PLOS ONE

RESEARCH ARTICLE

Glutathione-Induced Calcium Shifts in Chick Retinal Glial Cells

Hercules R. Freitas[1], Gabriel Ferraz[2], Gustavo C. Ferreira[1,3], Victor T. Ribeiro-Resende[1], Luciana B. Chiarini[4], José Luiz M. do Nascimento[5], Karen Renata H. Matos Oliveira[5], Tiago de Lima Pereira[5], Leonardo G. B. Ferreira[7], Regina C. Kubrusly[6], Robson X. Faria[8], Anderson Manoel Herculano[5☯], Ricardo A. de Melo Reis[1☯*]

1 Laboratory of Neurochemistry, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, **2** Institute of Biological Sciences, Center for Health Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, **3** Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, **4** Laboratory of Neurogenesis, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, **5** Institute of Biology, Federal University of Pará, Belém, PA, Brazil, **6** Laboratory Neuropharmacology, Dept Physiology and Pharmacology, Federal Fluminense University, Niterói, Brazil, **7** Laboratory of Inflammation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, **8** Laboratory of Toxoplasmosis, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil

☯ These authors contributed equally to this work.
* ramreis@biof.ufrj.br

Citation: Freitas HR, Ferraz G, Ferreira GC, Ribeiro-Resende VT, Chiarini LB, do Nascimento JLM, et al. (2016) Glutathione-Induced Calcium Shifts in Chick Retinal Glial Cells. PLoS ONE 11(4): e0153677. doi:10.1371/journal.pone.0153677

Editor: Henning Ulrich, University of São Paulo, BRAZIL

Received: August 29, 2015

Accepted: April 1, 2016

Published: April 14, 2016

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by grants from Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), (Instituto Nacional de Ciência e Tecnologia de Neurociência Translacional INCT-INNT), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to RAMR, VTRR, GCF and CAPES (PRO-AMAZONIA - 3288/2013) to AMH. HRF is recipient of a CAPES M.Sc. fellowship;

Abstract

Neuroglia interactions are essential for the nervous system and in the retina Müller cells interact with most of the neurons in a symbiotic manner. Glutathione (GSH) is a low-molecular weight compound that undertakes major antioxidant roles in neurons and glia, however, whether this compound could act as a signaling molecule in neurons and/or glia is currently unknown. Here we used embryonic avian retina to obtain mixed retinal cells or purified Müller glia cells in culture to evaluate calcium shifts induced by GSH. A dose response curve (0.1–10mM) showed that 5–10mM GSH, induced calcium shifts exclusively in glial cells (later labeled and identified as 2M6 positive cells), while neurons responded to 50mM KCl (labeled as β_{III} tubulin positive cells). BBG 100nM, a P2X7 blocker, inhibited the effects of GSH on Müller glia. However, addition of DNQX 70μM and MK-801 20μM, non-NMDA and NMDA blockers, had no effect on GSH calcium induced shift. Oxidized glutathione (GSSG) at 5mM failed to induce calcium mobilization in glia cells, indicating that the antioxidant and/or structural features of GSH are essential to promote elevations in cytoplasmic calcium levels. Indeed, a short GSH pulse (60s) protects Müller glia from oxidative damage after 30 min of incubation with 0.1% H_2O_2. Finally, GSH induced GABA release from chick embryonic retina, mixed neuron-glia or from Müller cell cultures, which were inhibited by BBG or in the absence of sodium. GSH also induced propidium iodide uptake in Müller cells in culture in a P2X7 receptor dependent manner. Our data suggest that GSH, in addition to antioxidant effects, could act signaling calcium shifts at the millimolar range particularly in Müller glia, and could regulate the release of GABA, with additional protective effects on retinal neuron-glial circuit.