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The role of the neuropeptide S system in addiction: Focus on its interaction with the CRF and hypocretin/orexin neurotransmission

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ABSTRACT

Recent behavioral, pharmacological and molecular findings have linked the NPS system to drug dependence. Most of the evidence supports the possibility that increased NPS activity may contribute to shaping vulnerability to addiction, especially relapse. However, data suggesting that the anxiolytic-like properties of NPS may have protective effects on addiction have been also published. In addition, evidence from conditioned place preference experiments, though not unequivocal, suggests that NPS *per se* is devoid of motivational properties. Intriguingly, several effects of NPS on drugs of abuse appear to be mediated by downstream activation of brain corticotrophin releasing factor (CRF) and hypocretin-1/orexin-A (Hcrt-1/Ox-A) systems. The major objective of the present article is to review the existing work on NPS and addiction. Particular attention is devoted to the interpretation of findings revealing complex neuroanatomical and functional interactions between NPS, CRF, and the Hcrt-1/Ox-A systems. Original data aimed at shedding light on the role of NPS in reward processing are also shown. Finally, existing findings are discussed within the framework of addiction theories, and the potential of the NPS system as a treatment target for addiction is analyzed.

Contents

Abbreviations: NPS, neuropeptide S; NPSR, neuropeptide S receptor; CRF, corticotrophin releasing factor; Hcrt-1/Ox-A, hypocretin-1/orexin-A; GPR154, G protein coupled receptor 154; ppNPS, prepropeptide NPS; Lys–Arg, lysine–arginine; mRNA, messenger RNA; LC, locus coeruleus; PeF, perifornical (area); EGFP, enhanced green fluorescent protein; KF, Kölliker–Fuse; GPCR, G protein coupled receptor; cAMP, cyclic adenosine mono phosphate; hNPSR-A, human NPSR form A; hNPSR B_{LONG}, human NPSR form B; VMH, hypothamalic ventromedial nucleus; DMH, dorsomedial hypothalamic nucleus; ICV, intracerebroventricular; LA, lateral amygdala; BLA, basolateral amygdala; EPM, elevated plus maze; PaV, paraventricular nucleus; ACTH, adrenocorticotropic hormone; AVP, arginine-vasopressin; EPN, endopiriform nucleus; PFC, prefrontal cortex; VTA, ventral tegmental area; DA, dopamine; GABA, Gamma aminobutyric acid; CPP, conditioned place preference; msP, Marchigian Sardinian (alcohol) preferring (rats); LH, lateral hypothalamus; OX_1, orexin 1 receptor; NMDA, N-methyl-D-aspartic acid; NA, noradrenaline; BNST, bed nucleus of stria terminalis; HPA, hypothalamic pituitary adrenal; CRF_1, CRF receptor 1; WT, wild type; KO, knock out; IP, intraperitoneal; CeA, central amygdala; LV, lateral ventricle; ACo, anterior cortical amygdaloid nucleus; M2, motor cortex; EN, endopiriform nucleus.

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1. Introduction

Neuropeptide S (NPS) is a 20 amino-acid peptide identified as the endogenous ligand for the deorphanized G-protein coupled receptor 154 (GPR 154), currently named the NPS receptor (NPSR) (Xu et al., 2004). This name was coined since the N-terminal residue, is a serine that is conserved in all species. The primary structure of NPS is highly conserved in higher vertebrates, but seems to be absent in fish (Reinscheid, 2007). NPS results from the cleavage of a prepropeptide (ppNPS) that shares the typical structural characteristics of other neuropeptide precursors. The cleavage of the precursor at a proteolytic processing site constituted by a Lys–Arg residue pair, releases the 20 amino acid mature peptide (Xu et al., 2007).

The NPS precursor gene is encoded by three small exons interrupted by two introns. The genomic organization of the NPS precursor gene seems to be identical in all species; only a few positions in the center and in the carboxy-terminal part of the peptide show sequence variations, indicating that the amino-terminus might contain structures critical for receptor binding (Reinscheid, 2007).

Detailed *in situ* hybridization studies have shown that ppNPS mRNA is expressed in only three rat brainstem regions; the peri-locus coeruleus (LC) area, the principal sensory trigeminal nucleus, and the lateral parabrachial nucleus (Xu et al., 2007). Confirming this finding, in a recent work using transgenic mice that express enhanced green-fluorescent protein (EGFP) under control of the endogenous ppNPS promoter, it was shown that there are about 500 NPS cells in the brainstem; all are localized between the Kölliker–Fuse nucleus (KF) of the lateral parabrachial area and the peri-LC area (Liu et al., 2011).

In the peri-LC area, NPS expressing neurons are predominantly glutamatergic, with a few cholinergic cells found in the lateral portion of this structure. In the principal sensory trigeminal nucleus, NPS co-localizes with glutamate, while in the lateral parabrachial nucleus, co-expression with CRF has been reported (Xu et al., 2004, 2007). Of note, in the EGFP mice a dense orexin/hypocretin fiber network surrounding NPS positive cells was described, thus suggesting the possibility of cross-talk between these two neuronal populations (Liu et al., 2011).

The NPSR has the typical consensus GPCR structure, with seven trans-membrane domains. In humans, the NPSR gene encodes at least eight receptor variants, but evidence suggests that only two of them, hNPSR-A and hNPSR B_{LONG}, that differ in their C-termini, produce functional receptors expressed on the cell membrane (Vendelin et al., 2005). The receptor variant hNPSR-A was found in two forms given by an A/T single-nucleotide-polymorphism (SNP) at position 107 coding for an Asn-Ile exchange (Laitinen et al., 2004). NPS showed a 10-fold higher potency on the NPSR Ile^{107} variant compared to NPSR Asn^{107} stimulating a higher mobilization of intracellular Ca^{2+} and cAMP formation (Reinscheid et al., 2005). Evidences demonstrate that the Asn-Ile^{107} SNP is related to panic disorders. In panic disorder (PD) patients the A/A genotype, encoding for NPSR Asn^{107}, was under-represented whereas both heterozygous (A/T) and homozygous (T/T) Ile^{107} variants were increased compared to healthy controls (Domschke et al., 2011; Okamura et al., 2007).

In rodents, only one variant of NPSR has been found, exhibiting high sequence homology with hNPSR-A (Pulkkinen et al., 2006). NPSR couples to both Gq and Gs proteins, hence receptor activation by NPS induces mobilization of Ca^{2+}, stimulates cAMP synthesis, and increases cellular excitability (Meis et al., 2008; Reinscheid and Xu, 2005; Xu et al., 2004; Yoshida et al., 2010).

Contrary to NPS, NPSR mRNA is widely expressed in many brain regions, including the olfactory regions, amygdala complex, and other limbic structures. NPSR mRNA is also expressed in the input and output regions of the hippocampal formation, and in multiple hypothalamic regions, such as the arcuate nucleus, hypothalamic ventromedial nucleus (VMH), and dorsomedial hypothalamic nucleus (DMH) (Liu et al., 2011; Xu et al., 2007).

Recently, the availability of specific antibodies has allowed analysis of the distribution of the NPSR protein in rat brain. Results revealed that the distribution of NPSR protein is consistent with the distribution of NPSR mRNA (Leonard and Ring, 2011). The widespread distribution of the NPSR and its mRNA in the brain indicates that the NPS system may be important in regulating a variety of physiological functions.

A seminal work by Xu et al. (2004) demonstrated that intra-cerebro-ventricular (ICV) injection of NPS increased wakefulness and reduced all stages of sleep in rats. In the same study it was shown that in mice, NPS stimulated locomotion in both naïve and habituated animals, and exerted an anxiolytic-like effect in a variety of behavioral tasks (*i.e.*, marble burying, elevated plus-maze, light-dark box, open field). These findings have been confirmed in follow-up studies, where the striking anxiolytic/pro-arousal traits of NPS were not only confirmed but expanded using a larger battery of behavioral tests, including the four-plate test and stress induced hyperthermia (Jungling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008). Interestingly, ICV as well as site specific injection of NPS into the lateral amygdala (LA) and baso-lateral amygdala (BLA) exerted an anxiolytic-like effect in the elevated plus-maze (EPM), light dark box, and open field, but did not increase locomotor activity (Jungling et al., 2008; Leonard et al., 2008; Meis et al., 2008). A role for NPS in the modulation of stress and arousal has also been documented, following ICV and intra-hypothalamic paraventricular nucleus (PaV) administration studies in which it was shown that NPS activates locomotion, increases plasma ACTH and corticosterone levels, and induces CRF and arginine-vasopressin (AVP) release (Smith et al., 2006). In another study, it was shown that ICV injection of NPS activates hypocretin-1/orexin-A (Hcrt-1/Ox-A) neurons in the hypothalamus (Niimi, 2006).

The NPS system has been shown to be involved in the modulation of conditioned fear behavior in mice (Jungling et al., 2008; Meis et al., 2008). Injection of NPS into the endopiriform nucleus (EPN) reduced freezing behavior during contextual fear memory retrieval, an effect probably obtained through activation of the BLA *via* stimulation of glutamatergic synaptic activity (Meis et al., 2008). Injection of NPS in the same area did not affect anxiety behavior in the EPM, suggesting that activation of NPSR in the EPN selectively modulates fear conditioned memory but is not involved in the modulation of general anxiety (Meis et al., 2008). In addition, when administered into the LA and BLA, NPS reduced expression of the conditioned fear response and facilitated fear extinction (Jungling et al., 2008; Meis et al., 2008).

The NPS possesses a unique pharmacological profile being able to mediate dissimilar and to a large extend opposite physiological actions such as arousal and anxiolysis. Unfortunately the data available so far do not allow a conclusive explanation for this dualistic role of NPS, but few speculations are possible.