Neurotensin and neurotensin receptors

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Neurotensin is a brain and gastrointestinal peptide that fulfils many central and peripheral functions through its interaction with specific receptors. Three subtypes of neurotensin receptors have been cloned. Two of them belong to the family of G protein-coupled receptors, whereas the third one is an entirely new type of neuropeptide receptor and is identical to gp95/sortilin, a 100 kDa-protein with a single transmembrane domain. In this review, the present knowledge regarding the molecular and pharmacological properties of the three cloned neurotensin receptors is summarized and the relationship between these receptors and the known pharmacological effects of neurotensin is discussed.

Neurotensin (NT) is a 13 amino acid peptide originally isolated from calf hypothalamus by Carraway and Leeman in 1973 (Ref. 1). Like many other neuropeptides, it fulfils a dual function of neurotransmitter or neuromodulator in the nervous system and of local hormone in the periphery. The biochemical and pharmacological properties of NT in the brain and in peripheral organs have been abundantly documented[2–7] and will only be summarized briefly here: NT is a neuromodulator of dopamine transmission and of anterior pituitary hormone secretion, and exerts potent hypothermic and analgesic effects in the brain. In the periphery, NT is a paracrine and endocrine modulator of the digestive tract and of the cardiovascular system of mammals and acts as a growth factor on a variety of normal or cancer cells.

The past 25 years have revealed a continued interest in NT, and significant advances have been made in our understanding of many aspects of NT functions. For example, cloning of the gene that encodes NT stands out as a landmark in the neurotensin field[8]. This achievement led to further work which showed that neurotensin and its structurally related analogue neuromedin N (Ref. 9) are synthesized from a common precursor[10] and differentially processed at dibasic sites by proprotein convertases[11] (see Box 1). Another important advance was made by Nakanishi and his group. They cloned the first NT receptor (NTS1, previously NTR-1) in 1990 and showed that it belonged to the family of G protein-coupled receptors[12]. Several years later, the first potent non-peptidic NT receptor antagonist SR48692 was developed by Sanofi and shown to block a number of central and peripheral effects of NT (Refs 13, 14), although it did not inhibit the hypothermic and analgesic effects of centrally injected NT. The second-generation antagonist SR142948A was found to be more potent than SR48692 and exhibited a wider spectrum of activity in that it antagonized hypothermia and analgesia induced by intracerebroventricular (i.c.v.) injection of NT (Ref. 15). Recently, two new NT receptors have been cloned; one of them (nts2) belongs to the G protein-coupled receptor superfamily[16–18], as does the NTS1 receptor, whereas the other (nts3) is structurally different from the NTS1 and nts2 receptors (Ref. 19). These three receptors all recognize the same C-terminal 8–13 sequence and display similar structure–function relationships towards NT.

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NTS1 receptor

Initial indications about the existence of NT receptor subtypes have come from binding data. Membranes prepared from brain[20] or gastrointestinal[21] tissues generally contain two different classes of NT binding sites. The high-affinity sites (K_d = 0.1–0.3 nM) are sensitive to Na^+ ions and GTP, which decrease the affinity of the receptor for NT. The low-affinity sites (K_d = 3–5 nM) are less sensitive to Na^+ ions and insensitive to GTP. The antihistamine-1 drug levocabastine, which is devoid of any NT-like pharmacological properties, can selectively block NT binding to the lower-affinity sites without changing the binding properties of the higher-affinity sites[22,23].

Cloning and characterization

The first molecular identification of an NT receptor was achieved by Nakanishi and colleagues[12]. These authors screened a cDNA library from rat brain using the oocyte expression technique and isolated a single functional cDNA clone of 3633 nucleotides. The longest open reading frame of the cDNA encodes a 424 amino acid protein with seven putative transmembrane domains (TMs) belonging to the family of G protein-coupled receptors (Fig. 1). The cloned receptor expressed in mammalian COS cells selectively binds NT with high affinity (K_d = 0.2 nM) and is insensitive to levocabastine[12]. The human NTS1 receptor has been cloned from the colonic adenocarcinoma cell line HT29 (Ref. 24) and shown to consist of a 418 amino acid protein that shares 84% homology with rat NTS1 (Fig. 2). The human *NTSR1* gene has been localized to the long arm (20q13) of chromosome 20. The gene occurs as a single copy and contains three introns in the coding region[25]. A tetranucleotide repeat polymorphism is located <3 kb from the gene and can serve as a useful marker to study the relationship between the NTS1 gene and neuropsychiatric disorders[25]. The rat *NTSR1* gene also occurs as a single copy in the rat haploid genome[26].

Localization

Northern-blot analysis has revealed that NTS1 mRNAs are expressed in the brain and intestine of rat[12] and human[24]. In the brain, *in situ* hybridization experiments showed that high levels of NTS1 mRNAs are found in neurones of the diagonal band of Broca, medial septal

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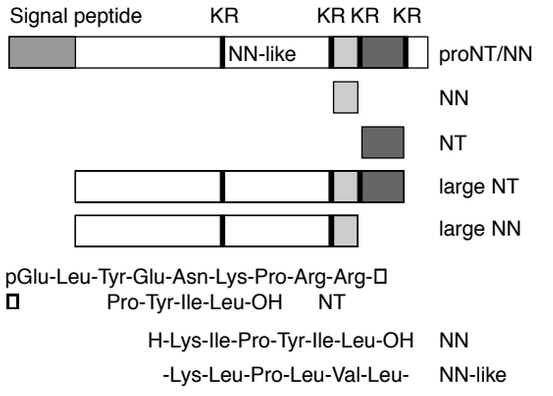
Box 1. Biosynthesis, processing and degradation of NT and related peptides



(Online: Fig. I)

Like all neuropeptides, neurotensin (NT) is synthesized as part of a larger precursor (Fig. I) which also contains neuromedin N (NN), a six amino acid neurotensin-like peptide[1]. NT and NN are located in the C-terminal domain of the precursor (pro-NT/NN), where they are flanked and separated by three Lys–Arg sequences. A fourth dibasic sequence preceding an NN-like sequence is present in the N-terminal domain. Dibasics are the consensus sites recognized and cleaved by processing endoproteases that belong to the recently identified family of proprotein convertases (PCs)[2]. In tissues that express pro-NT/NN, the three C-terminal Lys–Arg sites are differentially processed, whereas the dibasic site upstream of the NN-like sequence is not cleaved. In the brain, pro-NT/NN processing gives rise to NT and NN (Refs 3, 4). In the gut, processing leads mainly to the formation of NT and a large peptide ending with the NN sequence at its C-terminus (large NN)[3,4]. In the adrenal glands, NT, large NN and a large peptide ending with the NT sequence (large NT) are the major products[3]. Recent evidence indicates that PC1, PC2 and PC5-A are the prohormone convertases responsible for the processing pattern observed in the gut, brain and adrenal glands, respectively[5,6]. Both NT and NN are released upon depolarization of brain tissues[4]. Intestinal NT and large NN are secreted in the circulation after food ingestion[3]. The available pharmacological evidence indicates that both NT and NN bind with similar affinities (NN being slightly less potent than NT on the NTS1 and nts3 and equipotent with NT on the nts2) to the three NT receptor subtypes and that NN exhibits a similar biological profile to NT. Large NN has been shown to have NT-like biological activity on intestinal preparations[3]. However, its pharmacological properties have not been investigated in detail.

The mechanisms by which extracellular NT and NN are degraded and inactivated in the circulation and in tissues have been thoroughly investigated[4,7]. NN is rapidly inactivated by aminopeptidases, whereas NT is somewhat more slowly inactivated by metalloendopeptidases that cleave the peptide in its C-terminal domain. Although the degradation mechanism of large NN has not been studied, the peptide appears more stable than NT in the circulation[3]. It is tempting to speculate from these data that NN which is essentially found in the brain could function as a fast-acting neuropeptide, rather like the enkephalins, whereas large NN which is mainly produced by the intestine and released in the blood might act as a circulating hormone. NT would keep its definition as both a central neuromodulator and paracrine factor in the periphery.

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nucleus, nucleus basalis magnocellularis, suprachiasmatic nucleus, supramammillary area, substantia nigra and ventral tegmental area[27], as well as in the small dorsal root ganglion neurones of the spinal cord[28]. Superimposition of these data with those of autoradiographical[29] and immunohistochemical[30] studies provides useful information about the distribution of the NTS1 receptor. For example, there is a strong hybridization signal for NTS1 mRNA over neuronal perikarya within the substantia nigra whereas such signals are lacking over the striatum. By contrast, both brain regions contain the NTS1 protein as shown by autoradiography[29] and immunohistochemistry[30]. These findings support the hypothesis that dopamine neurones in the substantia nigra synthesize the NTS1 receptor, which is situated in their perikarya and dendrites and also in their terminals in the striatum following axonal transport.

Expression of the gene encoding the NTS1 receptor

The NTS1 receptor can be transiently or stably synthesized in a variety of cells, including *Xenopus* oocytes, which were used to clone this receptor[12]. Surprisingly, the Ca^{2+}-activated Cl^- current induced by NT in oocytes producing the rat NTS1 receptor was not blocked by SR48692 (Ref. 31). By contrast, the levocabastine-insensitive binding of NT to the rat or human NTS1 receptor transiently synthesized in COS cells can be completely inhibited by both SR48692 and SR142948A, with IC_{50} values of about 10 and 1 nM, respectively[13,15]. The rat NTS1 stably transfected in CHO or thymidine kinase defective (LTK) cells is efficiently coupled to phospholipase C (Refs 32–34) and the NT-induced increases in intracellular Ca^{2+} concentration and inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3] levels of transfected cells are antagonized by SR48692 (Ref. 34). NT also stimulates cAMP formation in CHO cells expressing the gene encoding the NTS1 receptor (Ref. 35). In addition, these transfected cells respond to NT by a prolonged activation of mitogen-activated protein kinases (MAPKs) and by an induction of the growth-related gene *krox-24*; both effects can be prevented by SR48692 (Ref. 36). These signalling properties of the NTS1 receptor can also be observed in the human colon carcinoma cell line HT29 and they provide a possible mechanism by which NT could promote cell growth.

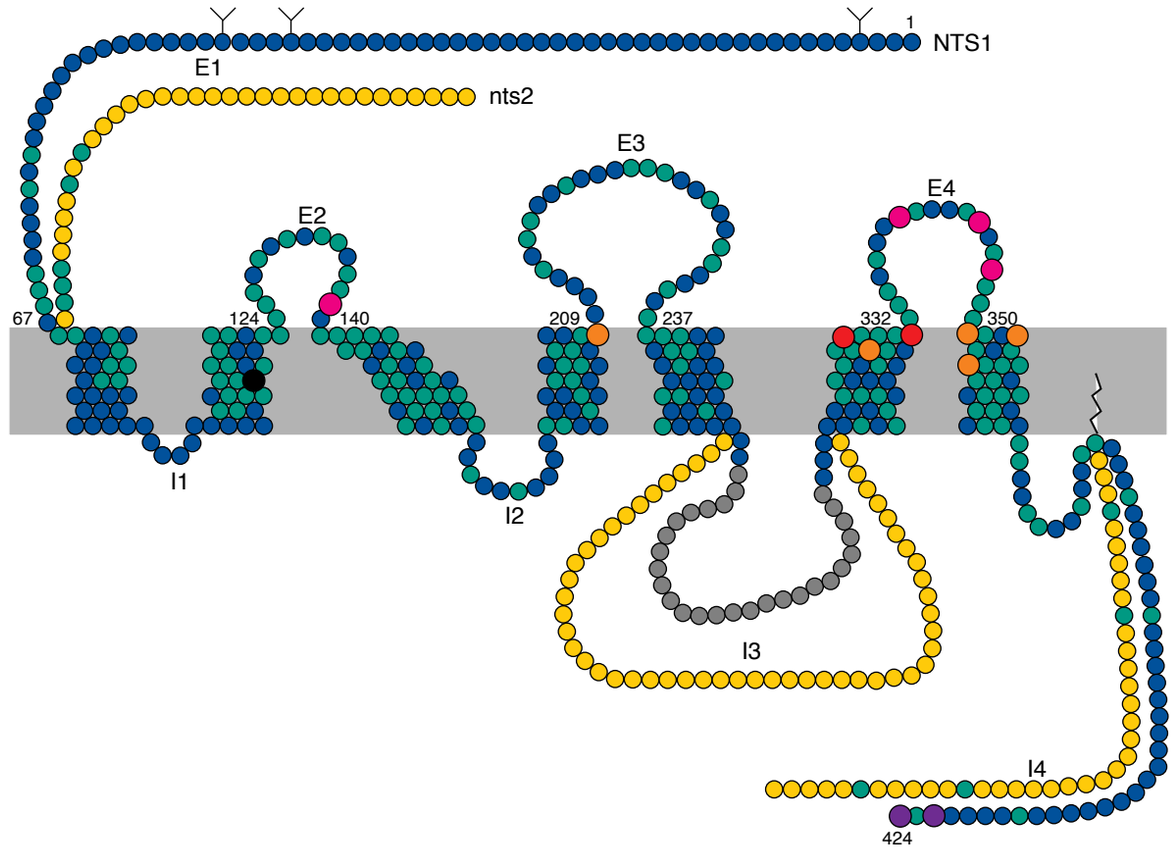

Fig. 1. The rat neurotensin receptor, NTS1 (blue). The invariant residues between the NTS1 and nts2 receptors are coloured in green. Regions of the nts2 receptor that strongly differ from NTS1 (E1, I3 and I4) are indicated in yellow. The N-terminal (1), C-terminal (424) and the first residues of each extracellular segments of the NTS1 receptor are numbered. The Na^+-binding site (Asp113) of NTS1 is shown in black. Residues involved in the binding of NT (pink), SR48692 (orange) or both (red) are indicated. Residues crucial for NTS1 internalization are in violet. The region of the third intracellular loop (I3) that is essential for coupling to phospholipase C is in grey. Glycosylation sites (Y) are highlighted.

Affinity labelling[34,37] and immunocytochemical[38] experiments performed on rat brain membranes or on cells transfected with the rat NTS1 cDNA have shown that this receptor type is produced as two different proteins with an apparent molecular mass of 50–60 kDa in total. This protein doublet stems from the existence of two initiation sites for the translation of the NTS1 mRNA. The first corresponds to the site initially identified as coding for Met1 (Ref. 12), whereas the second site is a non AUG codon that remains to be identified[39].

Regulation of NTS1 mRNA expression

NT receptor agonists have been shown to induce differential regulation of NTS1 mRNA in both HT29 (Ref. 40) and neuroblastoma[41] cells and of the tyrosine hydroxylase mRNA in neuroblastoma cells[41]. Although several arguments suggest that these effects are mediated via the NTS1 receptor, which is known to be expressed in these cell lines, the presence and possible role of other NT receptor subtypes (see below) cannot be ruled out and should be evaluated before a definitive conclusion can be drawn.

Functional residues of the NTS1 receptor

Site-directed mutagenesis and modelling of the rat NTS1 receptor has provided interesting results about the sites and amino acid residues involved in the binding of NT and SR48692, the coupling of the receptor to phospholipase C and the internalization process of this receptor.

NT and SR48692 binding sites

The agonist NT and the competitive antagonist SR48692 bind to distinct, albeit overlapping, regions of the NTS1 receptor. Thus, the SR48692 binding site lies in a pocket delimited by residues that belong mainly to TM6 and TM7 (Ref. 42). In particular, Arg327 and Tyr351 in TMs 6 and 7, respectively, are crucial for SR48692 binding. Arg327 is also essential for NT binding, as well as Tyr347 located in the third extracellular loop. In addition, Asp139 in the first extracellular loop[43] and an N-terminal extracellular segment near TM1 (Ref. 44) are important for NT binding. Modelling studies have provided further evidence that the third extracellular loop that connects TMs 6 and 7 interacts with NT (Ref. 45). Thus, part of the NT binding site appears to lie on top of the SR48692 binding pocket in a region delimited by the third extracellular loop and by TMs 6 and 7. This might explain the competitive nature of SR48692.

Transduction

Coupling of the NTS1 receptor to phospholipase C involves the third intracellular loop[46]. The Asp113 residue

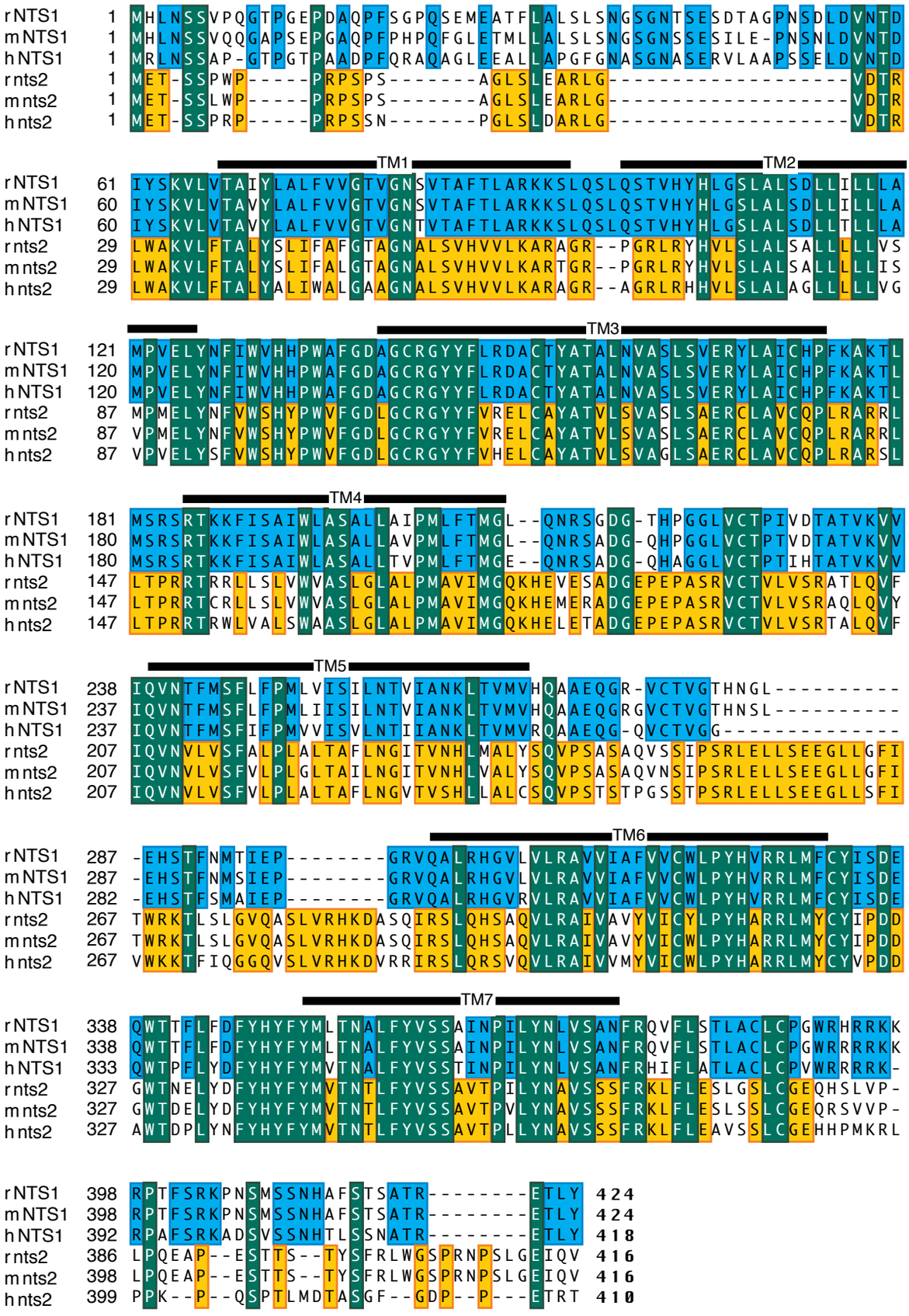

Fig. 2. Sequence alignment of the rat (r), mouse (m) and human (h) NTS1 and nts2 receptors. Invariant residues for all six receptors (green), for the three NTS1 (blue) or the three nts2 (yellow) receptors are boxed. Note that the sequence between residues 202 and 220 of the mouse nts2 has been corrected from Ref. 17. The mouse NTS1 sequence was obtained from GenBank (NID: G3551525).

Table 1. Agonist and antagonist activities of neurotensin receptor ligands on NTS1- and nts2-transfected cells

Ligand	NTS1				nts2			
	rNTS1 (XO)	rNTS1 (CHO)	rNTS1 (LTK)	hNTS1 (CHO)	mnts2 (XO)	mnts2 (HEK)	mnts2 (CHO)	hnts2 (CHO)
Neurotensin	+	+	+	+	(+)	0	(+)	–
SR48692	0	ND	–	–	(+)	ND	+	+
SR142948A	ND	ND	ND	–	ND	ND	ND	+
Levocabastine	0	0	0	0	(+)	ND	+	–

CHO, Chinese hamster ovary cells; h, human; HEK, human embryonic kidney cells; LTK, mouse fibroblasts deficient in thymidine kinase; m, mouse; r, rat; XO, *Xenopus oocyte*. +, agonist; (+), weak agonist; –, antagonist; 0, no effect (agonist and antagonist); ND, not determined.

located in TM2 (see Fig. 1) is responsible for the sodium sensitivity of NT binding and is also important for coupling to phospholipase C since its substitution by Ala leads to a 100-fold decrease in the potency of NT to stimulate Ins(1,4,5)P_3 formation[47].

Internalization

Following interaction with NT, 60–70% of the NTS1 receptor produced in COS or LTK cells internalizes according to a temperature-dependent process[34,48]. Biochemical evidence suggests that the receptor is not recycled to the plasma membrane after ligand-induced internalization[49]. The receptor region involved in the internalization process is the C-terminal intracytoplasmic tail and, more particularly, the Thr422 and Tyr424 residues located at the extremity of the tail[48].

nts2 receptor

As mentioned above, the existence of a levocabastine-sensitive receptor with a low affinity for NT was evident from binding data. The receptor protein corresponding to this binding activity (nts2) was cloned from rat[16], mouse[17] and human[18] brain using a strategy based on sequence homology with the known NTS1 receptor. The rat and mouse nts2 (416 amino acids) are slightly longer than their human counterpart (410 amino acids). The three receptors all have the 7TM structure of G protein-coupled receptors (Figs 1 and 2).

Comparison of NTS1 with nts2

The rat nts2 and NTS1 share 43% amino acid identity and 64% amino acid homology (Fig. 2). As illustrated in Fig. 1, the NTR2 has a shorter N-terminal extracellular tail and a longer third intracytoplasmic loop than the NTS1 receptor.

Other features of the nts2 receptor structure include the absence of putative N-glycosylation sites in the N-terminal domain and the replacement of a generally conserved Asp residue in TM2 by an Ala (Refs 16, 17) or by a Gly residue[18]. This substitution is responsible for the low sensitivity of the nts2 receptor to Na^+ ions: site-directed mutagenesis experiments have shown that replacing the corresponding Ala79 by an Asp improves the sensitivity of the nts2 to Na^+ ions but does not restore its sensitivity to GTP (Ref. 47). In the mouse, alternative splicing of the nts2 primary transcript leads to the production of a truncated receptor. This short (282 amino acids) non-functional receptor only contains five putative TMs and is expressed mainly in the spinal cord[50].

Binding properties

The full-length rat[16] and mouse[17] nts2 receptor transfected in COS cells binds NT and levocabastine with similar affinities (K_d = 2–10 nM). Receptors of both species differently recognize the Sanofi compounds, with high affinities for SR142948A (1–4 nM)[15] as compared to K_d values of 82 and 300 nM for SR48692 for rat[16] and mouse[17] nts2, respectively. Thus, SR142948A binds with similar affinities to the rat and mouse NTS1 and nts2 whereas SR48692 discriminates between both receptor types and preferentially recognizes the NTS1. The human nts2 expressed in COS cells[18] binds NT with an affinity similar to that of the rat and mouse nts2 (K_d = 2.6 nM). However, the affinity for levocabastine is much lower (K_d = 91 nM) and the human receptor does not discriminate between the two SR compounds, since K_d values are 49 and 67 nM for SR142948A and SR48692, respectively.

Transduction mechanisms

Xenopus oocytes transfected with the mouse nts2 respond to NT by induction of a Ca^{2+}-dependent Cl^- current[17]. However, the amplitude of the current is 10–100 times smaller than that induced by NT on NTS1-injected oocytes. Unexpectedly, both levocabastine and SR48692 behave as NT agonists and trigger an inward Cl^- current on oocytes expressing the gene encoding mouse nts2 (Ref. 31). These puzzling results have been confirmed in CHO cells synthesizing rat and human nts2. NT itself or JMV449, a potent peptidase-resistant NT receptor agonist, induce only a weak stimulation of intracellular Ca^{2+} mobilization in rat nts2-transfected CHO cells as compared to the stronger Ca^{2+} response triggered by SR48692 and levocabastine[51]. SR48692 and SR142948A also act as potent agonists at the human nts2 produced in CHO cells, triggering IP formation, Ca^{2+} mobilization, arachidonic acid release and stimulation of the MAP kinase activity. Surprisingly, these agonistic responses are antagonized by NT and levocabastine[18]. In addition, NT is unable to modify IP, cAMP and cGMP levels in HEK cells stably expressing the gene encoding mouse nts2, although the receptor is efficiently internalized after NT binding and

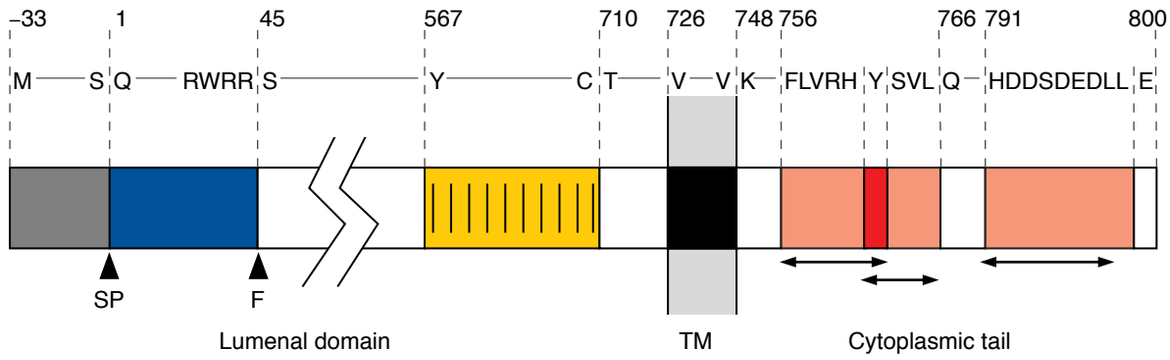

Fig. 3. The human nts3/gp95/sortilin. Colours indicate the peptide signal (grey), the (1–44) propeptide released by furin cleavage (violet), the cysteine-rich domain homologous to Vps10p, a yeast receptor for carboxypeptidase Y sorting[60] (yellow), the transmembrane domain (black) and the three internalization/sorting signals in the cytoplasmic tail homologous to the cation-independent mannose 6-phosphate/insulin growth factor-II receptor[60] (orange, underlined by arrows). Note the partial overlapping between two of these signal sequences.

can recycle to the plasma membrane in this cell system[49]. Taken together (Table 1), these results suggest that the nts2 receptor is only weakly coupled to phospholipase C, so that the resulting intracellular Ca^{2+} and Ins(1,4,5)P_3 responses can only be evidenced in the most sensitive cell models like the oocyte expression system. Moreover, both NT and levocabastine can act either as agonists of the mouse and rat nts2 or as antagonists of the human nts2, at least when these receptors are artificially expressed in *Xenopus* oocytes or CHO cells. It is clear that further studies are necessary to better characterize the transduction pathways of the nts2 receptor and to clearly delineate the mode of action of NT, levocabastine and SR compounds on this receptor type.

Localization of the nts2 receptor

Northern-blot analysis has revealed that nts2 mRNA is expressed mostly in the brain[16–18]. *In situ* hybridization data showed that nts2 mRNA is localized mainly in the olfactory system, the cerebral and cerebellar cortices, the hippocampal formation and selective hypothalamic nuclei of the mouse[52] and rat[53] brain. This distribution is different from that of NTS1 mRNA and only a few brain areas (e.g. diagonal band of Broca, medial septal nucleus and suprachiasmatic nucleus) are enriched in both NTS1 and nts2 mRNAs (Ref. 53). Ontogenic studies revealed that the mouse nts2 mRNA is detected only from postnatal day 14 and did not reach adulthood concentrations before day 30 (Ref. 52). This developmental pattern is markedly different from that reported for the NTS1 receptor mRNA (Refs 17, 54, 55), which is already expressed at birth, then transiently peaks between the first seven and ten days of life and decreases progressively to reach adult levels between 30 and 40 days. At the cellular level, the rat nts2 mRNA is expressed in neurones, glia and ependymal cells[53], whereas the mouse nts2 is mostly concentrated over neurones[52].

nts3 receptor

Purification, cloning and identification to sortilin

The first indications about the existence of an NT receptor structurally unrelated to the NTS1 and nts2 receptors have come from purification studies. Brains of mouse[56], rat, rabbit, horse, bovine and human[57] contain high-affinity NT binding sites that can be solubilized in an active form by the detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS). The ontogenic development, the binding properties and the molecular structure of the CHAPS-solubilized proteins bearing this activity are remarkably similar in all the mammalian species studied. Binding activities are maximal just after birth, affinities towards NT are between 0.1 and 0.5 nM and apparent molecular mass determined by affinity cross-linking are ~100 kDa. These NT receptors (nts3) have been purified after solubilization from mouse[58], rat and human[59] brain by affinity chromatography. The first 23 amino acids of the purified human nts3 were determined by Edman degradation and various nucleotides deduced from these sequence data were used to screen a human brain cDNA library[19]. Sequence analysis of positive clones revealed a homology of 100% with the previously cloned human gp95/sortilin[60]. The open reading frame of the nts3/gp95/sortilin cDNA encodes a protein of 833 amino acids containing an N-terminal signal peptide, a putative cleavage site for furin, a long luminal domain, a single TM and a short cytoplasmic tail (Fig. 3). The luminal domain contains a sequence similar to the two homologous domains of Vps10p, a yeast receptor for carboxypeptidase Y sorting, and the C-terminal tail exhibits marked sequence analogies with the corresponding segment of the cation-independent mannose 6-phosphate/insulin growth factor-II receptor CI-M6PR (Ref. 60). The peptide sequence determined as the N-terminus of the affinity-purified nts3 (Ref. 19) and sortilin[60] is not the N-terminus of the protein sequence deduced from the cDNA. Instead, this sequence is C-terminal to the furin cleavage site, indicating that the NTR3/sortilin is synthesized as a precursor which is processed into a 5 kDa peptide and a 95 kDa membrane protein.

The gene encoding human sortilin (designated *SOR1*) has been mapped to the proximal part of the short arm of chromosome 1 (Ref. 60). Two sortilin mRNA transcripts

of approximately 8 and 3.5 kb are expressed in human tissues. High levels of both forms are found in brain, spinal cord, heart, skeletal muscle, thyroid, placenta and testis[60].

Binding properties and subcellular localization

CHAPS-solubilized extracts of COS cells transfected with the cloned cDNA bind NT with an affinity of about 10 nM (Ref. 19). Co-transfection of the nts3/sortilin with furin partly converted the expressed receptor into a higher-affinity form (K_d = 0.3 nM). The low- and high-affinity NT binding sites probably correspond to the non-processed and furin-processed forms of the nts3/sortilin protein[19]. COS cells mainly express the nts3/sortilin into intracellular vesicles, in agreement with previous results obtained with both sortilin[60] and the nts3 receptor[19]. In rat adipocytes, sortilin is located in vesicles containing the glucose transporter GLUT4 and translocates to the plasma membrane in response to insulin[61,62]. Similarly, NT triggers insertion of the nts3 into the membrane from an intracellular compartment in mouse cortical neurons[63].

Mediation of NT functions by the three cloned receptors

Some of the pharmacological properties of NT have been ascribed to the selective activation of the NTS1 and nts2 receptors[64]. Intracerebroventricular injection in mice of antisense oligodeoxynucleotides directed against the nts2 decreases the nts2 mRNA and protein and concomitantly reduces NT-induced analgesia. This effect is specific in that the NTS1 levels are unaffected and sense or scramble oligodeoxynucleotides have no effect. In parallel experiments, NTS1-specific oligodeoxynucleotides selectively decrease expression of the NTS1 but not that of the nts2 receptor, and reduce the turning behaviour elicited by NT in mice[64]. Thus, NT-induced turning behaviour and analgesia are mediated by the NTS1 and nts2 receptors, respectively. These results are in agreement with structure–activity relationships of NT agonists[65,66] and antagonists[13,15]. For example, SR48692 blocks NTS1 but not nts2 and does not inhibit NT-induced analgesia[65], whereas SR142948A, which can block both NTS1 and nts2, is perfectly able to inhibit the analgesic properties of NT (Ref. 15).

No similar data are available for the nts3 and the physiological functions of the latest-cloned NT receptor remain hypothetical. Sequence similarities with the yeast sorting receptor Vps10p and the cation-independent mannose 6-phosphate/insulin growth factor II receptor as well as colocalization with the glucose transporter GLUT4 in adipocytes suggest that the nts3 receptor could either regulate the biosynthesis and the translocation of GLUT4-containing vesicles, or clear NT from the circulation or act as a sorting receptor. This NT receptor can also fulfil a classical function of transduction or serve as a vehicle to allow entrance of NT inside the cell where it could act as a regulator of transcription.

Concluding remarks

In 1996, a paper in this journal was entitled: *The neurotensin receptor: is there more than one subtype?*[67]. Today, this question can be answered in the affirmative. However, much progress remains to be done, in particular to define the physio-pathological roles associated with the three NT receptor subtypes that are the focus of this review. In this regard, it will be esssential to develop new pharmacological tools such as NT receptor agonists and antagonists specific for each class of receptor. Knockout mice lacking expression of either one of the three NT receptor subtypes might also prove useful in delineating the functions of these receptors. Finally, there is no reason to believe that all NT receptors have been characterized as the three known receptors do not account for all the observed properties of the peptide. In particular, the central hypothermic effects of NT and its analogues are not completely understood and could well involve an as yet unidentified NT receptor.

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Chemical names

JMV449: *H*-Lysψ(CH_2NH)-Lys-Pro-Tyr-Ile-Leu-OH

SR48692: 2-([1-{7-chloro-4-quinolinyl}-5-{2,6-dimethoxyphenyl}pyrazol-3-yl]carboxylamino)tricyclo(3.3.1.1.[3.7])decan-2-carboxylic acid

SR142948A: 2-(5,6-dimethylaminopropyl)-1-[4-{*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl}-2-ispropylphenyl]-1*H*-pyrazole-3-carbonyl)aminoadamantane-2-carboxylic acid

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