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Review

Molecular and cellular regulation of neurotensin receptor under acute and chronic agonist stimulation

Frédérique Souazé, Patricia Forgez *

INSERM U673-UMPC, Hôpital Saint-Antoine, Bâtiment Raoul Kourilsky, 184 rue du Faubourg Saint-Antoine, 75571 Paris Cedex 12, France

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ABSTRACT

Neurotensin is a tridecapteptide acting mostly in the brain and gastrointestinal tract. NT binds two G protein coupled receptors (GPCR), NTS1 and NTS2, and a single transmembrane domain receptor, NTS3/gp95/sortilin receptor. NTS1 mediates the majority of NT action in neurons and the periphery. Like many other GPCRs, upon agonist stimulation, NTS1 is internalized, endocytosed, and the cells are desensitized. It is tacitly acknowledged that the intensity and the lasting of cellular responses to NT are dependent on free and functional NTS1 at the cell surface. Understanding how NTS1 expression is regulated at the membrane should provide a better comprehension towards its function. This review analyzes and discusses the current cellular and molecular mechanisms affecting the expression of NTS1 at the cellular membrane upon acute and chronic NT stimulation.

Contents

0. Introduction

Neurotensin (NT) is a tridecapeptide localized primarily in the brain and in specialized endocrine cells (N-cells) of the small bowel [59]. NT is a neurotransmitter in the central nervous system (CNS) and a hormone in the periphery. Its actions are mediated through the stimulation of two specific G protein-coupled receptors (GPCR), NTS1 and NTS2, exhibiting high and

* *Corresponding author*. Tel.: +33 1 49 28 46 69; fax: +33 1 44 74 93 18.
E-mail address: forgez@st-antoine.inserm.fr (P. Forgez).
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low affinity for NT, respectively [17,66]. NT additionally binds to a single transmembrane domain receptor, NTS3 which is 100% homologous to gp95/sortilin [43]. NTS3 is predominantly localized in the trans Golgi network but the mature protein is also present in the plasma membrane [50].

In the CNS and in the periphery, the vast majority of known NT effects are mediated by NTS1, which is internalized and endocytosed upon agonist stimulation. During this process the cell becomes desensitized while the agonist message is transduced and NT cellular effects occur [32]. Cellular desensitization is an important feedback mechanism to prevent cellular over-stimulation. However, NT cell sensitization is dependent on the amount of free and functional NTS1 at the cell surface.

NT agonist induces different NTS1 responses invoking distinct trafficking and gene activation, dependent on the time and the dose of agonist exposure. On one hand, acute agonist stimulation leads to complete cell desensitization [34,42,72,74]. On the other hand, chronic stimulation with high agonist doses induces permanent cell sensitivity to NT [64,71]. *In vivo* the cellular response to NT is determined by the resultant of NT release, NT degradation, and the dynamic cellular regulation of NTS1 [16,37]. Defining the details of how NTS1 is regulated should provide a better understanding of NT function and cellular control. This review summarizes the evidence for NTS1-trafficking and for its regulation by diverse conditions of NT stimulation.

1. Trafficking of NT and NTS1 under acute and chronic agonist exposure

1.1. *NTS1 desensitization*

Cell desensitization arising after agonist exposure is a common mechanism for most GPCRs [26]. After a short exposure (several minutes) to a high concentration of agonist, NTS1 is activated, uncoupled, and internalized, leaving the cells in a desensitized state. Desensitization is characterized by the period of time during which the cells remain unresponsive to agonist. Carraway and Leeman [12], upon discovering space NT, also observed a lack of response to subsequent NT injections on the systemic NT hypotensive effect. Similar observations were made from cultured cells from different origins, such as neurons, transformed cells and cancer cells endogenously or transgenously expressing NTS1 [32]. Desensitization was observed at different levels, from effector activation to a final physiological effect, and was explained by a strong decrease of cell surface binding sites due to receptor internalization following initial exposure to NT [20,28,34]. The delay needed to reach the desensitized state varies from a few minutes for second messengers such as cGMP, Ca^{2+}, or IP_3 to several hours for NT mediated effects [1,28,72]. The desensitized state is caused by receptor uncoupling following receptor internalization. This state remains until NTS1 is recycled to the membrane, or new receptors reach the cell surface, or both [20,34]. Detailed NTS1 desensitization mechanisms have been extensively reviewed by Hermans and Maloteaux [32].

Receptor uncoupling from the heterotrimeric G protein is in response to receptor phosphorylation of specific serine/threonine residues by intracellular kinases dependent on the second messenger associated with the receptor, and the G protein-coupled receptor kinases that selectively phosphorylate NT-activated receptor [26]. The NTS1 C-terminal extremity contains the essential residues required for receptor uncoupling and internalization. The phosphorylation of the cluster serine-415, threonine-416, serine-417 is essential for NTS1 uncoupling [18,53]. The threonine-422 and the tyrosine-424 are crucial for the receptor internalization [15,33]. Uncoupling and phosphorylation of the NTS1 promotes the binding of cytosolic cofactor β arrestin, which engages the process of endocytosis [53,54].

1.2. *Receptor internalization and fate of internalized receptor*

NTS1 intemalization was first studied on neuronal cells and neurons and was shown to be induced by NT and NT fragments containing the active portion (residues 8–13). NTS1 internalization is time and temperature dependent, and can be suppressed by endocytosis blockers such as phenylarsine oxide, concanavalin A, and sucrose [42,25]. NTS1 internalization occurs via the clathrin endocytotic pathway since internalization is blocked by cell treatments such as hypertonic sucrose and potassium depletion [3,73]. Experiments examining the early steps involved in NTS1 endocytosis, using tagged receptor with green fluorescence protein (GPF), or epitope-tag, demonstrate that the receptor is most centrally located within cytoplasmic vesicles during the first 15–30 min [71,73].

The fate of NTS1 after internalization in early and late endosomes varies according to the cell type, the NT dose, and the time period for agonist exposure. Several situations have been described. In Cos-7 cells, the epitope-tagged NTS1 colocalized with lysosomal vesicle markers after 45 min of agonist exposure suggesting that NTS1 is degradated after stimulation and internalization [73]. Studies from neuroblastoma cell lines and rat primary cultured neurons confirmed this observation, since cells resensitization did not occur after agonist exposure and in the presence of protein synthesis inhibitors. It was concluded that internalized receptor was degradated and *de novo* synthesis receptor was responsible for cell resensitization [20,34]. These observations may, however, depend on the β-arrestin recruited to the plasma membrane upon agonist stimulation. NTS1 binds with strong affinity to both isoforms of β-arrestin 1 and 2, resulting in a delay of receptor dephosphorylation, and consequently causing delays in cellular resensitization and promoting the receptor targeting to the degradation pathway [54].

In synaptosomes from rat neo-striatum, a brain area where NTS1 is preferentially in presynaptic localization, NTS1 is internalized through a clathrin dependent pathway which is both time and temperature dependent. But in contrast to what was observed in NTS1 transfected or neuronal cells, NTS1 was recycled to the membrane [49]. This observation shows that NTS1 endocytosis and recycling is tissue dependent and suggests that different cellular regulatory molecules may contribute to endocytosis and trafficking processes.

Interestingly, cellular adaptation to NTS1 trafficking was observed upon continued and high concentration agonist