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Caffeine regulates GABA transport via A_1R blockade and cAMP signaling

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ABSTRACT

Caffeine is the most consumed psychostimulant drug in the world, acting as a non-selective antagonist of adenosine receptors A_1R and $A_{2A}R$, which are widely expressed in retinal layers. We have previously shown that caffeine, when administered acutely, acts on A_1R to potentiate the NMDA receptor-induced GABA release. Now we asked if long-term caffeine exposure also modifies GABA uptake in the avian retina and which mechanisms are involved in this process. Chicken embryos aged E11 were injected with a single dose of caffeine (30 mg/kg) in the air chamber. Retinas were dissected on E15 for *ex vivo* neurochemical assays. Our results showed that [^{3}H]-GABA uptake was dependent on Na^+ and blocked at 4 °C or by NO-711 and caffeine. This decrease was observed after 60 min of [^{3}H]-GABA uptake assay at E15, which is accompanied by an increase in [^{3}H]-GABA release. Caffeine increased the protein levels of A_1R without altering ADORA1 mRNA and was devoid of effects on $A_{2A}R$ density or ADORA2A mRNA levels. The decrease of GABA uptake promoted by caffeine was reverted by A_1R activation with N6-cyclohexyl adenosine (CHA) but not by $A_{2A}R$ activation with CGS 21680. Caffeine exposure increased cAMP levels and GAT-1 protein levels, which was evenly expressed between E11-E15. As expected, we observed an increase of GABA containing amacrine cells and processes in the IPL, also, cAMP pathway blockage by H-89 decreased caffeine mediated [^{3}H]-GABA uptake. Our data support the idea that chronic injection of caffeine alters GABA transport via A_1R during retinal development and that the cAMP/PKA pathway plays an important role in the regulation of GAT-1 function.

1. Introduction (963 words)

Caffeine is the main psychostimulant drug consumed in the world (Heishman and Henningfield, 1992; Temple et al., 2017). Caffeine is a methylxanthine able to affect several behavioral, cognitive and physiological functions of the CNS, such as sleep, cognition, learning, memory or promoting neuroprotection (Ribeiro and Sebastiao, 2010). Indeed, caffeine (50 μM) selectively interferes on synaptic transmission and plasticity in the hippocampus through adenosine receptor blockade, in a way that A_1R mediates the impact of caffeine on synaptic transmission while $A_{2A}R$ regulates its impact on long term potentiation (LTP) (Lopes et al., 2019). However, doses higher than 0.5 mM are able to inhibit cyclic nucleotides phosphodiesterase enzymes, mobilize internal calcium stores and also block $GABA_A$ receptors (Ferre, 2008; Fredholm et al., 1999; Ribeiro and Sebastiao, 2010; Williams and Jarvis, 1988). The adenosine effects are mostly linked to the activation of its metabotropic receptors, which can be classified into four cloned subtypes, namely A_1, A_{2A}, A_{2B}, and A_3 (Ribeiro et al., 2002). A_1R and

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