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NEUROTENSİN: A CENTRAL NERVOUS SYSTEM PEPTIDE

Gülberk UÇAR (*)

Summary: *Neurotensin (NT) is a tridecapeptide mainly distributed in specific areas of the brain and gastointestinal (GI) tract of many mammalian species. It acts as a local hormone in GI tract mediating the GI motility and secretion of insulin and glucagon; as a neurotransmitter or neuromodulator in the brain effecting cholinergic receptor activation and opioid peptide and catecholamine metabolism. These functions are initiated by specific NT receptors interacted with G proteins and are involved in modulation of second messengers as cAMP, cGMP and inositol phosphates.*

Keywords : *Neurotensin, Neurotensin binding sites, Neurotensin receptor, Central nervous system*

NÖROTENSİN: BİR MERKEZİ SİNİR SİSTEMİ PEPTİDİ

Özet: *Nörotensin (NT), birçok memeli türünün beyin ve mide barsak sisteminin özgül bölgelerinde bulunan 13 amino asitlik bir peptittir. Bu peptit mide-barsak sisteminde yerel hormon olarak görev yapıp mide-barsak hareketlerini ve insülin ve glukagonun salgılanışını düzenlemekte; beyinde sinirsel iletici olarak görev yapıp kolinerjik reseptör aktivasyonu ve opioid peptit, katekolamin metabolizmasını etkilemektedir. Bu işlevler, G proteinleri ile etkileşen ve inositol fosfatlar, cAMP ve cGMP gibi ikincil habercilerin modülasyonunu sağlayan özgül NT reseptörleri tarafından başlatılmaktadır.*

Anahtar Kelimeler : *Nörotensin, Nörotensin bağlanma yeri, Nörotensin reseptörleri, Santral sinir sistemi*

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(*) Hacettepe University, Faculty of Pharmacy, Department of Biochemistry, ANKARA.

Neurotensin (NT) is a 13 amino acid peptide originally isolated and sequenced first from bovine hypothalamus and subsequently from both bovine and human small intestine (1-4). Since its discovery and synthesis, several biological functions have been attributed to this peptide. In this review, the isolation and purification of neurotensin, its structure and distribution in mammalian tissues, NT binding sites and apparent biological actions were summarized.

ISOLATION

NT was discovered in the process of purifying substance P, a hypothalamic peptide, by ion exchange chromatography and clearly separated from the sialogogic activity as a seperate peak causing vasodilatation and cyanosis of exposed skin surfaces (1). Using the vasodilatory response to monitor purification procedures, Carraway and Leeman isolated NT and determined its amino acid sequence to be Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH (1, 2). Using a purification scheme involving gel filtration, ion exchange chromatography, and high voltage paper electrophoresis, the peptidic material obtained from 45 kg tissue was purified about 200 000 fold, yielding 150-200 nmoles pure NT (1).

Gel filtration on Sephadex G-25 column gave an active material with a molecular weight of 1,600 in a good agreement with the amino acid composition, indicating the presence of 13 amino acids in the peptide (1). Later, NT was isolated using radio-immunoassay (RIA) (5) when synthetic NT became available (6). Using immunogens prepared by coupling NT through its tyroglobulin and succinylated hemocyanin several antisera toward NT have been obtained. Recently a new antiserum (TG-1) spesific for the NH_2-terminal region of NT was generated. While native NT obtained from hypothalamic and intestinal extracts (7, 8) gave equal measurements with the different antisera, material extracted from other tissues gave disparate results (7). All NT-like substances reacted more strongly with COOH-terminal-directed antisera than with NH_2-terminal specific antisera and this suggested that they share in common with NT, its biologically active COOH-terminal region.

STRUCTURE

The structure of NT was deduced from sequence studies on the intact peptide and on its tryptic, chymotryptic and papain-generated fragments (2). It was suggested that NT differed slightly from this structure in situ while the pyrolidine carboxylic acid (<Glu^1) and glutamic acid (Glu^4) residues may have arisen by acid-catalyzed alterations of glutamine residues originally at these positions (9).

The amino acid sequence of NT doesn't resemble the other known peptides or proteins but aligned from their NH_2-terminal, vasopressin and luteinizing hormone-releasing hormone (LRH) are found to be more closely related to NT (Table 1). These sequence similarities might reflect a

Table 1. Comparison of the primary structure of neurotensin with other biologically active peptides

Neurotensin	<Glu	Leu	Tyr	Glu	Asn	Lys	Pro	Arg	Arg	Pro	Tyr	Ile	Leu - OH
Xenopsin						<Glu	(Gly)	(Lys)	[Arg]	[Pro]	(Trp)	[Ile]	[Leu] - OH
Vasopressin	H - Cys	Tyr	(Phe)	(Gln)	[Asn]	Cys	[Pro]	-----	[Arg]	(Gly)	- NH2		
LRH	[<Glu]	His	(Trp)	(Ser)	Tyr	----	(Gly)	Leu	[Arg]	[Pro]	Gly - NH2		
Oxytocin	H - Cys	Tyr	Ile	(Gln)	[Asn]	Cys	[Pro]	Leu	Gly - NH2				
TRH	[<Glu]	His	Pro - NH2										
Substance P	H - (Arg)	Pro	Lys	Pro	Gln	(Gln)	Phe	Phe	Gly	Leu	Met - NH2		
Bradykinin	H - (Arg)	Pro	Pro	Gly	Phe	Ser	[Pro]	Phe	[Arg] - OH				
Angiotensin I	H - (Asp)	Arg	Val	Tyr	Val	His	[Pro]	Phe	[His]	Leu - OH			

[ ] Identical residues ( ) acceptable codon substitutions

common origin for those three peptides (2). Only one non-mammalian peptide, xenopsin shows a remarkable resemblance to NT and exhibits a number of the biologic proporties of NT (10).

NT was chemically synthesized by using the Merrifield Solid Phase procedure in 1975 and extensive studies showed that the synthetic product was chemically and biologically indistinguishable from the native material (5). Studies with synthetic NT indicated that most of the biological actions of NT resides within its COOH-terminal five to six residues (Table 2).

A proposed model of a NT-receptor interaction is shown in Figure 1. The structure-activity data was found to be consistent with this model and it provided overwhelming support for the importance of the COOH-terminal residues as specific binding site and biological action. COOH-terminal sequences consisting five and more amino acids induced hypertension and hyperglycemia, increased vascular permeability and contracted the isolated guinea pig ileum while NH_2-terminal sequences, as large as NT (1-10) decapeptide, were ineffective. Although the NT (9-13) pentapeptide displayed reduced potency, it possessed full intrinsic biological activity in four test systems. This indicates that whole structure is necessary for complete receptor activation. Arginin[8] has an important role in binding since the NT (8-13) hexapeptide showed 30-50% biologic potency relative to NT. Dependence of receptor affinity on the presence of Arg^8, Arg^9 and a free carboxyl group of Leu^{13} indicates the involvement of strong ionic interactions between these groups and charged areas on the receptor (Fig 1) (9).

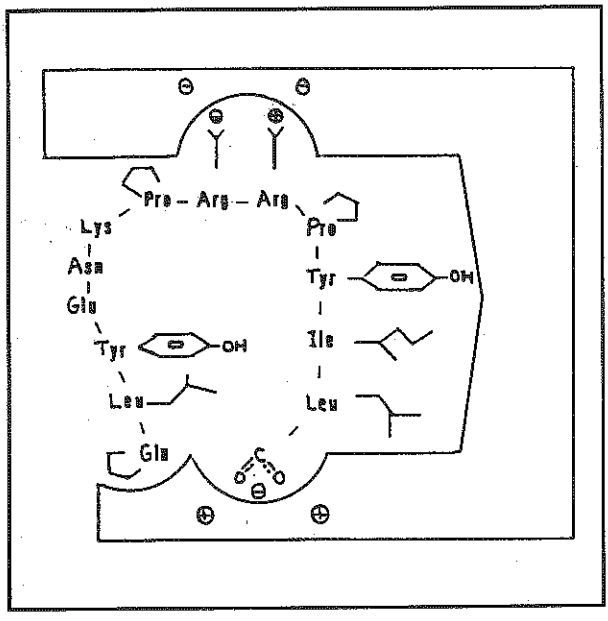

Figure 1. Proposed model for neurotensin - receptor interaction

Table 2. Relative biologic potencies of neurotensin fragments and analogs

Peptide	Percent of neurotensin activity	
	Hyperglycemia	Hypotension
<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH	100	100
<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile--OH	<0.5	<0.5
<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-OH	<0.2	<0.2
H-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH	100	80
H-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH	25	20
H-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH	20	10
H-Arg-Arg-Pro-Tyr-Ile-Leu-OH	55	60
H-Arg-Pro-Tyr-Ile-Leu-OH	1.0	0.5
H-Pro-Tyr-Ile-Leu-OH	<0.1	<0.1
H-Tyr-Ile-Leu-OH	<0.1	—
H-Ile-Leu-OH	<0.1	—
<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-NH2	<0.1	<0.1
H -Arg-Arg-Pro-Tyr-Ile-Gly-OH	<0.1	<0.1
H -Arg-Arg-Pro-Tyr-Ile-Asp-OH	<0.1	<0.1
(Xenopsin) <Glu-Gly-Lys-Arg-Pro-Trp-Ile-Leu-OH	20	20

A large number of NT analogs have been examined for their ability to interact with brain (11) or mast cell (12) receptors and for their various biological effects (13, 14). The COOH-terminal region is thought to be responsible for both binding and activity (15).

LOCALIZATION AND DISTRIBUTION

Central nervous system

Radioimmunological, immunohistochemical and radioautographic studies have shown that NT is widely distributed throughout the central nervous system of various mammalian species (16, 17). High ^{125}I-NT binding densities were observed in the bed nucleus of the stria terminalis, Celleja island, claustrum, olfactory tubercle and central nucleus of the amgydala with the exception of the central grey of the mesencephalon and interpeduncular nucleus. The remaining mesencephalon, medulla, pons, cerebellum and pineal gland have relatively low NT content (13-15).

NT has been shown to be present in the adrenal medullary cells of rat cow and cat mainly associated with chromaffin granules containing noradrenaline. It is secreted by splanchnic nerve stimulation simultaneously with cathecholamines, enkephalins and somatostatin, following cholinergic receptor activation or depolarization by potassium (16).

The nerves longitudinally oriented network around large cerebral arteries and appeared to be involved in modulating cerebrovascular tone in hypertension also have been shown to contain large amount of NT (17).

Gastrointestinal tract

NT is present in high concentrations in the mammalian gut, especially in endocrine cells of mucosa. Autoradiograms of ^{125}I-neurotensin bound to cross sections of the proximal and distal jejunum showed that the highest densities of silver grains were associated with the internal submucosal ganglia, external submucosal plexus and myenteric ganglia. A limited population of NT immunoreactive cells were found within the mucosal epithelium (18). Apical pole of cells containing NT in gut extends toward the intestinal lumen and these cells distributed along the villi with a characteristic morphology (19). Holzer et al. (20) reported that NT-like immunoreactivity corresponds to intact NT which was essentially distributed in the distal part of small intestine. Orci et al. (21) showed that this peptide was located mainly in discrete endocrine N cells of the mucosa. Specific NT receptor sites have been characterized in guinea pig and dog ileum smooth muscle tissues (18, 22) in which they are thought to modulate the contractility of gastrointestinal smooth muscle (23).

Plasma

Neurotensin has been detected in the range of 20 to 80 fmol/ml in extracts of

rat, bovine, dog, pig, rabbit, chicken and human plasma (7, 24, 25). Although the form of NT-like material found in plasma is not clearly known, some recent studies indicated that some of the immunoreactive materials obtained from plasma appeared to be authentic NT with the level of about 15-25 fmol/ml which is quite similar to the one identified in extracts of stomach mucosa (7).

NEUROTENSIN BINDING SITES

The existence of specific binding sites for NT has been established in the central nervous system (11, 14, 26-28). Strong hybridization signals for NT receptor mRNA were observed over neurons in the diagonal band, medial septal nucleus, nucleus basalis, magnocellularis, suprachiasmatic nucleus, substantia nigra, central tegmental area (29), hyperstriatum ventrale, archistriatum, neostriatum intermedium (30). Another study indicated that NT receptors were mainly localized in somatic sensory and motor regions of the human spinal cord suggesting that NT might play a role in modulating sensory-motor functions in human spinal cord (31).

The neurotensin receptor protein, solubilized from bovine cerebral cortex membrane exhibited saturable and specific bining of 3-11 NT with an affinity of K_d = 5.5 nM. It was shown that this receptor was consisted of a single polypeptide chain with a Mr of 50 000 and had intramolecular disulfide bonds (32).

In another study a soluble type of NT receptor with a molecular weight of 100 000 has been shown to contain a single binding site with a K_d of 0.36 nM and a Bm of 63 fmol/mg under both native and denaturating conditions (33). Nakagava et al. (34) examined NT binding on membrane fraction from neuroblastoma X glioma NG 108-15 hybrid cells and showed that there has been a single class of NT binding sites with a K_d of 0.86 nM and a B_{max} of 250 fmol/mg. Schotte et al. (35) demonstrated the presence of two binding sites for NT in rat brain: Site 1 was highly specific for NT and was homogenously distributed throughout all rat brain areas while site 2 had a lower affinity for NT and its binding was markedly different in separate brain regions. It is suggested that side 1 has to be considered as an acceptor for [3H] neurotensin. Structural studies of NT binding sites in membranes of rat, rabbit and mouse brains were found in good correlation with these data (26, 36).

High NT binding densities were observed in many areas of human basal forebrain, where acetylcholinesterase is widely distributed suggesting that endogenous NT might directly influence forebrain cholinergic function in the central nervous system (37, 38). NT-receptor sites were found to be associated with presynaptic nigrostriatal dopaminergic terminals in the caudate-putamen and with glial cells in brain (39, 40). A dopaminergic control of striatal and postynaptic NT systems were shown by several authors (40-42).

It has been previosly shown that NT receptor occupancy in the adenocarcinoma HT29 cell line leads to inositol phosphate formation (43). It was suggested that NT

stimulated inositol phospholipid metabolism and caused calcium mobilaziton. It was also demonstrated that GTP binding-proteins were involved in this process (44-47). It was reported that NT was able to regulate intracellular Ca^{2+} levels by using inositol triphosphate as a second messenger (48-51). It was concluded that NT attenuated cAMP production by exerting an inhibitory effect on adenylate cyclase through an interaction of the peptide receptors with the regulatory GTP-binding protein N_i (52) and stimulated intracellular cGMP formation (53, 54).

A functional cDNA clone for the NT receptor recently was isolated. This receptor consisted of 424 amino acids with seven putative transmembrane domains, shows selective, high affinity binding to NT. It was concluded that it belonged to the family of G-protein-coupled-receptors and mediated its neuronal and peripheral actions by effecting the G-protein-associated second messenger system (55).

NT receptors were also found in smooth muscle of small intestine (23, 56), gastric fundus (57, 58), duedonum, ileum (59), and jejunum (18). It was concluded that NT receptors in GI system were somehow linked to ionic channels, especially a voltage-dependent calcium channel (58, 60).

BIOLOGICAL ACTIONS

Gastrointestinal effects

It was previously reported that NT contracted guinea pig ileum, relaxed rat duodenum (1,61) and effected the motility of antral or fundic pouches (62). Kitabgi et al. (63) demonstrated a biphasic effect of NT application of the peptide induced rapid relaxation followed by contraction. Atropine partially inhibited NT-induced contractions of the ileum suggesting that NT might act directly on smooth muscle to relax ileum but indirectly to induce contractions. Recently, it was reported that picomolar concentrations of the NT caused concentration-dependent contractions of rat fundus. This potency was not modified with atropine and the contraction was followed by tachyphlaxis suggesting that NT activated a specific excitatory receptor located on smooth muscles of the rat fundus (58). There has been some reports indicating a central action of NT on gastric acid secretion. This inhibitory effect was thought to be occured within nucleus accumbens and be mediated by central nervous system alpha-adrenergic receptor activation (64).

Neurologic effects

NT is distributed widely throughout the central and the peripheral nervous system suggesting a role as neurotransmitter or neuromodulator. NT has been shown to be present in the adrenal medullary cells of many mammalians associated with chromaffin granules containing noradrenaline and is secreted by splanchic nerve stimulation simultaneously within cathecholamines, enkephalines and somatostatine following cholinergic receptor activation or depolarization by potassium. The presence of NT in the preganglionic nerves suggests a possible role for NT as a neuro-

transmitter (16). The relative potencies of various fragments of NT in competing for binding to brain membranes parallel in a general way their relative pharmacologic activities on peripheral tissues, but it is unclear whether the sites mediating pharmacological effects of NT in the periphery are identical with those in the central nervous system (11).

It is reported that NT directly influences the activity of magnocellular cholinergic neurons in the human forebrain (14, 37), and may be involved in the physiopathology of demending disorders such as Alzheimer's disease, in which these neurons have been shown to be effected (66). Significant decreases of NT receptors were found in brains of patients with Parkinson's disease (67-69). A relative NT definiency way shown is a subgroup of psychotic patients and significant increases in NT were found after neuroleptic treatment (42, 70, 71).

The possible involvement of NT in the regulation of respiratory drive has been tested on respiratory related neurons. A bilateral apneustic pattern was induced on the phrenic nerve activities by NT injection (0.23-0.54 pmol/s) and it was suggested that NT regulates respiratory rhythmogenesis by increasing the respiratory duration (72).

A significant fall in body temperature of animals by intracisternal injection of NT was reported earlier (73). The COOH-terminal of NT was found necessary for the production of hypothermia (74), since carboxyl fragment of the molecule was shown as essential both for binding to brain membranes and for biologic activity (27). Nemeroff et al. (75) found that central injection of NT increased sleeping time in mice treated with pentobarbital. Recently, when ethanol and NT were administered in combination, sedative and hypertermic effects of these two substances were found to be potentiated (76). Kalivas (77) reported that NT could act in the vental tegmental area and ventromedial mesencephalon to produce both a decrease in colonic temperature and an increase in spontaneous motor activity.

Endocrine effects

A single i.v. injection of NT induces dose-related hyperglycemia in animals (78-80). Carraway et al. (81) observed that the increase in plasma glucose was associated with dose-dependent increase in hepatic glycogen phopsporylase activity and reduction in liver glycogen content. They suggested that NT injection raised blood glucose by increasing hepatic glyconeogenesis. Wolfe et al. (79) observed a rise in plasma glucose levels and glucose production in glycogen-depleted animals following NT injection and concluded that this reflected an enhanced rate of gluconeogenesis. High doses of NT injection also reduces plasma insulin levels and increases circulating glucagon levels (78, 82, 83). The addition of NT to isolated pancreas preparations suppressed insulin secretion and stimulated glucagon release into the medium (84). However, a dual effect of NT on pancreatic hormone secretion was observed: at low concentrations, NT stimulated insulin, glucagon and somatostatin

release whereas at high concentrations it suppressed the release of these hormones (85). NT was shown to modulate the secretion of pancreatic hormones (83, 86).

The multiple effects of NT on glucoregulation may be mediated by histamine or catecholamines. Diphenhydramine, an H_1 receptor antagonist, blocked the hyperglycemia and hyperglucagonemia caused by the injection of NT or histamine while H_2 receptor blockade did not effect the response. NT injection also was found to be associated with a rise in plasma insulin levels (83, 87).

NT effects the release of several pituitary hormones. I.v. injection of NT caused increases at circulatory levels of prolactin, growth hormone, corticosteroids and ovarian steroids (88-92).

Vascular Effects

I.v injection of NT (0.16-1.0 mg/kg) caused a dose -related fall in arterial blood pressure in rats and acute tachyphylaxis accompanied the first administration of the peptide (1). Rapid vasodilatation of small blood vessels with NT injection has been reported previously and this response has been correlated with an increase in regional blood flow to the intestine (93). It was suggested that NT injection caused a rapid increase in vascular permeability to protein, an associated increase in hematocrite and a reversible cyanosis lasting 5-10 minutes (1). Induction of hypotension by NT was found to be mediated by histamine (16).

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