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Phosphodiesterase 10A controls D1-mediated facilitation of GABA release from striato-nigral projections under normal and dopamine-depleted conditions

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ABSTRACT

In the present study, we found that PDE10A inhibitor papaverine, alone or in combination with the D1 receptor agonist SKF38393, did not change spontaneous IPSCs (sIPSCs) frequency or amplitude in the substantia nigra pars reticulata (SNpr). An increase in frequency, but not in amplitude, of sIPSCs was only observed when SKF38393 and PDE10A inhibitors were associated to perfusion with higher extracellular K^+. On the other hand, the amplitude of evoked IPSCs (eIPSCs) of the striato-nigral projection to SNpr, was increased in response to co-administration of SKF38393 and papaverine in normal extracellular potassium. Of note, both an increase in sIPSCs frequency and eIPSC amplitude could be obtained either by a robust stimulation of adenylyl cyclase (AC) with forskolin (10 μM) or by a lower dose of forskolin (1 μM) associated to PDE inhibition. We next investigated the effects produced by dopamine (DA) depletion in the striatum. Under this condition, SKF38393 alone increased either sIPSCs frequency and eIPSC amplitude. In addition, in the striatum of DA-depleted mice we found reduced PDE10A levels and higher cAMP-dependent phosphorylation in response to D1 receptor stimulation. In accordance with these biochemical data, perfusion with papaverine had no effect on the SKF38393-induced changes of IPSCs in slices of DA-depleted mice. These findings reveal a dynamic interplay between PDE10A activity, level of neuronal network depolarization and degree of dopaminergic tone in the ability of D1 receptors to facilitate the GABAergic transmission to SNpr neurons from the direct nigro-striatal pathway.

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1. Introduction

The substantia nigra pars reticulata (SNpr) is a key output structure of the basal ganglia, whose overall activity is highly dependent on the GABAergic input originating from a large population of striatal medium-spiny neurons (MSNs). It is believed that the main function of the striato-nigral projection, which constitutes the so-called direct pathway (Alexander and Crutcher, 1990; Gerfen et al., 1990; Gerfen and Bolam, 2010) is to reduce the GABAergic output arising from SNpr and internal pallidus neurons, thereby disinhibiting target structures like the thalamus and superior colliculus (Deniau et al., 1978; Herkenham, 1979; Beckstead and Frankfurter, 1982; Nishimura et al., 1997; Bodor et al., 2008; Chuhma et al., 2011).

The release of GABA from striatal MSNs is modulated by the formation of cyclic adenosine monophosphate (cAMP) via the synthetic enzyme adenylyl cyclase (AC), leading to activation of cAMP-dependent protein kinase (PKA) and phosphorylation of the DA- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32)

Abbreviations: AC, adenylyl cyclase; ACSF, artificial cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; DA, dopamine; DARPP-32, DA- and cAMP-regulated phosphoprotein of 32 kDa; D1Rs, D1 receptors; eIPSCs, evoked IPSCs; IEI, inter-event interval; fsk, forskolin; MSNs, medium-spiny neurons; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PP, paired pulse; papav, papaverine; SCH, SCH23390; SKF, SKF38393; SNpr, substantia nigra pars reticulata; sIPSCs, spontaneous IPSCs.

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(Greengard, 2001; Neve et al., 2004; Missale et al., 1998; Fienberg et al., 1998).

Importantly, DA released by midbrain dopaminergic neurons in both the striatum and the substantia nigra (Geffen et al., 1976; Cheramy et al., 1981), controls GABA release through activation of D1 receptors (D1Rs) located at the somato-dendrites and terminals of striato-nigral MSNs (Barone et al., 1987; Le Moine et al., 1991; Jaber et al., 1996; Acosta-García et al., 2009). This regulation is thought to depend on stimulation of AC, through the heterotrimeric G-protein, Golf (Hervé et al., 1993; Nishi et al., 2011).

On the other hand, the level of intracellular cAMP depends not only on the activity of AC but also on that of phosphodiesterases (PDEs), which degrade cAMP to AMP. PDEs are highly expressed in the soma and terminals of GABAergic MSNs. 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; Xie et al., 2006; Nishi et al., 2008; Sano et al., 2008). Interestingly, recent data have shown that DA regulates PDE10A gene expression in the striatum, whereby a chronic lesion of the nigro-striatal DAergic pathway results in significant reduction of both PDE10A mRNA and protein in the striato-nigral MSNs of the direct pathway (Giorgi et al., 2011).

Considering that PDE10A and D1Rs are present in the fibers and synaptic boutons of striato-nigral MSNs (Coskran et al., 2006), we used papaverine, a specific inhibitor of PDE10A, to investigate the involvement of this enzyme in D1R-mediated GABA release in the SNpr. We also examined the effects of chronic DA denervation on the sensitivity of MSNs to D1R stimulation. We found that D1Rs require concomitant inhibition of PDE10A in order to facilitate GABA release. In contrast, following DA denervation, activation of D1Rs alone acquires the ability to enhance GABA release on SNpr neurons.

2. Methods

2.1. Animals

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) and 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.

2.2. Slice preparation

C57BL6/J mice (18–35 days old) were anaesthetized with intraperitoneal injection of chloral hydrate (400 mg/kg) and killed by decapitation. The brain was rapidly removed from the skull and sliced (250 μm) in cold (8–10 °C) artificial cerebrospinal fluid (ACSF), using a vibratome (Leica VT1200S, Leica Microsystems, Wetzlar, Germany) and left to recover in warm (33–34 °C) ACSF for at least 1 h. The slices, containing both the ventral midbrain and the striatum, were cut on the parasagittal plane at 15° relative to the midline, in order to preserve the striato-nigral direct pathway (Connelly et al., 2010).

Single slices were 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; saturated with 95% O_2, 5% CO_2 (pH 7.4).

2.3. Drugs

All drugs used in electrophysiological experiments were bath applied. Drugs used for behavioral experiments were dissolved in saline and injected intraperitoneally (i.p.) in a volume of 10 ml/kg. The following pharmacological agents were used: (5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cylcohepten-5,10-imine maleate (MK-801), 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt hydrate (CNQX), 3-isobutil-1-methyl-xanthine (IBMX), papaverine, (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide (SKF38393), (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), forskolin, 6-hydroxydopamine hydrochloride (6-OHDA), (±)-6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine hydrobromide (SKF81297). All drugs were from Sigma–Aldrich (Milan, Italy), apart from SKF81297 from Tocris (Bristol, UK).

2.4. Electrophysiology

SNpr neurons were visualized with infrared illumination and whole cell patch clamp recordings were obtained 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; Mg-ATP 4.0 (pH adjusted to 7.3 with KOH). Current signals were filtered at 3 kHz and digitized at 10 kHz using a Multiclamp 700B amplifier, operated by the pClamp10 software (Molecular Devices, Sunnyvale, CA, USA). Neurons were held at −70 mV and no series resistance compensation was implemented, in order to keep a low signal-to-noise ratio, however, recordings were discarded if series resistance changed by more than 15% from control. 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 were generated with a bipolar stimulating electrode in the striatum. Paired pulse (PP) ratio was measured using two stimuli at 50 ms interval.

2.5. 6-OHDA lesion

Twenty-one days 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 adapter. 6-hydroxydopamine (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 of 6-OHDA (2 μl per injection) into the right striatum as previously described (Santini et al., 2007), according to the following coordinates (mm) (Franklin and Paxinos, 1997): anteroposterior (AP), +1; mediolateral (ML), −2.1; dorsoventral (DV), −3.2; and AP +0.3; ML, −2.3; DV, −3.2. Animals were allowed to recover for 1 week before behavioral evaluation and drug treatment were carried out. This procedure leads to ≥80% decrease in striatal tyrosine hydroxylase immunoreactivity. Only mice with this TH depletion, as assessed by post-mortem Western Blotting quantification, were included in the analysis.

2.6. Western blotting

6-OHDA- and sham-lesioned mice were injected with the D1R agonist SKF81297 (3 mg/kg, i.p.) or vehicle and sacrificed by decapitation, 15 min later. The left and right striata were dissected out and sonicated in 750 μl of 1% SDS and boiled for 10 min. Aliquots (5 μl) of the homogenate were used for protein determination with a BCA (bicinchoninic acid) assay kit (Pierce Europe, Oud Beijerland, the Netherlands). Equal amounts of protein (25 μg) for each sample were loaded onto 10% polyacrylamide gels. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred overnight to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Uppsala, Sweden) (Towbin et al., 1979). The membranes were immunoblotted with antibodies against PDE10A (1:2000; FabGennix), phospho-Ser845-GluA1 (1:1000) (Cell Signaling Technology, Beverly, MA), and phosho-Thr34-DARPP-32 (1:1000; Snyder et al., 1992). Antibodies against β-actin (1:10000; Sigma–Aldrich) and not phosphorylation state-specific GluA1 (1:1000, Cell Signaling Technology) and DARPP-32 (1:1000; Snyder et al., 1992) were used as respective standard references. An antibody against tyrosine hydroxylase (1:1000, Chemicon International, Temecula, CA) was used to assess the severity of 6-OHDA lesions. For the analysis of TH and GluA1 levels, the detection was based on fluorescent secondary antibody binding and quantified with a Li-Cor Odyssey infrared fluorescent detection system (Li-Cor, Lincoln, NE). For the quantification of PDE10A levels, immunoreactive bands were stained with nitrobluetetrazolium (0.3 mg/ml) in the presence of 5-bromo-4-chloro-3-indolyl-phosphate (0.15 mg/ml). Densitometric analysis was then performed using the NIH ImageJ version l.29 program (NIH, Bethesda, MD, USA).

2.7. Data analysis

Drug effects on sIPSCs were evaluated as changes in their amplitude or inter-event interval (IEI) using the Kolmogorov–Smirnov (K–S) test on their mean cumulative distributions (bin size 50 ms for IEI and 10 pA for amplitude), or by comparison of their mean median values with the Student's *t*-test (*t*-test). Changes in eIPSCs amplitude (mean ± SEM) in response to specific pharmacological treatments were compared using the *t*-test. A $p < 0.05$ was considered significant. The biochemical effect of SKF81297 in 6-OHDA-lesioned striata on the pGluA1 levels was analyzed with a two-way (lesion and treatment) analysis of variance (ANOVA), followed by Fisher's post hoc comparison between groups. Student' *t*-test was used for comparisons between two groups, as in the case of PDE10 and TH.

3. Results

All electrophysiological data were obtained from neurons characterized by a tonic firing rate (>5 Hz), with no voltage *sag* when negative current steps were applied in current clamp-mode,