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Neuroscience Letters 373 (2005) 67–72
Neuroscience Letters
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Splice variants of the receptor for advanced glycosylation end products (RAGE) in human brain

Qunxing Ding, Jeffrey N. Keller*

Department of Anatomy and Neurobiology, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536-0230, USA

Received 12 July 2004; received in revised form 27 September 2004; accepted 27 September 2004

Abstract

Previous studies indicate that the receptor for advanced glycosylation end products (RAGE) plays an important role in multiple pathological processes, including Alzheimer's disease. Currently there are three established isoforms of the RAGE receptor, with each isoform generated as the result of alternative splicing. It is presently unclear which of the RAGE isoforms are normally expressed in the human brain, nor has it been determined if additional RAGE isoforms exist in the human brain. In the present study we demonstrate for the first time that each of the three established RAGE isoforms, as well as three previously unidentified RAGE splicing variants, are normally expressed in the human brain. These data suggest that RAGE may have multiple functions in the human brain, mediated by the individual or coordinated efforts of the different RAGE isoforms, with alternative splicing generating individual RAGE isoforms that specifically interact with the various ligands present in the brain.

Keywords: Alzheimer's disease; Amyloid-beta; Brain; Receptor for advanced glycosylation end products

The receptor for advanced glycosylation end products (RAGE), is a multiligand receptor of the immunoglobulin superfamily [1]. At present, there are three well-characterized isoforms of the RAGE receptor, two of which are individually generated as the result of alternative splicing [23]. These three RAGE isoforms are commonly referred to as the full-length RAGE receptor, secretory RAGE (sRAGE), and N-truncated RAGE (NtRAGE) [12,23]. The full-length RAGE receptor is an integral transmembrane protein with an extracellular domain containing one variable (V-type) and two constant (C-type) immunoglobulin domains [7]. Full-length RAGE also contains a short hydrophobic domain that corresponds to a single transmembrane domain, which is followed by a short cytoplasmic domain that is essential for RAGE-mediated signal transduction [7,17]. Secretory RAGE contains the same immunoglobulin domains present in full-length RAGE receptor mRNA, but also contains part of intron 9, which incorporates a stop codon within the sequence. Because of the insertion of this stop codon the sRAGE mRNA lacks exon 10 and 11, which encode the trasmembrane domain of RAGE, resulting in sRAGE being released [17,23]. The mRNA for NtRAGE retains intron 1, which like intron 9 contains a novel stop codon, resulting in the loss of both exon 1 and exon 2. This truncated version of full-length RAGE therefore lacks the V-type immunoglobulin domain, but is otherwise identical to full-length RAGE, and is retained in the plasma membrane [17,23]. As a result of the deletion of the V-type immunoglobulin domain NtRAGE is significantly impaired in its ability to bind RAGE ligands [23].

A growing number of studies now suggest that the role of RAGE signaling is not mediated by the sole action of any one type of RAGE receptor, but is summation of the effects derived from each of the different RAGE isoforms [9,18,19,22]. To add to this complexity, recent studies in the rat liver and rat kidney suggest that additional alternatively spliced RAGE isoforms exist [7]. Taken together, these data highlight the importance of understanding the totality of RAGE expression present within individual tissues, and suggest that each of

* Corresponding author. Tel.: +1 859 257 1412x333; fax: +1 859 323 2866.
E-mail address: Jnkell0@pop.uky.edu (J.N. Keller).

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doi:10.1016/j.neulet.2004.09.059

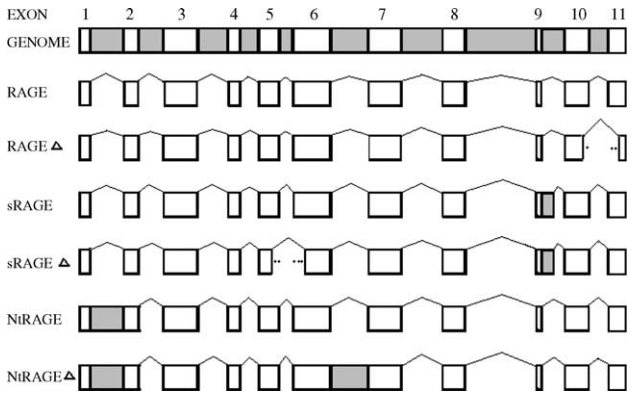
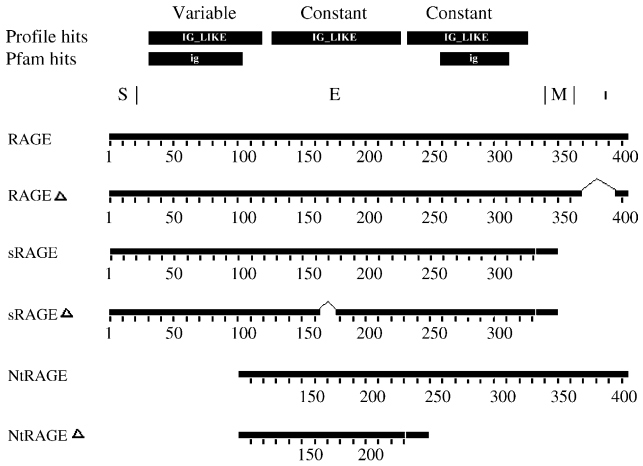


Fig. 1. Schematic illustration of the multiple RAGE splice isoforms found in human brain. Open boxes represent the exons and shaded boxes indicate introns in human RAGE sequence. The symbol (+) indicates the position of deletion occurred. Inserted shaded boxes represent read through introns.



S: signal sequence; E: Extracellular region; M: transmembrane region; I: Intracellular region

Fig. 2. Schematic representation of functional domains in RAGE splicing isoforms. There are three immunoglobulin-like domains (IG_LIKE) in full-length RAGE, with the first one being V-type (variable region), followed by two C-type (constant region). The first and third of these domains are observed to be highly homologous to actual immunoglobulin domains, as indicated by (ig). The numbers correspond to amino acid number and begin with first methionine resude. Connecting lines indicate the deletion positions, black bars in sRAGE and sRAGEΔ, and red bar in NtRAGEΔ represent the presence of a non-homologous additional amino acid sequence. S: signal sequence; E: extracellular region; M: transmembrane region; I: introcellular region.

the RAGE receptor isoforms is expressed in a tissue specific manner in order to cope with the specific ligands endogenous to an individual tissue.

RAGE receptors are predominantly studied for their role in diabetes, inflammation, atherosclerosis, and cancer [11,13,16,21]. A number of recent studies have also indicated that RAGE signaling may play an important role in multiple neurodegenerative conditions. For example, studies have indicated a probable role for RAGE in Crutzfield–Jakobs disease [16], ischemia reperfusion injury [20], and Alzheimer's disease (AD) [6]. At present the identification of which of the established RAGE receptor isoforms are normally present in the human brain, and the identification of potentially novel RAGE isoforms in the brain, has not been reported previously. Such data are essential in beginning to elucidate the specific role of RAGE signaling for both neurophysiological and neuropathological processes in the human brain.

In order to begin such studies we conducted studies using human brain tissue obtained from the brain tissue bank from the Sanders-Brown Center on Aging, with hippocampal brain tissue utilized for the generation of cDNA [4,5]. Two primers were used for the cloning of individual RAGE isoforms with the insertion of *Kpn*I and *Xba*I restriction enzyme sites (underlined): KpnS5: 5′-GGAGGTACCATGGCAGCCGGAACAGCAGTTGG-3′; XbaE3: 5′-CACTCTAGATCAAGGCCCTCCAGTACTACTCTCG-3′. The resulting fragments were isolated by agarose electrophoresis and then cloned into the pGEM-3Z vector (Promega) followed by sequencing (Davis Sequencing Inc.). Pfu DNA polymerase (Promega) was utilized in PCR amplification. The National Center for Biotechnology Information (Blast program) and ExPASy tools (http://www.us.expasy.org/tools/) were utilized for identification and analysis of individual sequences.

Our RT–PCR reaction generated six different PCR products, which were then cloned and sequenced. Sequence analysis indicated the presence of full-length RAGE and five splice variants of full-length RAGE (Fig. 1). The full-length RAGE in human brain was identical to the full-length human RAGE reported previously in human lung [14] (Fig. 1). The full-length RAGE in human brain possessed each of the established functional domains described previously [7,23] (Figs. 2 and 3). In addition to this established form of full-length RAGE, a slightly truncated version of full-length human RAGE was also identified in human brain (Fig. 1). This previously unidentified form of full-length RAGE possessed a 13 base pair deletion of exon 10 and a 35 base pair deletion in exon 11, resulting in a predicted deletion of 16 amino acids (Figs. 1 and 3). This unique form of full-length RAGE possessed an identical extracellular domain as full-length RAGE (Figs. 1 and 2), but lacked a significant substantial amount of the intracellular signal transduction domain (Fig. 2).

The sRAGE isoform identified in human brain (Fig. 1), was identical to the sRAGE sequence described in other tissues [7,9,23] (Fig. 3). An additional sRAGE variant was identified in the human brain (Fig. 1). This previously unidentified sRAGE splice variant lacked 16 base pairs of exon 5, and 20 base pairs in exon 6 (Figs. 1 and 2). This deletion results in a predicted deletion of 12 amino acids (Fig. 3), which was contained within the first constant immunoglobulin domain of RAGE (Fig. 2).

The NtRAGE isoform of RAGE was also identified in human brain (Figs. 1 and 2), and had a predicted amino acid sequence identical to the established NtRAGE sequence [7,23] (Fig. 3). An addition splice variant isoform of NtRAGE was also identified in human brain (Figs. 1 and 2). This variant of NtRAGE contained intron 1 as described previously for NtRAGE [23], but also contained intron 6, resulting in the

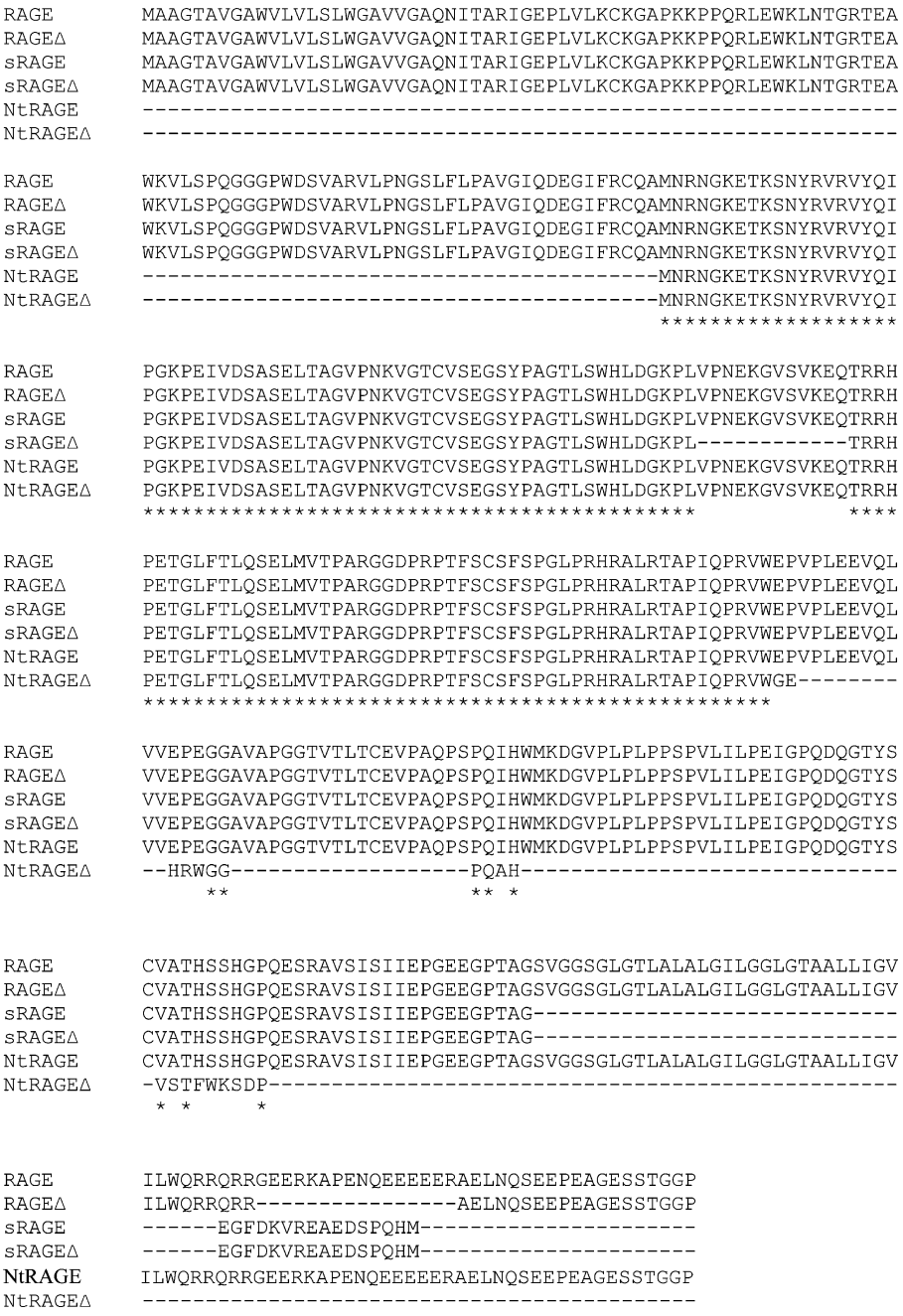


Fig. 3. Alignments of the amino acid sequences of the RAGE splicing isoforms. ExPASy tools are used to analyse the animo acid sequences of all the isoforms. Star (★) indicates the homologous amino acids. Broken dash lines represent the deletion locations or truncated regions.

predicited addition of 18 amino acids (Fig. 3), and a loss of exons 7–11 (Fig. 1). This novel NtRAGE isoform therefore lacked the second constant immunoglobulin domain of RAGE (Fig. 2).

We next sought to elucidate the relative abundance of the different RAGE isoforms in human brain. A one-step RT–PCR system (Invitrogen) was used to quantify the expression level of full-length RAGE, NtRAGE, and sRAGE as described previously by our laboratory [4,5]. Based on pilot experiments and real time RT–PCR curves, the optimal cycle number was established as being 21 cycles. Primers for analysis of full-length RAGE expression were identical to those described above and the primer pairs used for measuring NtRAGE and sRAGE are as followed: for NtRAGE, 5′-GTGCTGGTCCTCAGTCTGT-3′ and 5′-CCTTCTCATTAGGCACCAG-3′; for sRAGE, 5′-AGGCGAGGAGGGGCCAAC-3′ and 5′-AAGGTGGGGTTATACAGGAG-3′. The relative expression levels of individual RAGE isoforms data was obtained using the brains of four control patients (two males and two females). These patients

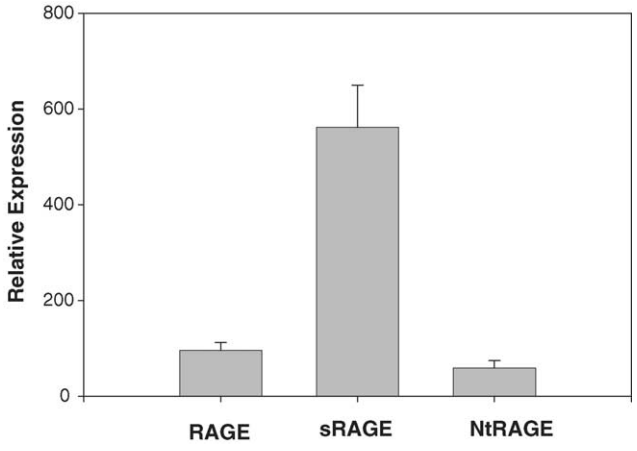


Fig. 4. Relative expression level of three RAGE isoforms. Total RNA were purified from four samples of human control brain (hippocampus) and semi-quantitive RT–PCR methods utilized to measure the expression level of full-length RAGE (RAGE), secretory RAGE (sRAGE), and N-truncated RAGE (NtRAGE). Bars represent the average value of four samples.

were devoid of dementia and any neuropathology not attributable to normal aging, with an average age of 83.2 ± 7.2, post mortem interval of less than 4 h, and average brain weight of 1217 ± 91 g. Analysis of brain tissue from four control individuals revealed that the levels of full-length RAGE and NtRAGE are nearly identical in control human brain (Fig. 4). In contrast, the level of sRAGE was ~4 fold higher than either full-length RAGE or NtRAGE (Fig. 4), indicating that sRAGE is the predominant RAGE isoform in the brains of non-demented and neuropathology-free individuals.

Our data demonstrate for the first time that multiple forms of RAGE are expressed in the human brain, with human brain possessing at least three previously unidentified isoforms of RAGE.

These data strongly suggest that the role of RAGE in both neurophysiological and neuropathological processes is mediated through a complex interplay between the various RAGE isoforms, and is not mediated solely by the full-length RAGE receptor. In future studies examining the role of RAGE in neurodegenerative conditions such as AD, it will therefore be of critical importance to examine the contribution of multiple RAGE isoforms, and not solely focus on full-length RAGE. In particular, it will be important to determine if the level of individual RAGE isoforms, or the ratio of RAGE isoforms, becomes altered in neurodegenerative conditions such as AD.

The presence of multiple RAGE isoforms in the human brain may indicate that individual RAGE isoforms have highly specialized functions in the human brain. For example, the novel full-length RAGE variant observed in the present study would be expected to bind the same ligands as normal full-length RAGE, but would not be expected to induce a similar pattern of signal transduction, based on the fact that this novel variant of full-length RAGE lacks a significant portion of the intracellular signal transduction domain. Studies are currently underway to determine the functional significance of this specific deletion to RAGE signaling. Similarly, the novel forms of sRAGE and NtRAGE observed in the present study lack significant portions of the constant immunoglobulin domain present in the established sRAGE and NtRAGE isoforms. It is highly likely that such modifications alter the ability of these novel RAGE isoforms to interact with specific ligands such as beta-amyloid. These data raise the very real possibility that the cells of the brain utilize alternative splicing as a means of selectively altering RAGE expression in order to cope with specific ligands present within the brain. It will be important in future studies to determine the functional relevance of splicing variations to the ability of individual RAGE isoforms to interact with brain resident RAGE ligands, such as beta-amyloid. Additionally, it will be important to elucidate the expression of these individual splice variants at the protein level, in order to understand the functional consequences of the splice variants observed in the present study.

The present study demonstrates that in the human brain there is a highly specialized regulation of RAGE expression, and presumably RAGE function, via alternative splicing. The multiple RAGE isoforms were not generated from a simple exon excision, but rather generated from at least two different forms of alternative splicing. For example, in the present study we observed the read through of introns as well as the deletion of specific exon sequences. It is interesting to note that when deletion of exon sequences was observed, it always occurred in two adjacent exons, and never was observed to occur in a single exon. In future studies it will be important to identify the molecular mechanisms responsible for the alternative splicing of RAGE.

Our data indicate that in the brains of control subjects, sRAGE is expressed at much higher levels than either full-length RAGE or NtRAGE. Previous studies have demonstrated that sRAGE can bind to RAGE ligands, and thereby prevent their binding to the full-length RAGE receptor located in the plasma membrane [23]. By inhibiting the binding of ligands to the full-length RAGE receptor, sRAGE prevents the initiation of full-length RAGE-induced signal transduction [15,23]. This inhibition may be important to maintaining brain homeostasis, given that RAGE-induced signal transduction is particularly potent at inducing NFκB activation, which then strongly induces the expression of the full-length RAGE receptor [8]. This feed forward pathway of full-length RAGE receptor activation promotes further RAGE-induced signal transduction, through the generation of full-length RAGE receptor expression, which is believed to play an important role in many pathological conditions such as atherosclerosis and diabetes [2,3,10] our data indicate that such a feed forward pathway may be prevented in the control human brain by the high level expression of sRAGE. It is interesting to note that at least two forms of sRAGE are evident in the control human brain, suggesting that sRAGE function is highly specialized within the brain. The novel NtRAGE variant observed in human brain lacks

the trasmembrane domain, and may serve as an additional secretory form of RAGE.

Taken together, these data suggest that in the healthy human brain a large amount of RAGE expression is generated for the purpose of generating a secreted form of RAGE. Once released, each of these secreted RAGE products would be expected to attenuate the ability of individual ligands to bind to the membrane-bound full-length RAGE receptor. Additionally these secreted products may aid in preventing the aggregation of potential RAGE ligands, such as beta-amyloid, which would decrease the propensity of ligands to stimulate RAGE-induced signal transduction. The ability of secreted RAGE to inhibit beta-amyloid aggregation would be expected to not only reduce RAGE-induced signal transduction but also provide a global benefit to the brain, possibly delaying or preventing the onset of beta-amyloid toxicity in the brain, which is believed to play a causal role in AD. Conversely, a loss of secreted RAGE expression may play a causal role in the initiation of beta-amyloid toxicity, and directly contribute to the manifestation of AD pathogenesis. Understanding the beneficial effects of secreted RAGE receptor isoforms towards beta-amyloid toxicity is therefore not only important to our understanding of RAGE signaling, but may provide a potential therapeutic benefit in the treatment of AD.

Acknowledgements

The authors wish to thank Dr William R. Markesbery for his continual support, and Dr Filomena Dimayuga for helpful discussions. The authors also thank the faculty and staff of the Sanders-Brown ADRC research group for providing valuable tissue and information. The ARDC brain tissue bank within Sanders-Brown is supported by the NIH grant AG05144. The present work was supported grants from the National Institutes of Health [AG018437 (J.N.K), AG005119 (J.N.K)].

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