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Opposite effects of the A_{2A} receptor agonist CGS21680 in the striatum of Huntington's disease *versus* wild-type mice

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Abstract

Huntington's disease (HD) is an inherited neurodegenerative disorder. Adenosine A_{2A} receptors (A_{2A}Rs) are involved in excitotoxic/neurodegenerative processes, and A_{2A}R ligands may be neuroprotective in models of HD. However, changes in the transcription, expression and function of A_{2A}Rs have been reported to occur in HD models. The aim of the present work was to verify whether A_{2A}R-mediated effects are altered in the striatum of transgenic HD (R6/2) *versus* wild-type (WT) mice. Extracellular field potentials (FPs) were recorded in corticostriatal slices from R6/2 mice in early (7–8 weeks) or frankly (12–13 weeks) symptomatic phases, and age-matched WT. In 12–13 weeks aged WT animals, the application of 75 μM NMDA induced a transient disappearance of the FP followed by an almost complete recovery at washout. In slices from HD mice, the mean FP recovery was significantly reduced ($P < 0.01$ *versus* WT). A_{2A}R activation oppositely modulated NMDA-induced toxicity in the striatum of HD *versus* WT mice. Indeed, the A_{2A}R agonist CGS21680 reduced the FP recovery in slices from WT mice, while it significantly increased it in slices from R6/2 mice. In early symptomatic (7–8 weeks) mice, no differences were observed between WT and HD animals in terms of basal synaptic transmission and response to NMDA. At the same age, the behavioural effects elicited by CGS21680 were qualitatively identical in WT and HD mice. These findings may have very important implications for the neuroprotective potential of A_{2A}R ligands in HD.

Keywords: Huntington's disease; R6/2 mice; Striatum; Adenosine A_{2A} receptors; NMDA

Huntington' s disease (HD) is an inherited neurodegenerative disease caused by a mutation (a CAG trinucleotide expansion) in exon 1 of the IT15 gene [19]. The expanded CAG repeat is translated into a polyglutamine (poliQ) expansion in the protein huntingtin (htt), that in this mutated version results very toxic for neurons. Excitotoxic mechanisms may play a role in HD [8]. In agreement, an increased sensitivity to NMDA-mediated toxicity has been reported in transgenic HD mice [4,21]. Besides NMDA, adenosine A_{2A} receptors (A_{2A}Rs) are thought to play a role in htt-mediated striatal degeneration. First, striatal neurons expressing A_{2A}Rs become dysfunctional early in HD [13]. Furthermore, in transgenic HD mice, the expression of A_{2A}Rs was found decreased before motor symptoms appearance [5]. Finally, changes in the transcription and function of A_{2A}Rs have been reported to occur in HD models [6,20]. These findings suggest that the effects of A_{2A}R ligands may be different in normal and pathological conditions. Since both A_{2A}R antagonists [2,3,16] and agonists [7] have been proposed as possible neuroprotective drugs for HD, it is very important to evaluate whether changes in A_{2A}R-mediated effects occur in HD *versus* wild-type (WT) mice.

The present work was aimed at verifying whether the effects elicited by A_{2A}Rs are altered in the striatum of transgenic R6/2 HD mice.

A colony of R6/2 mice and littermate controls was established at Charles River, Italy. This mouse strain develops a severe and progressive neurological phenotype starting from approximately 7–8 weeks of age [14]. Animal use and care followed the European Communities Council Directives (86/609/EEC).

Early (7–8 weeks) or frankly symptomatic (12–13 weeks) R6/2 mice and age-matched WT were used. Animals were decapitated under ether anaesthesia, the brains removed, and coronal corticostriatal slices (300 μm) cut with a vibratome. Slices were maintained in an artificial cerebrospinal fluid

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(ACSF; in mM: 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, 11 glucose, pH 7.3) for 1 h at 22–24 °C. A single slice was then transferred to a submerged recording chamber and continuously superfused at 32–33 °C. Extracellular field potentials (FPs) were recorded in the mediodorsal striatum by stimulation of the white matter between the cortex and the striatum. Responses were acquired and analyzed with LTP software [1]. In each experiment the mean FP baseline amplitude was taken as 100%. NMDA (50–100 μM) was applied over 5 min; ZM241385 (30–200 nM) or CGS21680 (30 nM) were applied over 10 min before and then co-applied with NMDA. A reduction of at least 90% of basal FP amplitude was defined as FP disappearance.

At 8 weeks of age, animals of both genotype received CGS21680 (0.5 mg/kg i.p.) or vehicle (10 ml/kg) ($N = 5$ veh-WT; 5 CGS-WT; 4 veh-R6/2; 5 CGS-R6/2) and, 30 min thereafter, they underwent a single 20 min session of open-field testing. Each subject was placed in the centre of an open-field arena (30 cm × 30 cm) subdivided into 6 cm × 6 cm, and *crossings* (the number of crossings of the square limits with both forepaws), *wall-rearing* (standing on the hind limbs and touching the walls of the apparatus with the forelimbs), and *rearing* (standing on the hind limbs away from the walls) were scored. Frequency of immobility and the time spent in the peripheral area and in the centre of the arena were also evaluated.

A mixed-model analysis of variance (ANOVAs) for repeated measures with two between-subject factors [genotype (wild-type *versus* R6/2) and treatment (vehicle *versus* CGS21680)] was used to analyze behavioural data. Post hoc comparisons were performed using Tukey's HSD test.

In a first series of experiments, frankly symptomatic R6/2 mice (12–13 weeks) and aged-matched controls were included. In corticostriatal slices from WT mice, the application of 75 μM NMDA induced the disappearance of the FP followed by an almost complete recovery after 50 min of washout ($N = 8$, Fig. 1). Conversely, in R6/2 mice the same treatment induced a toxic effect, as revealed by the only partial recovery at washout, which indicates a permanent impairment of synaptic activity ($N = 9$, Fig. 1). CGS21680 was tested at 30 nM, a concentration which did not affect FP amplitude by itself. When co-applied with NMDA in slices from WT mice, CGS21680 rendered the effects of NMDA frankly toxic, as revealed by the significant reduction of FP recovery *versus* NMDA alone ($N = 7$, Fig. 2). Interestingly, CGS21680 exerted the opposite effect (namely it significantly attenuated NMDA toxicity), in slices from R6/2 mice ($N = 6$, Fig. 3). The effects elicited by CGS21680 in mice from both genotypes were prevented by the $A_{2A}R$ antagonist ZM241385 (50 nM) ($N = 3$ in WT and 5 in R6/2, Figs. 2 and 3). ZM241385 alone (30–200 nM) did not influence the basal FP amplitude or NMDA-induced effects (not shown).

To verify whether the opposite effect of CGS21680 could depend on the different degrees of NMDA toxicity obtained in HD *versus* WT mice, we tested its effects towards different concentrations of NMDA. In WT slices, the application of 100 μM NMDA induced a toxic effect as revealed by the only partial recovery at washout (Fig. 4A). CGS21680 was still able to potentiate such a toxic effect of NMDA in WT slices ($N = 3$, Fig. 4A).

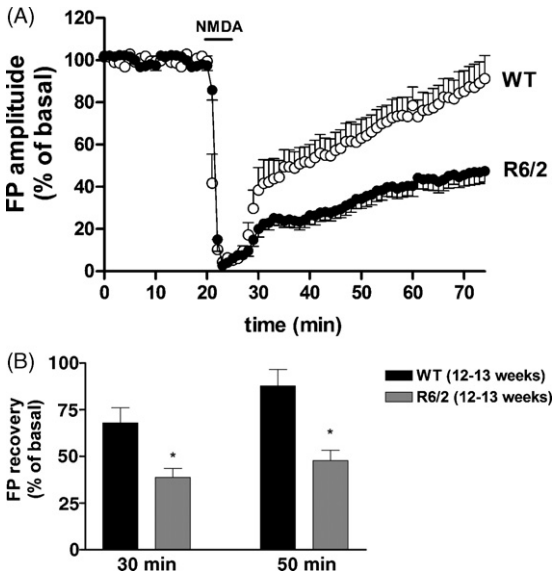

Fig. 1. Increased vulnerability to NMDA in slices from R6/2 *vs*. WT mice. In 12–13 weeks aged WT mice the application of NMDA (75 μM over 5 min) induced the disappearance of FP followed by an almost complete recovery of the response at washout. The recovery was significantly reduced in R6/2 mice of the same age. (A): time course of changes in FP amplitude. (B): mean FP amplitude after 30 and 50 min of washout. (*) = $P < 0.01$ *vs*. WT according to Mann–Whitney test.

In R6/2 mice, a milder effect was induced by applying 50 μM NMDA (Fig. 4B). Even in this condition (non-toxic effect of NMDA in HD slices), CGS21680 was unable to decrease the FP recovery (Fig. 4B). This indicates that the opposite effects

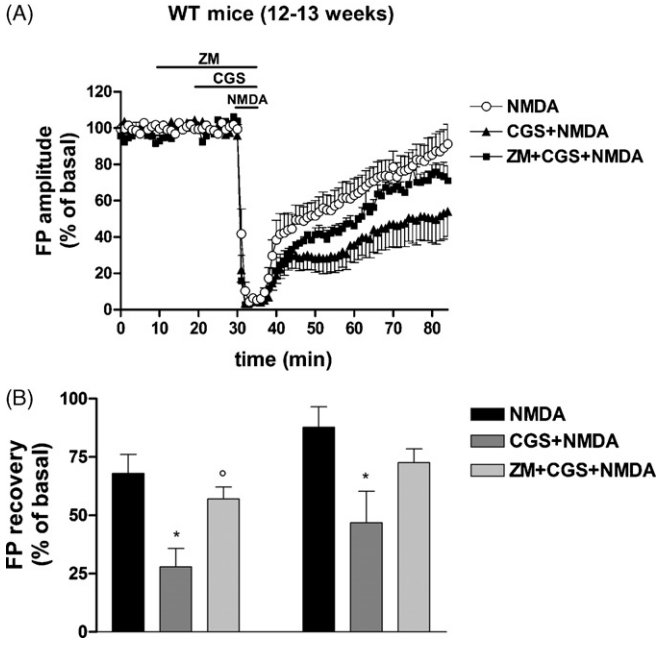

Fig. 2. The $A_{2A}R$ agonist CGS21680 significantly potentiated NMDA-mediated effects in WT mice of 12–13 weeks. Ten minutes of slices perfusion with CGS21680 (30 nM) followed by co-application with NMDA (75 μM) significantly reduced FP recovery. This effect was blocked by ZM241385 at the concentration of 50 nM. (A) and (B) as in Fig. 1. (*) = $P < 0.05$ *vs*. NMDA alone according to Mann–Whitney test. ° = $P < 0.05$ *vs*. CGS+NMDA according to Mann-Whitney test.