ELSEVIER

Journal of Neuroscience Methods 168 (2008) 127–133

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Detection of full-length and truncated neurokinin-1 receptor mRNA expression in human brain regions☆

Jian-Ping Lai[a], Avital Cnaan[b], Huaqing Zhao[b], Steven D. Douglas[a,*]

[a] *Division of Allergy and Immunology, Joseph Stokes Jr. Research Institute at the Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania Medical School, 34th Street & Civic Center Boulevard, ARC 1208, Philadelphia, PA 19104, USA*

[b] *Division of Biostatistics and Epidemiology, Joseph Stokes Jr. Research Institute at the Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania Medical School, 3535 Market Street, Philadelphia, PA 19104, USA*

Received 20 July 2007; received in revised form 3 October 2007; accepted 3 October 2007

Abstract

We have applied a newly developed SYBR green-based real-time RT-PCR assay for quantification of full-length and truncated neurokinin-1 receptor (NK1R) mRNA expression in nine regions of human brain tissues obtained from 23 subjects who died with no evidence of neurological or neurodegenerative disease. The following brain regions were examined: cingulate cortex, cerebellum, nucleus accumbens, caudate nucleus, putamen, pons, hippocampus, locus coeruleus, and basal ganglia. The SYBR green-based real-time PCR was more sensitive than TaqMan probe-based real-time PCR in amplifying both full-length and truncated NK1R mRNA. The real-time RT-PCR assay had excellent specificity and sensitivity, with a dynamic range of detection between 100 and 1,000,000 copies of the NK1R cDNA per reaction. The truncated NK1R mRNA levels were more abundant than those of the full-length NK1R in most of the regions examined and there was no significant difference in the truncated NK1R mRNA levels among the nine regions studied. There was, however, a significant difference in the expression of full-length NK1R mRNA levels among the nine regions ($P = 0.0024$), and the putamen region expressed the highest full-length NK1R mRNA. Further studies are needed in order to examine the differences between full-length and truncated NK1R in signal transduction and functional consequences in order to delineate the significance of the co-presence of the two forms of NK1R in the human brain.

Keywords: Full-length NK1R; Truncated NK1R; Real-time PCR; SYBR green; Human brain

1. Introduction

Substance P (SP), a member of the tachykinin family, is widely distributed in the mammalian CNS and peripheral tissue. The biologic responses to SP are mediated by its preferring neurokinin-1 receptor (NK1R). The human NK1R gene (Fong et al., 1992; Gerard et al., 1991; Hopkins et al., 1991; Takeda et al., 1991), localized to chromosome 2, has been cloned. NK1R has been identified on CNS and immune cells (Bost, 2004; Ho and Douglas, 2004; Ho et al., 1997; Lai et al., 1998a,b, 1999, 2000; Lucey et al., 1994; Payan et al., 1984; Shanahan et al., 1985; Stanisz et al., 1987; Wozniak et al., 1989). Mammalian NK1R has been studied by several methods and has been found in the dorsal striatum, nucleus accumbens, hippocampus, raphe nuclei, and medulla oblongata (Otsuka and Yoshioka, 1993). NK1R localization in specific regions of the human brain was observed in the striatum (Aubry et al., 1994) and cerebral cortex (Kus et al., 1998; Tooney et al., 2000).

NK1R, a member of the G-protein coupled receptor (GPCR) superfamily ($Gq/_{11}$), has a typical structure of seven transmembrane domains, an extracellular N-terminus and an intracellular C-terminus (Fong et al., 1992). A splice variant of the human NK1R mRNA with a truncated C-terminus (truncated NK1R) has been cloned and identified (Fong et al., 1992). The full-length NK1R is the predominant form reported to be expressed at sites in the human brain, whereas the truncated NK1R is widespread in peripheral tissues (Caberlotto et al., 2003). Both the full-length and the truncated NK1R protein have been detected in rat submaxillary glands (Kage et al., 1993) and parotid (Mantyh et al., 1996). A distinct distribution of these two NK1Rs has been observed in rat striatum, submaxillary

☆ Supported by NIH P01-MH076388 and R01-MH049981 to S.D.D.

* Corresponding author at: Section of Immunology, Division of Allergy and Immunology, The Children's Hospital of Philadelphia, 34th Street & Civic Center Boulevard, ARC 1208, Philadelphia, PA 19104, USA. Tel.: +1 215 590 1978; fax: +1 215 590 3044.

E-mail address: DOUGLAS@email.chop.edu (S.D. Douglas).

0165-0270/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2007.10.004

glands and parotid (Mantyh et al., 1996). The only sequence difference between the two forms NK1R is in the length of the C-terminal tail (Fong et al., 1992). The C-terminal region has been postulated to be of importance to the coupling to different second messenger systems and targeting of receptors on neurons. Our group has demonstrated that the C-terminal tail of NK1R is required for SP-induced intracellular calcium increase in a monocyte-macrophage cell line (THP-1) (Lai et al., 2006). Although activation of the truncated NK1R did not trigger a calcium increase in these cells, SP primes CCL5-mediated calcium increases via activation of the truncated NK1R (Lai et al., 2006). The binding and signaling properties of the truncated receptor and NK1R mutants have been compared with those of the full-length receptor that is expressed in CHO cells, xenopus oocytes and rat Kirsten virus-transformed kidney epithelial cells (KNRK) (Bohm et al., 1997; Fong et al., 1992; Li et al., 1997; Sasakawa et al., 1994). The truncated NK1R undergoes a less rapid desensitization (Li et al., 1997), suggesting a longer duration of the response evoked by SP agonists in those brain regions where the truncated form predominates. Thus, it is very likely that there are differences in signal transduction pathways and functional consequences for the expression of differences between the full-length and truncated NK1R in CNS. In order to delineate the role of these two forms of NK1R in the brain, we investigated the expression of the full-length and truncated NK1R in different brain regions.

Recently, Caberlotto et al., using a quantitative TaqMan PCR analysis, observed that the full-length NK1R was the most prevalent throughout the human brain, while in the peripheral tissue, the truncated form was most represented (Caberlotto et al., 2003). This study (Caberlotto et al., 2003) reported the distribution of NK1R mRNA in four post-mortem human brains. However, when the sensitivity of this TaqMan primer/probe assay (Caberlotto et al., 2003) was compared to our established real-time PCR assay which detects both full-length and truncated NK1R (Lai et al., 2005) using full-length NK1R plasmid cDNA as template, our assay was 4–16 times more sensitive than that of the TaqMan assay (Caberlotto et al., 2003) (data not shown). The sensitivity difference between these two assays is most likely due to the location of the primers and/or the Taqman probe used. We therefore modified this real-time PCR assay and examined the expression of the full-length and truncated NK1R in nine brain regions obtained from 23 post-mortem human brains. The regions studied included: cingulate cortex, cerebellum, nucleus accumbens, caudate nucleus, putamen, pons, hippocampus, locus ceruleus, and basal ganglia.

2. Materials and methods

2.1. Subjects and specimens

Twenty-three brain specimens were obtained from individuals who died with no evidence of neurological or neurodegenerative disease through the National NeuroAIDS Tissue Consortium (NNTC). Among the 23 subjects, 19 were HIV-positive and 4 were HIV-negative, 7 were females and 16 were males. These subjects died at ages 27–66 years old (average 46 years of age). Among these 23 subjects, 8 were Caucasian (35%) and 15 were non-Caucasian (65%). The specimens obtained from the 23 subjects, included: 23 cingulate cortex, 17 cerebellum, 9 nucleus accumbens, 8 caudate nucleus, 8 putamen, 8 pons with locus ceruleus, 7 hippocampus, 7 locus coeruleus, and 6 basal ganglia specimens.

2.2. RNA extraction

Total RNA was extracted from these brain tissues using Tri-Reagent (Molecular Research Center, Cincinnati, OH), as instructed by the manufacturer. After centrifugation at 13,000 × *g* for 15 min, RNA-containing aqueous phase was precipitated in isopropanol. RNA precipitates then were washed once in 75% ethanol and solubilized in 30 μl of RNase-free water (Lai et al., 2006). RNA concentration of pooled brain tissues was determined by UV spectroscopy at A_{260} (nm) and the RNA was used to generate GAPDH standard curve in order to quantitatively determine total RNA amount in each sample by real-time PCR.

2.3. Reverse transcription

Total RNA (4 μl) was subjected to reverse transcription. The final reaction mixture (20 μl) contained the following elements: 5 mM $MgCl_2$, 1× RT buffer, 500 μM of each dNTPs, 1 unit/μl recombinant RNasin, 10–15 units of AMV reverse transcriptase (Promega), and 50 ng random primers. Reverse transcriptase negative controls were used in order to control for genomic DNA contamination. RT was performed at 42 °C for 1 h. The reaction was terminated by holding the reaction mixture at 99 °C for 5 min. One-tenth (2 μl) of the resulting cDNA was used as a template for real-time PCR amplification.

2.4. Real-time PCR primers

The PCR primers used for quantitative measurement of the full-length and the truncated NK1R mRNA were modified from Caberlotto et al. (Caberlotto et al., 2003). The sequences of the primer pair used to amplify the full-length of NK1R were: (sense-L-F) 5′-TCTTCTTCCTCCTGCCCTACATC-3′; (antisense-L-R-967) 5′- AGCACCGGAAGGCATGCTTGAAGCCCA -3′, which is specific for the full-length NK1R sequence. The sequences of the primer pair for the truncated NK1R were: (sense-L-F) 5′-TCTTCTTCCTCCTGCCCTACATC-3′; (antisense-S-R-1083) 5′-TGGAGAGCTCATGGGGTTGGGATCCT -3′. The forward primer is identical to the one used by Caberlotto et al. (2003). However, the reverse primers for both full-length and the truncated NK1R are located further downstream from the forward primer in order to avoid potential non-specific amplification due to the 50% sequence homology of reverse primers of the full-length and truncated NK1R (Fig. 1). The sequences of the primer pair for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were: 5′-GGTGGTCTCCTCTGACTTCAACA-3′ (sense);