Invest Clin 64(4): 505 - 512, 2023 https://doi.org/10.54817/IC.v64n4a07

Nuclear and cytoplasmic expressions of the receptor for advanced glycation end products (RAGE) in the rat central nervous system.

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Keywords: RAGE; ligands; nucleus; cerebral cortex; cerebellum.

Abstract. The receptor for advanced glycation end products (RAGE) is a transmembrane protein involved in the induction of inflammatory processes and oxidative stress after interacting with its ligands on the cell surface. Localization on the cell surface is necessary for interaction with the ligands. This study aimed to determine the expression of RAGE in different parts of the normal rat brain and cerebellum using the immunofluorescence technique. Several cerebral cortex layers (molecular/granular layers: M/GL; pyramidal layer: PL) and the hypothalamus were analyzed, as well as the molecular layer (CML) and the granular layer (CGL) of the cerebellum. Cells with RAGE-positive nuclei were generally observed in the brain's cerebral cortex and cerebellum**.** In the M/GL, cells with different degrees of positivity in the nucleus and cytoplasm accompanied by RAGE-positive material in the adjacent extracellular space were observed, and RAGE-positive material in the neuropile. Pyramidal neurons presenting various degrees of nuclear RAGE-positive material budding and cells with different degrees of nuclear and cytoplasmic positivity were observed in PL. The hypothalamus showed a high number of cells with RAGE-positive granules adjacent to the nucleus and in the cytoplasm; nuclei remained negative. Many positive nuclei were observed in CML; they were scarce in CGL. These data suggest the storage of RAGE at the nuclear and cytoplasmic levels in healthy rats and hypothesize the possible translocation of this molecule to the cell surface in pathological conditions.

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Expresión nuclear y citoplasmática del receptor para compuestos de glicosilación avanzada en el sistema nervioso central de la rata.

Invest Clin 2023; 64 (4): 505 – 512

Palabras clave: RAGE; ligandos; núcleo; corteza cerebral; cerebelo.

Resumen. El receptor para compuestos de glicosilación avanzada (RAGE) es una proteína transmembrana involucrada en la inducción de procesos inflamatorios y en el estrés oxidativo después de su interacción con sus ligandos en la superficie celular. La localización de este receptor en la superficie celular es necesaria para su interacción con sus ligandos. El objetivo de este estudio fue determinar la expresión de RAGE en las diferentes partes del cerebro y cerebelo de la rata normal. Mediante la utilización de técnicas de inmunofluorescencia se analizaron varias capas de la corteza cerebral (capas molecular/granular: CM/G; capa piramidal: CP) y el hipotálamo. Las capas molecular (CMC) y la capa granular (CGC) del cerebelo fueron también analizadas. Se observaron células con el núcleo positivo para RAGE tanto en cerebro como cerebelo. En CM/G se apreciaron células con diversos grados de positividad para RAGE acompañadas de material positivo para RAGE en el espacio extracelular adyacente y en la neuropila. En la CP se observaron neuronas piramidales presentando diversos grados de gemación de material nuclear positivo para RAGE y diversas células con diferentes grados de positividad nuclear y citoplasmática. En el hipotálamo se apreciaron gran número de células expresando gránulos positivos a RAGE tanto adyacente al núcleo como en el citoplasma; el núcleo permaneció negativo. Alto número de núcleos positivos se apreciaron en la capa CMC a diferencia de la capa CGC del cerebelo. Estos hallazgos sugieren el almacenamiento del RAGE en el núcleo y en el citoplasma en la rata normal e hipotetizan una posible translocación de esta molécula a la superficie celular en condiciones patológicas.

Received: 26-11-2023 Accepted: 06-08-2023

INTRODUCTION

The receptor for advanced glycation end products (RAGE) is a transmembrane protein and a multireceptor belonging to the immunoglobulin superfamily, expressed on the cell surface and capable of binding to various ligands, inducing cell activation, cell dysfunction, and tissue damage [1, 2]. RAGE has been implicated in various pathophysiological processes such as neurodegenerative diseases and infectious processes [1-3]. After RAGE binds to its ligand, pro-inflammatory processes increase with the induction of pro-inflammatory cytokines and oxidative stress, determining a vicious circle of inflammation mediated by the overexpression of the nuclear transcription factor kB (NF-kB) that leads to cell damage [1,3]. Traditionally, the interaction of RAGE with its ligands at the cell surface level inducing intracellular signals leading to pro-inflammatory processes has been reported [4,

[5]. The interaction of RAGE with its ligands in the central nervous system is of paramount importance in the induction of neurodegenerative diseases [3, 6-8]; however, there is little information about the location of RAGE in central nervous system cells in non-pathological conditions. The present study is focused on determining by immunohistochemical methods the location of RAGE in the rat central nervous system under healthy conditions.

MATERIAL AND METHODS

This study used healthy male Sprague-Dawley rats (weight 150 to 200 g) (N=10). All rats had unlimited access to tap water and food. All animals were euthanized, and samples from rat cerebrum (including the hypothalamus) and cerebellum were embedded in Tissue-Tek (Miles, Inc, Diagnostic Division, Kankakee, Illinois, United States), frozen in acetone and dry ice, and stored at -70 ºC until use. Cryostat sections (4 μm) from samples were treated with a rabbit anti-rat RAGE antibody (5μg/mL: ab3611; Abcam, Cambridge, United Kingdom) to analyze RAGE brain expression. Rabbit immunoglobulin G (IgG) in tissues was localized by a secondary rhodamine-conjugated goat anti-rabbit IgG at 5μg/mL (Sigma-Aldrich, St. Louis, Missouri, USA). Antibody against a nonrelevant antigen or normal rabbit serum was used as the negative control. Sections were mounted in a solution of p-phenylenediamine in phosphate-buffered saline–glycerol and viewed under an epifluorescent microscope (Axioskop, Zeiss, Wetzlar, Germany). Positive cells were expressed as the number of cells per 0.0625 μ^2 from 20 randomly selected fields of the brain or cerebellum. Experiments were performed according to the ethical guidelines of the committee of bioethical and biosecurity of FONACIT (Caracas, Venezuela) following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 8023, revised 1978) and the committee of bioethics of the Universidad del Zulia School of Medicine.

Statistical analysis was performed using GraphPad Prism, version 7.0 (GraphPad Software, San Diego, USA). Measurement data with normal distribution is represented as mean ± standard deviation. For continuous variables that were normally distributed, differences between groups were determined by ANOVA and the posttest of Bonferroni. A p-value < 0.05 was considered to be statistically significant.

RESULTS

The analysis of different rat cerebrum and cerebellum areas showed high reactivity to the anti-RAGE antibody in several areas. At the level of the cerebral cortex, positive cells were observed in the molecular/granular layer (M/GL) and the pyramidal layer (PL). Positive cells were seen in the cerebellum in the molecular layer (CML) and less frequently in the cerebellar granular layer (CGL). A high number of positive cells was observed in the hypothalamus (Fig. 1). Histological analysis showed numerous cells with high nuclear reactivity to the anti-RAGE antibody in M/GL (Fig. 2). Likewise, cells with negative or scarcely positive nuclei were found accompanied by cytoplasmic and adjacent extracellular positive reactivity to RAGE, simulating a comet.

Interestingly, extracellular RAGE-positive areas without cell presence were observed (Fig. 3). Pyramidal neurons with highly positive nuclei and positive glial cells were observed in PL (Figs. 4 and 5). RAGE-positive nuclei presenting structures resembling nuclear buds in pyramidal neurons were observed (Fig. 5). The cells of the hypothalamus showed a high frequency of cytoplasmic RAGE-positive granules but no nuclear positivity (Figs. 1 and 6).

Nuclear expression of RAGE in the cerebellum was observed mainly in the CML, with a low frequency of positive nuclei in CGL; however, some cells showed granular cytoplasmic positivity in this layer. Purkinje cells were found to be negative (Figs. 1 and 7).

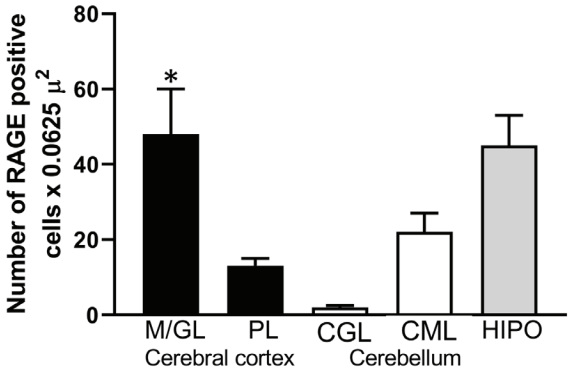

Fig. 1. Expression of the receptor for advanced glycation end products (RAGE) in the rat central nervous system. High nuclear expression of RAGE was observed in the molecular/granular layer of cerebral cortex and in the molecular layer of the cerebellum. Cells from the hypothalamus did not express nuclear RAGE but a high number expressed cytoplasmic RAGE in a granular form. M/GL: molecular/granular layer; PL: pyramidal layer; CGL: cerebellar granular layer; CML: cerebellar molecular layer; HIPO: hippocampus. * $p<0.01$ vs. PL, CGL, CML.

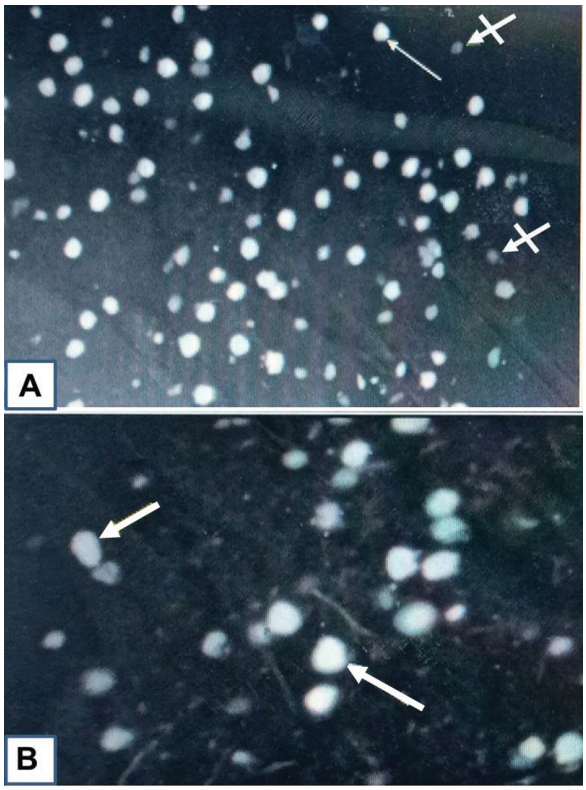

Fig. 2. Expression of the receptor for advanced glycation end products (RAGE) in the molecular/granular layer of the cerebral cortex. A) Panoramic view where a large number of positive nuclei can be observed. B) Detail. Arrows: positive nuclei. Cross arrows: negative nuclei. Original magnification: A: x600; B: x1000.

DISCUSSION

In this study, the expression of RAGE in the rat central nervous system was mainly limited to the cell nucleus and cytoplasm. Functionally, RAGE is expressed on the cell surface, where it interacts with various ligands to activate intracellular pathways that produce a pro-inflammatory and oxidative stress state [1-3, 6-8].

The presence of this receptor within the cell nucleus observed in this study suggests a nuclear function or represents a storage site for a subsequent trajectory of this receptor from the nucleus to the cell surface to exert its functions. Previous studies have shown the passage of intranuclear molecules to the cytoplasm [9-13], suggesting a possible cell surface expression pathway for RAGE. In this regard, the immunohistochemical findings of this report show cerebral cells showing decreased expression of nuclear RAGE accompanied by increased cytoplasm expression and adjacent extracellular RAGE-positive material. In addition, pyramidal neurons showed budding of RAGE-positive nuclear material, and cells with cytoplasm RAGE-positive granules were observed, suggesting a possible nuclear-to-cytoplasmic pathway.

The presence of RAGE in the nucleus suggests its nuclear localization prior to its synthesis, possibly as a storage site, as occurs with the non-histone chromosomal proteins "high mobility group" (HMG) that are present in the cell nucleus bound to DNA [10, 12] and perform functions such as determination of nucleosomal structure and stability, and binding of transcription factors to their cognate DNA sequences [14]. HMG can be localized in the nucleus, cytoplasm, and the extracellular space during some pathological processes where it can interact with

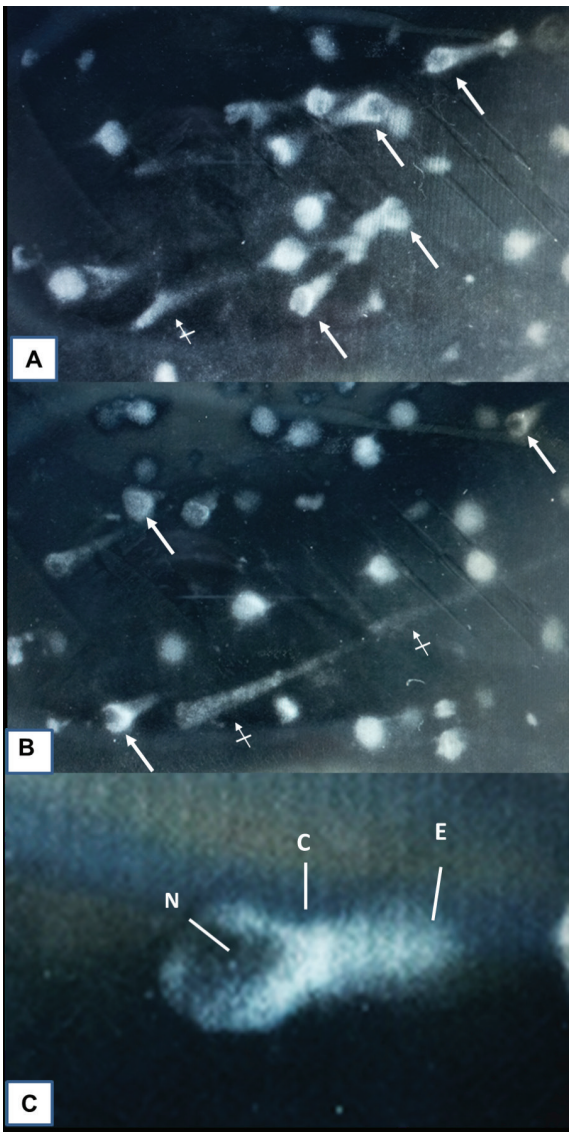

Fig. 3. Expression of the receptor for advanced glycation end products (RAGE) in the molecular/granular layer of the cerebral cortex. A and B) Cells with different degrees of nuclear and cytoplasmic positivity to RAGE (arrows). RAGE-reactive extracellular material is seen in the neuropile (crossed arrow). C) Detail of cell showing weak nuclear RAGE positivity and high positivity for cytoplasmic. Note the presence of RAGE-positive material adjacent to the cell. N: nucleus; C: cytoplasm; E: extracellular space. Original magnification: A and B: x600; C: x1000.

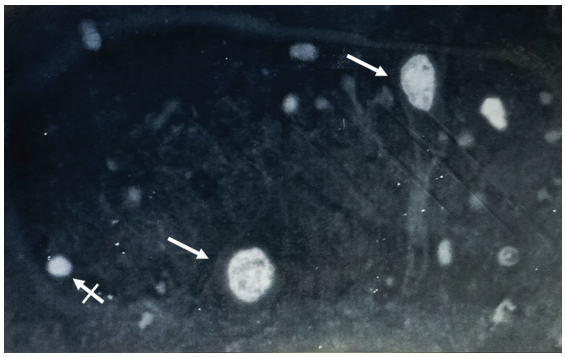

Fig. 4. Expression of the receptor for advanced glycation end products (RAGE) in the pyramidal layer of the cerebral cortex. Pyramidal neurons with high nuclear positivity for RAGE are appreciated. Arrows: Positive neurons. Cross arrow: probably a positive glial cell nucleus. Original magnification: x1000.

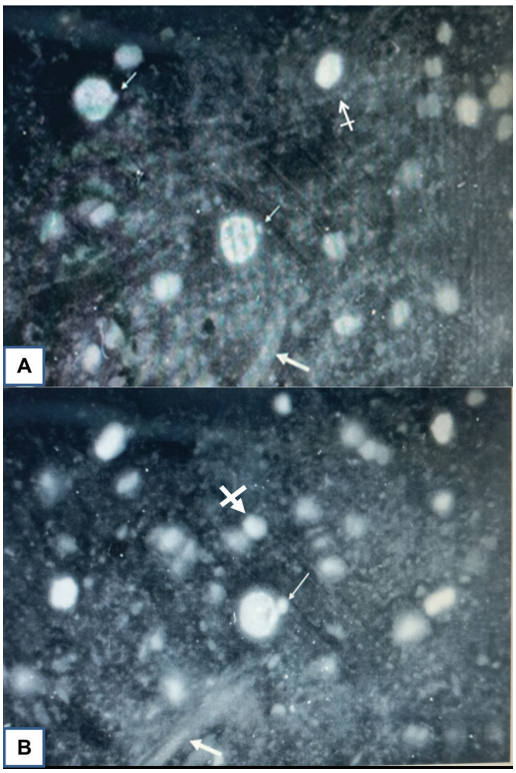

Fig. 5. Expression of the receptor for advanced glycation end products (RAGE) in the pyramidal layer of the cerebral cortex. A) Nuclear RAGE-positive pyramidal neurons showing nuclear budding (small arrows). Cross arrow: positive neuron without nuclear budding. Thick arrow: pyramidal neuron dendrite. B) Nuclear RAGE-positive pyramidal neuron showing nuclear budding (small arrow). Cross arrow: positive glial cell nucleus. Thick arrow: neuron axon. Original magnification: x1000.

RAGE [9, 11, 13]. There is no information available on the presence of RAGE in the nucleus, and possibly the expression of nuclear RAGE on the cell surface obeys mechanisms similar to those of HMG. Binding to DNA may represent the storage mechanism of RAGE in the nucleus. In this regard, RAGE's ability to bind to DNA [15] and its role in participating in DNA double-strand repair processes [16] has been reported. Another point of analysis is the intranuclear role of RAGE and HMG since the latter represents one of the RAGE's ligands [17].

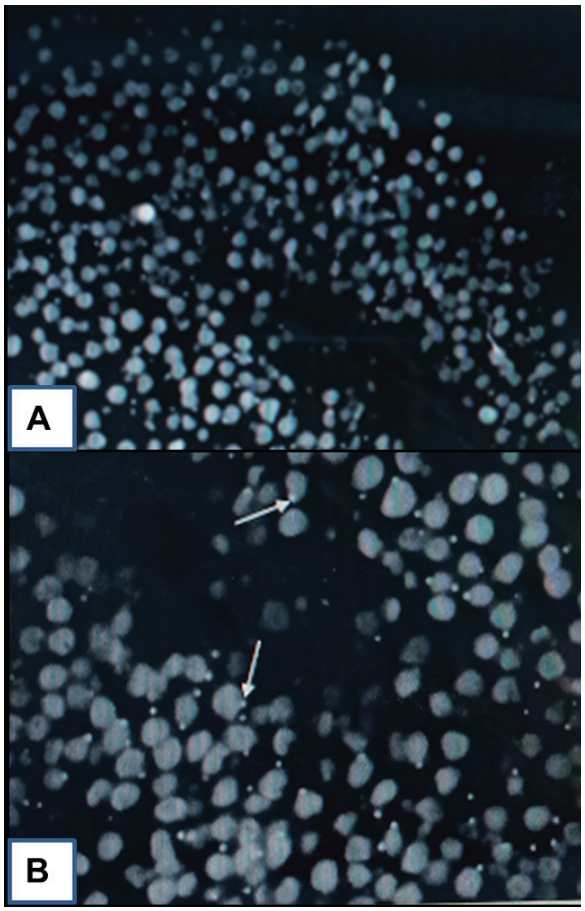

Fig. 6. Expression of the receptor for advanced glycation end products (RAGE) in hippocampus. A) Overview of hypothalamic cells, the majority of which are positive for cytoplasmic granular expression of RAGE. B) Hippocampal cells presenting positive RAGE granules adjacent to the nucleus or in the cytoplasm (arrows). Original magnification: A: x200; B:x600.

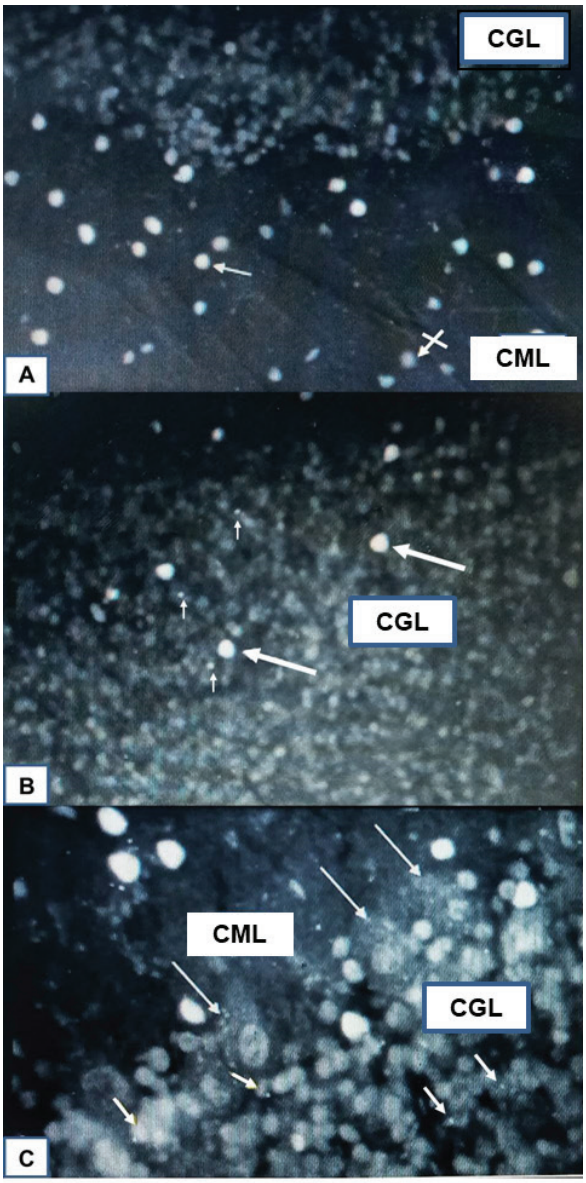

Fig. 7. Expression of the receptor for advanced glycation end products (RAGE) in the cerebellum of normal rats. A) High number of cells expressing RAGE-positive nuclei (arrow) in the molecular layer, compared to low number in the granular layer. Arrow: positive nucleus. Cross arrow: negative nucleus. B) Scarce presence of RAGE-positive nuclei (thick arrows) and RAGE-positive granules (small arrows) in cells of the granular layer. C) Scarce presence of RAGE-positive nuclei and RAGE-positive granules (small arrows) in the granular layer. Negativity was observed in Purkinje cells (arrows). Cerebellar molecular layer: CML. Cerebellar Granular layer: CGL. A and B: x200; C: x600.

The expression of RAGE in the central nervous system makes this tissue vulnerable to inflammatory processes. The role of RAGE in neurodegenerative processes, neuroinflammation, Parkinson's, and Alzheimer's diseases, among other encephalopathies, has been reported [1, 3, 18, 19]. Cerebral and cerebellar nuclear and cytoplasmic RAGE could play a role in these pathologies. Perhaps the factors that induce those pathologies induce the passage of nuclear and cytoplasmic RAGE to the cell surface.

In conclusion, this report demonstrates the presence of RAGE as a nuclear protein and its cytoplasmic expression in the cerebrum and cerebellum of normal rats. These data highlight possible studies on the translocation of RAGE from the nucleus to the cell surface, on nuclear functions, and on the interaction of RAGE with HMG in the nucleus.

Conflict of interest

Authors report no conflict of interest

Funding statement

This investigation has no financial support.

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Author Contributions

JMS: conceptualization, methodology, data curation, writing - original draft, writing - review & editing. AP: methodology, software, formal analysis, writing - review & editing. YC: resources, conceptualization, methodology, writing - review & editing. CP: methodology, software, formal analysis.

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