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Neurotensinergic augmentation of glutamate release at the perforant path-granule cell synapse in rat dentate gyrus: Roles of L-Type Ca^{2+} channels, calmodulin and myosin light-chain kinase

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ABSTRACT

Neurotensin (NT) serves as a neuromodulator in the brain where it is involved in modulating a variety of physiological functions including nociception, temperature, blood pressure and cognition, and many neurological diseases such as Alzheimer's disease, schizophrenia and Parkinson's disease. Whereas there is compelling evidence demonstrating that NT facilitates cognitive processes, the underlying cellular and molecular mechanisms have not been fully determined. Because the dentate gyrus expresses high densities of NT and NT receptors, we examined the effects of NT on the synaptic transmission at the synapse formed between the perforant path (PP) and granule cells (GC) in the rats. Our results demonstrate that NT persistently increased the amplitude of the AMPA receptor-mediated EPSCs at the PP-GC synapse. NT-induced increases in AMPA EPSCs were mediated by presynaptic NTS1 receptors. NT reduced the coefficient of variation and paired-pulse ratio of AMPA EPSCs suggesting that NT facilitates presynaptic glutamate release. NT increased the release probability and the number of readily releasable vesicles with no effects on the rate of recovery from vesicle depletion. NT-mediated augmentation of glutamate release required the influx of Ca^{2+} via L-type Ca^{2+} channels and the functions of calmodulin and myosin light chain kinase. Our results provide a cellular and molecular mechanism to explain the roles of NT in the hippocampus.

1. Introduction

Neurotensin (NT) is a tridecapeptide that is widely distributed in both the periphery such as the gastrointestinal tract, liver, pancreas, heart, lung and spleen, and the central nervous system including the hypothalamus, entorhinal cortex (EC), hippocampus, amygdala, septum, ventral tegmental area and substantia nigra (Boules et al., 2013; Caceda et al., 2006; Vincent et al., 1999). NT interacts with 3 types of receptors: NTS1, NTS2 and NTS3 (Hermans and Maloteaux, 1998; Mazella, 2001; Navarro et al., 2001; Nouel et al., 1999; St-Gelais et al., 2006; Vincent et al., 1999). NTS1 and NTS2 are G-protein-coupled receptors with seven-transmembrane domains and are distinguished based on their affinity for NT and sensitivity to the nonpeptide antagonist, SR48692, and the histamine receptor antagonist, levocabastine. NTS1 displays high affinity for NT and its effects are usually blocked by SR48692, whereas NTS2 shares only around 40% amino acid identities with NTS1, shows low affinity for NT and is sensitive to levocabastine (Pelaprat, 2006; St-Gelais et al., 2006; Vincent et al., 1999). NTS1 receptors are usually coupled to Gq proteins resulting in activation of phospholipase C (PLC) and the inositol phosphate signaling cascade (Watson et al., 1992), although other signaling molecules including cyclic GMP (Mistry and Vijayan, 1987; Slusher et al., 1994), cyclic AMP (Yamada et al., 1993), MAP kinase (Ehlers et al., 2000; Poinot-Chazel et al., 1996) and Akt (Liu et al., 2004) have been implicated. Unlike NTS1 receptors, the pharmacological and signaling properties of NTS2 receptors are still controversial and it is still uncertain as to whether NT is an agonist, inverse agonist or antagonist for this receptor type (Hwang et al., 2009; Mazella et al., 1996; Sarret et al., 2002). NTS3 receptors are originally identified as the intracellular sorting protein, sortilin, and they bind NT with high affinity once converted to the mature form upon cleavage by the protein convertase furin. This non G protein-coupled receptor appears to be involved in molecule sorting between the cell surface and intracellular compartments (Mazella, 2001; Navarro et al., 2001).

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NT has been implicated in modulating many physiological functions including nociception, temperature, blood pressure and cognition, and a variety of neurological diseases such as Alzheimer's disease (AD), schizophrenia and Parkinson's disease (Boules et al., 2013; Tyler-McMahon et al., 2000). Among these physiological functions and pathological disorders, there is compelling evidence demonstrating that NT modifies the cognitive processes and functional alterations of NT may undergo the pathology of AD that is characterized as declination of memory. For example, NTS1 polymorphisms are significantly associated with variation in working memory performance among healthy adults (Li et al., 2011); NT binding sites are negatively correlated with age and cognitive status (Rowe et al., 2006); application of NT receptor agonists *in vivo* enhances (Azmi et al., 2006; Laszlo et al., 2010; Ohinata et al., 2007; Xiao et al., 2014) whereas administration of NT receptor antagonists decreases (Laszlo et al., 2010; Tirado-Santiago et al., 2006) cognitive functions; whereas NTS2 knockout (KO) mice showed reduced fear memory (Yamauchi et al., 2007), NTS1 receptors are usually considered to be responsible for the effects of NT on memory (Azmi et al., 2006; Laszlo et al., 2010; Tirado-Santiago et al., 2006). Paradoxically, the cellular and molecular mechanisms whereby NT modifies the processes of cognition have not been fully determined. In the previous study, we have demonstrated that activation of NTS1 receptors in layer II principal neurons in the EC persistently increases the excitability of these neurons (Xiao et al., 2014). Because the axons of layer II neurons form the perforant path (PP) that synapses onto the dentate gyrus granule cells (GC) of the hippocampus, we examined the effects of NT on synaptic transmission at the PP-GC synapse. Our results demonstrate that NT persistently increases glutamate release at the PP-GC synapse via activation of NTS1 receptors. NT-induced persistent glutamate release involves increases in the readily releasable pool size and release probability without altering quantal size. The effects of NT require an increase in intracellular Ca^{2+} mediated by the influx of Ca^{2+} via L-type Ca^{2+} channels and the functions of calmodulin and myosin light chain kinas (MLCK). Our results further the understanding of the cellular and molecular mechanisms whereby NT facilitates learning and memory in the hippocampal formation.

2. Materials and methods

2.1. Slice preparation

The ages of the animals used for electrophysiological recordings were postnatal 3–5 weeks for Sprague–Dawley rats and mice in which NTS1 gene or NTS2 gene were deleted as described previously (Xiao et al., 2014). Pairs of homozygous NTS1 or NTS2 knockout (KO) mice were initially provided by Dr. Etsuko Wada from the Department of Degenerative Neurological Diseases, National Institute of Neuroscience, Tokyo, Japan. The mice used for the experiments in this study were bred in the animal facility of the University of North Dakota. Detailed experimental procedures for generating and genotyping the KO mice were described previously (Maeno et al., 2004). Horizontal brain slices (400 μm) including the hippocampus, subiculum and EC were cut using a vibrating blade microtome (VT1000S; Leica, Wetzlar, Germany) as described previously (Deng and Lei, 2008; Wang et al., 2011; Xiao et al., 2009) with the following modifications (Xiao et al., 2014). After being deeply anesthetized with isoflurane, animals were decapitated and their brains were dissected out in ice-cold saline solution that contained (in mM) 130 N-methyl-D-glucamine (NMDG)-Cl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 5.0 $MgCl_2$, and 10 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4, adjusted with HCl). Slices were then incubated in the above solution except NMDG-Cl was replaced with NaCl at 35 °C for 1 h for recovery and then kept at room temperature (~24 °C) until use. All animal procedures conformed to the guidelines approved by the University of North Dakota Animal Care and Use Committee.

2.2. Recordings of synaptic currents

Whole-cell patch-clamp recordings using a Multiclamp 700B amplifier in voltage-clamp mode were made from dentate gyrus GCs in hippocampal slices. Cells in the slices were visually identified with infrared video microscopy and differential interference contrast optics (Ramanathan et al., 2012; Wang et al., 2012, 2013). Recording electrodes were filled with the solution containing (in mM) 100 Cs-gluconate, 0.6 EGTA, 5 $MgCl_2$, 8 NaCl, 2 ATP_2Na, 0.3 GTPNa, 40 HEPES and 1 QX-314 (pH 7.3). The extracellular solution comprised (in mM) 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$ and 10 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4). Bicuculline (10 μM) was included in the extracellular solution to block $GABA_A$ receptors. The holding potential was at −60 mV. AMPA receptor-mediated EPSCs were evoked by placing a stimulation electrode in the middle to the inner one third of molecular layer of dentate gyrus to stimulate the medial PP. Under these conditions, the recorded currents were completely blocked by application of DNQX (10 μM) or GYKI 52466 (100 μM) at the end of experiments confirming that they were mediated by AMPA receptors. Series resistance was rigorously monitored by delivering a 5 mV voltage step after each evoked current. Experiments were discontinued if the series resistance changed by >15%. Data were filtered at 2 kHz, digitized at 10 kHz, acquired on-line and analyzed after-line using pCLAMP 9 or 10 software (Molecular Devices, Sunnyvale, CA). To avoid potential desensitization induced by repeated bath applications of NT, one slice was limited to only one application of NT and only one cell was recorded from each slice.

2.3. Data analysis

Data are presented as the means ± S.E.M. Concentration-response curve of NT was fit by Hill equation: $I = I_{max} \times \{1/[1 + (EC_{50}/[ligand])^n]\}$, where I_{max} is the maximum response, EC_{50} is the concentration of ligand producing a half-maximal response, and n is the Hill coefficient. Student's paired or unpaired t test or analysis of variance (ANOVA) was used for statistical analysis as appropriate; P values are reported throughout the text and significance was set as $P < 0.05$. N numbers in the text represent the number of cells examined unless stated otherwise.

3. Results

3.1. NT increases glutamatergic transmission at the PP-GC synapses via activation of NTS1 receptors

We examined the effects of NT on glutamatergic transmission by recording, from the dentate gyrus GCs, AMPA EPSCs evoked by stimulating the PP. Bath application of NT (0.5 μM) for 5 min enhanced AMPA EPSCs (Fig. 1A_1–A_2). The amplitude of AMPA EPSCs began to increase gradually and reached maximal in ~20 min after the beginning of NT application (150 ± 12% of control, n = 10, $P = 0.002$ vs. baseline, Fig. 1A_1–A_2). NT-induced increases in AMPA EPSCs persisted for at least 1 h in our whole-cell recording configuration (136 ± 6% of control, n = 10, $P < 0.001$ vs. baseline, Fig. 1A_1–A_2). This phenomenon resembles long-term potentiation (LTP) suggesting that it may be related to learning and memory. However, bath application of the heat-inactive NT (0.5 μM, inactivated by heating at 100 °C for 20 min) for 5 min failed to significantly increase the amplitude of AMPA EPSCs (101 ± 2% of control, n = 5, $P = 0.78$ vs. baseline, Fig. 1B_1–B_2) suggesting that it is unlikely that NT-induced increases in AMPA EPSCs are due to the nonspecific action of the NT preparation. Furthermore, bath application of the active fragment of NT (NT8-13, 0.5 μM), also enhanced AMPA EPSCs (171 ± 18% of control, n = 5, $P = 0.016$ vs. baseline, Fig. 1C) whereas application of the inactive fragment of NT (NT1-8, 0.5 μM) did not alter AMPA EPSCs (101 ± 7% of control, n = 6, $P = 0.89$ vs. baseline, Fig. 1C) suggesting that NT-induced facilitation of AMPA EPSCs is mediated by activation of NT receptors. The EC_{50} of NT was measured to be 94 nM (Fig. 1D).

We next tested the roles of NT receptors in NT-mediated facilitation of AMPA EPSCs. Pretreatment of slices with and continuous bath application of NTS1 antagonist, SR48692 (1 μM), blocked NT-induced increases in AMPA EPSCs (109 ± 7% of control, n = 5, $P = 0.24$ vs. baseline, Fig. 2A). However, application of the NTS2 antagonist, levocabastine (30 μM), failed to alter the effect of NT significantly (148 ± 16% of control, n = 6, $P = 0.034$ vs. baseline; $P = 0.89$ vs. NT alone, Fig. 2A). Furthermore, application of PD149163 (0.5 μM), a NTS1 agonist, also significantly augmented AMPA EPSCs (136 ± 6% of control, n = 6, $P = 0.002$ vs. baseline, Fig. 2B). These results demonstrate that NT increases AMPA EPSCs by activation of NTS1 receptors. We further probed the involvement of NT receptors by utilizing NTS1 and NTS2 KO mice. Application of NT (0.5 μM) to slices cut from NTS1 KO mice failed to significantly