ELSEVIER

European Journal of Pharmacology 437 (2002) 27–30

www.elsevier.com/locate/ejphar

The human tachykinin NK_1 (short form) and tachykinin NK_4 receptor: a reappraisal

Nigel M. Page *, Nicola J. Bell

School of Animal and Microbial Sciences, The University of Reading, Reading RG6 6AJ, UK

Received 8 October 2001; received in revised form 3 January 2002; accepted 11 January 2002

Abstract

Excessive secretion of placental neurokinin B into the circulation during the third trimester of pregnancy is seen in women with preeclampsia. To determine a role for neurokinin B, we have used a number of different animal models to ascertain the expression of the three tachykinin receptors (NK_1—both short and long forms, NK_2 and NK_3) and the putative human tachykinin NK_4 receptor in the placenta. Human and rat placenta express all three classical tachykinin receptors. However, we failed to reveal the expression of the short tachykinin NK_1 receptor or the tachykinin NK_4 receptor in any of 24 human tissues examined including the placenta. We conclude that the proposed short form of the tachykinin NK_1 receptor is a truncated genomic clone and that the human tachykinin NK_4 receptor is in fact, the guinea pig tachykinin NK_3 receptor. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Neurokinin B; Tachykinin NK_1 receptor, short; Tachykinin NK_1 receptor, long; Tachykinin NK_4 receptor

1. Introduction

Neurokinin B is a member of the tachykinins, which includes substance P and neurokinin A. We have found that the human placenta expresses the preprotachykinin-B mRNA transcript, which encodes neurokinin B at levels greater than in the brain and spinal cord (Page et al., 2001b). Conversely, we failed to detect the presence of any of the four alternatively spliced preprotachykinin-A transcripts, which encode substance P and neurokinin A at any stage of pregnancy (Page et al., 2001b). Therefore, we conclude that neurokinin B is the only tachykinin produced by the placenta to interact with peripheral tachykinin (NK) receptors. Neurokinin B activates three tachykinin receptors with an order of affinity as follows: NK_1, substance P > neurokinin A > neurokinin B; NK_2, neurokinin A > neurokinin B > substance P; and NK_3, neurokinin B > neurokinin A > substance P. Earlier, we proposed that the increased levels of neurokinin B secreted from the placenta during preeclampsia may be an emergency signal to increase blood flow to the utero-placental unit (Page et al., 2000b). This present study was undertaken to determine which of the tachykinin receptors are available targets for this neurokinin B and, hence, capable of contributing to the changes in utero-placental haemodynamics. We examined the expression of all three tachykinin receptors in the human and rat placenta. In humans, we differentiated between the reported shorter form of the tachykinin NK_1 receptor (Fong et al., 1992) and the native longer form. The truncated form is found to be 96 amino acids shorter at its carboxyl terminus, effectively removing the entire carboxyl tail after the end of the seventh transmembrane domain. This has been shown to alter the receptor's ligand binding efficiency (Fong et al., 1992; Mantyh et al., 1996) and effector systems (Fong et al., 1992), suggesting that the two forms may be differentially expressed (Mantyh et al., 1996). Also included in this study was the novel tachykinin NK_4 receptor, originally cloned from a human placenta cDNA library (Xie et al., 1992) and shown to be 80% similar at the amino acid level to the human and rat tachykinin NK_3 receptor (Donaldson et al., 1996). More importantly, this receptor is shown to respond potently to the physiological ligand, neurokinin B (Donaldson et al., 1996; Krause et al., 1997), and could be a candidate receptor for neurokinin B in the placenta. However, Sarau et al. (2000) have reported that although the tachykinin NK_4 receptor has nearly identical pharmacology to the known human tachykinin NK_3 receptor, their use of various

* Corresponding author. Tel.: +44-118-9875123; fax: +44-118-9310180.

E-mail address: sasnpage@reading.ac.uk (N.M. Page).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)01278-5

molecular biological techniques has so far been unsuccessful in detecting the tachykinin NK_4 receptor sequence in the human genome. They also failed to detect this receptor in the genome of a range of other species, including monkey, mouse, rat, hamster and guinea pig. The debate as to the existence and identity of the tachykinin NK_4 receptor has therefore remained undecided.

2. Materials and methods

2.1. RNA extraction and first strand cDNA synthesis

Total placenta RNA was isolated from rats (CD strain) at day 17 and guinea pig (Dunkin-Hartley) at day 41 of gestation as previously described (Page et al., 2000a). Total human RNA from each of 24 tissues (brain, heart, kidney, liver, lung, colon, bone marrow, small intestine, spleen, stomach, thymus, prostate, uterus, fetal brain, fetal liver, spinal cord, placenta, adrenal gland, pancreas, salivary gland, trachea, mammary gland, skeletal muscle and testis) was obtained from a human RNA master panel (Clontech). This panel contained pure high-quality total RNA, guaranteed to be genomic DNA-free and quantified accurately at 1 μg/μl. One and a half micrograms each of purified total RNA was subjected to first strand cDNA synthesis using the SMART RACE amplification kit (Clontech) to produce 3′ SMART RACE cDNA as previously described (Page et al., 2001a).

2.2. Polymerase chain reaction

Gene specific primers were designed to amplify the human NK_1 (accession numbers: M84425 long form, M84426 short form) (forward 5′ -GGCCATGAGCTCCAC CATGTACAACCCC; reverse long form 5′ -GCATGAAGG GAGGCAGGTCAAAGGCAGTGG; reverse short form 5′ -AATCAGTCTACTCCGGGCTCCCATTCCTGG), human NK_2 (accession number: M57414) (forward 5′ -TGCTGGT GGTGCTGACGTTTGCCATCTGCT; reverse 5′ -CTGTTG ACTCTCGTGGAGAGGGAGGTCGT), human NK_3 (accession number: M89473) (forward 5′ -GGCTGGCAATGA GCTCAACCATGTACAATCCCA; reverse 5′ -GGTGAGC TTATGAAACTTGAAGTGGCGGAGGCA), rat NK_1 (accession number: NM_012667) (forward 5′ -AGCAGGCTG CATCCCGGCTCTGAGAGCAGC; reverse 5′ -AGCTTTC GCTTCCAGTCACATCTGAGACGG), rat NK_2 (accession number: M31838) (forward 5′ -ATGGCAGGATTGCCGTG AGGCTTGTTGGA; reverse 5′ -GGCGCAGTGAACTCTG GGAAAGCTCAGGTGA) and rat NK_3 (accession number: J05189) (forward 5′ -CCCAAGGTGATAGCTATCATGA GGCCACCG; reverse 5′ -AACCCCTCTGCTCTTCTGCG GTCACAAAGG) tachykinin receptors. Primers were also chosen for the human tachykinin NK_4 receptor (accession number: M84605) (forward 5′ -GAGGGACGACCAGA GACGTAGGCTCCA; reverse 5′ -ATCAGTGTCACAAAT GTGCACAGTGACT). Polymerase chain reaction (PCR) amplification was performed at 95 °C for 30 s and 68 °C for 1 min for 40–50 cycles, using each set of gene specific primers. Reactions were performed in triplicate and controls containing no reverse transcriptase and no template were included. The human tachykinin NK_4 receptor primers were also used cross-species, in an attempt to amplify the novel tachykinin NK_4 receptor from the rat and guinea pig placenta. The full-length guinea pig tachykinin NK_3 receptor was amplified using two gene specific primers derived from the human tachykinin NK_4 receptor sequence (forward 5′ -CGGCCACGGCGGCTCCCCGACCTGCC reverse 5′ -GAGAAGGGGTGCCTGCAGTGGCCTTGAC) and performed in conjunction with the advantage-GC cDNA polymerase mix by following the manufacturer's protocol (Clontech). Amplified PCR products were visualised following gel electrophoresis as previously described (Page et al., 2000b).

2.3. Cloning, sequencing and bio-informatic analysis of PCR products

PCR products were excised from agarose gels, purified, cloned and sequenced to confirm specificity of amplification as previously described (Page et al., 2001a). The BLASTn2.1 computer programme (Altschul et al., 1997) was used to align nucleotide and amino acid sequences in order to calculate identities and homologies.

3. Results

We detected the presence of single bands of the expected size for each of the three tachykinin receptors in the human (NK_1, 549 bp; NK_2, 292 bp; NK_3, 343 bp) and rat (NK_1, 360 bp; NK_2, 366 bp; NK_3, 336 bp) placenta (Fig. 1A). Control reactions performed with total RNA and with no cDNA template all produced clean tracks with no visible bands (data not shown). We failed to amplify the tachykinin NK_4 receptor from either the human or rat placenta even after 50 cycles of PCR (Fig. 1A). As the tachykinin NK_4 receptor could not be amplified from human placenta cDNA, expected to be an abundant source of this receptor (Sarau et al., 2000), we decided to screen a panel of 24 human tissues (Section 2.1). No expression of the human tachykinin NK_4 receptor was found in any of these human tissues, even when a different pair of tachykinin NK_4 specific primers was used (data not shown). Neither could we amplify this sequence from human genomic DNA nor find matching large genomic DNA contigs or expressed sequence tags (ESTs) from within the Genbank database. Moreover, the tachykinin NK_4 receptor primers produced a band of the expected size (429 bp) only when used to amplify guinea pig placenta cDNA (Fig. 1A). This band, on sequencing, revealed the intervening sequence between the gene specific primers to be 100% identical to that of the proposed human tachykinin NK_4 receptor at both the

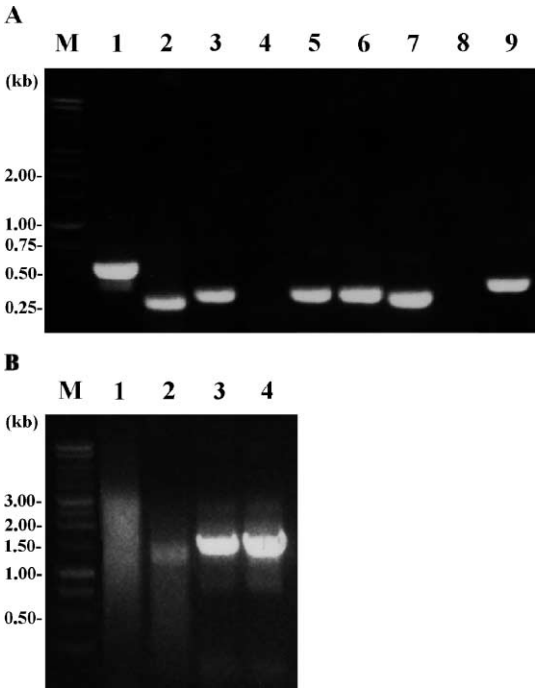

Fig. 1. (A) PCR amplification of human (lanes 1–4), rat (lanes 5–8) and guinea pig (lane 9) placenta cDNA. Gene specific primers were designed for the human tachykinin NK_1 (lane 1), human tachykinin NK_2 (lane 2), human tachykinin NK_3 (lane 3), human tachykinin NK_4 (lane 4), rat tachykinin NK_1 (lane 5), rat tachykinin NK_2 (lane 6), rat tachykinin NK_3 (lane 7), human tachykinin NK_4 (lane 8) and human tachykinin NK_4 (lane 9) receptors. (B) PCR amplification of the full-length guinea pig tachykinin NK_3 receptor using human NK_4 primers. Reactions were performed in the presence of a GC-melt buffer (Clontech) at final concentrations of 0.0 M (lane 1), 0.5 M (lane 2), 1.0 M (lane 3) and 1.5 M (lane 4). The DNA size markers are shown on the left.

nucleotide and amino acid level. We then designed primers to encompass the whole of the coding region of the human tachykinin NK_4 receptor and used these to amplify a band of the expected size from the guinea pig cDNA (Fig. 1B). This 1323 base pair coding region, on sequencing, was again found to be 100% identical to the complete human tachykinin NK_4 receptor sequence. We used the BLASTn2.1 computer programme to compare this guinea pig receptor cDNA sequence to those of the nonredundant DNA database held within the Genbank database. The receptor was found to match closely with the tachykinin NK_3 receptor of other species with very similar scores (rabbit 86%, human 85%, mouse 85%, rat 85% and dog 84%). Homology matching of the human tachykinin NK_3 receptor against the known tachykinin NK_3 receptors of the different species also produced similar percentage scores.

We looked for the expression of the short form of the tachykinin NK_1 receptor in the whole human brain as it was cloned originally from this tissue (Fong et al., 1992) before also looking in the spinal cord and placenta. We failed to detect this transcript in any of these tissues even after 50 cycles of PCR (Fig. 2A). Similarly, PCR on the remaining 21 tissues of the human panel failed to show any expression (data not shown). We only successfully amplified a band of the expected size (250 base pairs) from human genomic DNA (Fig. 2A). However, the native long form of the tachykinin NK_1 receptor was detected in the whole brain, spinal cord and placenta (Fig. 2A) and in the remaining 21 human tissues of the panel after 45 cycles as an expected 404 base pair PCR product (data not shown). Amplification of the long form from genomic DNA produced a 1927 base pair fragment as the chosen primers spanned an intron across exons 4 and 5 (Fig. 2A). Analysis of the genomic contig AC007400, revealed that the longer form of the tachykinin NK_1 receptor is encoded on five exons, while the proposed shorter form is encoded on the same first four exons (Fig. 2B). The splice donor site at the end of exon 4 appears not to be recognised and also encodes the premature stop codon. While the 3′ untranslated region of the long form is represented in the Genbank database, we have found no ESTs in the Genbank database corresponding to the 3′ untranslated region of the short transcript nor was it found to contain a recognisable polyadenylation signal (e.g. AATAAA).

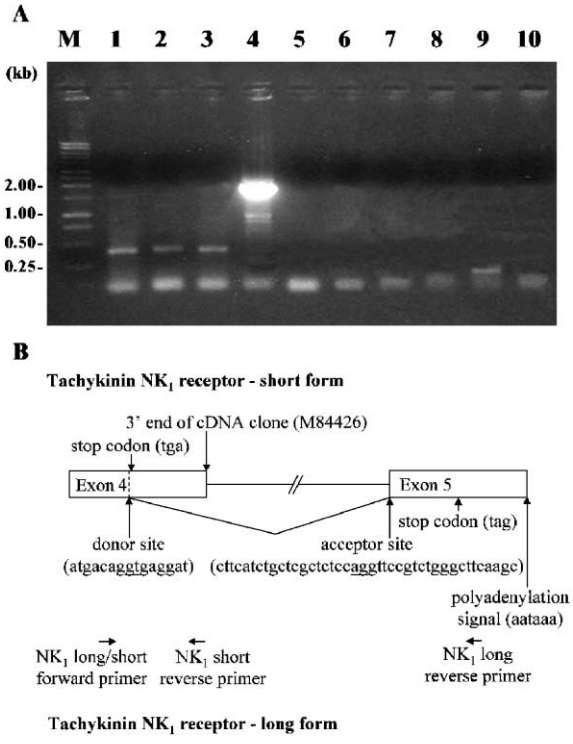

Fig. 2. (A) PCR amplification of the human tachykinin NK_1-long form (lanes 1–5) and the NK_1-short form (lanes 6–10) receptor performed on human whole brain (lanes 1 and 6), spinal cord (lanes 2 and 7), placenta (lanes 3 and 8), genomic DNA (lanes 4 and 9) and water (lanes 5 and 10). The DNA size markers are shown on the left. (B) The gene structure and differential splice site of the short and long form of the tachykinin NK_1 receptor. The donor and acceptor sites are illustrated to show the extended exon 4 of the truncated tachykinin NK_1-short form receptor. The stop codons are illustrated as well as the extreme ends of the 3′ untranslated region of each receptor. The location of each of the gene specific primers for the PCR is also shown.

4. Discussion

Neurokinin B is found to be the only tachykinin expressed by the placenta capable of signalling through peripheral tachykinin receptors. It was therefore important to decipher which of the tachykinin receptors is expressed in the placenta. All three of the characterised tachykinin receptors (NK_1, NK_2 and NK_3) are present, though further studies will be needed to determine their precise location and function. It is possible that these receptors may not only be located to the trophoblast, which makes up the majority of the cell population of the placenta, but to cells such as the endothelium of blood vessels or to resident macrophages.

The expression of the shorter variant of the tachykinin NK_1 receptor was not observed in any of the 24 human tissues examined. The shorter variant could only be amplified from human genomic DNA, while the putative tachykinin NK_4 receptor was neither expressed in any human tissue examined nor found to be present in the human genome. The sequence of this receptor was determined to be a guinea pig tachykinin receptor. As the variation at the amino acid sequence and nucleotide sequence level are similar between the known tachykinin NK_3 receptor sequences of a range of different species, it is likely that this receptor is in fact the guinea pig tachykinin NK_3 receptor. We can only speculate that perhaps Xie et al. (1992), who originally cloned the tachykinin NK_4 receptor from the human placenta as a putative opioid receptor, cross-contaminated their isolated human cDNA with that of guinea pig. This is probable as they were also isolating opioid receptors from the guinea pig brain at around the same time (Xie et al., 1994). Nevertheless, Donaldson et al. (1996) have reported the expression by Northern blot analysis of the tachykinin NK_4 receptor in human skeletal muscle, lung and liver, but not in the placenta or brain. It is likely that the tachykinin NK_4 receptor sequence, used as a labelled probe, sharing an 85% identity to the human tachykinin NK_3 receptor sequence, would have cross-hybridised with the endogenous tachykinin NK_3 receptor sequence explaining the long exposure time of 6 days needed to reveal the expression. No comparison using a tachykinin NK_3 receptor sequence as a labelled probe was performed to consolidate this result. Recently, we have subsequently shown that human skeletal muscle, lung and liver are all sites of tachykinin NK_3 receptor expression (Bell et al., 2001). It is also worthy of note that Sarau et al. (2000) have previously screened the guinea pig genome for the tachykinin NK_4 receptor sequence. While they used Southern blot analysis to rule out the presence of the tachykinin NK_4 receptor sequence in the genome of the rat, dog, rabbit, monkey, mouse, cow and chicken, they used PCR to determine the existence of the tachykinin NK_4 receptor in the guinea pig and hamster genome. It is possible that this latter approach prevented them from detecting the presence of the tachykinin NK_4 receptor in the guinea pig genome as their PCR primers may have been designed to span a large intron, preventing an efficient PCR amplification. The intron and exon map of the tachykinin NK_4 receptor is unknown.

Our results demonstrate that at the present time, there are only three known tachykinin receptors (NK_1–NK_3) and that there are no other known isoforms. The nomenclature for these receptors therefore remains unchanged at the current time. Consequently, the search for the existence of new tachykinin receptors or different variants remains to be determined.

Acknowledgements

The Medical Research Council of the United Kingdom supported this work as a programme grant awarded to Philip J. Lowry. Nicola J. Bell is the recipient of a prize studentship from the British Society for Endocrinology.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Bell, N.J., Graham, G.J., Gibbins, J.M., Lowry, P.J., Page, N.M., 2001. The activation of peripheral neurokinin receptors in the pathology of pre-eclampsia. Endocr. Abstr. 2, OC24.

Donaldson, L.F., Haskell, C.A., Hanley, M.R., 1996. Functional characterization by heterologous expression of a novel cloned tachykinin peptide receptor. Biochem. J. 320, 1–5.

Fong, T.M., Anderson, S.A., Yu, H., Huang, R.R., Strader, C.D., 1992. Differential activation of intracellular effector by two isoforms of human neurokinin-1 receptor. Mol. Pharmacol. 41, 24–30.

Krause, J.E., Staveteig, P.T., Mentzer, J.N., Schmidt, S.K., Tucker, J.B., Brodbeck, R.M., Bu, J.Y., Karpitskiy, V.V., 1997. Functional expression of a novel human neurokinin-3 receptor homolog that binds [3H]senktide and [125I-MePhe7]neurokinin B, and is responsive to tachykinin peptide agonists. Proc. Natl. Acad. Sci. U.S.A. 94, 310–315.

Mantyh, P.W., Rogers, S.D., Ghilardi, J.R., Maggio, J.E., Mantyh, C.R., Vigna, S.R., 1996. Differential expression of two isoforms of the neurokinin-1 (substance P) receptor in vivo. Brain Res. 719, 8–13.

Page, N., Butlin, D., Manyonda, I., Lowry, P., 2000a. The development of a genetic profile of placental gene expression during the first trimester of pregnancy; a potential tool for identifying novel secreted markers. Fetal Diagn. Ther. 15, 237–245.

Page, N.M., Woods, R.J., Gardiner, S.M., Lomthaisong, K., Gladwell, R.T., Butlin, D.J., Manyonda, I.T., Lowry, P.J., 2000b. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. Nature 405, 797–800.

Page, N.M., Butlin, D.J., Lomthaisong, K., Lowry, P.J., 2001a. The human apolipoprotein L gene cluster: identification, classification and sites of distribution. Genomics 74, 71–78.

Page, N.M., Woods, R.J., Lowry, P.J., 2001b. A regulatory role for neurokinin B in placental physiology and pre-eclampsia. Regul. Pept. 98, 97–104.

Sarau, H.M., Mooney, J.L., Schmidt, D.B., Foley, J.J., Buckley, P.T., Giardina, G.A., Wang, D.Y., Lee, J.A., Hay, D.W., 2000. Evidence that the proposed novel human "neurokinin-4" receptor is pharmacologically similar to the human neurokinin-3 receptor but is not of human origin. Mol. Pharmacol. 58, 552–559.

Xie, G.X., Miyajima, A., Goldstein, A., 1992. Expression cloning of cDNA encoding a seven-helix receptor from human placenta with affinity for opioid ligands. Proc. Natl. Acad. Sci. U.S.A. 89, 4124–4128.

Xie, G.X., Meng, F., Mansour, A., Thompson, R.C., Hoversten, M.T., Goldstein, A., Watson, S.J., Akil, H., 1994. Primary structure and functional expression of a guinea pig kappa opioid (dynorphin) receptor. Proc. Natl. Acad. Sci. U.S.A. 91, 3779–3783.