Role of adenosine A1 receptor in Huntington's Disease

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Huntington's disease (HD) is an inherited and incurable neurodegenerative disorder characterized by marked striatal atrophy. Adenosine receptors may represent a possible therapeutic target for HD. The localization of A_{2A} receptors (A_{2A}Rs) in medium-sized spiny neurons, which are selectively lost in HD suggested that these receptors could play an important pathogenetic role. Unfortunately, conflicting data have been reported so far on their potential use as therapeutic target, since the stimulation/blockade of A_{2A}Rs can exert both neuroprotective and neurotoxic effects according to their synaptic localization and to the phase of the disease (Popoli et al. 2008). On the contrary, A_1 receptor (A_1R) stimulation exerts neuroprotective effects both at pre- and post-synaptic level in response to various insults to the brain (Sebastiao and Ribeiro, 2009), thus indicating them as a better target. Interestingly, indeed, A_1R stimulation was found neuroprotective in a lesional model of HD (Blum et al., 2002). However, whether the expression, pharmacology and functions of A_1Rs are altered in genetic models of HD has never been explored.

In the present study we characterized the expression, affinity and functional effects of A_1Rs in R6/2 mice (the most widely used transgenic model of HD) and in cellular models of HD using different experimental approaches.

Electrophysiological recordings of extracellular field potentials in corticostriatal slices demonstrated that the selective A_1R agonist ciclopentyladenosine (CPA, 300 nM for 20') was significantly more effective in reducing synaptic transmission in symptomatic (11-13 weeks of age) R6/2 than in age-matched WT mice (mean FP amplitude at the end of CPA application: 27.03±4.67% and 51.22±6.30% of basal in R6/2 and WT, respectively; $P<0.05$). Both paired-pulse stimulation protocol and K^+-evoked glutamate efflux from synaptosomes demonstrated that the increased effect of CPA in R6/2 mice was due to a higher inhibition of glutamate release from the pre-synaptic terminal. All the above reported CPA effects were fully prevented by the A_1R antagonist DPCPX. Binding studies revealed that the density of A_1Rs was significantly reduced in the cortex (Bmax 347±3 vs 467±14 fmol/mg protein) and the striatum (Bmax 250±6 vs 373±2 fmol/mg protein) of R6/2 vs. WT mice, while receptor affinity was not affected. To try to understand the mechanisms of the increased A_1R-mediated effects, we evaluated (western blot) the expression of adenosine deaminase (ADA), that (besides regulating adenosine levels) can modulate the functions of A_1Rs. ADA levels were not significantly changed in membranes, while they were significantly reduced in the cytosol of R6/2 mice. The different functional activity of A_1Rs in HD mice was associated also to a different intracellular signaling pathway involved in the synaptic effect of CPA. In fact, while the PKA pathway was involved in both genotypes, p38 MAPK inhibitor SB203580 partially prevented synaptic CPA effect in R6/2 mice (51.90±8.76% of basal; $P<0.05$ vs. CPA alone) but not in WT; moreover, CPA differently modulated the phosphorylation status of p38 in the two genotypes. Finally, we analysed the ability of CPA to modulate motor activity in R6/2 and WT mice and, at least at presymptomatic stage (6 weeks), we found no changes. *In vitro* studies confirmed a different behavior of A_1Rs in HD: CPA (500 nM for 5 hours) modulated cell viability in ST14A/Q120 (HD cells), without affecting the viability of Q15 (control) cells.

In conclusion, our results demonstrate that in presence of the HD mutation A_1Rs undergo profound changes in terms of expression, pharmacology and functional activity. These changes have to be taken in due account when considering A_1Rs as a potential therapeutic target.

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