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Research Report

Serum albumin induces osmotic swelling of rat retinal glial cells

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ABSTRACT

Edema in the ischemic neural tissue develops by increased vascular permeability associated with extravasation of albumin, and by glial swelling. Here, we show that bovine serum albumin acutely administered to slices of the rat retina causes swelling of glial somata under hypoosmotic conditions. The effect of albumin was dose-dependent, with half-maximal and maximal effects at 10 nM and 1 μM, respectively, and was mediated by activation of transforming growth factor-β receptor type II, oxidative stress, and the production of arachidonic acid and prostaglandins. Albumin-induced glial swelling was prevented by glutamate and purinergic receptor agonists. The data suggest that serum albumin may induce glial swelling in the presence of osmotic gradients.

1. Introduction

Ischemia–reperfusion of the neural tissue is commonly accompanied by the development of edema which is a major pathogenic factor contributing to neuronal degeneration and, via compression of blood vessels, tissue hypoxia. Generally, edema is caused by disruption of the blood–brain barrier and/or fluid accumulation within cells resulting in cellular swelling (cytotoxic edema) (Kimelberg, 2004). In the brain, swelling of astrocytes usually occurs concomitantly in vasogenic edema, and represents a major mechanism of edema formation under ischemic and other conditions such as hyponatremia (Kimelberg, 2004). Cerebral ischemia–reperfusion is associated with a biphasic swelling of glial cells; early swelling occurs during the ischemic episode, and late swelling occurs concomitantly with vasogenic edema within hours or days after reperfusion (Rumpel et al., 1997). A similar time dependence of tissue swelling was described in the ischemic retina (Stefánsson et al., 1987). In addition to vasogenic edema, a swelling of glial cells is suggested to contribute to

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Abbreviations: AOPCP, adenosine-5′-O-(α,β-methylene)-diphosphate; ARL-67156, 6-N,N-diethyl-d-β,γ-dibromomethylene ATP; BAPTA-AM, bis-(o-aminophenoxy)ethane-N,N,N′,N′-tetra-acetic acid acetoxymethyl ester; BSA, bovine serum albumin; CSC, 8-(3-chlorostyryl) caffeine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; H-89, N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide; LY341495, (2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; MRS2179, N6-methyl-2′-deoxyadenosine-3′,5′-bisphosphate; NBTI, N-nitrobenzylthioinosine; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; SB431542, 4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide; TGF, transforming growth factor

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edema formation in the ischemic retina (Bringmann et al., 2004).

There is little knowledge regarding the relationship between vasogenic and cytotoxic edema in the neural tissue. An increase in vessel permeability is associated with extravasation of serum proteins such as albumin. It has been shown that local inflammation in the retina causes an increased vesicular transport of serum proteins through vascular endothelial cells, and that these proteins are accumulated in pericytes, perivascular microglia, and retinal glial (Müller) cells, suggesting that retinal glial cells act as a secondary barrier to extravasated serum proteins (Claudio et al., 1994). Serum albumin is a multifunctional protein with neurotrophic and neuroprotective properties (Emerson, 1989; Zoellner et al., 1996; Belayev et al., 2001). However, serum albumin may have also detrimental effects in the neural tissue such as induction of hyperexcitability and epileptiform activity (Ivens et al., 2007). It is not known whether albumin may influence glial swelling, a major component of edema in the neural tissue.

Extracellular fluid accumulation and cellular swelling indicate that cellular mechanisms of fluid transport and removal are disturbed. Normally, edema is resolved from the neural tissue by the dehydrating action of glial cells. Retinal glial cells maintain the water homeostasis of the neural retina (Bringmann et al., 2004). The transglial water transport is coupled to a transport of ions, in particular of potassium, and is facilitated by the co-localization of inwardly rectifying potassium (Kir) channels and water channels in perivascular membranes (Nagelhus et al., 1999). It has been shown that retinal glial cells downregulate perivascular Kir channels after transient ischemia of the retina, during ocular inflammation, and in diabetes (Pannicke et al., 2004, 2005, 2006); this downregulation should disrupt both the glial cell-mediated potassium clearance of the retina and the water transport through the cells (Bringmann et al., 2004). The downregulation of Kir channels in retinal glial cells is associated with an induction of cellular swelling under anisoosmotic conditions (Pannicke et al., 2004, 2005, 2006). Because extravasation of albumin is a characteristic of edema, we investigated whether serum albumin alters the osmotic swelling characteristics of retinal glial cells. We found that albumin evokes acute glial swelling under hypotonic conditions (a situation that resembles hypoxia-induced cytotoxic edema in the brain), and that the swelling was prevented by a glutamatergic–purinergic receptor signaling that results in opening of ion channels in glial membranes.

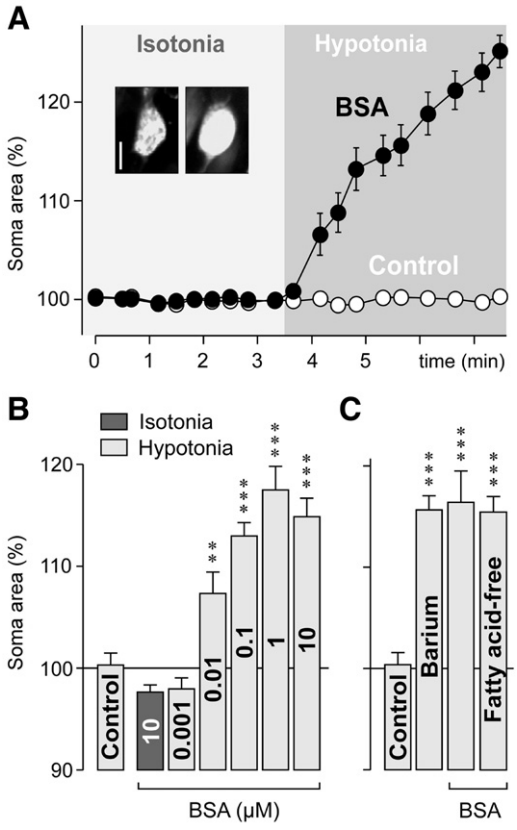

Fig. 1 – Bovine serum albumin (BSA) evokes osmotic swelling of glial somata in slices of the rat retina. (A) Superfusion of a retinal slice with a hypotonic solution (60% of control osmolarity) had no effect on the glial soma size under control conditions. However, the cells displayed time-dependent swelling of their somata when BSA (5 μM) was administered simultaneously with the hypotonic solution. The images show a dye-filled glial soma recorded before (left) and during (right) hypotonic exposure in the presence of BSA. The data were obtained in 9 and 10 cells, respectively. (B) BSA administered simultaneously with the hypotonic solution evoked a dose-dependent swelling of glial somata, whereas BSA application in isotonic solution had no effect. The concentration of BSA (in μM) is given in the bars. (C) Glial soma area which was recorded in the absence (control) and presence of the following agents: barium chloride (1 mM), normal BSA (5 μM), and fatty acid-free BSA (5 μM). Bar diagrams show the mean (±SEM) glial soma area (n=6–20) measured after a 4-minute superfusion of retinal slices with the hypotonic solution, and expressed in percent of the soma area before osmotic challenge (100%). Significant difference *vs.* control: **$P<0.01$; ***$P<0.001$. Bar, 5 μm.

2. Results

2.1. *Albumin induces osmotic glial cell swelling*

Acute swelling of the somata of retinal glial (Müller) cells was investigated by superfusion of freshly isolated retinal slices with a hypoosmolar solution (containing 60% of control osmolarity). As shown in Fig. 1A, superfusion of the slices with isoosmolar solution, or with hypoosmolar solution, for 4 min did not evoke a swelling of glial somata. Thus, the degree of swelling after 4 min was taken for comparison with other conditions. However, hypotonic challenge in the presence of bovine serum albumin (BSA) induced a rapid swelling of glial cell bodies (Fig. 1A). Albumin did not evoke glial swelling under isotonic conditions (Fig. 1B). The swelling-inducing effect of albumin under hypotonic conditions was dose-dependent (Fig. 1B). The half-maximal and maximal effects were at 10 nM and 1 μM, respectively. It has been shown that inflammatory lipids such as arachidonic acid induce a swelling of retinal glial cells under hypotonic conditions