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Neuropeptide S: A transmitter system in the brain regulating fear and anxiety

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ABSTRACT

The recently discovered Neuropeptide S (NPS) and its cognate receptor represent a highly interesting system of neuromodulation with unique physiological effects. On one hand, NPS increases wakefulness and arousal. On the other, NPS produces anxiolytic-like effects by acutely reducing fear responses as well as modulating long-term aspects of fear memory, such as attenuation of contextual fear or enhancement of fear extinction. The main sources of NPS in the brain are a few clusters of NPS-producing neurons in the brainstem. NPS binds to a G-protein-coupled receptor that is highly conserved among vertebrates and stimulates mobilization of intracellular Ca^{2+} as well as activation of protein kinases. In synaptic circuits within the amygdala, which are important for processing of acute fear as well as formation and expression of fear memories, NPS causes increased release of the excitatory transmitter glutamate, especially in synaptic contacts to a subset of GABAergic interneurons. Polymorphisms in the human NPS receptor gene have been associated with altered sleep behavior and panic disorder. In conclusion, the NPS system displays a unique physiological profile with respect to the specificity and time course of its actions. These functions could provide interesting opportunities for both basic research and clinical applications.

1. Introduction

About 10–15% of adults are experiencing clinically relevant symptoms of anxiety disorders or post-traumatic stress disorders. Chronic anxiety disorders significantly reduce the quality of life for the affected patients. Research investigating the neurobiological basis of anxiety disorders and stress is therefore not only an academic challenge but could also have significant clinical and public health-related impact. Most importantly, better understanding of intra- and intercellular signal pathways in the critically involved synaptic networks of the brain are expected to yield novel insights for the development of focused drug-based therapies. Besides the "classical" transmitter systems, neuropeptides have received increasing attention since they appear to play a central role in modulation of information processing particularly in those brain areas that are involved in expression of fear or stress. The recently discovered neuropeptide S (NPS) and its cognate receptor (NPSR) display a spectrum of effects that are highly interesting to basic research on anxiety disorders. NPS produces anxiolytic-like effects in rodents while increasing wakefulness at the same time (Xu et al., 2004; Vitale et al., 2008; Rizzi et al., 2008). The anxiolytic-like effect of NPS has been confirmed in a number of preclinical mouse and rat models predictive of anxiolytic action, including the four-plate test, elevated zero maze, stress-induced hyperthermia, and defensive burying (Leonard et al., 2008; Vitale et al., 2008). While NPS is thus considered a potent anxiolytic compound, the studies to date have not revealed it to alter depression-related behavior (Leonard et al., 2008). In any case, and of particular interest here, are genetic studies indicating that a variant of human NPSR with reduced agonist efficacy might be associated with panic disorder (Reinscheid et al., 2005; Okamura et al., 2007). This paper presents recent findings to explain NPS-induced effects at the molecular, cellular and behavioral level, with a focus on circuits of the amygdala and related modulation of fear expression and memory.

2. Neuropeptide S and the NPS receptor

NPS and its receptor NPSR were first described in 2004 and represent a novel transmitter system that is found mainly expressed in the brain (Xu et al., 2004). NPS contains 20 amino acids, is highly conserved among tetrapods, and was termed after its aminoterminal serine residue (S) found in all species examined

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so far (Reinscheid, 2007) (Fig. 1 A). It is processed from a larger precursor protein (89 amino acids in rat). In the rat brain, NPS precursor mRNA expression is detected by *in situ* hybridization in three nuclei of the brain stem: a previously uncharacterized area between the locus coeruleus and Barrington's nucleus (Fig. 1 B), an area near the lateral parabrachial nucleus and in the principle sensory 5 nucleus of the trigeminal system (Xu et al., 2004, 2007). NPS is co-expressed with excitatory transmitters such as glutamate, acetylcholine or corticotropine releasing factor (CRF). The projection areas of these NPS-producing neurons are currently unknown, but are a focus of current studies. Outside of the central nervous system, the NPS precursor is expressed mainly in endocrine tissues (Xu et al., 2004).

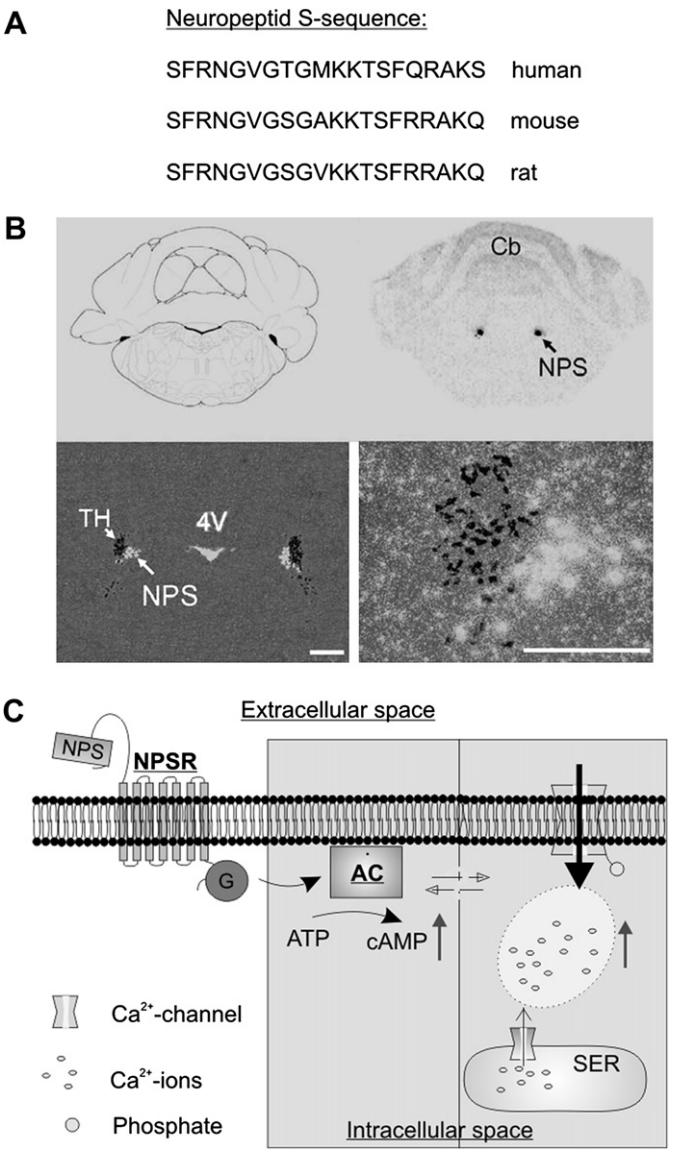

Fig. 1. NPS and the NPS-receptor. A) Neuropeptide S consists of 20 amino acids and displays significant sequence-homology in human, mouse and rat. B) Identification of NPS-producing neurons in the brain stem of rats through *in situ*-hybridization against the mRNA of the NPS-precursor peptide (white). NPS-positive neurons are found next to tyrosine hydroxylase-positive neurons (TH) of the locus coeruleus, but do not colocalize. 4V = 4. ventricle, Cb = cerebellum. Scale bars 500 μm (left), 250 μm (right). C) The binding of NPS to the NPS-receptor triggers the synthesis of intracellular cAMP via a G-protein (G) adenylyl cyclase (AC) pathway. Furthermore, the NPS–NPS-receptor interaction leads to a significant increase in intracellular Ca^{2+}-concentration, either by activation of Ca^{2+}-channels in the plasma membrane and/or the endoplasmatic reticulum. (A–C, modified from Xu et al., 2004).

The NPSR is a typical member of the G-protein-coupled receptor superfamily. It is also known as GPR154, VRR1 or GPRA (Gupte et al., 2004; Laitinen et al., 2004). It is mainly expressed in the brain (Xu et al., 2007) and its mRNA can be detected in brain areas involved in olfactory processing, such as anterior olfactory bulb, prepiriform and endopiriform cortex. NPSR expression was also found in brain areas critical for fear processing (e.g. amygdala and paraventricular nuclei of the hypothalamus) as well as brain regions involved in sleep-wake modulation (e.g. thalamic intralaminar nuclei, preoptic nucleus or tuberomammillary nucleus). Intracellularly, NPSR couples to G-proteins. Based upon the *in vitro* pharmacology of the NPSR, the G_q and G_s-type of G proteins are likely candidates, although alternative pathways including $G_{a(olf)}$ or $G_{ßγ}$-signaling cannot be excluded. Agonist binding leads to elevated intracellular Ca^{2+} concentration and increase of cAMP formation via activation of adenylyl cyclase (Reinscheid et al., 2005). In addition, NPSR activation stimulates mitogen-activated protein kinase (MAPK) phosphorylation (Reinscheid et al., 2005). In essence, NPS binding to its receptor induces a number of intracellular signaling cascades which might produce numerous effects inside the cell. Recently, bicyclic piperazines (SHA 66 and SHA 68; Okamura et al., 2008) and NPS peptide analogues (Camarda et al., 2009; Guerrini et al., 2009) were identified as effective NPSR antagonists. Details of NPS-induced intracellular signal transduction events are currently under investigation.

The human NPSR gene is encoded by at least 9 exons and is located on chromosome 7p14. A number of polymorphisms have been identified in the human NPSR gene and associated with increased risk of asthma (Laitinen et al., 2004) or circadian phenotypes (Gottlieb et al., 2007). One single nucleotide polymorphism leads to an Asn/Ile exchange in position 107 (N107I), which results in an increase in the potency of NPS at NPSR-Ile^{107} (Reinscheid et al., 2005; Bernier et al., 2006). Structural characterization of NPS and mutagenesis studies showed that the NH(2)-terminal third of NPS are necessary and sufficient for activation of NPSR (Bernier et al., 2006). Furthermore, part of a nascent helix within the peptide might act as a regulatory region that inhibits receptor activation. This inhibition is absent in the Ile^{107} variant of NPSR, suggesting that residue 107 interacts with the regulatory region of NPS (Bernier et al., 2006). Of particular interest here is a study reporting that the less active isoform NPSR-Asn^{107} is underrepresented in a male cohort of panic disorder patients, while samples from healthy volunteers, patients diagnosed with schizophrenia or attention deficit/hyperactivity disorder displayed no association with any of the two NPSR alleles (Okamura et al., 2007). Therefore, the NPSR gene might be involved in the pathogenetic mechanisms of anxiety or panic disorders in a gender-specific manner.

3. NPS-mediated control of transmitter release in the amygdala

The amygdala – located in the temporal lobe of the brain – is a key structure of the limbic system and is composed of anatomically and functionally distinct nuclei. The amygdala plays a central role in processing and expression of emotions, especially during formation and consolidation of fear-related memory traces (reviewed in Maren and Quirk, 2004). Fig. 2 provides an overview about the functional and structural organization of amygdalar nuclei that have been proven relevant for NPS action so far. The lateral (LA), basolateral (BLA) and basomedial amygdala form the basolateral amygdaloid complex. The LA receives afferent inputs from various brain regions (e.g. thalamus, prefrontal cortex (PFC), brainstem, and hippocampus) and is axonally connected to BLA and the central amygdala (CeA), which provides the major output