Neuroscience Letters 751 (2021) 135776

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Arundic acid (ONO-2526) inhibits stimulated-S100B secretion in inflammatory conditions

Adriana Fernanda K. Vizuete [a,*], Juliana de Lima Cordeiro [b], Juliana Dalibor Neves [b], Marina Seady [a], Lucas Kich Grun [b], Florencia María Barbé-Tuana [c], Marina Concli Leite [a], Carlos Alexandre Netto [a,b], Carlos-Alberto Gonçalves [a,b]

[a] Graduate Program in Biochemistry, Institute of Basic Health Sciences, UFRGS, Porto Alegre, Brazil
[b] Graduate Program in Neuroscience, Institute of Basic Health Sciences, UFRGS, Porto Alegre, Brazil
[c] Graduate Program in Cellular and Molecular Biology, School of Sciences, PUCRS, Porto Alegre, Brazil

ARTICLE INFO

Keywords:
Arundic acid
Astrocyte
Neuroinflammation
S100B
GFAP

ABSTRACT

Astrocytes respond to injury by modifying the expression profile of several proteins, including the S100 calcium-binding protein B (S100B), assumed to be a marker as well as a mediator of brain injury. AA is an inhibitor of S100B synthesis and plays a protective role in different models of brain injury, as decreases in S100B expression cause decreases in extracellular S100B. However, S100B mRNA expression, S100B protein content and S100B secretion do not always occur in association; as such, we herein investigated the effect of AA on S100B secretion, using different approaches with three stimulating conditions for S100B secretion, namely, low potassium medium, TNF-α (in hippocampal slices) and LPS exposure (in astrocyte cultures). Our data indicate that AA directly affects S100B secretion, indicating that the extracellular levels of this astroglial protein may be mediating the action of this compound. More importantly, AA had no effect on basal S100B secretion, but inhibited stimulated S100B secretion (stimulated either by the proinflammatory molecules, LPS or TNF-α, or by low potassium medium). Data from hippocampal slices that were directly exposed to AA, or from animals that received the acid by intracerebroventricular infusion, contribute to understanding its neuroprotective effect.

1. Introduction

Astrocytes interact with neurons, forming the synapse tripartite with pre- and post-neurons, supporting and regulating neuronal activity (see [1] for a review). These cells also play key role in the immune system and are active elements in neuroinflammatory signaling [2,3]. Astrogliosis and astrocyte reactivity are related to brain injury and the development of psychiatric disorders and neurologic diseases [4,5]. Glial fibrillary acidic protein (GFAP), a specific component of the intermediate cytoskeleton filaments of astrocytes [6], and S100 calcium-binding protein B (S100B) [7] are widely used as markers of astroglial activity and injury.

S100B is not exclusively astroglial, but astrocytes are mainly responsible for the synthesis and secretion of this protein in the brain [8]. The intracellular action of S100B regulates cell metabolism and stimulates differentiation, mitosis and plasticity of the cytoskeleton [9], while the extracellular effect of S100B is dependent on its concentration (see [10] for a review). At nanomolar levels, the protein acts as a neurotrophic factor, but higher levels of S100B lead to brain damage and stimulate tissue injury signaling pathways, inflammatory responses and apoptosis.

Arundic acid' ((2R) -2-propyloctanoic acid), also denominated ONO-2526, has been proposed as a protective drug in many brain disorders including hypoxia, chronic hypoperfusion, ischemic stroke and intracerebral hemorrhage [11–14]. The mechanism of action of this molecule was initially proposed to occur *via* the inhibition of S100B mRNA synthesis and, consequently, the reduction of its protein level [15]. However, other studies have suggested that this compound affects other targets in reactive astrocytes, besides S100B synthesis [13,16,17]. Therefore, the mechanism action of AA needs clarification. Despite

Abbreviations: AA, arundic acid; GFAP, glial fibrillary acidic protein; ICV, intracerebroventricular; LPS, lipopolysaccharide; S100B, S100 calcium-binding protein B; TNF-α, tumor necrosis factor.

* Corresponding author at: Department of Biochemistry, UFRGS, Ramiro Barcelos, 2600-Anexo, 90035-003, Porto Alegre, Brazil.
E-mail address: adrianavizuete@gmail.com (A.F.K. Vizuete).

https://doi.org/10.1016/j.neulet.2021.135776
Received 25 November 2020; Received in revised form 21 February 2021; Accepted 23 February 2021
Available online 13 March 2021

uncertainties, there is no doubt about the involvement of S100B in the mechanism of action of AA, especially considering that the inhibition of the protein may be accompanied by a reduction in its secretion. However, S100B mRNA expression, S100B protein content and S100B secretion do not always occur in association [7] and, therefore, it is necessary to determine the effect of AA on S100B secretion.

In order to study the specific effect of AA on basal and stimulated S100B secretion, we used three different experimental approaches. Firstly, we exposed acute hippocampal slices to AA, then astrocyte cultures and finally we used hippocampal slices of animals after intracerebroventricular (ICV) administration.

2. Materials and methods

2.1. Material

AA was purchased from Tocris (Bristol, United Kingdom). A complete list of material, as well as details of the methodology, are provided in Supplement 1.

2.2. Animals

Fifty male Wistar rats, at postnatal day 90, were obtained from our breeding colony (Department of Biochemistry, UFRGS) and maintained under controlled light and environmental conditions. Procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and Committee of Animal Use of UFRGS (number 34,321).

The animal study was divided into two parts. Firstly, we observed S100B secretion in acute hippocampal slices exposed to AA. Secondly, we analyzed hippocampalS100B at 24 h after ICV administration of AA or vehicle. See also graphical abstract.

2.3. Surgical procedure

Animals were divided into the following groups: Naive (anesthesia alone), sham (surgery + vehicle) and AA dissolved in saline solution (0.1, 1 or 10 μg/Kg in 5 μL). They were anesthetized with isoflurane and placed in a stereotaxic frame for ICV administration of AA [18]. Rats received a single unilateral infusion of 5 μL AA (0.1, 1, 10 and 100 μg/Kg) or sham (vehicle, 0.9 % NaCl). See methodological details in Supplement 1.

2.4. Preparation and incubation of hippocampal slices

Animals were euthanized by decapitation and the hippocampi were dissected and transverse slices of 0.3 mm were obtained using a McIlwain Tissue Chopper. Slices were incubated with AA at 10, 50 and 100 μM, or without AA, in normal saline (basal) or low potassium (0.2 mM) medium. Hippocampal slices from animals that received ICV AA were incubated at 24 h after administration in basal medium, low potassium medium or in saline medium containing TNF-α (10 ng/mL) [19,20]. Details are described in Supplement 1.

2.5. Astrocyte cultures

In order to observe the direct effect of AA on astrocytes and the S100B protein, we performed studies on astrocyte cultures under basal and inflammatory conditions induced by lipopolysaccharide (LPS), as previously described [21]. Details are described in Supplement 1. Cells were grown until confluence and used at 21 days. At this time, these primary astrocyte cultures were characterized as being cells that were positive for GFAP and negative for NeuN and Iba-1 [22]. For the S100B secretion assay, medium was replaced by DMEM without FBS, and in the presence of LPS (10 μg/mL) or not (basal). The effect of 100 μM AA was evaluated at 1 and 24 h.

2.6. Cell integrity and viability

Cell integrity and viability of cell cultures and brain slices were evaluated by measuring extracellular lactate dehydrogenase (LDH) activity and (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) reduction assay [23].

2.7. S100B and GFAP measurement

Contents of S100B and GFAP were measured by ELISA as previously described [24,25].

2.8. Determination of mRNA S100B expression by quantitative real time RT-PCR

The analysis of S100B gene expression was performed by quantitative real time reverse transcription polymerase chain reaction (RT-qPCR) using SYBR Green I (Molecular Probes) as the fluorescent detector in astrocyte culture. All reactions were carried out in a StepOnePlus® real-time PCR system. Relative ratios were calculated using the comparative cycle threshold (CT) method (ΔΔCT method) [26]. Detailed methodology, including primer sequences, is described in Supplement 1 and Table 1.

2.9. Statistical analysis

All results are expressed as means ± standard error mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. For observing the associations of mRNA with protein and secretion of S100B, data were analyzed by Pearson correlation coefficient. The level of statistical significance was set at $P < 0.05$. All analyses were performed using Prism 5.0 (GraphPad).

3. Results

3.1. AA abrogates low potassium-induced increases in S100B secretion

Initially, we exposed acute hippocampal slices from animals to AA (10, 50 or 100 μM) (Fig. 1). Only AA at 100 μM *per se* increased the S100B secretion (Fig. 1A, $P = 0.0023$; $F_{(3,20)} = 6.858$). Moreover, 10 and 50 μM AA prevented the increment in S100B secretion induced by the low potassium concentration in the incubation medium (Fig. 1B, $P = 0.0247$; $F_{(4,29)} = 3.278$). Cell viability and integrity were not changed by AA at these concentrations, in either media (Figs. 1C and D, $P = 0.8451$ and $P = 0.7675$; $F_{(4,25)} = 0.3447$ and $F_{(4,\ 24)} = 0.4555$, respectively).

3.2. AA prevents the effect of LPS on S100B secretion in astrocyte cultures

Firstly, we studied AA effect in astrocytes under basal conditions (See Supplement 2). We then investigated the effect of AA on LPS-modulated S100B secretion at 1 h and 24 h later. It is important notice that LPS induced a biphasic response, as it increases S100B secretion at 1 h and decreases S100B secretion at 24 h (Fig. 2A and 2B, respectively). Fig. 2A shows that LPS increased S100B secretion, while AA at 100 μM per se did

Table 1
Primer sequences for RT-qPCR.

Gene Id	Accession number	5′- 3′Sequence	Size
S100b	NM_013191	Forward: GAGGAAATCAAAGAGCAGGAA Reverse: TGGAGACGAAGGCCATAA	103 bp
Actb	NM_031144.3	Forward: GCAGGAGTACGATGAGTCCG Reverse: ACGCAGCTCAGTAACAGTCC	74 bp

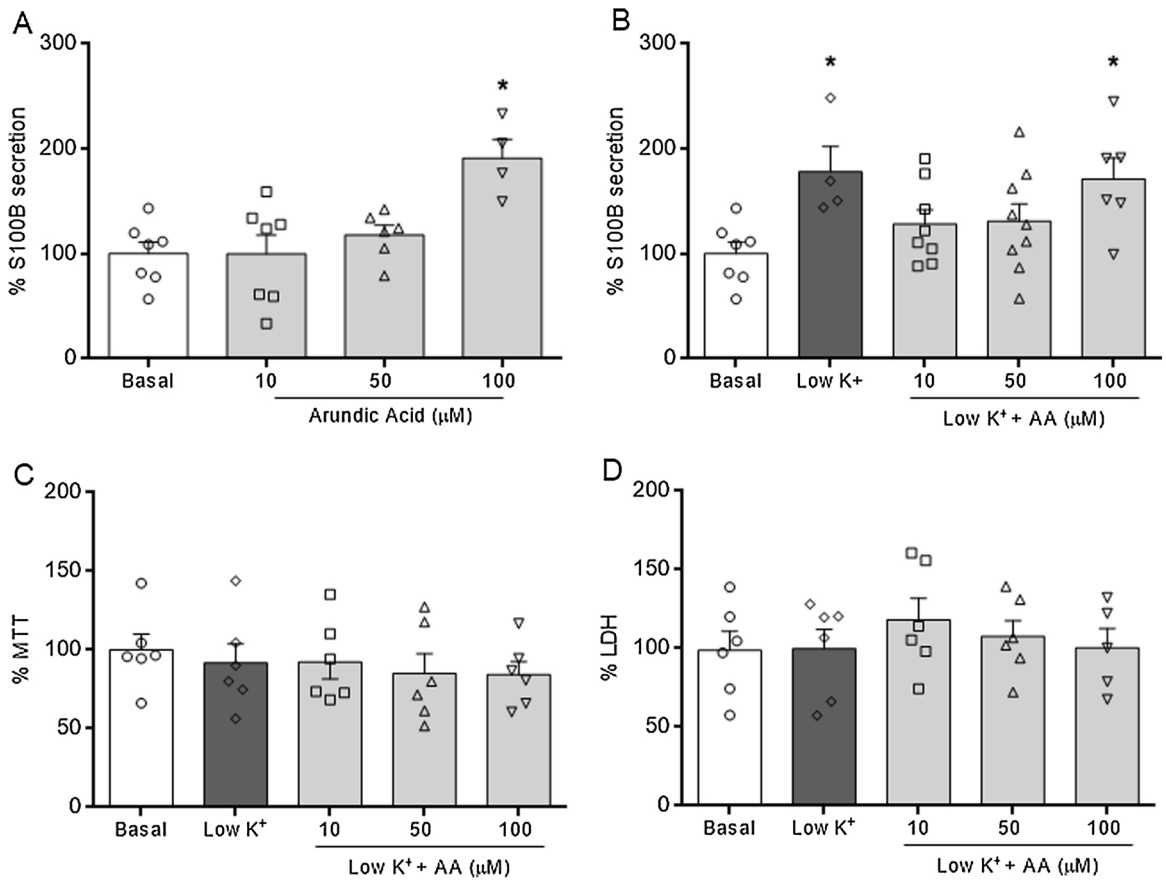

Fig. 1. Effect of AA on S100B secretion in acute hippocampal slices. In A, basal S100B secretion in the presence of AA (10-100 μM) was increased with AA only at the 100 μM concentration. *In B*, when hippocampal slices were maintained in a low-potassium medium (containing 0.2 mM KCl), AA (10 and 50 μM) reverted the augmentation of S100B secretion induced by low K^+. These treatments did not affect cell viability (measured by MTT reduction assay) (in panel C) or cell integrity (measured LDH activity) (in panel D). Values are expressed as means ± SEM of 6-8 animals per group; independent experiments were performed in triplicate, assuming the control value as 100 %. Data were analyzed by ANOVA, followed by the Tukey's test, assuming $p < 0.05$. * indicates difference from other groups.

not affect S100B secretion, but prevented LPS-stimulated S100B secretion (Fig. 2A, $P = 0.0110$; $F_{(3,9)} = 7.332$). Moreover, Fig. 2B demonstrates that AA also did not affect S100B secretion, but prevented the LPS-induced decrease in S100B secretion at 24 h ($P = 0.0065$; $F_{(3,9)} = 8.046$). The S100B cell content did not change at 24 h after LPS exposure and/or AA exposure (Fig. 2C, $P = 0.9553$; $F_{(3,12)} = 0.1055$), neither was GFAP content altered (data not shown). However, interestingly, LPS stimulated an increase in S100B mRNA at 24 h and AA prevented this change (Fig. 2D, $P = 0.0048$; $F_{(3,11)} = 7.681$).

We took the opportunity to evaluate the possible correlations between mRNA expression, protein content and S100B secretion, in astrocyte cultures. The gene expression of S100B did not correlate with intracellular S100B protein levels under any of the conditions tested (See Supplement 3).

3.3. ICV AA alters S100B secretion in acute hippocampal slices

We analyzed basal and stimulated S100B secretion (in low potassium medium or in the presence of TNF-α) in acute hippocampal slices from rats at 24 h after ICV administration of AA (Fig. 3). There was no difference in basal S100B secretion between the groups, including in the naïve animals (Fig. 3A, $P = 0.9285$; $F_{(4,32)} = 0.2145$), after different ICV doses of AA (0.1, 1 or 10 μg/kg). When acute hippocampal slices were incubated in low-potassium medium, S100B secretion augmented (Fig. 3B), but this effect was prevented in slices from animals that had previously received AA at a dose of 1 or 10 μg, but not at 0.1 μg ($P = 0.0470$; $F_{(3,11)} = 3.675$). A similar profile was observed when hippocampal slices were incubated in the presence of TNF-α (Fig. 3C). Previous exposure to AA (ICV, at 1 and 10 μg) prevented the augmentation of S100B secretion induced by incubation with this inflammatory cytokine (Fig. 3C, $P = 0.0051$; $F_{(3,17)} = 6.117$).

A low dose of ICV AA, which did not prevent low potassium or TNF-α- stimulated S100B secretion, increased hippocampal S100B content (Fig. 3D, $P = 0.0005$; $F_{(4,17)} = 8.664$). This effect also was observed for GFAP content (Fig. 3E, $P = 0.0191$; $F_{(4,24)} = 3.620$).

4. Discussion

AA is proposed as a neuroprotective compound in many models of brain injury [11–14]. In all these studies, it is assumed that the protective activity of AA involves the inhibition of S100B synthesis and that this in turn leads to a change in S100B extracellular activity. Recently, we evaluated the direct and protective effect of AA through ICV administration in a model of intracerebral hemorrhage [14,27], but S100B secretion was not directly evaluated. Herein, we investigated the direct effect of AA on S100B secretion, which is not always related to protein synthesis or expression [7].

The mechanism of S100B secretion remains unclear [28], but some secretagogues have been identified, such as LPS [21], pro-inflammatory cytokines such as IL-1β and TNF-α [29] and low potassium medium [19], which were used in this study. Our results show that AA did not alter basal S100B secretion in hippocampal slices or astrocyte cultures; however, AA (10 or 50 μM) prevented low potassium medium-induced S100B secretion in hippocampal slices. AA at 100 μM *per se* increased S100B secretion, making it difficult to identify any inhibition of low-potassium induced S100B secretion at this concentration. As

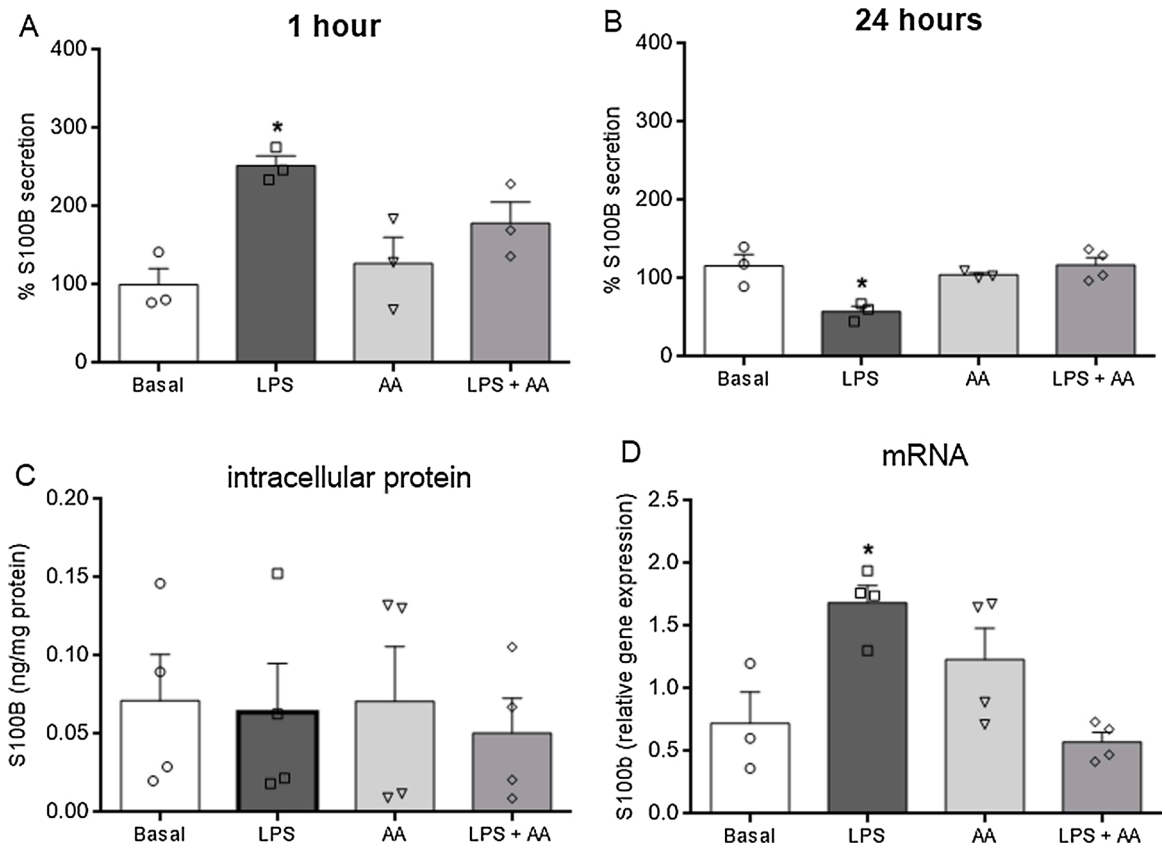

Fig. 2. Effect of AA on S100B secretion, S100B protein and S100B mRNA in astrocyte cultures exposed to LPS. Astrocyte cultures were incubated with LPS (10 μg/mL) or not (basal) for 1 h (Panel A) or 24 h (Panel B). AA at 100 μM was added in both conditions. Secretion assays were performed, assuming the control value as 100 %. Note that LPS increased S100B secretion at 1 h and decreased it at 24 h. Panel C depicts cellular S100B protein content, measured by ELISA at 24 h. Panel D shows S100B mRNA, measured by RT-qPCR at 24 h. Values are expressed as means ± SEM of 3-4 animals; independent experiments were performed in triplicate. Values of S100B protein are expressed in ng/mg protein. Values of gene expression of S100B are expressed as gene expressions relative to beta actin (S100B/beta actin). Data were analyzed by ANOVA, followed by the Tukey's test, assuming $P < 0.05$. * indicates difference from other groups.

observed for hippocampal slices, AA did not alter basal S100B secretion in astrocyte cultures (even at 100 μM). AA also did not affect basal secretion in astrocyte cultures. However, this molecule caused a reduction in the LPS-induced S100B secretion observed during the first hour of LPS exposure.

On the other hand, in astrocyte cultures, 24 h of LPS exposure reduces extracellular levels of S100B [23], and AA was also able to prevent this effect of LPS. Therefore, AA prevented the biphasic effect of LPS on S100B secretion in astrocytes, *i.e.* the increase at 1 h and the decrease at 24 h. In addition, rats that received ICV AA exhibited no alterations in basal S100B secretion in acute hippocampal slices at 24 h after administration. However, previous ICV administration of AA at 1 or 10 μg/Kg prevented the S100B augmentation induced by low potassium and TNF-α in hippocampal slices. Therefore, *in vivo* or *in vitro* administration of AA prevented S100B secretion in an inflammatory scenario, which could be induced by LPS (in isolated astrocytes) or by TNF-α (in hippocampal slices).

When rats received ICV AA at 0.1 μg/kg, they exhibited increased S100Blevels in hippocampal slices, as well as an increase in GFAP. This was not observed when 1 or 10 μg of AA was administered. No changes were observed in naïve and sham animals. The astrogliosis induced by the lowest dose of AA (based on the GFAP content increment) seems to be brain region-dependent, as preliminary results in the striatum show a contrasting decrease in GFAP (data not shown). At this point, we can only speculate on an *in vivo* double effect of AA on the hippocampal tissue, where at low doses it could cause astrogliosis, but where this effect is absent / blocked at high doses. However, this hypothesis should be further explored in further studies.

No changes in S100B content were observed in astrocyte cultures exposed only to AA. Although the S100B protein content was not altered by LPS at 24 h, S100B mRNA expression increased at this time and this augmentation was prevented by AA. As such, AA prevented S100B secretion and gene expression under inflammatory conditions. However, AA *per se* did not affect the gene expression in astrocyte cell cultures, in contrast to the pioneer study in astrocyte cultures that reported that S100B gene and protein expression were downregulated by AA [15].

5. Conclusions

This study indicates that AA directly affects S100B secretion, indicating that the extracellular levels of this astroglial protein may be mediating the action of the acid. More importantly, the effect of AA was not seen on basal S100B secretion, but on stimulated S100B secretion (in response to inflammatory stimuli with LPS or TNF-α or the presence of a medium with low potassium). Data were obtained using different experimental approaches; acute hippocampal slices and astrocyte cultures exposed to AA, as well as hippocampal slices from animals that received the compound by intracerebroventricular administration. These data contribute to understanding the protective effect of AA in brain tissue.

Note

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

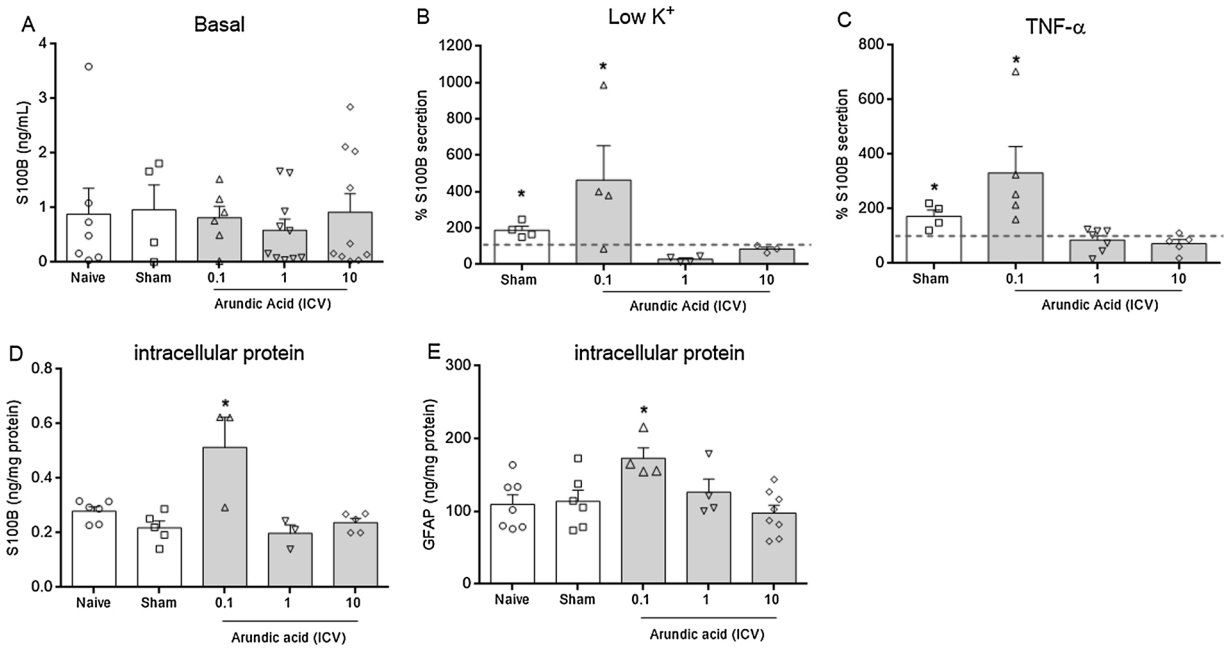

Fig. 3. Effect of intracerebroventricular AA administration on S100B secretion in acute hippocampal slices. In Panel A, basal secretion of S100B in hippocampal slices from rats that received ICV AA (0.1, 1 or 10 μg/Kg) or saline (sham) or were not submitted to stereotaxic injection (naïve). In Panel B, low potassium-induced S100B secretion in hippocampal slices from rats that received ICV AA (0.1, 1 or 10 μg/Kg) or saline (sham). In Panel C, TNF-α-induced S100B secretion in hippocampal slices from rats that received ICV AA (0.1, 1 or 10 μg/Kg) or saline (sham). Secretion assays in B and C panels were performed assuming the control value as 100 % in normal potassium medium and without TNF-α, respectively. S100B (Panel D) and GFAP (Panel E) hippocampal contents (expressed in ng/ mg of protein) from rats that received ICV AA (0.1, 1 or 10 μg/Kg) or saline (sham) or were not submitted to stereotaxic injection (naïve). Values are expressed as means ± SEM of 3-8 animals per group; independent experiments were performed in triplicate. Data were analyzed by ANOVA, followed by the Tukey's test, assuming $P < 0.05$. * indicates difference from other groups.

CRediT authorship contribution statement

Adriana Fernanda K. Vizuete: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Project administration, Visualization. **Juliana de Lima Cordeiro:** Methodology, Investigation. **Juliana Dalibor Neves:** Methodology, Investigation. **Marina Seady:** Methodology, Investigation. **Lucas Kich Grun:** Methodology, Investigation. **Florencia María Barbé-Tuana:** Methodology, Investigation. **Marina Concli Leite:** Investigation, Resources. **Carlos Alexandre Netto:** Resources, Funding acquisition. **Carlos-Alberto Gonçalves:** Resources, Writing - review & editing, Project administration, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflicts of interests.

Acknowledgments

The authors gratefully acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and National Institute of Science and Technology for Excitotoxicity and Neuroprotection (INCTEN/CNPq).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neulet.2021.135776.

References

[1] G. Perea, M. Navarrete, A. Araque, Tripartite synapses: astrocytes process and control synaptic information, Trends Neurosci. 32 (2009) 421–431, https://doi.org/10.1016/j.tins.2009.05.001.
[2] R.N. Saha, X. Liu, K. Pahan, Up-regulation of BDNF in astrocytes by TNF-α: a case for the neuroprotective role of cytokine, J. Neuroimmune Pharmacol. 1 (2006) 212–222, https://doi.org/10.1007/s11481-006-9020-8.
[3] E. Colombo, C. Farina, Astrocytes: key regulators of neuroinflammation, Trends Immunol. 37 (2016) 608–620, https://doi.org/10.1016/j.it.2016.06.006.
[4] Y. Koyama, Functional alterations of astrocytes in mental disorders: pharmacological significance as a drug target, Front. Cell. Neurosci. 9 (2015) 1–12, https://doi.org/10.3389/fncel.2015.00261.
[5] H. Phatnani, T. Maniatis, Astrocytes in neurodegenerative disease, Cold Spring Harb. Perspect. Biol. 15 (2015) 1–18.
[6] Z. Yang, K.K.W. Wang, Glial Fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker, Trends Neurosci. 38 (2015) 364–374, https://doi.org/10.1016/j.tins.2015.04.003.Glial.
[7] C.A. Gonçalves, M. Concli Leite, P. Nardin, Biological and methodological features of the measurement of S100B, a putative marker of brain injury, Clin. Biochem. 41 (2008) 755–763, https://doi.org/10.1016/j.clinbiochem.2008.04.003.
[8] S.S. Pinto, C. Gottfried, A. Mendez, D. Gonçalves, J. Karl, C.A. Gonçalves, S. Wofchuk, R. Rodnight, Immunocontent and secretion of S100B in astrocyte cultures from different brain regions in relation to morphology, FEBS Lett. 486 (2000) 203–207, https://doi.org/10.1016/S0014-5793(00)02301-2.
[9] E. Raponi, F. Agenes, C. Delphin, N. Assard, J. Baudier, C. Legraverend, J.-C. Deloulme, S100B expression defines a state in which GFAP- expressing cells lose their neural stem cell potential and acquire a more mature developmental stage, Glia. 55 (2015) 165–177, https://doi.org/10.1002/glia.20445.
[10] R. Donato, G. Sorci, F. Riuzzi, C. Arcuri, R. Bianchi, F. Brozzi, C. Tubaro, I. Giambanco, S100B's double life: intracellular regulator and extracellular signal, Biochim. Biophys. Acta - Mol. Cell Res. 1793 (2009) 1008–1022, https://doi.org/10.1016/j.bbamcr.2008.11.009.
[11] I. Fukushi, K. Takeda, S. Yokota, Y. Hasebe, Y. Sato, Effects of arundic acid, an astrocytic modulator, on the cerebral and respiratory functions in severe hypoxia, Respir. Physiol. Neurobiol. 226 (2016) 24–29, https://doi.org/10.1016/j.resp.2015.11.011.
[12] R. Ohtani, H. Tomimoto, H. Wakita, H. Kitaguchi, K. Nakaji, R. Takahashi, Expression of S100 protein and protective effect of arundic acid on the rat brain in chronic cerebral hypoperfusion, Brain Res. 5 (2007) 5–10, https://doi.org/10.1016/j.brainres.2006.11.084.
[13] H. Higashino, Æ.A. Niwa, Æ.T. Satou, Y. Ohta, Æ.S. Hashimoto, Æ.M. Tabuchi, S. B.Á. Shrsp, Immunohistochemical analysis of brain lesions using S100B and glial

fibrillary acidic protein antibodies in arundic acid- (ONO-2506) treated stroke-prone spontaneously hypertensive rats, J. Neural Transm. 116 (2009) 1209–1219, https://doi.org/10.1007/s00702-009-0278-x.
[14] J.L. Cordeiro, J.D. Neves, A.F. Vizuete, D. Aristimunha, T.A. Pedroso, E.F. Sanches, C.A. Gonçalves, C.A. Netto, Arundic acid (ONO-2506), an inhibitor of S100B protein synthesis, prevents neurological deficits and brain tissue damage following intracerebral hemorrhage in male wistar rats, Neuroscience. 440 (2020) 97–112, https://doi.org/10.1016/j.neuroscience.2020.05.030.
[15] T. Asano, T. Mori, T. Shimoda, R. Shinagawa, S. Satoh, N. Yada, S. Katsumata, S. Matsuda, Y. Kagamiishi, N. Tateishi, Arundic acid (ONO-2506) ameliorates delayed ischemic brain damage by preventing astrocytic overproduction of S100B, Curr. Drug Targets CNS Neurol. Disord. 4 (2005) 127–142, https://doi.org/10.2174/1568007053544084.
[16] N. Tateishi, T. Mori, Y. Kagamiishi, S. Satoh, N. Katsube, E. Morikawa, T. Morimoto, T. Matsui, T. Asano, Astrocytic activation and delayed infarct expansion after permanent focal ischemia in rats. Part II : suppression of astrocytic activation by a novel agent (r) - (−) -2-propyloctanoic acid (ONO-2506) leads to mitigation of delayed infarct expansion, J. Cereb. Blood Flow Metab. 22 (2002) 723–734.
[17] M. Hanada, R. Shinjo, M. Miyagi, T. Yasuda, K. Tsutsumi, Y. Sugiura, Arundic acid (ONO-2506) inhibits secondary injury and improves motor function in rats with spinal cord injury, J. Neurol. Sci. 337 (2014) 186–192, https://doi.org/10.1016/j.jns.2013.12.008.
[18] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, sixth edition, Elsevier Acad. Press, 2007, https://doi.org/10.1016/0143-4179(83)90049-5.
[19] P. Nardin, L. Tortorelli, A. Quincozes-Santos, L.M. V De Almeida, M.C. Leite, A. P. Thomazi, C. Gottfried, S.T. Wofchuk, R. Donato, C.A. Gonçalves, S100B secretion in acute brain slices: modulation by extracellular levels of Ca2+ and K+, Neurochem. Res. 34 (2009) 1603–1611, https://doi.org/10.1007/s11064-009-9949-0.
[20] D.F. de Souza, K. Wartchow, F. Hansen, P. Lunardi, M.C. Guerra, P. Nardin, C. A. Gonçalves, Interleukin-6-induced S100B secretion is inhibited by haloperidol and risperidone, Prog. Neuro-Psychopharmacology Biol. Psychiatry. 43 (2013) 14–22, https://doi.org/10.1016/j.pnpbp.2012.12.001.
[21] M.C. Guerra, L.S. Tortorelli, F. Galland, C. Da Ré, E. Negri, D.S. Engelke, L. Rodrigues, M.C. Leite, C. Gonçalves, Lipopolysaccharide modulates astrocytic S100B secretion : a study in cerebrospinal fluid and astrocyte cultures from rats, J. Neuroinflammation 8 (2011) 128, https://doi.org/10.1186/1742-2094-8-128.
[22] F. Galland, M. Seady, J. Taday, S.S. Smaili, C.A. Gonçalves, M.C. Leite, Astrocyte culture models: molecular and function characterization of primary culture, immortalized astrocytes and C6 glioma cells, Neurochem. Int. 131 (2019) 104538, https://doi.org/10.1016/j.neuint.2019.104538.
[23] M. Hansen, S. Nielsen, K. Berg, Re-examination and further development of a precise and rapid dye method for measuring cell growth / cell kill, J. Immunol. Methods 119 (1989) 203–210, https://doi.org/10.1016/0022-1759(89)90397-9.
[24] M.C. Leite, F. Galland, G. Brolese, M.C. Guerra, J.W. Bortolotto, R. Freitas, L.M. V. De Almeida, C. Gottfried, C.-A. Gonçalves, A simple, sensitive and widely applicable ELISA for S100B: methodological features of the measurement of this glial protein, J. Neurosci. Methods 169 (2008) 93–99, https://doi.org/10.1016/j.jneumeth.2007.11.021.
[25] F. Tramontina, M.C. Leite, K. Cereser, D.F. de Souza, A.C. Tramontina, P. Nardin, A.C. Andreazza, C. Gottfried, F. Kapczinski, C.A. Goncalves, Immunoassay for glial fibrillary acidic protein: antigen recognition is affected by its phosphorylation state, J. Neurosci. Methods 162 (2007) 282–286, https://doi.org/10.1016/j.jneumeth.2007.01.001.
[26] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[27] J.L. Cordeiro, J.D. Neves, F. Nicola, A.F. Vizuete, E.F. Sanches, C.A. Gonçalves, C. A. Netto, Arundic acid (ONO-2506) attenuates neuroinflammation and prevents motor impairment in rats with intracerebral hemorrhage, Cell. Mol. Neurobiol. (2020), https://doi.org/10.1007/s10571-020-00964-6.
[28] M. Leite, S100B secretion is mediated by Ca2+ from endoplasmic reticulum: a study using DMSO as a tool for intracellular Ca2+ mobilization, Glia 65 (2017) E103–E578, https://doi.org/10.1002/glia.23157.
[29] D.F. De Souza, M.C. Leite, A. Quincozes-santos, P. Nardin, L.S. Tortorelli, M. M. Rigo, C. Gottfried, R.B. Leal, C. Gonçalves, S100B secretion is stimulated by IL-1 β in glial cultures and hippocampal slices of rats : likely involvement of MAPK pathway, J. Neuroimmunol. 206 (2009) 52–57, https://doi.org/10.1016/j.jneuroim.2008.10.012.