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Regular Article

Adenosine A1 receptor stimulation reduces D1 receptor-mediated GABAergic transmission from striato-nigral terminals and attenuates L-DOPA-induced dyskinesia in dopamine-denervated mice

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ABSTRACT

γ-Aminobutyric acid A receptor ($GABA_AR$)-mediated postsynaptic currents were recorded in brain slices from substantia nigra pars reticulate neurons. The selective adenosine A1 receptor (A1R) antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), increased the frequency, but not the amplitude of spontaneous inhibitory post-synaptic currents (IPSCs) in the presence of the dopamine D1 receptor agonist SKF 38393 (SKF) and phosphodiesterase 10A inhibitors (papaverine or AE90074). Under these conditions, DPCPX also increased the amplitude of evoked IPSCs (eIPSCs). The effect of DPCPX was also examined in a mouse model of Parkinson's disease (PD), generated by unilateral denervation of the dopaminergic input to the striatum. In this model, SKF alone was sufficient to increase sIPSCs frequency and eIPSCs amplitude, and these effects were not potentiated by DPCPX. To confirm a depressive effect of A1Rs on the synaptic release of GABA we used the selective A1R agonist 5′-chloro-5′-deoxy-N^6-(±)-(*endo*-norborn-2-yl)adenosine (5′Cl5′d-(±)-ENBA) which has limited peripheral actions. We found that 5′Cl5′d-(±)-ENBA decreased sIPSCs frequency, without affecting their amplitude, and decreased eIPSCs amplitude. Importantly, in the PD mouse model, 5′Cl5′d-(±)-ENBA prevented the increase in sIPSC frequency and eIPSC amplitude produced by SKF. Since exaggerated DA transmission along the striato-nigral pathway is involved in the motor complications (e.g. dyskinesia) caused by prolonged and intermittent administration of L-DOPA, we examined the effect of A1R activation in mice with unilateral DA denervation. We found that 5′Cl5′d-(±)-ENBA, administered in combination with L-DOPA, reduced the development of abnormal involuntary movements. These results indicate the potential benefit of A1R agonists for the treatment of L-DOPA-induced dyskinesia and hyperkinetic disorders providing a mechanistic framework for the study of the interaction between DA and adenosine in the striatonigral system.

Introduction

The substantia nigra pars reticulata (SNpr) is an important relay network that connects the striatum to the thalamus, the superior colliculus (Di Chiara et al., 1979; Yasui et al., 1995) and the brain stem (Smith et al., 1998; Takakusaki, 2013).

A key regulatory role on the activity of SNpr neurons is played by the γ-aminobutyric acid (GABA)ergic afferents of striatal medium spiny neurons (MSNs). Thus, the inhibition exerted by MSNs on SNpr GABAergic neurons promotes the activity of glutamatergic thalamic neurons, maintaining an excitatory synaptic drive to the cerebral cortex (Beckstead and Frankfurter, 1982; Bodor et al., 2008; Chuhma et al., 2011; Deniau et al., 1978; Herkenham, 1979; Nishimura et al., 1997). Interestingly, the release of GABA in the SNpr is modulated by many receptors located on MSNs (Bergevin et al., 2002; Grillner et al., 2000; Lu and Ordway, 1997; Szabo et al., 2002; Wu et al., 1995; Zheng et al., 2002), critically involved in functional and dysfunctional aspects of basal ganglia transmission through modification of SNpr neurons' discharge.

Abbreviations: ACSF, artificial cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; DA, dopamine; DARPP-32, DA- and cAMP regulated phosphoprotein of 32 kDa; D1R, D1 receptor; A1R, A1 receptor; eIPSCs, evoked IPSCs; sIPSCs, spontaneous IPSCs; IEI, inter-event interval; fsk, forskolin; MSNs, medium-spiny neurons; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PP, paired pulse; papav, papaverine; SKF, SKF 38393; SNpr, substantia nigra pars reticulata.

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In Parkinson's disease (PD), the loss of the dopamine (DA) input to the basal ganglia reduces the response of D1 receptors (D1Rs) expressed on the MSNs of the striatonigral pathway, thereby contributing to the emergence of akinesia (De Long, 1990). On the other end, DA depletion produces a sensitization of D1R transmission (Gerfen et al., 2002), which, in the presence of dopaminergic drugs, may lead to an excessive release of GABA on SNpr cells. This, in turn, results in thalamic disinhibition and cortical over excitation, ultimately involved in motor complications associated with prolonged administration of L-DOPA, such as dyskinesia (Cenci et al., 2009; Feyder et al., 2011; Santini et al., 2008). We have recently demonstrated that the stimulation of D1Rs increases GABA-mediated synaptic events in SNpr cells, only in association with permissive factors such as: phosphodiesterase (PDE) 10A inhibition and presynaptic depolarization. On this regard it should be noted that there are conflicting reports on D1-mediated facilitation (Cameron and Williams, 1993; Chuhma et al., 2011; de Jesús Aceves et al., 2011; Radnikow and Misgeld, 1998) and inhibition (Miyazaki and Lacey, 1998) of IPSCs. Several factors may explain the lack of effect by the sole stimulation of D1Rs that we observed, including our animal model (mice vs. rats), the metabolic state of the GABAergic due to temperature settings, level of extracellular K^+, the use of a potassium- vs. a cesium-based intracellular solution, or agonist and antagonist concentrations.

Anatomical data show that PDE10A is present in the direct D1R-bearing (striato-substantia nigra and striato-internal pallidus) and indirect D2R-bearing (striato-external pallidus) MSNs, but not in striatal interneurons (Coskran et al., 2006; Nishi et al., 2008; Sano et al., 2008; Xie et al., 2006). We have also reported that, following DA denervation, D1R stimulation alone increases synaptic GABAergic events in the SNpr (Mango et al., 2014). This phenomenon is most likely caused by the development of sensitized D1R-transmission on nigrostriatal MSNs and may be implicated in L-DOPA-induced dyskinesia.

A large amount of data indicates that adenosine antagonizes D1R-mediated transmission via stimulation of A1 receptors (A1Rs) (Ferré, 1997; Ferré et al., 2001), which are densely expressed in the SNpr (Fastbom et al., 1987). It has been shown that D1Rs and A1Rs are co-localized in striatum (Ferré et al, 1991); thus, an opposing interaction between A1 and D1 receptors may take place at the level of second messengers and beyond (Abbracchio et al, 1987; Fuxe et al, 2007).

Thus, by using patch-clamp electrophysiological techniques, we examined the effects of the A1R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and the potent and highly selective A1R agonist 5′-chloro-5′-deoxy-N^6-(±)-(*endo*-norborn-2-yl) adenosine (5′-chloro-5′-deoxy-(±)-ENBA, 5′Cl5′d-(±)-ENBA) which has limited peripheral actions (Franchetti et al., 2009; Luongo et al., 2012, 2014) on the GABAergic synaptic transmission in the normal and DA-depleted SNpr. Moreover, we studied the impact of the A1R–D1R interaction in vivo, using a mouse model of PD and L-DOPA-induced dyskinesia.

Materials and methods

Animals

Male C57BL/6 mice (Taconic, Tornbjerg, Denmark) were housed under a 12-hour light-dark cycle with food and water ad libitum. Behavioral experiments were carried out during the light phase. All experiments followed international, as well as local guidelines on the ethical use of animals from the European Communities Council Directive of 24 November 1986 (86/609/EEC), the ethical committee of the University of Tor Vergata (Rome, Italy) and the Swedish Animal Welfare Agency. All efforts were made to minimize animal suffering and the number of animals used.

Slice preparation

C57BL/6 mice (about 30 days old) were anesthetized with intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg) and killed by decapitation. Slices containing both the ventral midbrain and the striatum were cut as previously described (Mango et al., 2014). A single slice was then placed in a recording chamber (~0.5 ml volume), on the stage of an upright microscope (Axioscope FS, Carl Zeiss, Germany) and submerged in a continuously flowing (3 ml/min) ACSF at 34–35 °C, composed of (in mM): NaCl 126; KCl 2.5; $MgCl_2$ 1.2; $CaCl_2$ 2.4; NaH_2PO_4 1.2; $NaHCO_3$ 19; glucose 11; and saturated with 95% O_2 and 5% CO_2 (pH 7.4).

Drugs

The following pharmacological agents were used (from Sigma-Aldrich, Milan, Italy): (5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801), 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt hydrate (CNQX), papaverine, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), SCH 23390, (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide (SKF 38393), 6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol (SKF 81297), 6-hydroxydopamine hydrochloride (6-OHDA), and 3,4-dihydroxy-L-phenylalanine (L-DOPA) (administered i.p. together with benserazide hydrochloride). AE90074 was a kind gift of Dr Jan Kehler, Lundbeck (Copenhagen, Denmark) and N^6-(±)-*endo*-norbornyl-9*H*-(5-chloro-5-deoxy-β-D-ribofuranosyl)adenine (5′-chloro-5′-deoxy-(±)-ENBA, 5′Cl5′d-(±)-ENBA) was synthesized at the University of Camerino, as previously reported (Franchetti et al., 2009). All drugs used in the electrophysiological experiments were bath applied, while those for the behavioral experiments were dissolved in saline and injected i.p. in a volume of 10 ml/kg, except for 5′Cl5′d-(±)-ENBA that was dissolved in 5% DMSO.

Electrophysiology

Whole cell patch-clamp recordings were obtained as previously described (Mango et al., 2014) using borosilicate glass electrodes (3–5 MΩ) filled with (in mM): KCl (145), $CaCl_2$ (0.05), EGTA (0.1), HEPES (10), Na_3-GTP (0.3), and Mg-ATP (4.0) (pH adjusted to 7.3 with KOH). Spontaneous IPSCs (sIPSCs) were recorded in the continuous presence of MK-801 (10 μM) and CNQX (10 μM), in order to block ionotropic glutamate receptors, and captured off-line from 3 min traces using Clampfit (Molecular Devices, Sunnyvale, CA, USA). Stimulus-evoked IPSCs (eIPSCs) were generated with a bipolar stimulating electrode in the striatum. A paired-pulse protocol (PPR) was employed with an inter-pulse interval of 50 ms to evaluate changes in the paired-pulse ratio (PPR) of eIPSCs (PPR = 2nd IPSC/1st IPSC).

6-OHDA lesions

Twenty-one day (young) or 2 month (adult) old mice were anesthetized with a mixture of Hypnorm Solution (VetaPharma Ltd, Leed, UK), Midazolam 5 mg/ml (Hameln Pharmaceuticals GmbH, Hameln, Germany) and water (1:1:2) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) equipped with a mouse adaptor. 6-OHDA was dissolved in saline containing 0.02% ascorbic acid at the concentration of 3 μg/μl *free base*. Each mouse received two unilateral injections (2 μl each) of 6-OHDA into the right striatum as previously described (Santini et al., 2007), according to the following coordinates (mm) (Franklin and Paxinos, 1997): for young mice — anteroposterior (AP) +1, mediolateral (ML) −1.9, dorsoventral (DV) −2.9 and AP +0.5, ML −2.2, DV −2.9; for adult mice — AP +1, ML −2.1, DV −3.2 and AP +0.3, ML −2.3, DV −3.2. This procedure leads to an ≥80% decrease in striatal tyrosine hydroxylase (TH) immunoreactivity (see Fig. 5A), as assessed post-mortem by Western blotting in all mice. Only the animals with this level of depletion were included in the analysis. One week after the lesion, young mice were subject to an electrophysiological investigation. In preliminary experiments, a group of young mice prepared according to the same procedure was also