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Cloning and distribution of neuropeptide S and its receptor in the pig

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ABSTRACT

Neuropeptide S (NPS) precursor gene is present in most vertebrates. However, the genes of NPS and its receptor (NPSR), and their functions in the pig are currently not well understood. In order to clarify their physiological functions, it is essential to characterize in detail the distribution of NPS and NPSR. In this report, the cDNAs of NPS and NPSR were cloned and sequenced. Homology and phylogenetic analysis of NPS gene sequences were performed. The expression of NPS and NPSR mRNA in the pig was systemically investigated using semiquantitative reverse transcription polymerase chain reaction (RT-PCR), while the distribution of NPS was determined by immunohistochemistry. Our results demonstrated that the gene and predicted amino acid sequences of both NPS and NPSR were highly conserved. Phylogenetic analysis showed that NPS coding sequences from related species display high degrees of homology. NPS and NPSR mRNAs were widely expressed in various tissues of the pig. NPS mRNA was highly expressed in CNS, while NPSR mRNA was widely expressed in many tissues, with high expression in the hypophysis, endocrine tissues and glands. NPS protein also exhibited the different distribution in various organs. In the pig brain, NPS immunoreactive cells were mainly found in the diencephalon, pons and hypophysis, while immunoreactive fibres were widely distributed in the hypothalamus and olfactory bulb. In the peripheral organs, NPS immunoreactive cells were observed in the respiratory tract, alimentary tract, endocrine organs, genitourinary tract, lymphatic organs, muscle tissue, skin and skin appendages. By showing gene sequences and distribution of NPS and NPSR, these results suggest that NPS and NPSR in the pig might play important role in modulating a variety of physiological functions as in human and other animals. This research provided molecular and morphological data for further study of physiological function of NPS–NPSR system.

1. Introduction

Neuropeptide S (NPS), a 20 amino acid residue neurotransmitter, affects many physiological processes in mammals. In humans, NPS is part of a larger precursor molecule that contains several proteolytic processing sites (Reinscheid and Xu, 2005). The primary sequence of NPS is highly conserved in different species (Reinscheid, 2007, 2008; Xu et al., 2004). NPS is the endogenous ligand of the previously orphan G-protein coupled receptor (WO 0231145 A1; Sato et al., 2002; Xu et al., 2004), which is newly named NPS receptor (NPSR) (Bernier et al., 2006; Roth et al., 2006). NPS and NPSR form a newly discovered neuropeptide system that modulates many physiological and pathological activities. The central addition of NPS increased motor activity (Okamura et al., 2008; Roth et al., 2006; Xu et al., 2004), produced anxiolytic-like effects (Rizzi et al., 2008; Xu et al., 2004), potently promoted wakefulness and suppressed all stages of sleep (Xu et al., 2004). NPS and NPSR may also be involved in food intake (Beck et al., 2005; Cline et al., 2007; Niimi, 2006; Smith et al., 2006), stress (Smith et al., 2006) and macrophage immune responses (Pulkkinen et al., 2006). Therefore, further research of NPS and its receptor might have wide applications, because they are probably important in developing efficacious medicines for anaphylactic diseases, wakefulness and stress (Lei et al., 2008).

Although NPS appears to be absent from fish genomes, sequence coding for NPS are present in pigs and other vertebrate genomes (Reinscheid, 2007). The expression of both NPS precursor and NPSR genes have been analyzed by in situ hybridization in the brain of the rat (Xu et al., 2007). These experiments showed that the NPSR mRNA was widely expressed throughout the nervous system, with the highest levels showing up in the cerebral cortex, thalamus, hypothalamus and amygdala, and low levels in the brainstem. In contrast, the NPS precursor mRNA was mainly expressed in brainstem nuclei such as in the locus coeruleus area, the principal sensory trigeminal nucleus, and the lateral parabrachial nucleus. A small number of scattered NPS-positive cells were found in other brain areas, such as in the amygdale and hypothalamus (Xu et al., 2007). The regional distribution of NPS and NPSR expression in the rat suggested that activation of the NPS system might influence behavioral arousal and possibly modulate sleep-wakefulness cycles (Xu et al., 2004). Strong expression of NPSR

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Table 1
The primers used in PCR.

Primers	5′–3′ sequence
S (+)	GGTGTCCGGAAAACCTGATTACT
S(−)	CACAGCTTTGCTCTTCGAAAGGA
R(+)	TTGCCGAGTGGTCCGCTATT
R(−)	CCTTTGAGATGAGTCCTCGGTT
H(+)	CGGACATCTAAGGGCATCA
H(−)	AAGACGGACCAGAGCGAAA

"S", pig neuropeptide S; "R", pig neuropeptide S receptor; "H", pig 18S rRNA; " + ", sense primer, "−", anti-sense primer.

mRNA in the amygdala, the dorsal endopiriform nucleus, and various hypothalamic nuclei suggested that the NPS system might influence emotional behaviors such as stress or anxiety responses (Reinscheid, 2008). Anatomical substrates for NPS-mediated effects on feeding behavior might be the arcuate nucleus of the hypothalamus that expressed high levels of NPSR (Xu et al., 2007).

To our knowledge, there is no relevant paper on pig NPS and NPSR genes. It is lack of information to report the distribution and biological role of NPS in pigs. In this study, we reported identification of a NPS cDNA and a NPSR cDNA in the pig. The genes were cloned using specific primers with the polymerase chain

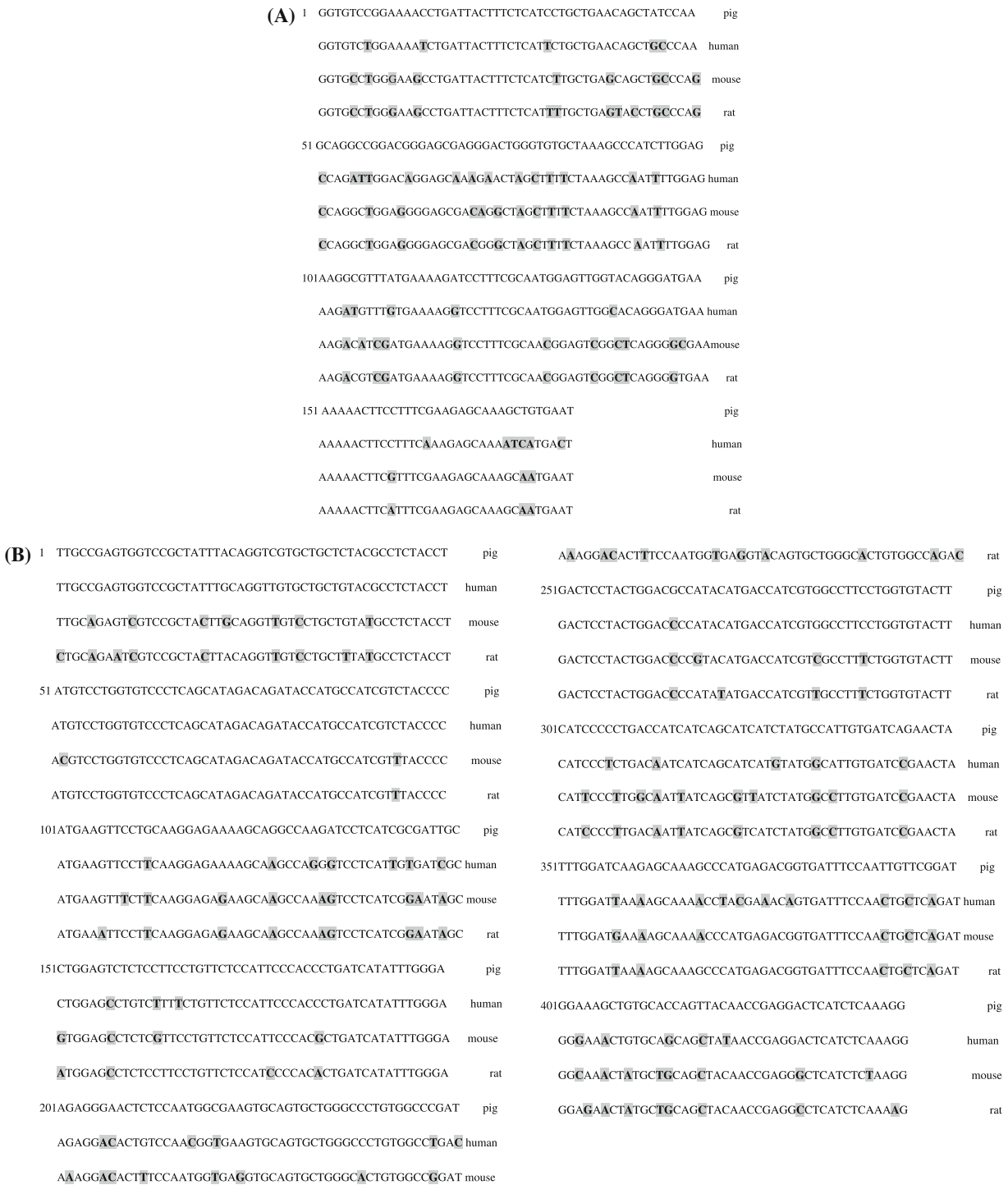

Fig. 1. Nucleotide sequences of NPS (A) and NPSR (B) from pig, which were compared with the human, mouse and rat sequences. Nucleotide sequences different from the pig NPS and NPSR sequences are shaded.