Neuroscience 337 (2016) 285–294

CAFFEINE ALTERS GLUTAMATE–ASPARTATE TRANSPORTER FUNCTION AND EXPRESSION IN RAT RETINA

ADRIANA PINTO DE FREITAS, [a]
DANIELLE DIAS PINTO FERREIRA, [a]
ARLETE FERNANDES, [a] **ROBERTTA SILVA MARTINS,** [a]
VLADIMIR PEDRO PERALVA BORGES-MARTINS, [a]
MATHEUS FIGUEIREDO SATHLER, [a]
MAURÍCIO DOS-SANTOS-PEREIRA, [b]
ROBERTO PAES-DE-CARVALHO, [c]
ELIZABETH GIESTAL-DE-ARAUJO, [d]
RICARDO AUGUSTO DE MELO REIS [e] **AND**
REGINA CELIA CUSSA KUBRUSLY [a]*

[a] *Laboratório de Neurofarmacologia, Departamento de Fisiologia e Farmacologia, Instituto Biomédico, Universidade Federal Fluminense, Niterói, Brazil*

[b] *Laboratorio de Neurofisiologia Molecular, Departamento de Fisiologia, Faculdade de Medicina de Ribeirao Preto, Universidade de São Paulo, Ribeirão Preto, Brazil*

[c] *Laboratório de Neurobiologia Celular, Departamento de Neurobiologia, Instituto de Biologia, Universidade Federal Fluminense, Niterói, Brazil*

[d] *Laboratório de cultura de tecidos Hertha Meyer, Departamento de Neurobiologia, Instituto de Biologia, Universidade Federal Fluminense, Niterói, Brazil*

[e] *Laboratório de Neuroquímica, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil*

Abstract—L-Glutamate and L-aspartate are the main excitatory amino acids (EAAs) in the Central Nervous System (CNS) and their uptake regulation is critical for the maintenance of the excitatory balance. Excitatory amino acid transporters (EAATs) are widely distributed among central neurons and glial cells. GLAST and GLT1 are expressed in glial cells, whereas excitatory amino acid transporter 3/excitatory amino acid carrier 1 (EAAT3/EAAC1) is neuronal. Different signaling pathways regulate glutamate uptake by modifying the activity and expression of EAATs. In the present work we show that immature postnatal day 3 (PN3) rat retinas challenged by L-glutamate release [^{3}H]-D-Aspartate linked to the reverse transport, with participation of NMDA, but not of non-NMDA receptors. The amount of [^{3}H]-D-Aspartate released by L-glutamate is reduced during retinal development. Moreover, immature retinae at PN3 and PN7, but not PN14, exposed to a single dose of 200 or 500 μM caffeine or the selective A2A receptor (A2AR) antagonist 100 nM ZM241385 decreased [^{3}H]-D-Aspartate uptake. Caffeine also selectively increased total expression of EAAT3 at PN7 and its expression in membrane fractions. However, both EAAT1 and EAAT2 were reduced after caffeine treatment in P2 fraction. Addition of 100 nM DPCPX, an A1 receptor (A1R) antagonist, had no effect on the [^{3}H]-D-Aspartate uptake. [^{3}H]-D-Aspartate release was dependent on both extracellular sodium and DL-TBOA, but not calcium, implying a transporter-mediated mechanism. Our results suggest that in the developing rat retina caffeine modulates [^{3}H]-D-Aspartate uptake by blocking adenosine A2AR.

Key words: adenosine, A2A receptor, glutamate transport, NMDA receptors, retina.

INTRODUCTION

The amino acid L-glutamate is considered a major mediator of excitatory signaling in the central nervous system (CNS), including the retina from different species (Martins and Pearson, 2008; de Souza et al., 2012). Glutamatergic activity is mediated by a variety of ionotropic and metabotropic receptors in the CNS. Ionotropic N-methyl-D-aspartate (NMDA) receptors are involved in many events during development, including dendritic spine formation, maintenance and remodeling (McKinney, 2010). Prolonged activation of ionotropic glutamate receptors can lead to excitotoxicity (Ferreira et al., 1996). Therefore its extracellular levels must be highly regulated in order to avoid neuronal injury (Ishikawa, 2013).

Glutamate-aspartate transporters or excitatory amino acid transporters (EAATs) are essential for the maintenance of glutamate homeostasis. EAATs are widely distributed in central neurons and glial cells (Danbolt, 2001; Martinez-Lozada et al., 2011). They are driven by Na^+ and K^+ gradients (Jiang and Amara, 2011) and five different 'high-affinity' glutamate transporters have been cloned: GLAST (EAAT1) (Storck et al., 1992), GLT-1 (EAAT2) (Pines et al., 1992), EAAC1

*Corresponding author. Address: Laboratório de Neurofarmacologia, Universidade Federal Fluminense, Rua Hernani de Mello, 101/213 São Domingos, Niterói, RJ 24210-130, Brazil. Fax: +55-21-2629-2400.
E-mail addresses: dudadefreitas@ig.com.br (A. P. de Freitas), ferreiradanielle@id.uff.br (D. D. P. Ferreira), arletefernandes@id.uff.br (A. Fernandes), romartins@id.uff.br (R. S. Martins), vladimirppbm@gmail.com (V. P. P. Borges-Martins), msathler@id.uff.br (M. F. Sathler), msp_biomed@yahoo.com.br (M. dos-Santos-Pereira), robpaes@vm.uff.br (R. Paes-de-Carvalho), egiestal@vm.uff.br (E. Giestal-de-Araujo), ramreis@biof.ufrj.br (R. A. de Melo Reis), kubrusly@vm.uff.br (R. C. C. Kubrusly).

Abbreviations: cAMP, cyclic adenosine monophosphate; CNS, central nervous system; DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine; EAA, excitatory amino acid; EAAT, excitatory amino acid transporter; NMDA, N-methyl-D-aspartate; TTBS, Tween 20 Tris-Buffered Saline.

http://dx.doi.org/10.1016/j.neuroscience.2016.09.028
0306-4522/