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Characterisation, localisation and expression of porcine *TACR1, TACR2* and *TACR3* genes

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ABSTRACT: Substance P is involved in many physiological and pathophysiological processes. This functional diversity is mediated by three neurokinin receptor subtypes (NK1R, NK2R and NK3R) encoded by the *TACR1*, *TACR2* and *TACR3* genes, respectively. Despite the increasing interest in using pigs (*Sus scrofa*) to study human disease mechanisms, the sequences of these receptors are still unconfirmed or in the case of the NK1 receptor, not yet even unpredicted. We employed *in silico* analysis to define the localisation of the porcine tachykinin receptor genes, and to predict the structures and amino acid sequences of the respective proteins. A reverse transcription polymerase chain reaction (RT-PCR) assay was performed to analyse the expression of tachykinin receptor genes in different porcine tissues. The data show that the *TACR1* gene is located on chromosome 3, *TACR2* on chromosome 14 and *TACR3* on chromosome 8. All three genes encode proteins with structures that incorporate features of G-protein-coupled receptors with sizes of 407, 381 and 464 amino acids, respectively. The receptors display a high degree of similarity to other mammalian neurokinin receptors. The NK1R subtype is expressed in both the central nervous system and peripheral tissues, while NK2R expression seems to be localised mostly to peripheral tissues. The expression of NK3R is found mainly in the central nervous system. This report provides for the first time the results of a comprehensive analysis of the structure and distribution of porcine NK1R, as well as other porcine neurokinin receptors and their genes. We hope that our data may offer an invaluable foundation for the future studies on the function of diverse tachykinin peptides in the central nervous system and peripheral tissues.

Keywords: NK1 receptor; NK2 receptor; NK3 receptor; pig; substance P; tachykinin receptors

Tachykinins are a family of closely related peptides that share a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH. They are widely distributed in both the central nervous system and peripheral tissues, and function as neurotransmitters and neuromodulators (Otsuka and Yoshioka 1993). For many years, the most well-known members of the tachykinin family were substance P, neurokinin A (NKA) and neurokinin B (NKB). However, in 2000, a fourth mammalian tachykinin (hemokinin-1) was found in the mouse. This discovery has shed light on a completely new and species-divergent group of novel tachykinins (the endokinins) and more unexpectedly, tachykinin gene-related peptides (Page 2005).

Substance P has been implicated in various behavioural responses and in the regulation of neuronal survival and degeneration (Hasenohrl et al. 2000). It has been shown to be involved in a variety of neurodegenerative disorders such as Alzheimer's (Sakurada et al. 1990), Parkinson's (Halliday et al. 1990), and Huntington's disease (Augood et al. 1996). Substance P also regulates cardiovascular (Hoover et al. 2000) and respiratory function and is involved in activation of the emetic reflex (Diemunsch and Grelot 2000). It participates in the neurotransmission of pain by mediating the process of nociceptive transmission (Zubrzycka and Janecka 2000), stimulates haematopoiesis (Rameshwar et al.

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1993), motility and secretion in the gastrointestinal tract (Improta and Broccardo 2006). At the level of the genitourinary tract, it is involved in smooth muscle contraction, and modulates release of inflammatory mediators as well as steroid secretion by the gonads (Candenas et al. 2005).

These disparate activities of substance P are mediated by three tachykinin receptor subtypes also known as neurokinin receptors (NK1R, NK2R and NK3R), which all belong to the G protein-coupled receptor (GPCR) superfamily and are encoded by the *TACR1*, *TACR2* and *TACR3* genes, respectively. These receptors are glycoproteins with seven α-helical transmembrane segments, an extracellular amino-terminus and an intracellular carboxyl tail (Pin and Bockaert 1999). The conserved carboxyl terminal domain of tachykinins interacts with the tachykinin receptors, while the unique amino terminal sequence dictates receptor specificity. Each tachykinin appears to preferentially activate a specific tachykinin receptor, although at high ligand concentration, each tachykinin can activate each of the tachykinin receptors. Substance P has a high affinity for NK1R, whereas NKA and NKB have been observed to bind preferentially to NK2R and NK3R, respectively (Nakanishi 1991).

Nowadays, pigs are increasingly being used as laboratory animals, because of their close anatomical and physiological similarity to humans. As a result, they are often used as models to study a variety of disease mechanisms and different pathophysiological conditions in humans, such as obesity and gastrointestinal dysfunctions (Spurlock and Gabler 2008; Pidsudko et al. 2011; Zhang et al. 2013). Substance P was reported to be localised in the porcine central nervous system (Barnes et al. 1993; Holm et al. 1993) and in the nerve fibres innervating many porcine organs (Schmidt et al. 1991; Happola et al. 1993; Czaja et al. 2001; Lamb and Sparrow 2002; Podlasz et al. 2003; Pidsudko et al. 2008; Podlasz and Wasowicz 2008; Kaleczyc et al. 2010). In the pig, substance P can stimulate both endocrine and exocrine pancreatic functions through NK-1 receptors (Schmidt et al. 2000). It also has a modulatory effect on ovarian function (Pitzel et al. 1991). Pigs can be useful models for the study of human cystic fibrosis as it has been shown that substance P induces liquid secretion from bronchial submucosal glands of pigs (Trout et al. 2001).

Despite the important physiological roles substance P plays in pigs, the identities and functions of porcine neurokinin receptors remain largely unknown. In this paper, we report the distribution of neurokinin receptors in porcine tissues using the RT-PCR technique. Using *in silico* analysis, we have designed specific primers for PCR, identified the sequence of genomic DNA encoding the NK1 receptor as well as predicted the mRNA sequence for NK2 and NK3 receptors. Finally, we have defined the amino acid sequence of the neurokinin receptors.

MATERIAL AND METHODS

The study was performed on female pigs (*Sus scrofa*) of the Polish Large White breed weighing approximately 30 kg. The experiment was accepted by the Local Ethical Commission of the University of Warmia and Mazury in Olsztyn, Poland (affiliated to the National Ethics Commission for Animal Experimentation, Polish Ministry of Science and Higher Education). Animals were deeply anaesthetised with pentobarbital (Vetbutal, Biowet, Poland; 30 mg/kg body weight *i.v.*). Sixteen different tissues including skeletal muscle, stomach wall, spleen, kidney, adrenal glands, hippocampus, skin, lung, heart, spinal cord, hypothalamus, liver, pituitary gland, thymus, ovary and jejunum were collected and placed into RNALater (Ambion, USA) overnight at 5 °C and then frozen at –20 °C. Total RNA was isolated using the Total RNA Mini Kit (A&A Biotechnology, Poland) according to the manufacturer's instructions.

Primer design. All sequences required for designing specific primers were obtained from the GenBank database. For this purpose, the Primer-BLAST tool was used (http://www.ncbi.nlm.nih.gov/). The predicted nucleotide sequence of the porcine NK1 receptor was based on *Sus scrofa* breed Ellegaard Gottingen minipig Contig39424, whole genome shotgun sequence (GenBank accession No. AOCR01039423.1) in alignment with the predicted *Bos taurus* tachykinin receptor 1 (TACR1) mRNA sequence (GenBank accession No. XM_002691188.1).

Primers for NK2 and NK3 receptors were designed using predicted *Sus scrofa* tachykinin receptor 2 (TACR2) mRNA sequence (GenBank accession No. XM_001929073.5) and predicted *Sus scrofa* tachykinin receptor 3 (TACR3) mRNA sequence (GenBank accession No. XM_003482466.2).

Tissue distribution of NK1R, NK2R and NK3R transcripts. RT-PCR assays were performed to de-

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tect the expression of neurokinin receptor genes in different porcine tissues. Based on the coding sequences obtained, gene-specific primers (Table 1) for NK1R, NK2R, NK3R and GAPDH (AF017079; served as an internal control) were designed to amplify cDNA fragments of 404, 393, 298 and 320 bp, respectively. Reverse transcription (RT) was performed using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR for 10 min at 25 °C followed by 15 min at 50 °C and terminated by heating at 85 °C for 5 min in a total volume of 20 µl, consisting of 1.3 µg total RNA from different tissues, 5x Reaction Mix (Thermo Scientific, USA), Maxima Enzyme Mix (Thermo Scientific, USA) and water (nuclease-free). PCR amplification was performed with 0.5 µl of the cDNA templates under the following conditions: 2 min at 95 °C, followed by 35 cycles (NK1R, NK2R, NK3R: 95 °C for 10 s, 62 °C for 10 s, and 75 °C for 30 s; GAPDH: 95 °C for 10 s, 60 °C for 10 s, and 75 °C for 30 s). The PCR products were visualised in 2% agarose gel under UV illumination (Pharmacia LKB Macro Vue, Sweden) after ethidium bromide staining. A GeneRuler™ 100 bp DNA Ladder (100–1000 bp; Fermentas, Lithuania) was used as a size marker. The results were documented by photography with a CCD camera (Tayama, Japan).

Extraction of PCR products and sequencing. After PCR product visualisation, bands of the appropriate size were isolated and DNA was purified using the Gel Out Kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. Purified samples were sent for sequencing (Genomed, Poland) with the specific primers listed in Table 1. The obtained sequences were analysed using the BLAST tool available in the BioEdit software, version 7.2.5.

Multi-sequence alignment and phylogenetic analyses. Predicted nucleotide sequences of porcine neurokinin receptors were translated into protein sequences using BioEdit software. Other neurokinin receptor amino acid sequences of various species were retrieved from the GenBank database. The assumed amino acid sequences of NK1R, NK2R and NK3R were aligned with those of cow, human and mouse using the Clustal Omega online programme. The putative transmembrane domains were predicted using the TMHMM 2.0 online protein topology prediction tool. Phylogenetic analysis was computed using the MEGA6 program, in which the phylogenetic tree was constructed using the Neighbor-Joining method.

RESULTS

Localisation of the neurokinin receptor genes

Bioinformatic comparison of the predicted porcine NK1R sequence with porcine genome databases (Ensembl, GenBank) was unsuccessful. Analysis of the *TACR1* gene in human and bovine genomes and collation of the neighbouring genes suggested that the porcine *TACR1* gene is probably located in the region between the *GCFC2* and *POLE4* genes on chromosome 3 (Figure 1A). The porcine *TACR2* gene was mapped to chromosome 14 (Figure 1B) between the *HK1* gene and its paralogue (ENSSSCG00000027658), while the *TACR3* gene is located on chromosome 8 (Figure 1C) between the *CXXC4* and *CENPE* genes.

Characterisation of the amino acid sequences of the predicted neurokinin receptors

Using the Contig39424, whole genome shotgun sequence (GenBank accession No. AOCR01039423.1) from the *Sus scrofa* Ellegaard Gottingen minipig breed in alignment with the predicted *Bos taurus* tachykinin receptor 1 (TACR1) mRNA sequence (GenBank accession No. XM_002691188.1), the nucleotide sequence of the porcine NK1 receptor was predicted and translated into amino acids. *In silico* analysis revealed that porcine NK1R is a protein of 407 amino acids. As shown in the multi-sequence alignment (Figure 2), the predicted NK1 receptor in pig shares 96% amino acid sequence

Table 1. Primers used for RT-PCR assays

Gene	Primer sequence (sense)	Primer sequence (antisense)	Size (bp)
NK1R	GTCATCGTGGTGACTTCCGT	GGCATGGTCTCTGTGGTTGA	404
NK2R	CAACCACAGGTTTCGCTCTG	CACTGCCTGAGAGGGATGAC	393
NK3R	CCATTGCGGTGGACAGGTAT	GCTCCCCACAGAGTGATTCC	298
GAPDH	ACATTGTCGCCATCAATG	ATGCCCATCACAAACATG	320

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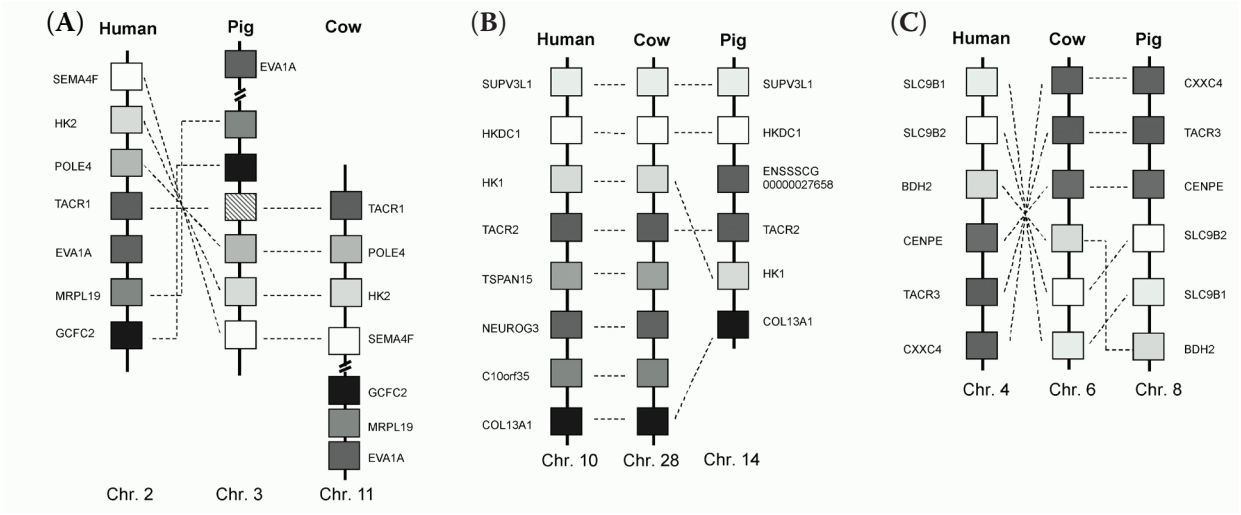

Figure 1. Synteny analysis showing the chromosomal localisation of (**A**) neurokinin 1 receptor gene (TACR1), (**B**) neurokinin 2 receptor gene (TACR2) and (**C**) neurokinin 3 receptor gene (TACR3) in the human, cow and pig genomes (**A**) SEMA4F: sema domain, immunoglobulin domain (Ig), transmembrane domain and short cytoplasmic domain, (semaphorin) 4F; HK2: hexokinase 2; POLE4: polymerase (DNA-directed), epsilon 4, accessory subunit; EVA1A: eva-1 homolog A (*C. elegans*); MRPL19: mitochondrial ribosomal protein L19; GCFC2: GC-rich sequence DNA-binding factor 2. White square with red stripes is the predicted localisation of the TACR1 gene. (**B**) SUPV3L1: SUV3-like helicase; HKDC1: hexokinase domain containing 1; HK1: hexokinase 1; TSPAN15: tetraspanin 15; NEUROG3: neurogenin 3; C10orf35: chromosome 10 open reading frame; COL13A1: collagen, type XII, alpha 1; ENSSSCG00000027658 is a paralogue of the HK1 gene. (**C**) SLC9B1: solute carrier family 9, subfamily B (NHA1, cation proton antiporter 1), member 1; SLC9B2: solute carrier family 9, subfamily B (NHA2, cation proton antiporter 2), member 2; BDH2: 3-hydroxybutyrate dehydrogenase, type 2; CENPE: centromere protein E, 312 kDa; CXXC4: CXXC finger protein 4; ENSSSCG00000028101 is a paralogue of the SLC9B2 gene

identity with its homologue from human, 95% from cow and 93% from mouse. Amino acid residues in the seven transmembrane helices (TM), intra- and extracellular loops are highly conserved, whereas sequences in the N- and C-terminal domains are less conserved. The NK2 receptor is a protein of 381 amino acids. As in the case of NK1R, NK2R shares high similarity with its human (85%), cow (90%) and mouse (84%) NK2R orthologs (Figure 3). The biggest differences in sequence conservation are found in the amino- and carboxy-terminuses. Also, TM V is not fully conserved in the analysed species, because mouse TM V is shifted to the right by four amino acids. The 464-amino acid porcine NK3R protein (Figure 4) shows 85–93% identity with other analysed NK3R homologues. The highest divergences are between the compositions and length of the N-terminal domains. The extracellular amino-terminus is longest in human NK3R and consists of 89 amino acids, whereas in the porcine N-terminus one amino acid is missing, in the cow two are missing and the mouse NK3R N-terminus is shorter by 17 amino acids.

The phylogenetic tree was constructed based on the amino acid sequences of porcine and other mammalian neurokinin receptors (Figure 5). The overall tree topology revealed high similarity between receptors of each type forming three main branches. All of the porcine neurokinin receptors are clustered close to the bovine neurokinin receptors.

Tissue distribution of NK1R, NK2R and NK3R transcripts

Using RT-PCR assays, we examined the mRNA expression of NK1R, NK2R and NK3R in 16 tissues from female pigs including the skeletal muscle, stomach wall, spleen, kidney, adrenal glands, hippocampus, skin, lung, heart, spinal cord, hypothalamus, liver, pituitary gland, thymus, ovary and jejunum (Figure 6). The expression of NK1R (as judged by the presence of a 404 bp band) was detected in the stomach wall, kidney, hippocampus, heart, hypothalamus, pituitary gland, ovary and jejunum. A weaker signal was detected in the skeletal muscle, lung, spinal cord and

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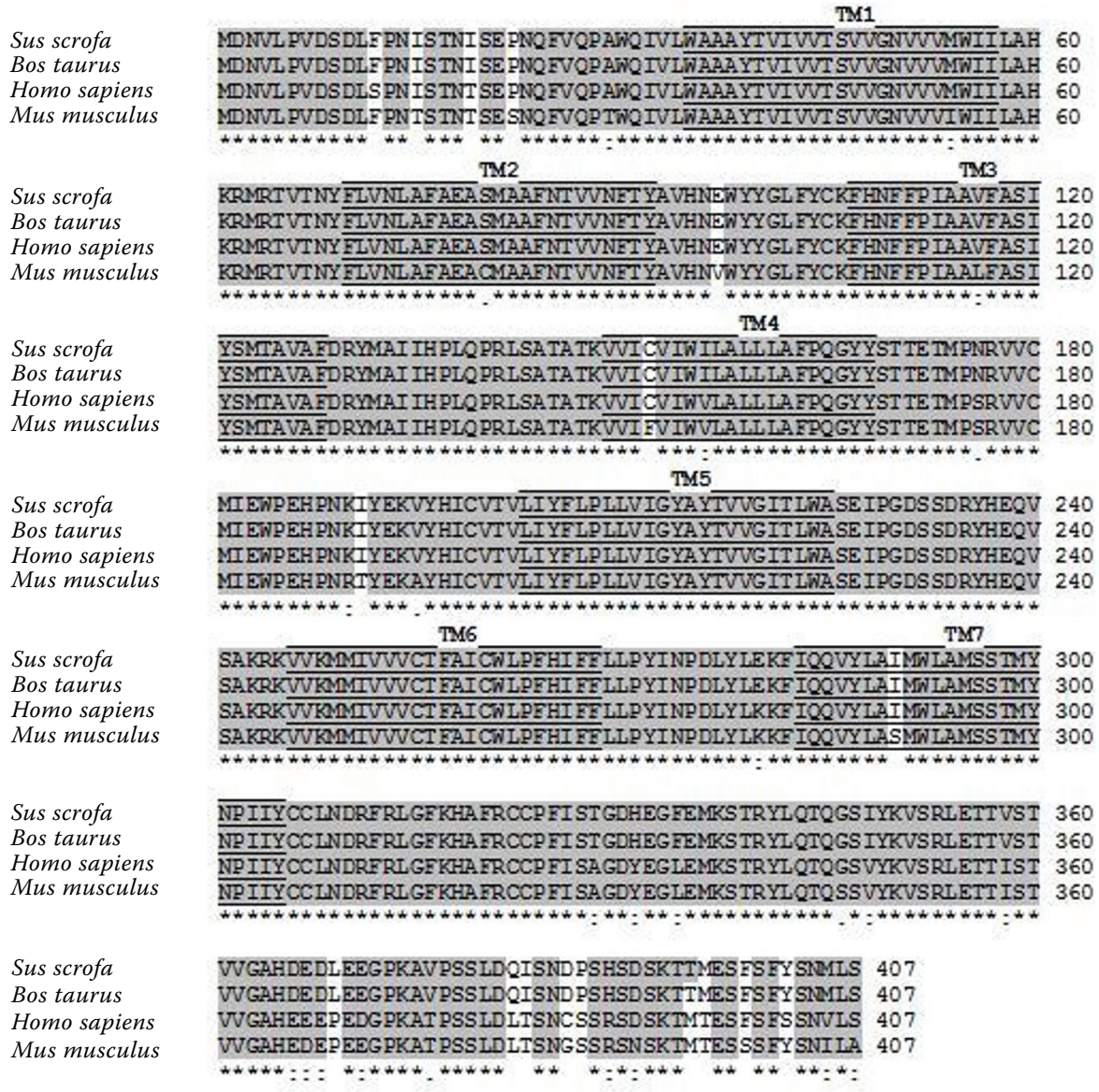

Figure 2. Amino acid sequence alignment of the predicted porcine neurokinin type I receptor with homologues from other species. The porcine NK1R amino acid sequence aligned with cow NK1R (XM_002691188.1), human NK1R (AAA60601.1) and mouse NK1R (CAA44707.1)

Conserved amino acid residues are shaded and the strength of conservation is denoted: asterisk indicates positions which have a single, fully conserved residue; colon – conservation between groups of strongly similar properties; period – conservation between groups of weakly similar properties. Seven putative transmembrane domains (TM) are highlighted under the sequences

thymus, whereas very faint bands were found in the spleen, adrenal gland, skin and liver samples after 35 cycles of PCR amplification. Similar to NK1R, NK2R expression (as judged by the presence of a 393 bp band) could also be detected in the stomach wall, kidney, heart, hypothalamus, pituitary gland, ovary, jejunum as well as in the skeletal muscle, spleen, adrenal gland, skin and lung. Very weak PCR bands were detected in the hippocampus and spinal cord after 35 cycles of PCR amplification. The expression of NK3R (as judged by the presence of a 298 bp band), similarly to NK1R and NK2R, was detected in the hypothalamus, pituitary gland and ovary. PCR products were also amplified from the spinal cord, adrenal gland and hippocampus. The weakest signals were from the skeletal muscle, kidney, skin, lung, jejunum and thymus. No bands were detected in the heart and liver after 35 cycles of PCR amplification. In all the porcine tissues examined, no bands of the expected sizes (NK1R, 404 bp; NK2R, 393 bp and

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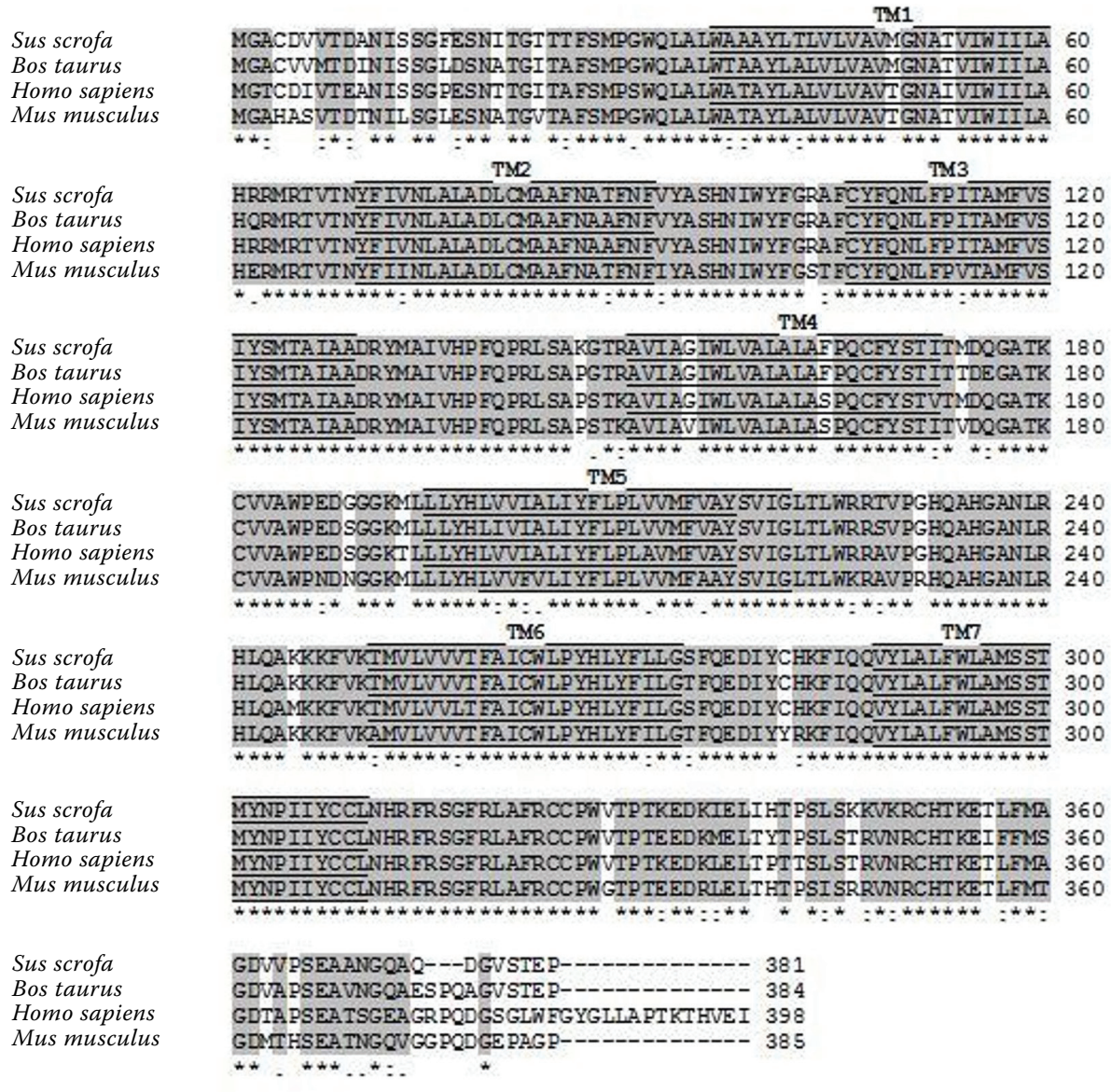

Figure 3. Amino acid sequence alignment of the predicted porcine neurokinin type II receptor with homologues from other species. The porcine NK2R amino acid sequence aligned with cow NK2R (NP_776894.1), human NK2R (NP_001048.2) and mouse NK2R (NP_033340.3)

Conserved amino acid residues are shaded and the strength of conservation is denoted: asterisk indicates positions which have a single, fully conserved residue; colon – conservation between groups of strongly similar properties; period – conservation between groups of weakly similar properties. Seven putative transmembrane domains (TM) are highlighted under the sequences. Dashes represent gaps in the aligned sequences

NK3R, 298 bp, GAPDH, 320 bp) were detected in the RT-negative controls.

Sequencing

Three PCR products of 404, 393 and 298 bp, corresponding to portions of predicted porcine NK1R, NK2R and NK3R mRNA, respectively, were sent for sequencing. The NK1R mRNA was isolated from stomach wall, NK2R mRNA from thymus and NK3R mRNA was obtained from the pituitary gland. Sequencing confirmed the compatibility of these amplicons with fragments of predicted sequences encoding putative receptor proteins.

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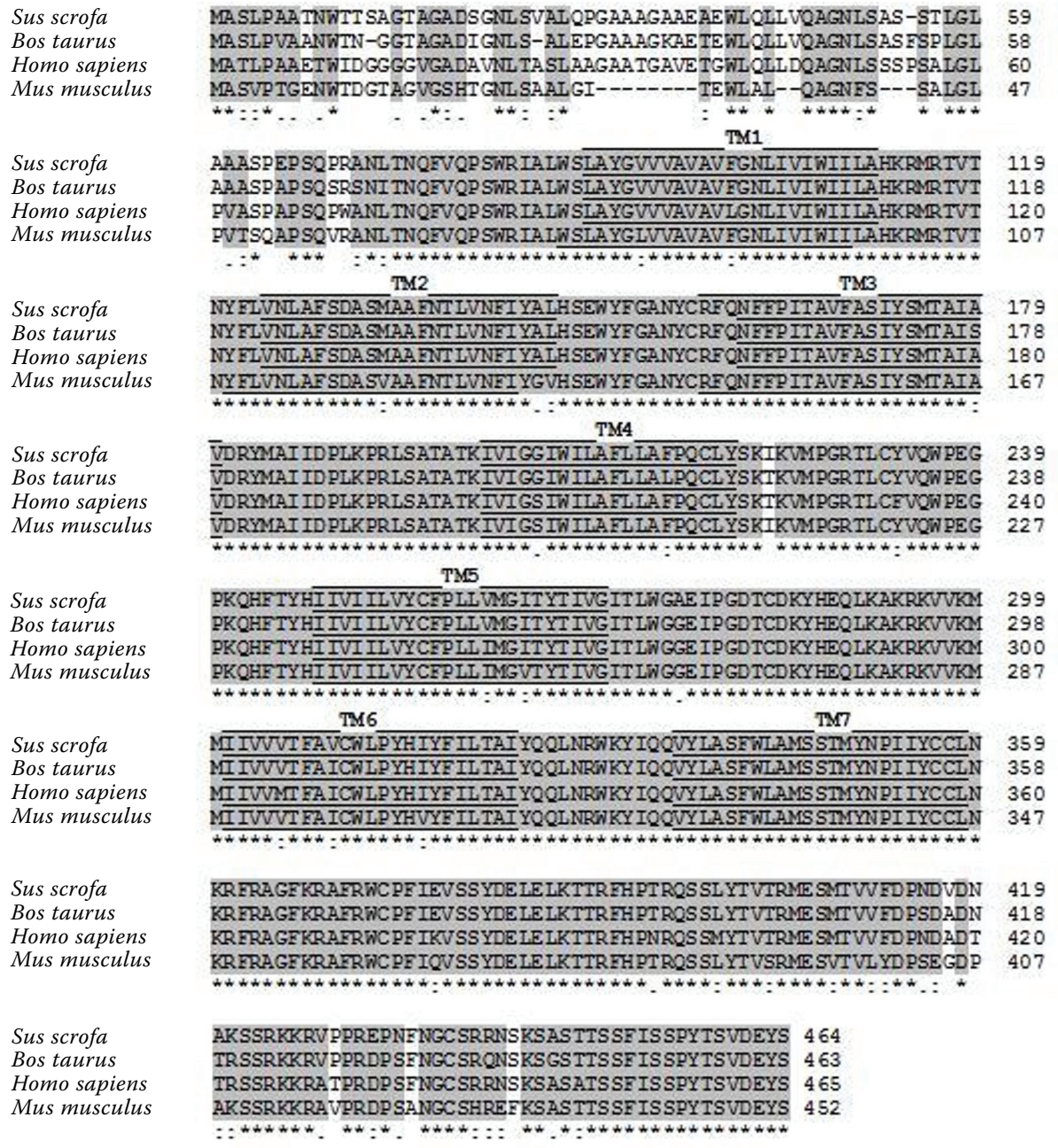

Figure 4. Amino acid sequence alignment of the predicted porcine neurokinin type III receptor with homologues from other species. The porcine NK3R amino acid sequence aligned with bovine NK3R (NP_001179262.1), human NK3R (NP_001050.1) and murine NK3R (NP_067357.1)

Conserved amino acid residues are shaded and the strength of conservation is denoted: asterisk indicates positions which have a single, fully conserved residue; colon – conservation between groups of strongly similar properties; period – conservation between groups of weakly similar properties. Seven putative transmembrane domains (TM) are highlighted under the sequences. Dashes represent gaps in the aligned sequences

DISCUSSION

The neurokinin receptor family is known to have three distinct members, NK1R, NK2R and NK3R, which have been isolated and characterised in humans, mice and rats. In this report, we have identified and described these receptors in pig. Moreover, we have shown that the mRNAs of neurokinin re-

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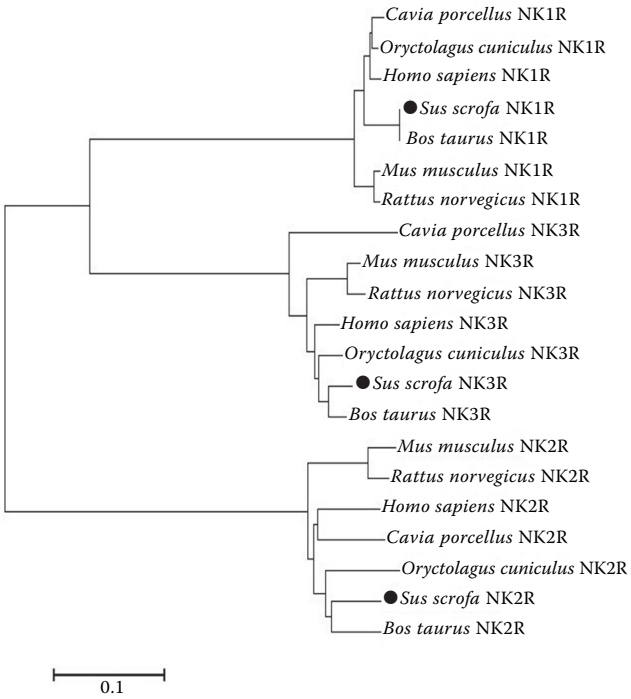

Figure 5. Phylogenetic tree showing the vertebrate lineage of the neurokinin receptor family, constructed using the Neighbor-Joining method. Sequences of the identified porcine neurokinin receptors (NK1R, NK2R, NK3R) are marked with filled circles (●)

Amino acid sequences of the identified neurokinin receptors along with the deduced species homologues were retrieved from the GenBank database, in which the accession numbers are listed as followed: NK1R of *Cavia porcellus* (CAA45608.1); *Oryctolagus cuniculus* (XP_002709748.1); *Homo sapiens* (AAA60601.1); *Sus scrofa* (predicted); *Bos taurus* (XM_002691188.1); *Mus musculus* (CAA44707.1); *Rattus norvegicus* (AAA42176.1). NK2R of *Cavia porcellus* (XP_003473635.1); *Mus musculus* (NP_033340.3); *Rattus norvegicus* (NP_542946.1); *Homo sapiens* (NP_001048.2); *Oryctolagus cuniculus* (NP_001075800.1); *Sus scrofa* (XM_001929073.5); *Bos taurus* (NP_776894.1). NK3R of *Mus musculus* (NP_067357.1); *Rattus norvegicus* (NP_058749.1); *Homo sapiens* (NP_001050.1); *Cavia porcellus* (NP_001166200.1); *Oryctolagus cuniculus* (NP_001075524.1); *Sus scrofa* (XM_003482466.2); *Bos taurus* (NP_001179262.1)

ceptors are broadly distributed in different porcine tissues. In this context, we provide for the first time, information regarding the localisation, structure and expression of the TACR1 gene in the pig.

Structural analysis of the porcine neurokinin receptors investigated in this study revealed the presence of the most characteristic features of G protein-coupled receptors, including seven transmembrane domains consisting of 20–23 amino acids, extra- and intracellular loops, an amino-terminus outside the cell and a cytoplasmic carboxy-terminus (Maggi 1995; Kobilka 2007). The mRNAs of tachykinin receptors have been cloned in different mammalian species (Masu et al. 1987; Gerard and Gerard 1991; Takeda et al. 1991; Sundelin et al. 1992; Takahashi et al. 1992; Sarau et al. 2001; Brylla et al. 2005). In all of them, including the pig, NK1R is a protein of 407 amino acids. Porcine NK2R has 381 residues and is shorter than NK2R from the cow (384 amino acids), human (398 amino acids) and mouse (385 amino acids). In all species, the NK3 receptor (464, 463, 465 and 452 amino acids in pig, cow, human and mouse, respectively) is extended at the amino-terminal region and therefore longer than the NK1 and the NK2 receptors. All deduced NKRs sequences share a high degree of amino acid sequence homology with the corresponding receptors in other species, such as the cow (NK1R 95%, NK2R 90%, NK3R 93%), human (NK1R 96%, NK2R 85%, NK3R 93%)

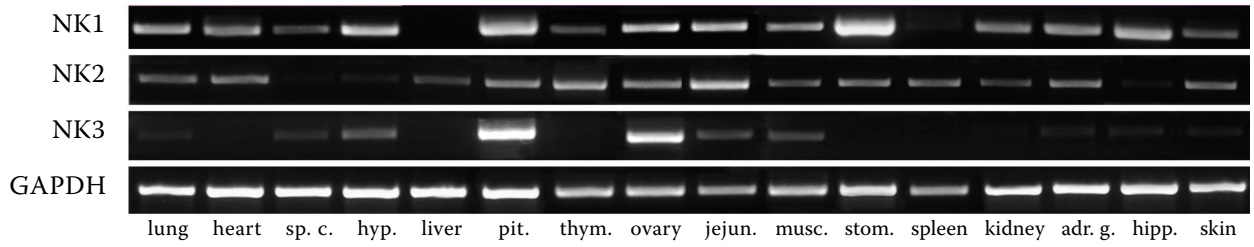

Figure 6. RT-PCR analysis of neurokinin receptor distribution in porcine tissues. Amplified GAPDH fragments (320 bp) were used as an internal control and samples without cDNA served as negative controls (not shown). The result is representative of three independent experiments

adr. g. = adrenal gland; hipp. = hippocampus; hyp. = hypothalamus; jejun. = jejunum; musc. = skeletal muscle; pit. = pituitary gland; sp. c. = spinal cord; stom. = stomach wall; thym. = thymus

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and mouse (NK1R 93%, NK2R 84%, NK3R 85%). It is noteworthy that the porcine NK1 receptor is more similar to its human homologue. The most conserved regions of the protein among different species are transmembrane segments that form a ligand-binding pocket. The greatest diversity in NK1 between species is observed at the amino-termini. Critical differences in these structures must exist in order to confer specificity and for the high-affinity binding of receptor ligands (Fong et al. 1992). The C-terminus is also a region of the receptor which is variable between species.

The structure and localisation of the neurokinin receptor genes (*TACR1, TACR2* and *TACR3*) was determined by *in silico* comparison with pig genomic DNA sequences retrieved from the Ensembl and NCBI databases. In the case of NK1R, there is no gene identified as *TACR1* in the pig genome. After localizing the *TACR1* gene in human and cow genomes and correlating with the pig genome, the putative position of the porcine *TACR1* gene was mapped between the *GCFC2* and *POLE4* genes on chromosome 3. This position was indicated by the order of neighbouring genes (*POLE4, HK2, SEMA4F*) in the bovine genome, which coincides with that in the pig. Moreover, the contiguity of these genes is the same in the human genome, but due to a partial chromosome inversion, *POLE4, HK2* and *SEMA4F* are located before the *TACR1* gene.

The *TACR2* gene was mapped to chromosome 14 where it is flanked by the *HK1* gene and its paralogue. During evolution, the *HK1* gene was probably duplicated in the porcine genome as evidenced by the high identity of the *HK1* gene and ENSSSCG00000027658. The high similarity between chromosome fragments containing *SUPV3L1, HKDC1* and *HK1* (and its paralogue in the pig) genes in the compared species confirmed the localisation of the *TACR2* gene. Based on the presence of the *TSPAN15, NEUROG13* and *C10orf35* genes between the *TACR2* and *COL13A1* genes in cow and human genomes, it can be inferred that the fragment of the porcine chromosome with those genes was deleted.

The *TACR3* gene was mapped to chromosome 8 between the *CENPE* and *CXXC4* genes. The positions of neighbouring genes in the other analysed animals are very similar. The same arrangement of genes is present in the cow and pig genomes except that the *BDH2* gene is located next to the *SLC9B1* gene, while on chromosome 6 in the cow it is located between the *CENPE* and *SLC9B2* genes. As a result of a chromosome inversion in the human genome, the location of the gene location has been altered. However, the order of genes remains the same.

The porcine genes encoding porcine tachykinin receptors have the same structural organisation as the tachykinin receptor genes of other mammalian species (Gerard et al. 1993) and contain five exons, with introns that interrupt the protein-coding sequence at identical positions. Considering the tachykinin receptor genes, exon 1 encodes the entire 5'-untranslated region and the coding region through the end of transmembrane domain III (TM III). Exon 2 contains intracellular loop 2, TM IV and extracellular loop 2. Exon 3 contains TM V and intracellular loop 3. Exon 4 codes for TM VI, extracellular loop 3 and TM VII. Exon 5 contains the cytoplasmic C-terminus and the entire 3'-untranslated region (Almeida et al. 2004).

The sequences encoding porcine NK1R, NK2R and NK3R analogues were identified in all analysed species and form three distinct clusters corresponding to the three subtypes of neurokinin receptors (Severini et al. 2002). The amino acid sequences in each cluster are highly conserved, suggesting stringent evolutionary control over the genes encoding the investigated molecules.

This study shows that the mRNAs of tachykinin receptors are broadly distributed in porcine tissues. NK1 receptor mRNA was present in almost all tissues assayed. This is in agreement with previous data reported in rats (Tsuchida et al. 1990) and in humans (Pinto et al. 2004). The fact that NK1R is strongly expressed in a wide range of the tissues assayed suggests that it plays a broad and indispensable role in mediating the effects of tachykinins in the pig. A few porcine tissues (liver, spleen) were characterised by a very low, or undetectable, expression of NK1R. The same results were obtained in human and rat tissue (Vigna et al. 1994; Pinto et al. 2004). The high levels of NK1R expression in the pituitary gland, confirmed also in the rat anterior pituitary (Larsen et al. 1989), suggests that it may play an important role in regulation of the hypothalamo-pituitary-adrenal axis (Nussdorfer and Malendowicz 1998). The presence of NK1R in the hippocampus is associated with the regulation of the release of other neurotransmitters which may cause pathopsychological states like anxiety or depression. For this reason, NK1 receptor antago-

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nists are used as antidepressants (Fuchs et al. 2005). Several reports have demonstrated the presence of NK1R in different neuronal and non-neuronal cells in the gastrointestinal tract (Sternini et al. 1995; Goode et al. 2000). In pathological states, such as inflammation in Crohn's disease, NK1R expression is up-regulated (Renzi et al. 2000). Various parts of the genitourinary tract, such as the urinary bladder or the kidney, also exhibit neurokinin 1 receptor expression (Meini et al. 1994; Chen and Hoover 1995). Application of NK1 receptor antagonists may be effective for the treatment of urge incontinence (Green et al. 2006).

In their study regarding the contribution of muscarinic, tachykinin and purinergic receptors to bladder function, Bahadory et al. (2013) tried to detect the expression of the NK1 receptor at the mRNA level in the fresh tissue layers of the porcine female urinary bladder and their cultured cell counterparts. In both cases the detection of NK1R was unsuccessful because of the lack of knowledge about the porcine *TACR1* gene and its transcript. The primers for the RT-PCR assay could be designed based only on the homology of the NK1 receptor mRNA sequences in different mammalian species. For the porcine NK1 receptor, this method enabled the detection of NK1R transcripts only once in the porcine female reproductive system (Bukowski 2014).

The tachykinin NK2 receptor is mainly expressed in peripheral tissues and its expression in the central nervous system appears to be restricted to specific brain nuclei (Saffroy et al. 2003). In this study, we observed the presence of *TACR2* mRNA in the hippocampus, hypothalamus, pituitary gland and spinal cord but its expression was markedly lower than the expression of NK1R and NK3R. As in another report (Pinto et al. 2004), we confirmed the expression of NK2R in a wide range of porcine peripheral tissues. NK2 receptor (as well as NK1 receptor) expression was detected in several structures in human central airways, including smooth muscle, glands, vessels, and pulmonary arteries (Mapp et al. 2000). In the respiratory system, the NK2 receptor seems to play an important role during inflammatory states (Bai et al. 1995). In the gastrointestinal tract (oesophagus, stomach, small and large intestine), the neurokinin 2 receptor is primarily expressed on effector cells which are involved in the regulation of intestinal motor functions (Portbury et al. 1996). This effect may contribute to some defined intestinal diseases, for example, irritable bowel syndrome, in which NK2 receptor antagonists could prove beneficial (Holzer and Holzer-Petsche 2001).

The NK3 receptor is primarily expressed in the central nervous system but its presence was also detected in specific peripheral tissues, such as human ovary, uterus (Roman et al. 2012), kidney, lung (Pinto et al. 2004), rat and mouse oesophagus (Mann et al. 1997; Wang et al. 2002). The present results seem to confirm the above findings because *TACR3* mRNA was strongly expressed in the hippocampus, hypothalamus, pituitary gland and spinal cord while its expression in peripheral tissues was very low or undetectable. However, the contribution of NK3R to some peripheral effects of tachykinins cannot be excluded. Functional studies using selective agonists or antagonists of the NK3 receptor in the central nervous system show that it might play mechanistic roles in depression and anxiety (Massi et al. 2000) as well as schizophrenia (Spooren et al. 2005). In the peripheral tissues, the NK3 receptor is an alternative target in the treatment of female health disorders (uterine fibroids, endometriosis) because of its important role in hypothalamic-pituitary-gonadal axis modulation. Robust expression of the NK3 receptor together with NK1 and NK2 receptors in the ovary suggest that these factors play important roles during reproduction. At this level, tachykinins are, through their receptors, involved in modulation of hormonal steroid secretion and stimulation of smooth muscle which facilitates transport of the shed oocyte inside the Fallopian tubes. They may also be implicated in regulation of granulosa cell function, as well as in age-related decline in ovary function (Pitzel et al. 1991; Alvarez et al. 2002; Garcia-Ortega et al. 2014). We failed to observe any NK3R mRNA signal in the gastrointestinal tract, or detected it only faintly. Other reports have revealed its presence, but only in nerve cell bodies of the plexus (according to the strict definition of the latter; Mann et al. 1997). Together with NK1R and NK2R, NK3R regulates intestinal contractility (Valero et al. 2011). There is also clear evidence from functional studies that tachykinin NK3 receptors play an important role in the respiratory system, where they control pulmonary functions and may increase neuronal activity and the responsiveness of target cells (Myers and Undem 1993; Myers et al. 1996).

To conclude, in the present study we have characterised the localisation, structure and expression

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of the porcine *TACR1, TACR2* and *TACR3* genes. The porcine tachykinin receptor genes and their corresponding protein products show similar arrangements, structures, sequences and tissue distributions to other mammalian species, including human. These similarities indicate that the pig can be a good model for studying the functions of neurokinins and their receptors.

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