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CAFFEINE POTENTIATES THE RELEASE OF GABA MEDIATED BY NMDA RECEPTOR ACTIVATION: INVOLVEMENT OF A_1 ADENOSINE RECEPTORS

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Abstract—Caffeine, a methylated derivative of xanthine and widely consumed psychoactive substance, acts in several targets in the nervous system. We investigated its role in retinal explants of chick embryo analyzing the role of purinergic receptors in [^{3}H]-GABA release induced by D-aspartate (D-asp). D-Asp increases GABA-release 4.5-fold when compared to basal levels from 13-day-old chick embryo retina explants. Caffeine 500 μM elevated D-asp-induced GABA release in 60%. The release was inhibited in the presence of NNC-711, a GABA transporter-1 (GAT-1) blocker or by MK-801, an N-methyl-D-aspartate receptor (NMDAR) antagonist. Caffeine did not modify [^{3}H]-GABA uptake carried out for 5, 10, 30 and 60 min and did not increase the release of D-asp or glutamate at basal or stimulated conditions. The caffeine effect was mimicked by the adenosine A_1 receptor antagonist DPCPX and by the adenylyl cyclase (AC) activator forskolin. It was also blocked by the protein kinase A (PKA) inhibitor H-89, tyrosine kinase inhibitor genistein or by the src family kinase (SFK) inhibitor PP1. Forskolin-stimulated cyclic adenosine monophosphate (cAMP) levels were reduced in the presence of the A_1 receptor agonist CHA. Western blot analysis revealed that 500 μM caffeine increased phosphoGluN2B expression levels in approximately 60% when compared to total GluN2B levels in embryonic E13 retina. The GluN2B subunit-containing NMDAR antagonist ifenprodil inhibited the caffeine effect. Our results suggest that caffeine potentiates D-asp-induced GABA release, which is mediated by GAT-1, via inhibition of adenosine A_1 receptor and activation of the PKA pathway. Regulation of NMDAR by phosphorylation of GluN2B subunit by a SFK may also be involved in the effect promoted by caffeine. © **2014 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: adenosine receptors, caffeine, GABA, retina, NMDA receptor.

INTRODUCTION

Caffeine is a methylated derivative of xanthine and is considered the most widely consumed psychoactive substance in the world (Ogawa and Ueki, 2007). Caffeine stimulates motor activity (Ferre, 2008), modulates onset and quality of sleep (Diaz-Munoz and Salin-Pascual, 2010), improves attention/vigilance, increases memory retention (Cunha and Agostinho, 2010) and is also a cognitive enhancer (Daly, 2007). Many studies have reported a potential therapeutical role for caffeine in several neurodegenerative disorders, including Parkinson and Alzheimer diseases (Arendash and Cao, 2010; Marques et al., 2011). Pharmacological mechanisms underlying caffeine effects are primarily via a nonselective antagonism of adenosine receptors, with A_1 and A_{2A} receptors as preferential targets (Fredholm et al., 1999; Ferre, 2008).

The embryonic retina has been used for the past 40 years as a model for development and neurochemical signaling, since the major neurotransmitter systems are present in the cellular components of this tissue. Among these, dopamine, adenosine, γ-aminobutyric acid (GABA) and glutamate predominate as major transmitters (Reis et al., 2007). Adenosine is a purine nucleoside present in all cells. This neuromodulator has many roles in the nervous system, including neuroprotection, synapse development and modulation of neurotransmitter circuitry in the developing nervous system (Ferreira and Paes-de-Carvalho, 2001; Paes-de-Carvalho et al., 2003; Fredholm, 2010). Adenosine is able to modulate synaptic transmission through activation of four distinct G protein-coupled adenosine receptors (A_1R, A_{2A}R, A_{2B}R, A_3R) (Paes-De-Carvalho, 2002). A_1Rs are classically involved with the inhibition of neurotransmitter release, whereas A_{2A}Rs facilitate it. A_1R activation inhibits adenylyl cyclase (AC), whereas A_{2A}R activates this enzyme (Ribeiro et al., 2002; Pearson et al., 2003), leading to a decrease and increase in cyclic adenosine mono-

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Abbreviations: AC, adenylyl cyclase; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; D-asp, D-aspartate; E#, embryonic day #; EDTA, ethylenediamine tetraacetic acid; GABA, gamma-aminobutyric acid; GAT, GABA transporter; GluN1, GluN2A, GluN2B, subunits of NMDA receptor; HPLC, high-performance liquid chromatography; MEM, minimum essential medium; NMDAR, N-methyl-D-aspartate receptor; PKA, protein kinase A; SFK, src family kinase; TCA, trichloroacetic acid.

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