Published in final edited form as:

J Neurol Sci. 2008 September 15; 272(1-2): 174–177. doi:10.1016/j.jns.2008.04.025.

Neurokinin-1 Receptor mRNA Expression Differences in Brains of HIV-Infected Individuals

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Abstract

The neurokinin-1 receptor, a G-protein coupled receptor, is present in cells of the nervous system and the immune system. Utilizing our recently developed SYBR green-based RT-PCR, we quantified full-length and truncated NK1R mRNA expression in the cingulate cortex and cerebellum of autopsy brains from HIV-negative and -positive individuals. In the cingulate cortex, the expression of the full-length NK1R was greater in HIV-negative individuals (n=3) in comparison to HIV-positive individuals (n=21; p-value=0.026). There were no observed differences in expression of the truncated NK1R in cingulate cortex between HIV-positive and -negative individuals. The expression of NK1R isoforms, both truncated and full-length, was similar between HIV-negative and -positive individuals in the cerebellum. It was not possible to directly relate the magnitude of NK1R expression to impairment in neuropsychological impairment. This small cohort and none of the subjects had HIV Encephalopathy. These preliminary data support the concept that the full-length form of NK1R may have important significance in cognitive functions and deficiency of this isoforms may be relevant in neurologic and psychiatric manifestations of neuroAIDS.

Keywords

Neurokinin-1 receptor; Isoforms; HIV brain

INTRODUCTION

The neurokinin-1 receptor (NK1R), the substance P preferring receptor, is a member of the G-protein coupled receptor (GPCR) superfamily (Gq/11) with a typical structure of seven transmembrane domains, an extracellular N-terminus and an intracellular C-terminus (1). NK1R is present in cells of the nervous system and immune system, and a splice variant of the human NK-1R mRNA with a truncated C-terminus (truncated NK1R) has been cloned and identified (1). The full-length NK1R is the predominant form expressed at sites in the human brain, whereas the truncated NK1R occurs throughout the central nervous system and in peripheral tissues (2). Both forms of NK1R protein occur in rat submaxillary glands (3) and parotid (4). A distinct distribution of the two isoforms of NK1R was observed in rat striatum,

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submaxillary glands and parotid (4). The binding and signaling properties of the truncated receptor or mutants have been compared with those of the full-length receptor that expressed in several cell systems and differences have been observed (1,5–7). We have recently applied a SYBR green-based real-time RT-PCR for quantification of full-length and truncated NK1R mRNA in 9 regions of human brain tissues (8). The findings suggest that the expression levels and function of the full-length NK1R are anatomic region-dependent (8). We demonstrated that truncated NK1R has higher expression levels than that of the full-length NK1R, and that there are significant differences in expression in different brain regions (8). Further, the full-length NK1R functions as a complete receptor in a monocyte-derived cell line (9).

We have demonstrated differences between SP-induced calcium mobilization between the full-length and truncated NK1R in monocyte-derived macrophages (9). The differentiated macrophage expresses both forms of NK1R, and induces a calcium increase directly, whereas the truncated isoform primes CCL5-mediated calcium increases (9). We have examined the expression of the full-length and truncated NK1R in the cingulate cortex and cerebellum in 24 autopsy brain samples from 21 HIV-positive and 3 HIV-negative adults.

In examining the role of the neurokinin-1 receptors (NK1R) in HIV and in psychiatric disorders, we hypothesize that there are differences in expression between full-length and truncated isoforms of NK1R in various parts of the brain. We further hypothesize that there are differences in expression of the forms of NK-1R in the cingulate cortex, in comparison to the cerebellum. We have previously observed differences in levels of plasma substance P between HIV + and HIV- men (10) and women (11).

METHODS

Subjects and Specimens

Twenty-three brain specimens, 16 men and 7 women ages 31–58 years, were made available through a request to the National NeuroAIDS Tissue Consortium. Total RNA was extracted from these brain tissues using Tri-Reagent (Molecular Research Center, Cincinnati, OH), as instructed by the manufacturer (9). 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 ul of RNase-free water (9). RNA concentration of pooled brain tissues was determined by UV spectroscopy at A₂₆₀ 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.

Real Time PCR Primers

The PCR primers used for quantitative measurement of the full-length and truncated isoforms of NK1R mRNA were modified from Caberlotto *et al.* (2,8). The sequences of the primer pair used to amplify the long isoform of NK-1R were: (sense) 5'-TCTTCTTCCTCCTGCCCTACATC-3'; (antisense) 5'-AGCACCGGAAGGCATGCTTGAAGCCCA-3', which is specific for the full length NK1R sequence. The sequences of the primer pair for the truncated NK1R were: (sense) 5'-TCTTCTTCCTCCTGCCCTACATC-3'; (antisense) 5'-TGGAGAGCTCATGGGGTTGGGATCCT-3'. The sequences of the primer pair for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were: 5'-GGTGGTCTCCTCTGACTTCAACA-3' (sense); 5'-GTTGCTGTAGCCAAATTCGTTGT-3' (antisense) (12). The primers were resuspended in TE buffer and stored at -30° C.

Reverse Transcription

Total RNA (4 μ l) was subjected to reverse transcription. The final reaction mixture (20 μ l) contained the following elements: 5 mM $MgCl_2$, 1X RT buffer, 500 μ M of each dNTPs, 1 unit/ μ l 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 μ l) of the resulting cDNA was used as a template for real-time PCR amplification (8).

Real-Time PCR Assay

The MyiQ iCycler system (Bio-Rad Laboratories, Inc., Hercules, CA) was used for real-time PCR analysis. Thermal cycling conditions were designed as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Fluorescent measurements were recorded during each annealing step. At the end of each PCR run, data were automatically analyzed by the system and amplification plots were obtained. For each PCR, 2 μ l of cDNA template was added to 23 μ l of iQTM SYBR Supermix or iQ Supermix (for TaqMan probe) (Bio-Rad) containing the primer pair for either the full-length or the truncated NK-1R, or for GAPDH. An NK-1R full-length plasmid (a gift from Dr. Norma Gerard, Harvard University, Boston, MA) was used to prepare standard curves and used as a specificity control for real-time PCR. The full-length plasmid was amplified by the primer pair for the full-length NK-1R, but not by the primer pair for the truncated NK-1R. In our preliminary experiments, using serial diluted cDNA derived from pooled brain tissues, the amplification efficiency of the full-length and truncated NK1R primer pairs were very similar (103% for truncated and 102% for the full-length NK1R, respectively). We therefore used the full-length NK1R standards to measure the truncated NK1R expression levels. All amplification reactions were performed in duplicate and the average threshold cycle (Ct) numbers of the duplicates were used to calculate the expression levels (copy numbers) of full-length and truncated NK-1R using the standard curve generated with the full-length NK1R plasmid. In order to control for the integrity of the RNA and normalize NK-1R mRNA levels in these samples, a GAPDH mRNA fragment in the RNA also was amplified using our established real-time RT-PCR with iQTM SYBR green Supermix (Bio-Rad), as previously reported (12). In brief, in order to generate a total RNA standard curve with GAPDH primers, a known amount of total RNA standard extracted from pooled brain tissues was reverse transcribed and serial diluted (ranging from 32 to 2000 ng), and the GAPDH fragment was amplified for 40 cycles. The mRNA in the brain samples was amplified in the same plate with the standard under the identical conditions. The quantity (ng) of total RNA in the samples was automatically calculated by The MyiQ iCycler system based on the data obtained from the total RNA standard curve. All amplification reactions were performed in duplicate and an average RNA quantity (ng) of the duplicates was used to normalize the full-length and truncated NK1R mRNA levels in the brain tissue samples examined.

The expression levels of the full-length and truncated NK-1R cDNA were normalized to an endogenous cDNA, GAPDH. In order to normalize the full-length and truncated NK1R mRNA levels, the full-length and truncated NK1R copy numbers in brains tissues samples were divided by the total RNA quantity (ng) determined by the GAPDH real-time RT-PCR in the same sample and then multiplied by 1000 to convert the unit to the copy number of NK-1R full-length or truncated mRNA per microgram (μ g) of total RNA.

All measures exhibited considerable skewness, so we applied a $\log_{10}$ transformation prior to summarizing via means and standard deviations (Table 1). With the exception of the truncated form in the cerebellum, the standard deviations for the HIV positive group were at least twice as large as for the HIV negative group, so we report Kruskal-Wallis tests in order to examine

whether expression levels were different between full-length and truncated isoforms separately for each of the two brain regions.

RESULTS

Table 1 shows the means and standard deviations for the log₁₀-transformed full-length and truncated NK1R mRNA levels, together with the p-values from the Kruskal-Wallis tests.

NK1R expression levels are similar for the truncated isoform for both brain regions examined (Table 1). The report is limited to data for 21 HIV positive autopsy brains. This limitation is related to the availability of autopsy brain material. There are, however, significant differences in the expression of full-length NK1R between HIV positive and HIV negative patients in the cingulate cortex, with higher expression levels observed for the HIV negative subjects ($p=0.026$). In the HIV positive subjects there was no apparent relationship between the reduction in expression of full length NK1R in the cingulate cortex and neuropsychologic impairment neuropathologic diagnosis. None of the first HIV subjects studied had HIV-encephalopathy.

DISCUSSION

There is a difference in expression levels of full-length NK1R based on HIV status in the cingulate cortex. The observation of a decrease in expression of the full-length isoform in cingulate cortex in relationship to HIV status, without a corresponding change in the cerebellum is a striking finding. This finding is of particular interest, in view of several recent observations. Several studies relate alterations in prefrontal and parietal tissue, which correlate with cognitive motor defects (13). Further, and of considerable interest, are the findings that cingulate cortex has an important role of executive processes and vital cognitive function, such as a site for integration of information sources, including cognitive, emotional, and interoceptive signals (14–16). The transcription factor Δ FosB down-regulates the substance P gene in a rat model. Δ FosB reduces depression-like behavior and inhibits stress-induced release of substance P, further indicating an important role for NK1R in depression (17).

Our investigation is the first investigation of neurokinin-1 expression of full-length and truncated forms of NK1R in relationship to HIV infection in autopsy human brains. We previously observed significant differences between full length NK1R mRNA expression levels among the nine regions studied. (8). The functional and signaling differences between the full-length and truncated NK1R have not been fully investigated. The sequence difference between the two forms of NK1R is in the length of the C-terminus (1). The presence of the C-terminus tail is the structural basis for the observed SP-induced calcium increase (9). Activation of the truncated NK1R primes CCL5-mediated calcium increase, indicating that the truncated NK1R also has functional activity (9). Thus, the truncated NK1R very possibly has different signal transduction pathway(s) in comparison to the full-length NK1R. We therefore speculate that the expression and function of the truncated NK1R is universal in these two regions studied, while the expression level of the full-length NK1R is inducible by several factors including NF- κ B (16,17). Our studies therefore supports the concept that there are unique characteristics of NK1R expression, and that NK1R-mediated signaling between undifferentiated (macrophage cell lines) THP-1 cells and differentiated THP-1 cells results in unique changes in NKIR (9). We have recently demonstrated the importance of full-length NKIR in signaling and functions of the receptor in the macrophage cell line (9). In another context, the full-length and truncated NKIR has been studied in several cell systems and in signaling functions, the full-length NKIR is required. In addition, we further propose that the interaction between NKIR and β -chemokine receptors, in particular, CCR5 receptor, is important in neuroimmune interactions in the brain.

Acknowledgements

Supported by R01-MH049981 and P01-MH076388 to S.D.D. Brain tissue specimens, data, and support were provided by the National NeuroAIDS Tissue Consortium (request R085). The NNTC is funded by the National Institutes of Health N01MH32002.

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Table 1

NK1R mRNA *

	HIV-positive (n=21)	HIV-negative (n=3)	KW test P
Cingulate Cortex			
Truncated NK1R	4.70±0.22	4.71±0.07	0.76
Full-length NK1R	4.79±0.37	5.24±0.13	0.026
Cerebellum			
Truncated NK1R	4.79±0.33	4.92±0.31	0.57
Full-length NK1R	3.81 ±0.35	3.89±0.16	0.63

* log₁₀ mRNA copies/ng ± standard deviation