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INCREASED DESENSITIZATION OF DOPAMINE D_2 RECEPTOR-MEDIATED RESPONSE IN THE VENTRAL TEGMENTAL AREA IN THE ABSENCE OF ADENOSINE A_{2A} RECEPTORS

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Abstract—G-protein coupled receptors interact to provide additional regulatory mechanisms for neurotransmitter signaling. Adenosine A_{2A} receptors are expressed at a high density in striatal neurons, where they closely interact with dopamine D_2 receptors and modulate effects of dopamine and responses to psychostimulants. A_{2A} receptors are expressed at much lower densities in other forebrain neurons but play a more prominent yet opposing role to striatal receptors in response to psychostimulants in mice. It is, therefore, possible that A_{2A} receptors expressed at low levels elsewhere in the brain may also regulate neurotransmitter systems and modulate neuronal functions. Dopamine D_2 receptors play an important role in autoinhibition of neuronal firing in dopamine neurons of the ventral tegmental area (VTA) and dopamine release in other brain areas. Here, we examined the effect of A_{2A} receptor deletion on D_2 receptor-mediated inhibition of neuronal firing in dopamine neurons in the VTA. Spontaneous activity of dopamine neurons was recorded in midbrain slices, and concentration-dependent effects of the dopamine D_2 receptor agonist, quinpirole, was compared between wild-type and A_{2A} knockout mice. The potency of quinpirole applied in single concentrations and the expression of D_2 receptors were not altered in the VTA of the knockout mice. However, quinpirole applied in stepwise escalating concentrations caused significantly reduced maximal inhibition in A_{2A} knockout mice, indicating an enhanced agonist-induced desensitization of D_2 receptors in the absence of A_{2A} receptors. The A_{2A} receptor agonist, CGS21680, did not exert any effect on dopamine neuron firing or response to quinpirole, revealing a novel non-pharmacological interaction between adenosine A_{2A} receptors and dopaminergic neurotransmission in midbrain dopamine neurons. Altered D_2 receptor desensitization may result in changes in dopamine neuron firing rate and pattern and dopamine release in other brain areas in response to persistent dopamine release and administration of psychostimulants.

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Abbreviations: aCSF, artificial cerebrospinal fluid; CGS21680, 4-[2-[[6-amino-9-(N-ethyl-β-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzene propanoic acid hydrochloride; GIRK, G-protein coupled inward rectifying potassium; NSB, non-specific binding; SCH23390, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; VTA, ventral tegmental area.

Key words: adenosine A_{2A} receptor, dopamine D_2 receptor, dopamine neuron, D_2 receptor desensitization, A_{2A} receptor knockout mice, ventral tegmental area.

Increasing numbers of studies show that G-protein coupled receptor (GPCR) signaling can occur through the formation of oligodimers or multimers in neurons (Dalrymple et al., 2008; Ferre et al., 2008). Adenosine A_{2A} receptors are $G_{s/olf}$-coupled receptors for the endogenous neuromodulator adenosine (Fredholm et al., 2011). The expression of A_{2A} receptors in the brain is at the highest density in the soma of the GABAergic medium spiny neurons in the striatum, with significantly lower levels of expression elsewhere in the brain (Rosin et al., 1998, 2003; Svenningsson et al., 1999). In the striatum, A_{2A} receptors co-localize with dopamine D_2 receptors (Ferre et al., 2008). Via different G-protein coupling and second messenger systems, the activation of the adenosine A_{2A} receptors increases cAMP levels and cellular excitability, while the activation of the dopamine D_2 receptors decreases cAMP levels and cellular excitability. Antagonistic interactions between the two receptor systems are also manifested by direct receptor–receptor crosstalk in the form of heterodimers, where stimulation of the adenosine A_{2A} receptors causes a reduction in the affinity of dopamine D_2 receptor agonists and vice versa (Ferre et al., 1991; Kull et al., 1999; Azdad et al., 2009). The functional interactions between adenosine A_{2A} receptors and dopamine D_2 receptors are thought to underlie the involvement of adenosine A_{2A} receptors in the control of several behavioral functions (Sebastiao and Ribeiro, 1996; Fredholm et al., 2005). Most notably, A_{2A} receptor agonists inhibit the motor and rewarding effects of psychostimulants, similar to effects produced by dopamine D_2 receptor antagonists; whereas A_{2A} receptor antagonists potentiate effects of psychostimulants, in agreement with the activation of dopamine D_2 receptors (Rimondini et al., 1997; Shimazoe et al., 2000; Knapp et al., 2001; Filip et al., 2006).

In adenosine A_{2A} receptor knockout mice (Ledent et al., 1997), however, both the motor and reward effects of psychostimulants were reduced (Chen et al., 2000; Fredholm et al., 2005; Soria et al., 2006; Castane et al., 2006; Shen et al., 2008), contrary to the enhancing effects of A_{2A} receptor antagonists in wild-type mice. The discord between effects of A_{2A} receptor ligands and genetic silencing raises potential issues regarding the long-term use of A_{2A} receptor antagonists for the treatment of Parkinson's dis-

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ease (Schwarzschild et al., 2006) and A_{2A} agonists for schizophrenia (Ferre, 1997). Compensatory alterations in neural circuits are one of the possible causes for the different effects of psychostimulants in the A_{2A} receptor knockout mice. However, extrastriatal A_{2A} receptors, for example, those located on glutamatergic inputs to the striatum from the cerebral cortex (Schiffmann et al., 2007), albeit at low expression density, were found to mediate a prominent excitatory effects of psychostimulants, opposite to the effects of postsynaptic striatal A_{2A} receptors on striatopallidal neurons (Shen et al., 2008). The use of striatum and forebrain-specific A_{2A} receptor knockout mice has revealed that extrastriatal A_{2A} receptors in the forebrain predominantly mediate the excitatory effects of psychostimulants, whereas striatal A_{2A} receptors mediate inhibitory effects of psychostimulants (Shen et al., 2008). These findings may suggest that A_{2A} receptors that are expressed outside the striatum, albeit at low densities, could play significant roles in neuronal functions.

The ventral tegmental area (VTA) is a midbrain region closely involved in dopaminergic neurotransmission, as dopaminergic neurons in the VTA form ascending mesocorticolimbic projections to forebrain regions. In addition, the VTA is suggested to be the site important for the development of sensitization to repeated exposures to drugs of abuse, via effects of dopamine on dopamine receptors (Wise, 1996) and neuroplastic changes at glutamatergic synapses (Bonci and Malenka, 1999). Dopamine D_1- and D_2-like receptors (referred to as D_1 and D_2 receptors thereafter) are highly expressed in the VTA (Bouthenet et al., 1987; Wamsley et al., 1989; Chen et al., 1991; Adell and Artigas, 2004), where activation of D_2 receptors inhibits the firing activity of dopamine neurons, carrying out an important autoinhibitory function (Bunney et al., 1973; White and Wang, 1984; Sibley et al., 1993; Mercuri et al., 1997; Adell and Artigas, 2004).

We examined the effects of A_{2A} receptor deletion on D_2 receptor-mediated autoinhibition of VTA dopamine neuron firing activity in brain slices. Concentration-dependent effects of the D_2 receptor-selective agonist, quinpirole, were investigated in wild-type and the global adenosine A_{2A} receptor knockout mice. Expression of dopamine receptors in the VTA of the A_{2A} knockout mice and effects of the A_{2A} receptor agonist CGS21680 on neuronal firing and responses to quinpirole were also assessed. We found that the maximal effect of quinpirole was reduced in the A_{2A} receptor knockout mice when treated with rising concentrations of quinpirole, but not single concentrations, which may indicate enhanced agonist-induced desensitization of D_2 receptors in the absence of A_{2A} receptors. A novel role of A_{2A} receptors in modulating dopaminergic neurotransmission is revealed.

EXPERIMENTAL PROCEDURES

All animal breeding and experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

VTA slice preparation and electrophysiological recordings

The adenosine A_{2A} receptor knockout mice on the out-bred CD1 background were originally created by Ledent et al. (1997) and heterozygous breeding colonies are maintained at the University of Surrey. Midbrain slices were prepared from wild-type and adenosine A_{2A} receptor knockout mice aged 3–4 weeks. The mice were killed by cervical dislocation and the whole brains were immediately dissected and immersed in ice cold sucrose-containing buffer and saturated with 95% O_2/5% CO_2. The sucrose-containing buffer contained: 200 mM sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 25 mM $NaHCO_3$, 10 mM $MgSO_4$, 2.5 mM KCl, 1.2 mM NaH_2PO_4 and 0.5 mM $CaCl_2$. The midbrain block was dissected and mounted with cyanoacrylic glue to a glass cutting stage of a vibratome (Series 1000 Vibratome, St. Louis, MO, USA). Single coronal sections (300 μm) containing the VTA were harvested (3–4 per animal) and placed in an incubation chamber for at least 1 h at 27–28 °C in oxygenated artificial cerebrospinal fluid (aCSF), which contained NaCl 123 mM, $NaHCO_3$ 22 mM, NaH_2PO_4 1.25 mM, KCl 3.75 mM, D-Glucose 10 mM, $CaCl_2$ 2.5 mM and $MgSO_4$ 1.2 mM.

Electrophysiological recordings were performed as previously detailed for rat VTA slice preparations (Chen et al., 2005; de Filippi et al., 2010). A single slice was placed in the recording chamber and continuously perfused with oxygenated aCSF at a flow rate of 2 ml/min and at 32±0.5 °C. The VTA area was identified as a grey area medial to the substantia nigra and the medial lemniscus. Borosilicate glass microelectrodes with a tip of approximately 1 μm, pulled with a P-97 Flaming/Brown micropipette puller (Sutter Instrument Company, Novato, CA, USA), were filled with aCSF to give an impedance of 3–6 MΩ and used for recording from single neurons. Extracellular recordings of the spontaneous action potentials were amplified (AxonPatch 1D, Molecular Devices Corp., Silicon Valley, CA, USA) initially by 10 times in the I=0 mode with a low-cutoff frequency at 5 kHz and then by 100 times in the AC mode (Neurolog systems, Digitimer, Welwyn Garden City, UK) without any further filtering. Signals were digitized using CED1401 Micro Mark 2 (Cambridge Electronic Design Ltd., Cambridge, UK), and captured with Spike 2 Software Version 5.2 (Cambridge Electronic Design Ltd.).

Two types of spontaneously firing neurons in the VTA were recorded and identified. Dopamine neurons were classically defined by their pacemaker-like spontaneous firing, with frequencies at 0.5–4 Hz and an action potential waveform of 2.5–3 ms in duration with a large negative phase. The firing frequency was significantly suppressed by 50 μM dopamine (−74.2±4.8%, n=29) or by the D_2 agonist, quinpirole (Grace and Onn, 1989; Johnson and North, 1992). The other type of neurons displayed higher spontaneous firing frequencies (4–15 Hz) with action potential waveforms of a shorter duration (~2 ms). Dopamine did not inhibit the firing of these classically defined non-dopaminergic or GABAergic neurons (Grace and Onn, 1989; Johnson and North, 1992). A recent study confirmed that almost all electrophysiologically classified dopaminergic neurons are tyrosine hydroxylase-positive (Brown et al., 2009), despite discrepancies found in some other studies (Margolis et al., 2006, 2010). “Dopamine neurones” referred to in this study are therefore those that conform to electrophysiological and pharmacological criteria, but not necessarily to the content of dopamine.

Autoradiographic binding

The brains of wild-type and adenosine A_{2A} receptor knockout mice were removed, snap frozen in isopentane (−35 °C) and stored at −80 °C until sectioning. Adjacent 20-μm coronal sections were cut at an interval of 300 μm for the determination of total and non-specific binding (NSB). Quantitative autoradiography was performed as detailed previously for dopamine D_1 and D_2 receptor