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Systemic Inflammation Changes the Site of RAGE Expression from Endothelial Cells to Neurons in Different Brain Areas

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Abstract

The receptor for advanced glycation endproducts (RAGE) is a transmembrane, immunoglobulin-like receptor that interacts with a broad repertoire of extracellular ligands. RAGE belongs to a family of cell adhesion molecules and is considered a key receptor in the inflammation axis and a potential contributor to the neurodegeneration. The present study aimed to investigate the content and cell localization of RAGE in the brain of Wistar rats subjected to systemic inflammation induced by a single dose of lipopolysaccharide (LPS, 5 mg/kg, i.p.). Fifteen days after LPS administration, the content of RAGE was analyzed in the prefrontal cortex (PFC), hippocampus (HIPP), cerebellum (CB), and *substantia nigra* (SN) were investigated. RAGE levels increased in all structures, except HIPP; however, immunohistochemistry analysis demonstrated that the cell site of RAGE expression changed from blood vessel-like structures to neuronal cells in all brain areas. Besides, the highest level of RAGE expression was found in SN. Immunofluorescence analysis in SN confirmed that RAGE expression was mainly co-localized in endothelial cells (RAGE/PECAM-1 co-staining) in untreated animals, while LPS-treated animals had RAGE expression predominantly in dopaminergic neurons (RAGE/TH co-staining). Decreased TH levels, as well as increased pro-inflammatory markers (TNF-α, IL-1β, Iba-1, GFAP, and phosphorylated ERK1/2) in SN, occurred concomitantly to RAGE stimulation in the same site. These results suggest a role for RAGE in the establishment of a neuroinflammation-neurodegeneration axis that develops as a long-term response to systemic inflammation by LPS.

Keywords RAGE · Endothelial cells · Dopaminergic cells · Neuroinflammation · Neurodegeneration · *Substantia nigra*

Introduction

The receptor for advanced glycation endproducts (RAGE) is a transmembrane immunoglobulin-like receptor belonging to a subfamily of the cell adhesion molecules (CAMs) superfamily [1]. RAGE can be constitutively or inducibly expressed in a wide variety of cells such as neurons microglia and brain endothelial cells [2–4]. In skin and lung cells, RAGE expression is constitutive and high levels of RAGE expression during development and adulthood are normally maintained during cell homeostasis [5]. In contrast, RAGE presents distinct characteristics in other cell types in adult life, such as neurons, where under physiological conditions it is expressed in a regulated manner and generally in response to specific stimulation [2].

RAGE is considered a class of pattern recognition receptors with the capacity to bind a wide number of ligands with different structures, sizes, and molecular organizations [6, 7]. The accumulation of RAGE ligands, such as amyloid β-peptide, DNA-binding protein high-mobility group box 1 (HMGB1)/amphoterin, S100/calgranulins [2], bacterial lipopolysaccharide (LPS) [8], or 70-kDa heat shock protein (HSP70) [9, 10], induces inflammatory signaling in a variety of cells and tissues [11].

Chronic inflammation is consistently present in neurodegenerative diseases including Alzheimer's and Parkinson's

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disease (PD) [12]. LPS, an endotoxin from gram-negative bacteria, has been used as an agent to induce a progressive neurodegenerative model in rodents. A single systemic LPS (5 mg/kg, i.p.) injection induces a physiopathology very similar to PD, with progressive and cumulative neuroinflammation in the SN culminating in the loss of dopaminergic neurons responses in the course of months following its administration [13].

Recent works have demonstrated an important role of RAGE in mediating inflammation-induced neurodegeneration. Inhibition of RAGE in hippocampus prevents sepsis-induced cognitive impairment [14] and antibody-mediated blocking of RAGE prior to systemic LPS injection inhibits neuroinflammation [15]. Considering the previously demonstrated capacity of LPS to generate neuroinflammation and neurodegeneration and the increasing role of RAGE as a mediator of such processes, we investigated the effect of systemic inflammation induced by LPS on RAGE expression and cellular localization in the brain. Fifteen days after LPS injection, RAGE was evaluated in the prefrontal cortex (PFC), hippocampus (HIPP), cerebellum (CB), and *substantia nigra* (SN) by immunohistochemistry and immunofluorescence approaches. Our results indicate that systemic inflammation induces significant changes in the homeostasis of RAGE in the brain, remarkably in the SN.

Methods

Ethics Statement

The research protocol was approved under project number 33493 by the Ethical Committee for Animal Experimentation of the Universidade Federal do Rio Grande do Sul, Brazil (CEUA-UFRGS). All experimental procedures were performed in accordance with the guidelines of the National Institutes of Health [16] and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. All efforts have been made to minimize animal suffering.

Animals

Male Wistar rats (60 days old) bred in our facilities were maintained at constant temperature of 21 ± 1 °C and 12-h light-dark cycle. They were caged in groups of four animals with free access (ad libitum) to water and standard commercial food (Chow Nuvilab CR-1 type; PR, Brazil). The rats were anesthetized with a single dose of ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.) for all surgical procedures.

Experimental Design

The rats were randomly divided into two groups:

Group 1: control group received saline solution (*v*/*v*) intraperitoneal (i.p.)
Group 2: received LPS 5 mg/kg (i.p.)

Fifteen days after LPS administration, six animals from each group were decapitated and PFC, HIPP, CB, and SN were dissected for western blotting (WB) analysis; six animals per group were intracardially perfused for IF assessment; six animals per group were intracardially perfused for IHC assessment.

Western Blotting

For immunoblotting experiments, tissues were prepared using a radioimmunoprecipitation assay buffer protocol [17]. Total proteins (30 μg/well) were fractionated by SDS-PAGE and electroblotted onto nitrocellulose membranes using a Bio-Rad trans-blot semi-dry electrophoretic transfer cell. Protein loading and electroblotting efficiency were verified through Ponceau S staining. After washing the membranes with TTBS (100 mM Tris-HCl, pH 7.5, containing 0.9% NaCl, and 0.1% Tween-20), they were incubated with primary antibodies (1:500 dilutions) for 20 min at room temperature in SNAP i.d. 2.0 Protein Detection System (MERCK). The membranes were washed again with TTBS. Polyclonal and monoclonal antibodies used were as follows: anti-p-ERK-44/42 (Thr202/Tyr204) (9101), anti-ERK-44/42 (9102), TH (2791), Myd88 (4283), synaptophysin (4329), anti-NFκB-p65 (8242), and anti-Phospho-NFκB-p65 (3033) from Cell Signaling Technology®; GFAP (G3893) from Sigma-Aldrich®; Iba-1 (NCNP24) from FUJIFILM Wako Pure Chemical; NeuN (MAB377) from MERCK; TNF-α (ab6671); and IL-1β (ab9787) from ABCAM. RAGE (PA1-84173) was from Thermo Fisher Scientific. The blots were incubated with anti-rabbit, anti-goat, or anti-mouse peroxidase-linked secondary antibody for an additional 20 min in SNAP (1:5000 dilution) and washed again. The immunoreactivity was detected from enhanced chemiluminescence using Supersignal West Pico Chemiluminescent kit from Thermo Fisher Scientific. The chemiluminescence was captured with an ImageQuant LAS 4000 (GE Healthcare). Densitometric analysis of the images was performed using ImageJ software (ImageJ v1.49, National Institute of Health, USA). Blots were developed to be linear in the range used for densitometry. All results were expressed as a relative ratio to β-actin (A1978) from MERCK or total protein content. Six animals per group were used to statistical analysis.

Animal Perfusion Fixation

Rats were perfused via the vascular system with descending aorta clamped. Sterile saline was administered for 10 min followed by perfusion with 4% paraformaldehyde (PFA)

solution in PBS, pH 7.4, for 10 more minutes. The brains were carefully recovered and maintained in 4% PFA for 24 h at 4 °C. They were then transferred into 15% sucrose solution for 24 h at 4 °C followed by immersion in 30% sucrose for additional 24 h at 4 °C. The brains were lightly dried and frozen at − 20 °C [18].

Immunohistochemical Assay

Serial coronal sections (30 μm) of PFC, HIPP, CB, and SN were obtained using a cryostat at − 20 °C (Jung Histoslide 2000R; Leica; Heidelberg, Germany). The free-floating sections were washed with PBS and then the endogenous peroxidases were blocked with 5% hydrogen peroxide in methanol. The sections were then preincubated in 2% bovine serum albumin (BSA) diluted in PBS-Triton X-100 0.3% for 1 h. RAGE antibody (AB37647) from ABCAM (diluted 1:500 in BSA 1% and PBS-Triton X-100 0.3%) was incubated for 48 h at 4 °C. Negative controls were performed omitting the primary antibody. After incubation, an HRP-labeled polymer conjugated (87-8983) from Thermo Fisher Scientific was added and incubated for 60 min at room temperature. Afterwards, the slices were washed and exposed to a solution of diaminobenzidine (0.06%) for 5 min and then washed with water. Next, sections were transferred to slides and then dehydrated with alcohol, cleared in xylene, mounted with Eukitt quick-hardening mounting medium (03989-MERCK), and covered with coverslips. The images were acquired using a Microscopy EVOS® FL Auto Imaging System (AMAFD1000 - Thermo Fisher Scientific). The brownish color was considered as a positive expression of RAGE. The mean was calculated using five slices from each region per animal. Six animals per group were used to statistical analysis.

Immunofluorescence Assay

Serial coronal sections (13 μm) of PFC, HIPP, CB, and SN were obtained using a cryostat at − 20 °C (Jung Histoslide 2000R; Leica; Heidelberg, Germany). A total of five slices per region of brain rats were collected in PBS containing 0.1% Triton X-100. The free-floating sections were incubated in 5% albumin for 2 h to block nonspecific binding. The sections were then incubated with antibodies for 48 h at 4 °C. The details of the antibody source and dilutions are as follows. Anti-RAGE (1:200; PA1-84173) was from Thermo Fisher Scientific, anti-TH (1:200; 2792S) was from Cell Signaling Technology®, anti-PECAM-1 (1:50; sc-1506) was from Santa Cruz Biotechnology, Inc., and Iba-1 (1:500; NCNP24) was from FUJIFILM Wako Pure Chemical. Antibodies were diluted in PBS containing 2% bovine serum albumin. Primary antibodies were excluded from the incubation of negative controls. After washing four times with 0.1% PBS, tissue sections were incubated with secondary antibodies, which included anti-goat 555, anti-rabbit 488, or anti-mouse 488 from Cell Signaling Technology®, all of them diluted 1:500 in PBS containing 2% BSA. After incubation in secondary antibodies for 1 h and DAPI for 5 min (D9542, MERCK) at room temperature (21 ± 3 °C), the sections were washed several times in 0.1% PBS, transferred to gelatinized slides, mounted with FluorSave™ (345789- MERCK), and covered with coverslips. The images were acquired using a Microscopy EVOS® FL Auto Imaging System (AMAFD1000 - Thermo Fisher Scientific). The mean was calculated using five slices from each region per animal. Six animals per group were used to statistical analysis.

Protein Assay

Total protein was quantified by Bradford assay and used to normalize all data [19].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 7 (GraphPad Software Inc., CA, USA). Data were evaluated by unpaired *t* test. The results are expressed as mean ± SD. Differences were considered significant when $p < 0.05$.

Results

The RAGE Content Is Increased by LPS in Different Brain Regions

Fifteen days after a single intraperitoneal injection of LPS (5 mg/kg), the brain of animals was dissected and the prefrontal cortex (PFC), hippocampus (HIPP), cerebellum (CB), and *substantia nigra* (SN) were analyzed for total content of RAGE by WB. LPS increased RAGE in PFC (Fig. 1a), CB (Fig.1c), and SN (Fig. 1d). HIPP had no significant changes (Fig. 1b).

LPS Induced Changes in RAGE Cellular Localization

The cellular localization of RAGE in the brain was investigated via an IHC analysis of PFC (Fig. 2a), HIPP (Fig. 2b), CB (Fig. 2c), and SN (Fig. 2d). In control animals, positive staining for RAGE was observed only in blood vessels, indicating localization of endothelial cells. On the other hand, in animals that received LPS injection, positive staining for RAGE was observed only in cells with neuronal morphology, but no staining of blood vessels was observed. Counting of endothelial cells with positive RAGE staining demonstrated that LPS significantly decreased the number of $RAGE^{+}$ endothelial cells/field in HIPP and SN (Fig. 2e, upper panel). However,

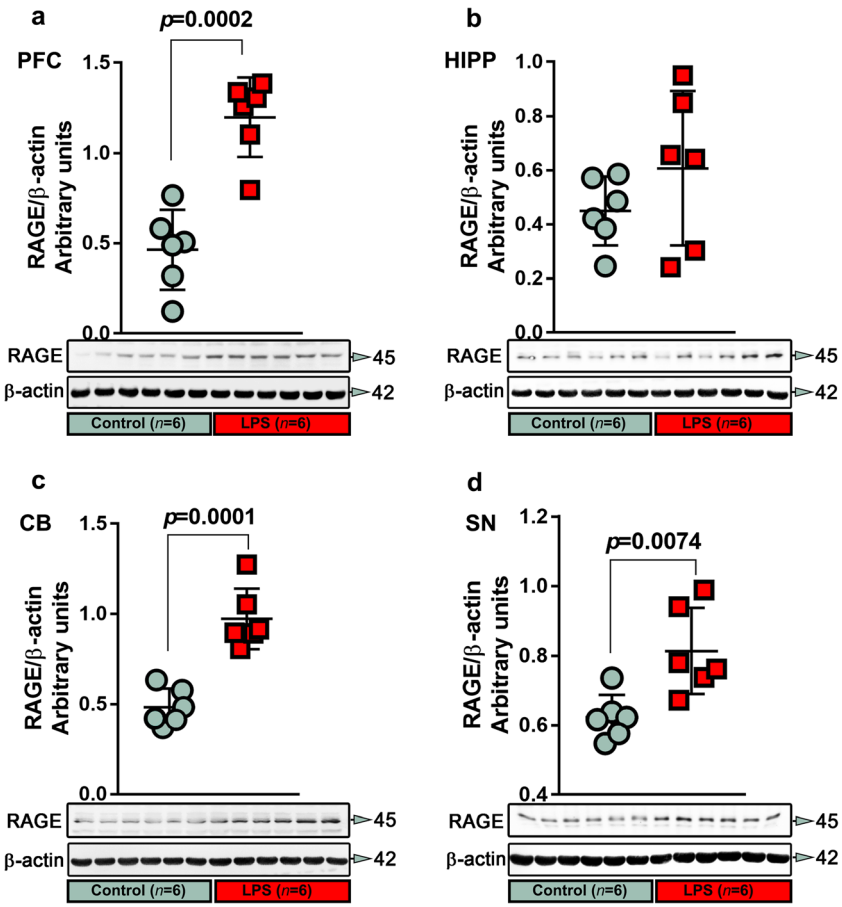

Fig. 1 RAGE content in different brain regions of Wistar rats 15 days after intraperitoneal injection of LPS (5 mg/kg). Graphs demonstrate WB quantification of RAGE in **a** PFC (prefrontal cortex), **b** HIPP (hippocampus), **c** CB (cerebellum), and **d** SN (*substantia nigra*). Values represent mean ± SD from 6 rats per group. The unpaired *t* test was applied to all data. *p* values are embedded in the figure. Western blot panels of RAGE and β-actin are shown below the graphs

counting of neuronal cells demonstrating positive staining for RAGE showed an increased number of $RAGE^+$ neurons/field in PFC, HIPP, and SN in the animals that received LPS (Fig. 2e, lower panel).

RAGE Localization Is More Expressive in SN

Interestingly, SN was the structure which presented the highest number of RAGE positive cells/field in both control ($RAGE^+$ endothelial cells) and LPS-treated animals ($RAGE^+$ neuronal cells). Further analyses to confirm RAGE cellular localization was performed using this structure. The IF analysis (Fig. 3) in control animals also demonstrated the presence of RAGE only in structures resembling blood vessels, consistent with IHC results. Higher magnification revealed a transient profile of staining that resembles vesicular pattern in some structures (pigmented characteristics in Fig. 3a, left side). Rats that received systemic LPS injection showed increased RAGE staining in cells with neuronal morphology, absent in control rats. Subcellular structures clearly resembling axons and neurites can be identified with positive RAGE staining (Fig. 3b). In addition to that, RAGE staining in blood vessel-like structures decreased significantly, although structures in which RAGE staining is diffused along blood vessels and neurons (Fig. 3b, right side) have been observed.

RAGE Is Co-localized with Endothelial Cell Marker PECAM-1 in Untreated Animals

To further confirm that RAGE cellular localization changes from endothelial to neuronal cells in rats subjected to systemic inflammation by LPS, double staining for RAGE and specific markers for either endothelial or neuronal cells were analyzed via IF. In control animals, RAGE staining was co-localized with the platelet and endothelial cell adhesion molecule-1 (PECAM-1), confirming the presence of this receptor in endothelial cells (Fig. 4a). RAGE staining has also been observed in cells negative for PECAM-1 (Fig. 4a, right side), and in some blood vessels, endothelial cells with staining for either RAGE or PECAM-1 have been observed. On the other hand, LPS-treated animals showed a decreased number of cells with double staining for RAGE and PECAM-1 (Fig. 4b). Although some cells in blood vessels presented RAGE and PECAM-1 double staining, most cells in blood vessels of LPS-treated animals presented single reactivity to PECAM-1 antibodies, and RAGE staining localized in different sites.

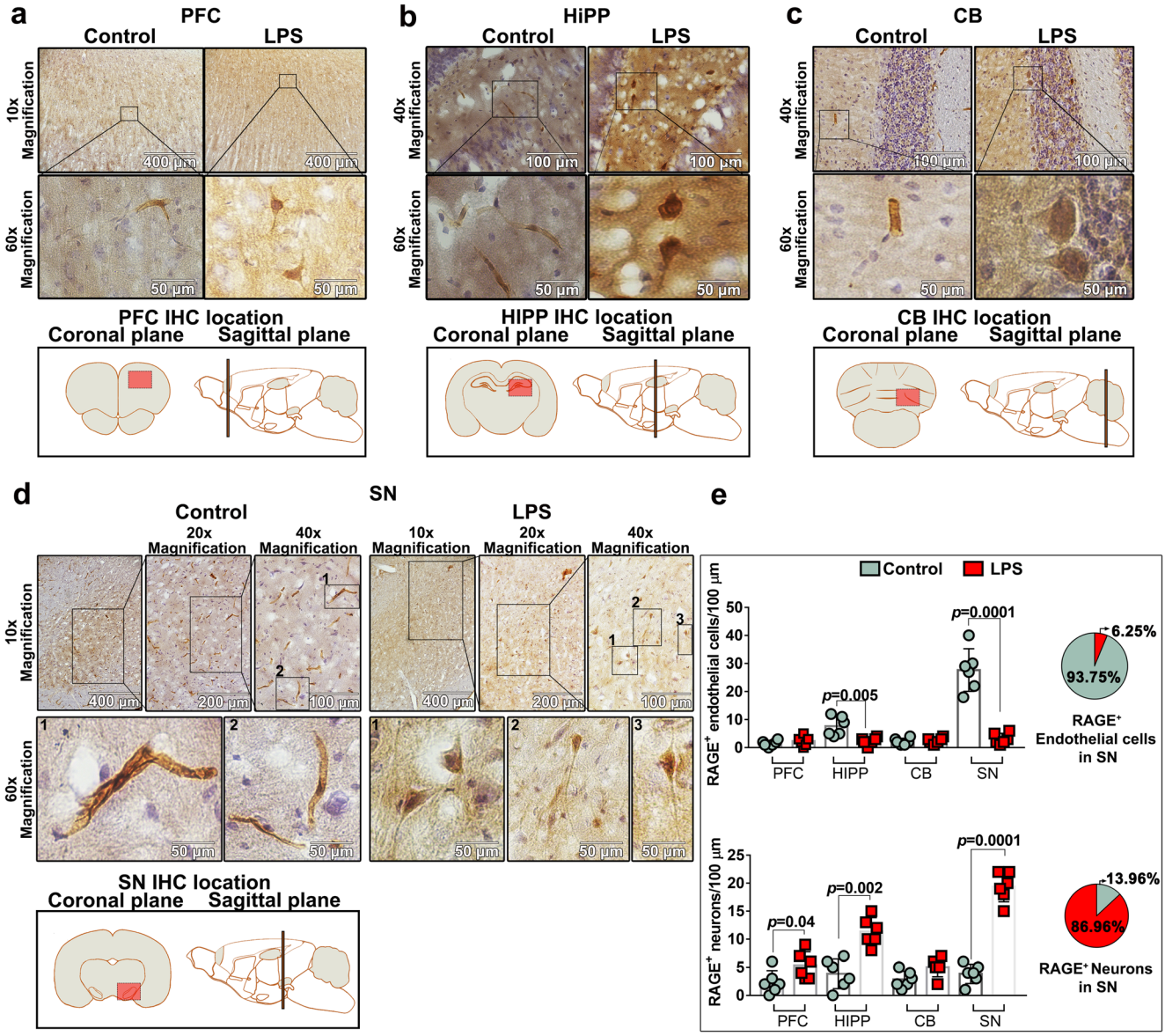

Fig. 2 Immunohistochemistry analysis of RAGE localization in different brain regions of Wistar rats 15 days after intraperitoneal injection of LPS (5 mg/kg). RAGE immunostaining was evaluated in **a** PFC (prefrontal cortex), **b** HIPP (hippocampus), **c** CB (cerebellum), and **d** SN (*substantia nigra*). Representative images from control and LPS groups ($n = 6$ per group) at increasing magnifications are shown. **e** Quantification of $RAGE^+$ endothelial cells (upper panel) and quantification of $RAGE^+$ neuronal cells (lower panel). Values represent mean ± SD. The unpaired *t* test was applied to all data. *p* values are embedded in the figure. The illustration figures were created in the Mind the Graph platform. The brain coordinates were from [20]

RAGE Is Co-localized with Dopaminergic Neurons in Animals Subjected to Systemic Inflammation by LPS

Next, the double staining for RAGE and the marker for dopaminergic neurons tyrosine hydroxylase (TH) were analyzed via IF. In untreated animals, RAGE staining and TH staining have been observed in distinct types of cells (Fig. 5a). The pattern of TH staining is consistent with the distribution of dopaminergic neurons in the SN pars compacta, whereas RAGE has been observed in structures resembling blood vessels. On the other hand, RAGE and TH were localized in the same cell type in animals treated with LPS, although RAGE single staining in some endothelial cells has also been observed (Fig. 5b, c). Visualization in higher magnification reveals a vesicular pattern of double staining for RAGE and TH in distinct areas of the same cells. (Fig. 5c, inserts).

Systemic Inflammation Induced by LPS Activates Neuroinflammation in the SN

A single systemic LPS administration induces systemic inflammation and affects the inflammatory state of different brain regions in a time-dependent manner. To explore the

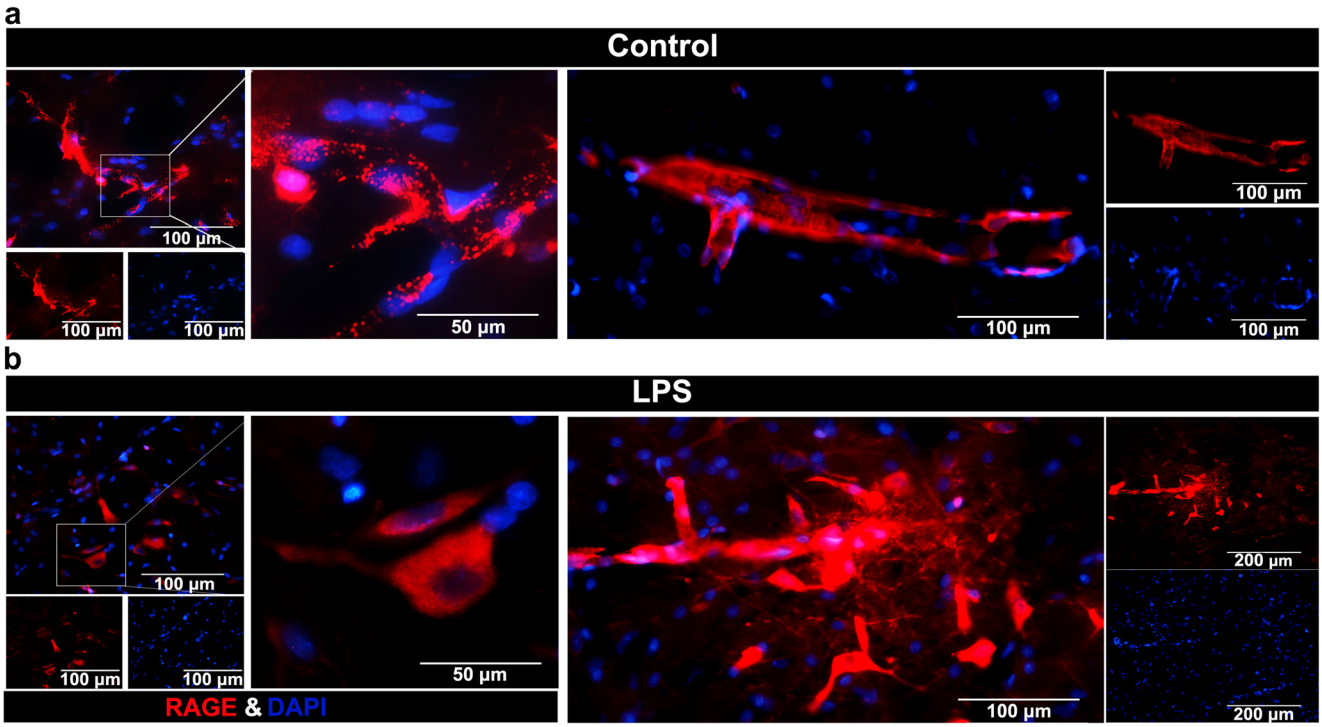

Fig. 3 Immunofluorescence analysis of RAGE in the SN (*substantia nigra*) of Wistar rats 15 days after intraperitoneal injection of LPS (5 mg/kg). RAGE immunostaining and DAPI staining to the nucleus are shown in **a** control group and **b** LPS group. Representative immunofluorescence images of SN immunostained for RAGE and DAPI from six different animals per group ($n = 6$) are demonstrated

mechanism by which systemic LPS induces this change in the cellular localization of RAGE in the brain, we analyzed the content of pro-inflammatory cytokines in SN. The levels of TNF-α and IL-1β increased in the SN of LPS-treated animals (Fig. 6a, b). Besides, the increased levels of GFAP and Iba-1 suggest that systemic inflammation is modulating the activation state of astrocytes (Fig. 6c) and microglia in the SN (Fig. 6d and Supplementary Fig. 1). The activation state of ERK1/2, a major MAPK involved in the modulation of RAGE-mediated inflammatory signaling, increased as well (Fig. 6e). On the other hand, MyD88, an adaptor protein that is involved in TLR4-mediated activation of pro-inflammatory transcription factors, has not changed in LPS-treated animals (Fig. 6f). Nuclear factor kappa B (NFκB) that regulates the expression of multiple inflammatory and immune genes was increased by LPS in total fraction (cytosol and nucleus) analyzed (Fig. 6g). Quantification via WB confirmed that systemic inflammation reduces TH levels in SN (Fig. 6h), but other neuronal markers (NeuN and synaptophysin) had no differences compared to those in control animals (Fig. 6i, j).

Discussion

RAGE has been considered a key receptor in the inflammation axis [21]. Our results demonstrate that RAGE content was increased by LPS in PFC, CB, and SN, but not in HIPP. However, although total levels of RAGE did not change in HIPP, the IHC results showed that RAGE staining also changed from blood vessels to neurons in this structure. The WB technique does not distinguish RAGE localization, thus reinforcing the importance of microscopy-based detection of proteins in the brain. Particularly when considering that RAGE expression is associated with the inflammatory state of a given cell type, the site of its expression becomes very relevant to understand the tissue physiological state.

The RAGE presence in brain endothelial cells has been previously associated to transport across the blood-brain barrier [22]. The cell adhesion role of RAGE enables cells to adhere to components of the extracellular matrix and also to other cells through homophilic interactions [1] as RAGE presents a high degree of structural homology to neural cell adhesion molecules (N-CAM) [23, 24]. Endothelial cells lining the intima of blood vessels are the first cells to be exposed to pathogens and xenobiotics in the bloodstream [25]. Vascular endothelium forms a vast network which dynamically regulates inflammation and maintains vascular integrity, and dysregulation of endothelial inflammatory pathways may promote neuronal failure [25].

Based on RAGE adhesion characteristics, the presence of this receptor in blood vessels was expected; however, the increased staining of RAGE in SN compared to that in other brain regions was surprising. In addition, the co-localization of RAGE with another adhesion molecule constituent of endothelial cells (PECAM-1) in SN may be an important

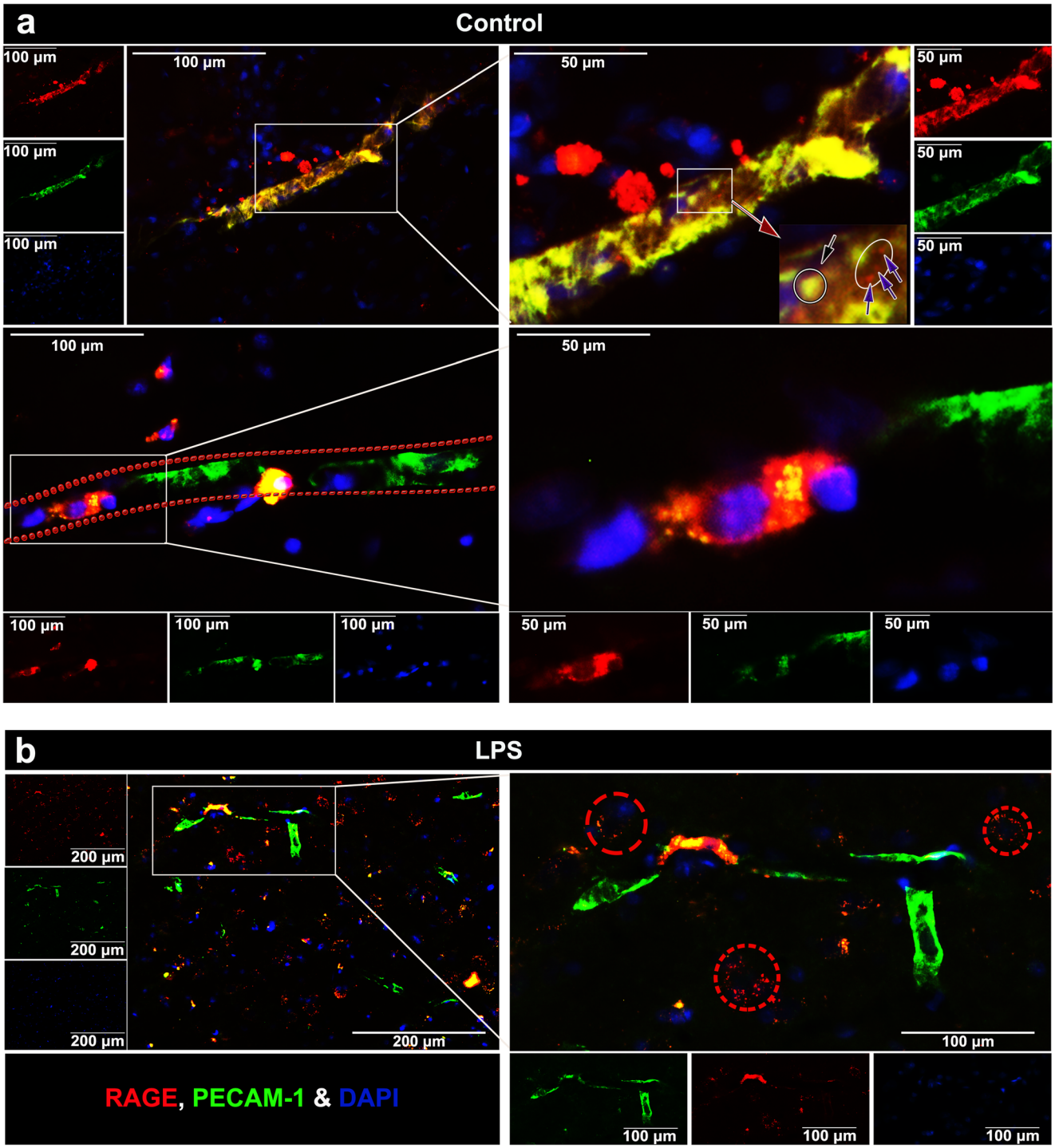

Fig. 4 Immunofluorescence analysis of RAGE and PECAM-1 in the SN (*substantia nigra*) of Wistar rats 15 days after intraperitoneal injection of LPS (5 mg/kg). The immunostaining to RAGE, PECAM-1, and DAPI was analyzed for co-localization in the SN of **a** control group and **b** LPS group. Merged images reveal localization of RAGE and PECAM-1 immunostaining in the same cells. Blue arrows indicate RAGE staining (red) and black arrows show the co-localization of RAGE and PECAM-1. Images are shown at increasing magnification. Dotted lines in control panel represent blood vessel limits. Dotted circles highlight RAGE staining in non-endothelial cells. Representative images from $n = 6$ per group)

indicator of a role in inflammatory response, since RAGE is a receptor for leukocyte integrins that contribute to the recruitment of inflammatory cells [26]. The high levels of RAGE in endothelial cells may promote adhesive interactions of circulating monocytes with the endothelial surface and upregulation of pro-inflammatory factors such as TNF-α and IL-1β and may eventually result in transendothelial migration [26, 27]. This, in turn, may induce a response in brain resident cells such as

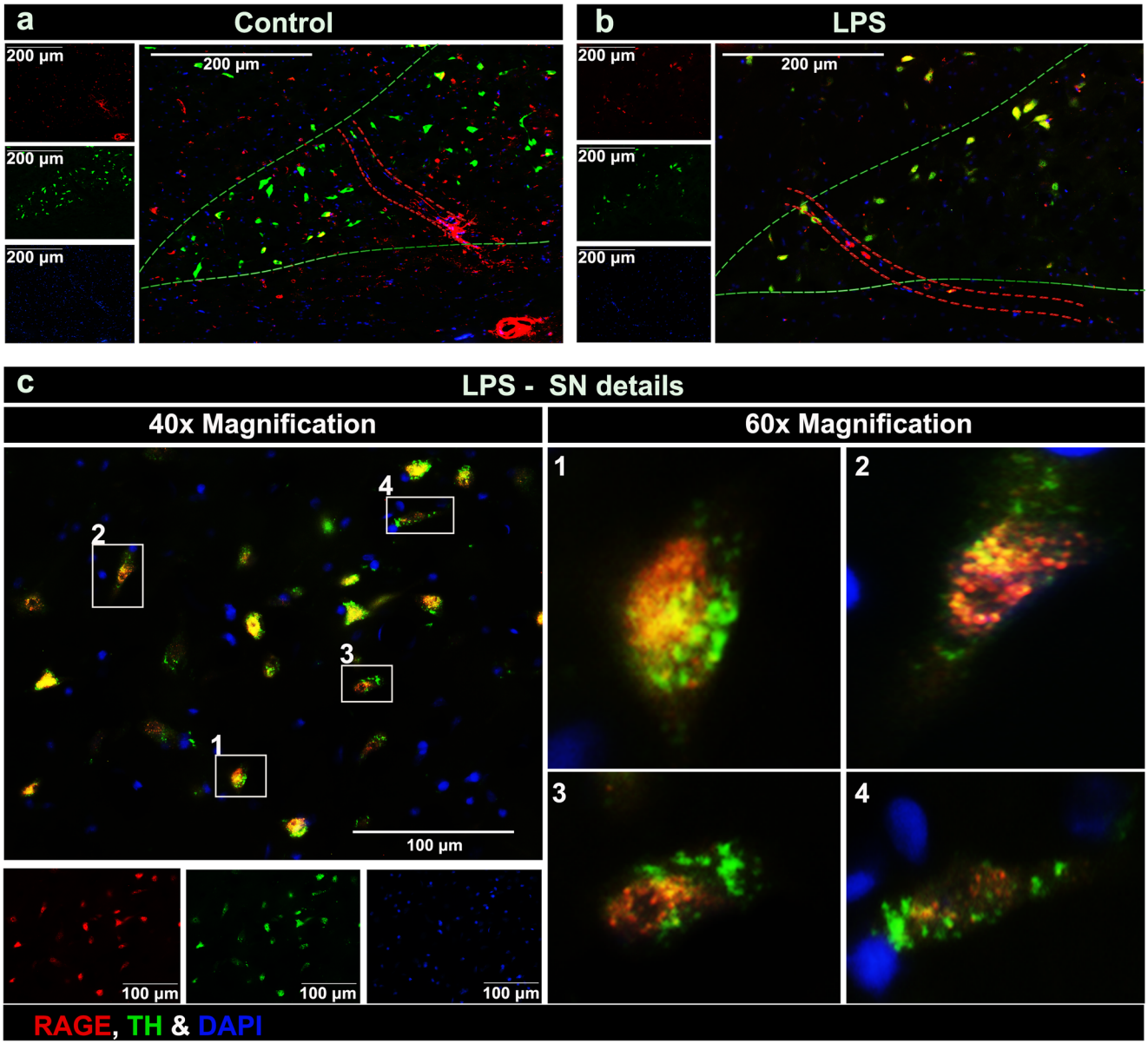

Fig. 5 Immunofluorescence analysis of RAGE and TH in the SN (*substantia nigra*) of Wistar rats 15 days after intraperitoneal injection of LPS (5 mg/kg). The immunostaining to RAGE, TH, and DAPI was analyzed in the SN of **a** control and **b** LPS groups. Dotted green lines represent the limits of SN region. Dotted red lines represent blood vessel limits, and **c** visualization of LPS group at higher magnification. Numbers represent each cell in detail. Representative images ($n = 6$ per group)

astrocytes and microglia. LPS administration results in rapid brain TNF-α and IL-1β accumulation (24 h) [15] and our results have demonstrated that these cytokines remain elevated for 15 days in SN. Generally, TNF-α and IL-1β present a rapid transitory increase induced by a stressor agent and decrease after the physiological anti-inflammatory response. These cytokines may remain increased in the SN via processes that may include glial activation.

Microglia are highly dynamic resident innate immune cells of the central nervous system; under both physiological and pathological conditions, they scan their environment and regulate tissue homeostasis [28]. When activated, microglia release pro-inflammatory cytokines, which contribute to sustain the neuroinflammation [29]. According to our results, microglia remained increased 15 days after LPS administration (Fig. 6d and supplementary figure 1), probably via RAGE signaling, but more experiments are necessary to confirm a prominent role for RAGE in sustaining microglia activation. Astrocytes, which exert important support function to neurons, also play an important role in neuroinflammation and response to stress. We observed that systemic inflammation induced by polymicrobial sepsis led to an increased activation state of microglia and astrocytes in the brain cortex and hippocampus of Wistar rats, which was reversed by hippocampal

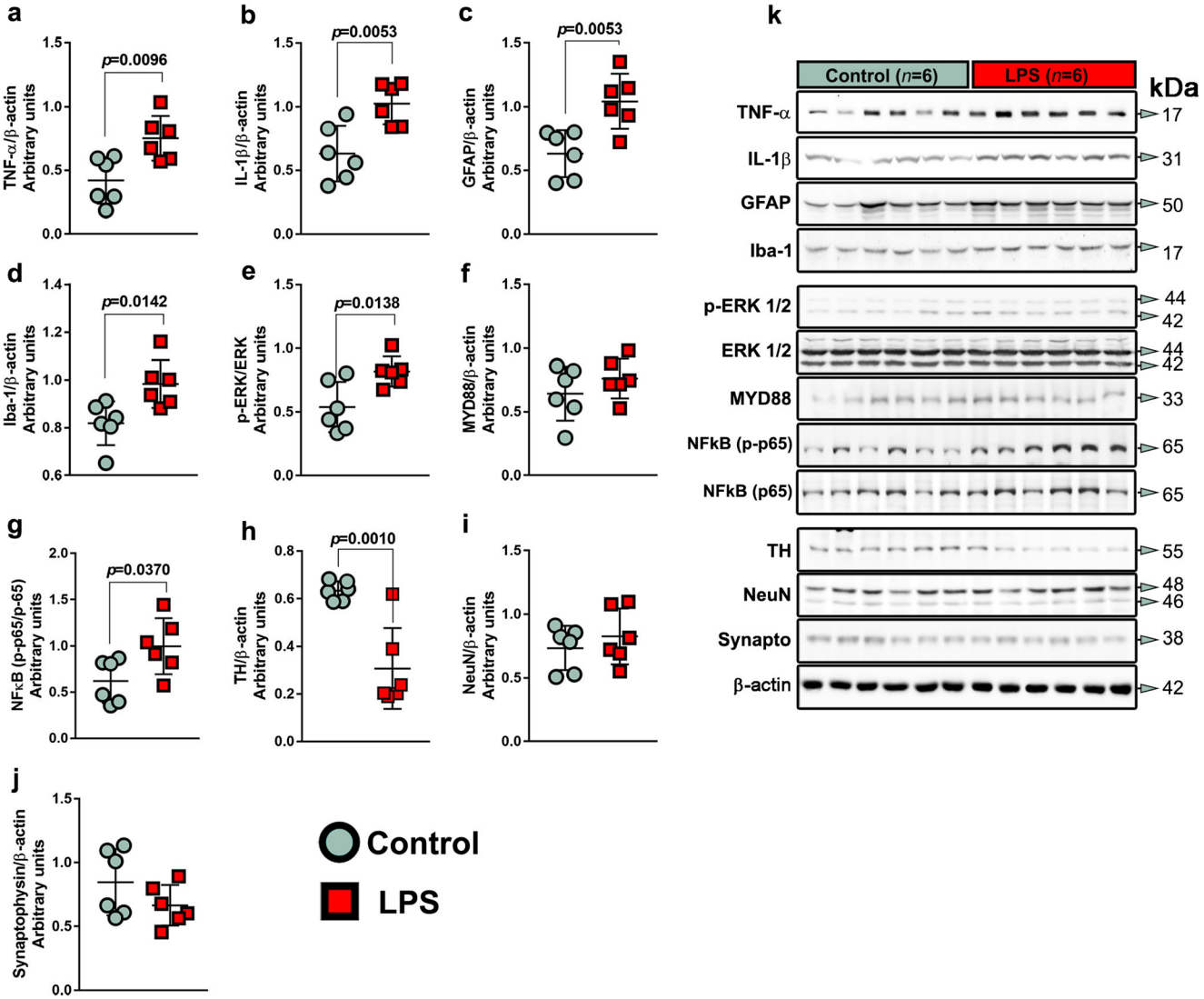

Fig. 6 Western blot analyses of inflammation and neuron markers. Fifteen days after intraperitoneal injection of LPS (5 mg/kg), SN (*substantia nigra*) was isolated for WB. **a** TNF-α, **b** IL-1β, **c** GFAP, **d** Iba-1, **e** p-ERK1/2, **f** MYD88, **g** NFκB (p65), **h** TH, **i** NeuN, and **j** synaptophysin were quantified. **k** Original WB panels. Data were normalized per β-actin content. Values represent mean ± SD from 6 rats per group. The unpaired *t* test was applied to all data. *p* values are embedded in the figure

immune-neutralization of RAGE [30]. This suggests an important role of RAGE in the pro-inflammatory activation of microglia and astrocytes in our model as well.

The multiligand nature of RAGE provides this receptor with the ability to engage a broad range of pro-inflammatory ligands [26] including damage-associated molecular pattern molecules (DAMPs). These molecules consist of multiple heat shock proteins, S100 molecules, and HMGB1, among others, which can prime the assembly and activation of the inflammasome [31]. Once these molecules bind to RAGE, they trigger a vicious circle of perpetuated RAGE self-stimulation [7], leading to the recruitment of multiple intracellular signaling molecules, including MAP kinases, which are regulatory signaling molecules in inflammation [32]. The increase in ERK1/2 phosphorylation (Fig. 6e) in SN is consistent with RAGE-dependent pro-inflammatory signaling since RAGE directly binds to ERK through a D-domain-like docking site [32]. The ERK1/2-mediated activation of NFκB-dependent pro-inflammatory gene transcription is considered the major axis of RAGE inflammatory signaling [33]. When RAGE expression is increased, a series of signal transduction cascades are initiated and lead to the activation of transcription factor NFκB as well as increased expression of cytokines, chemokines, and adhesion molecules, which in turn leads to the pathogenesis of various inflammatory-related diseases [2].

According to its adhesion capacity, RAGE released by blood vessels could easily bind to other receptors in a paracrine mechanism of signaling. The increased presence of RAGE in dopaminergic neurons may somehow be associated with increased susceptibility to blood-brain barrier (BBB) transient

disruption in this region. Our group previously showed that systemic injection of LPS (5 mg/kg, the same dose used in this work) destabilizes the BBB, increasing its permeability to high molecular weight constituents in blood and susceptibility to brain inflammation during the first 24 h [15]. The overactivation of this receptor during endotoxemia may trigger and contribute to sustaining chronic inflammation [2]. This, in turn, could contribute to the increased vulnerability of SN to systemic inflammation induced by a single intraperitoneal injection of LPS observed in previous works, according to which sustained neuroinflammation leads to progressive neurodegeneration in dopaminergic areas during the course of months after a single systemic LPS administration [13, 34]. Corroborating a possible role for RAGE in this process, previous data have demonstrated that RAGE inhibition prevents dopaminergic cell loss of SN in a hemiparkinsonism model [3].

Inflammation is implicated in the progressive nature of neurodegenerative diseases, such as PD, but the mechanisms are poorly understood [13]. As mentioned earlier, this LPS model usually induces systemic inflammation and neuroinflammation, followed by loss of dopaminergic neurons after 7 months [13]. Interestingly, our results have demonstrated a significant decrease in TH levels (Fig. 6h) in SN. RAGE can exert a direct additional stimulus resulting in a permanent pro-inflammatory response which leads to neuronal damage [7]. This early decrease in TH levels may be transient, not associated with neuronal loss, whereas the decrease observed in TH levels at 6 to 7 months after LPS injection is permanent and directly associated to neurodegeneration. In our model, we have also observed that TH levels did not differ from those in control when analyzed 30 and 60 days after LPS injection (unpublished data). Nonetheless, further investigation is necessary to understand both this process and the role of RAGE in such process.

Conclusion

Our results have demonstrated that systemic inflammation triggered by a single LPS administration induces a change in the cell site of RAGE expression (from endothelial cells to neurons) in different brain areas, markedly in the SN. This brain region may be more vulnerable to neuroinflammation induced by systemic LPS administration, this susceptibility may be associated with the high content of RAGE in the blood vessels of this region. Future investigations are required for understanding (i) why this change in the site of expression occurs and (ii) the role of RAGE in the development of long-term neuroinflammation and neurodegeneration in response to systemic LPS. These answers may help determine the role of RAGE in the pathogenesis of PD and the potential application of RAGE pharmacological inhibition for therapy development.

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Compliance with Ethical Standards

The research protocol was approved under project number 33493 by the Ethical Committee for Animal Experimentation of the Universidade Federal do Rio Grande do Sul, Brazil (CEUA-UFRGS). All experimental procedures were performed in accordance with the guidelines of the National Institutes of Health and the Brazilian Society for Neuroscience and Behavior recommendations for animal care.

Competing Interests The authors declare that they have no competing interests.

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