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Astroglial S100B Secretion Is Mediated by Ca^{2+} Mobilization from Endoplasmic Reticulum: A Study Using Forskolin and DMSO as Secretagogues

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Abstract: S100B, a homodimeric Ca^{2+}-binding protein, is produced and secreted by astrocytes, and its extracellular levels have been used as a glial marker in brain damage and neurodegenerative and psychiatric diseases; however, its mechanism of secretion is elusive. We used primary astrocyte cultures and calcium measurements from real-time fluorescence microscopy to investigate the role of intracellular calcium in S100B secretion. In addition, the dimethyl sulfoxide (DMSO) effect on S100B was investigated in vitro and in vivo using Wistar rats. We found that DMSO, a widely used vehicle in biological assays, is a powerful S100B secretagogue, which caused a biphasic response of Ca^{2+} mobilization. Our data show that astroglial S100B secretion is triggered by the increase in intracellular Ca^{2+} and indicate that this increase is due to Ca^{2+} mobilization from the endoplasmic reticulum. Also, blocking plasma membrane Ca^{2+} channels involved in the Ca^{2+} replenishment of internal stores decreased S100B secretion. The DMSO-induced S100B secretion was confirmed in vivo and in ex vivo hippocampal slices. Our data support a nonclassic vesicular export of S100B modulated by Ca^{2+}, and the results might contribute to understanding the mechanism underlying the astroglial release of S100B.

Keywords: S100B secretion; calcium signaling; astrocytes

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1. Introduction

S100B protein is a glial marker widely used in the investigation of brain damage and neurodegenerative and psychiatric diseases in patients and experimental models [1–3] in which its extracellular levels are evaluated in cerebrospinal fluid (CSF), blood serum, or cell culture medium. Changes in CSF and serum S100B are related to the inflammatory response in multiple sclerosis [4], and extracellular levels of S100B appear to have prognostic value for outcomes in traumatic brain injury [5].

S100B is a small (10.5 kDa) protein belonging to the S100 family of Ca^{2+}-binding proteins [6], which comprises more than 20 members expressed in a cell-specific manner. Within cells, S100B exists as a homodimer in which the two subunits are held together by noncovalent bonds [7,8]. Astrocytes represent the major S100B-containing cell type in the gray matter of the central nervous system [1,9]. This protein has many putative intracellular

targets but is also secreted (less than 1%) and has autocrine and paracrine effects on glia, neurons, and microglia. The effect of extracellular S100B on target cells can be trophic or toxic, depending on its local concentration, redox environment, and activated signaling pathways [7,10].

Many S100B secretagogues have been identified, including forskolin, lysophosphatidic acid [7], fluoxetine [11], kainate [12], huperzine A [13], and carbenoxolone [14]. Moreover, it is known that metabolic stress conditions affect S100B secretion, such as elevated concentrations of glutamate [15], glucose, beta-hydroxy-butyrate, and ammonia [1]. However, the underlying secretion mechanism remains unknown and possibly involves a nonclassical vesicular export [1,7,16].

S100B secretagogues have been studied in astrocyte cultures without fetal serum, used to grow and maintain cultured cells (e.g., [17]). However, serum deprivation per se is able to stimulate S100B secretion [7,18]. Therefore, some putative secretagogues might act via a mechanism triggered by serum deprivation, a complex event where Ca^{2+} might be a key mediator. For example, serum deprivation in astrocyte cultures induces Ca^{2+} release from the endoplasmic reticulum, which, in turn, causes the release of fibroblast growth factor-1 [19]. In addition, serum deprivation induces an early transient increase in cAMP [18], which could be associated with intracellular Ca^{2+} changes. However, a clear connection between intracellular Ca^{2+} in astrocytes and S100B release is still lacking.

We found that Ca^{2+}-free medium or exposure to EGTA in acute hippocampal slices causes a significant increase in S100B secretion [20], in agreement with previous observations in brain slices [15], possibly due to the mobilization of internal stores of Ca^{2+}. In fact, treatment with EGTA in a serum-free medium has been reported to cause an increase in S100B secretion from the U87 glioblastoma cell line [16]. In addition, Ca^{2+} channel blockers reduced S100B secretion in acute hippocampal slices [20], again suggesting an involvement of intracellular Ca^{2+}.

During the course of our study which aimed to investigate the role of extra- and intracellular Ca^{2+} in S100B secretion in primary astrocytes, we serendipitously observed that dimethyl sulfoxide (DMSO), a widely used vehicle in biological assays, is a powerful S100B secretagogue, and it is able to mobilizes intracellular Ca^{2+}. Many other studies reported the biological activities of this compound (e.g., [21]). Herein, using different strategies to regulate intracellular Ca^{2+} (i.e., serum deprivation, forskolin, and DMSO), we characterized the mechanistic relation between S100B secretion and Ca^{2+} mobilization from the endoplasmic reticulum.

2. Results

2.1. Serum Deprivation, External Calcium, and Forskolin Stimulate S100B Secretion

Generally, S100B secretion has been studied in glial cultures under serum-free conditions, in which Ca^{2+} and cAMP are putatively involved messengers [7,22,23]. A time-dependent increase in S100B secretion from astrocytes was observed after serum deprivation for 15 min, 1 h, and 6 h (Figure 1A). Figure 1B shows that EGTA (1–3 mM) significantly increased S100B secretion after 1 h of serum deprivation. This secretion was negatively correlated with external free Ca^{2+}. In addition, ionophores (1 µM ionomycin or 1 µM A23187) were able to increase S100B secretion within 1 h; this increase was equivalent to or larger than that induced by forskolin, a well-known secretagogue for S100B (Figure 1C). Similar results were observed after 15 min for all these compounds (Figure S1). However, the secretion induced by ionophores, within 6 h, affected cell integrity (as evaluated by propidium iodide—Figure S2).