 (7)	0.65	100	27.0 ± 3.0 (7)	4.1
[Ala^9]-NT	100	75.0 ± 12.0 (7)	1.5	100	34.0 ± 4.0 (7)	2.6
[D-Pro^{10}]-NT	100	900.0 ± 60.0 (8)	0.1	100	210.0 ± 20.0 (7)	0.4
[Gly^{10}]-NT	50–100	3800 ± 24000 (8)	~0.01	50–100	2700 – 23000 (7)	~0.01
[D-Tyr^{11}]-NT	75–100	1650 – 3100 (6)	~0.05	70–100	7850 – 19000 (4)	~0.01
[Phe^{11}]-NT	100	6.7 ± 1.8 (8)	16	100	6.1 ± 0.9 (8)	15
[D-Phe^{11}]-NT	60–100	3000 – 9000 (6)	~0.02	70–100	4250 – 18000 (4)	~0.01
[Ala^{11}]-NT	97	8400 ± 1200 (6)	0.01	>40	–(6)	<0.01
[Leu^{11}]-NT	70–100	3000 – 6300 (6)	~0.03	80–100	7100 – 24000 (6)	~0.01
[Trp^{11}]-NT	100	1.0 ± 0.1 (7)	130	100	28.0 ± 4.0 (6)	3.2

these various assay systems. The results obtained with NT derivatives modified in position 11 also support this hypothesis (see below).

The results obtained with analogues of NT substituted in position 10 were interpreted as an indication that Pro^{10} contributes to the affinity of NT for its cardiac and smooth muscle receptors, probably by influencing the conformation of the whole molecule. Pro^{10} may be essential for providing the critical orientation of C-terminal chemical groups involved in receptor activation. Such an interpretation is consistent with the conformational role attributed to Pro and Gly in other biologically active peptides (Rudinger, 1971; Regoli, Park & Rioux, 1974). [D-Pro^{10}]-NT also exhibited a large decrease of potency in the hypothermic assay (rel. potency = <0.1% compared to 100% for NT) (Rivier *et al.*, 1977). In the 'mast cell binding' assay, [D-Pro^{10}]-NT was only 8 to 9 times less potent than NT (Rivier *et al.*, 1977).

Analogues of NT in which Tyr^{11} was replaced with D-Tyr or D-Phe exhibited a very low potency. Similar results were observed with [Ala^{11}]-NT and [Leu^{11}]-NT. These results indicate the importance of having an aromatic side chain with the proper spatial configuration in position 11 for optimizing the affinity of NT for its cardiac and smooth muscle receptors. The results obtained with [Phe^{11}]-NT suggest that the hydroxyl group in the *para* position of Tyr^{11} may contribute to the affinity of NT for its receptors by forming a hydrogen bond with NT receptors. The replacement of Tyr^{11} with Trp slightly increased the potency of NT in the rat stomach strip thus suggesting that, in this tissue, NT receptors can accommodate a rather large aromatic side chain. Cardiac NT receptors appear slightly different in this respect since [Trp^{11}]-NT was much less potent than NT in guinea-pig atria.

The results obtained with [D-Tyr^{11}]-NT and [D-Phe^{11}]-NT deserve further discussion. These two compounds were found to be 10 times more potent than NT in the hypothermia test but to exhibit a relatively reduced affinity (a factor of 10 for [D-Tyr^{11}]-NT and 150 for [D-Phe^{11}]-NT in the 'mast cell binding' assay (Rivier *et al.*, 1977)). In our preparations, the potency of these compounds was reduced by a factor of 5000 to 10000 compared to NT. These discrepancies may be related to different rate and/or extent of degradation and/or to the existence of different NT receptors in the various tissues. Our analogues [D-Tyr^{11}]-NT and [D-Phe^{11}]-NT were also tested for their hypothermic effect in the rat and found more potent than NT (Drs F. Jolicoeur and A. Barbeau, personal communication) which confirms previous results published by Rivier *et al.* (1977) for these analogues.

In conclusion, we believed that Arg^9 and Pro^{10} mainly contribute to the affinity of NT for its cardiac and smooth muscle receptors while Tyr^{11} may be more closely involved in the process of NT receptor activation. Our data also raise the possibility of the existence of different NT receptors in various tissues. Further studies are needed to substantiate these interesting hypotheses.

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