BRAIN RESEARCH 1635 (2016) 95–104

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Brain Research

Research Report

Suppression of outward K^+ currents by activating dopamine D1 receptors in rat retinal ganglion cells through PKA and CaMKII signaling pathways

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ARTICLE INFO

Article history:
Accepted 21 January 2016
Available online 27 January 2016

Keywords:
Dopamine D1 receptor
Outward K^+ currents
Retinal ganglion cell
Patch-clamp

ABSTRACT

Dopamine plays an important role in regulating neuronal functions in the central nervous system by activating the specific G-protein coupled receptors. Both D1 and D2 dopamine receptors are extensively distributed in the retinal neurons. In the present study, we investigated the effects of D1 receptor signaling on outward K^+ currents in acutely isolated rat retinal ganglion cells (RGCs) by patch-clamp techniques. Extracellular application of SKF81297 (10 μM), a specific D1 receptor agonist, significantly and reversibly suppressed outward K^+ currents of the cells, which was reversed by SCH23390 (10 μM), a selective D1 receptor antagonist. We further showed that SKF81297 mainly suppressed the glybenclamide (Gb)- and 4-aminopyridine (4-AP)-sensitive K^+ current components, but did not show effect on the tetraethylammonium (TEA)-sensitive one. Both protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase II (CaMKII) signaling pathways were likely involved in the SKF81297-induced suppression of the K^+ currents since either Rp-cAMP (10 μM), a cAMP/PKA signaling inhibitor, or KN-93 (10 μM), a specific CaMKII inhibitor, eliminated the SKF81297 effect. In contrast, neither protein kinase C (PKC) nor mitogen-

Abbreviations: 4-AP, 4-aminopyridine; AC, adenylate cyclase; Bis IV, bisindolylmaleimide IV; BK_{Ca}, large-conductance Ca^{2+}-activated K^+ channels; CaMKII, calcium/calmodulin-dependent protein kinase II; CNS, central nervous system; DA, dopamine; DMSO, dimethyl sulfoxide; EGTA, ethylene glycol-bis(β-aminoethyl ether) N, N, N', N'-tetraacetic acid; ERK, extracellular signal-regulated kinase; Gb, glybenclamide; HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; I_h, hyperpolarization-activated cation currents; K_{ATP}, ATP-sensitive K^+ channels; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; RGC, retinal ganglion cell; RITC, rhodamine-B-isothiocyanate; TEA, tetraethylammonium; TTX, tetrodotoxin

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http://dx.doi.org/10.1016/j.brainres.2016.01.039
0006-8993/

activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway seemed likely to be involved because both the PKC inhibitor bisindolylmaleimide IV (Bis IV) (10 μM) and the MAPK/ERK1/2 inhibitor U0126 (10 μM) did not block the SKF81297-induced suppression of the K^+ currents. These results suggest that activation of D1 receptors suppresses the Gb- and 4-AP-sensitive K^+ current components in rat RGCs through the intracellular PKA and CaMKII signaling pathways, thus modulating the RGC excitability.

1. Introduction

Dopamine (DA), an important neuromodulator, is involved in diverse functions in the central nervous system (CNS) by activating the specific G-protein coupled receptors (Bissière et al., 2003; Gulledge and Jaffe, 1998; Kebabian and Calne, 1979; Missale et al., 1998; Paspalas and Goldman-Rakic, 2005; Penit-Soria et al., 1987; Yang and Seamans, 1996). Based on the biochemical and pharmacological properties, five DA receptor types (D1-D5) are classified into two groups: D1 receptors (D1, D5) that couple to the G_s and activate adenylate cyclase (AC), and D2 receptors (D2, D3 and D4) that negatively couple to AC through the $G_{i/o}$ (Enjalbert and Bockaert,1983; Kebabian and Greengard, 1971; Missale et al., 1998; Sibley and Monsma, 1992).

In the retina, DA is released by dopaminergic amacrine cells and interplexiform cells. By regulating the retinal neuronal network, DA may be involved in a variety of retinal functions (Witkovsky, 2004). For example, DA might indirectly regulate retinal ganglion cell (RGC) spiking by changing the balance of excitatory and inhibitory inputs (Gustincich et al., 1997; Hokoc and Mariani, 1987; Pourcho, 1982; Thier and Alder, 1984). DA is also found to regulate the surrounding of the RGC receptive field mediated by the D1 receptors (Jensen 1989). On the other hand, both D1 and D2 receptors are expressed in RGCs (Bjelke et al., 1996; Hayashida et al., 2009; Nguyen-Legros et al., 1997; Tran and Dickman, 1992; Veruki and Wässle, 1996; Wagner et al., 1993). Direct actions of DA on RGCs have been also reported. For instance, in the dissociated cell preparations, DA could regulate the excitability of RGCs by inhibiting discharges of the cells in turtle and goldfish through modulating voltage-gated Ca^{2+} or Na^+ channels (Hayashida et al., 2009; Hayashida and Ishida, 2004; Liu and Lasater, 1994; Vaquero et al., 2001). In rat retinal slices, it was reported that activation of D1 receptors by DA regulated RGC spikes through modulation of hyperpolarization-activated cation currents (I_h) (Chen and Yang, 2007).

RGCs express many types of voltage-gated K^+ channels (Clark et al., 2009; Ettaiche et al., 2001; Fohlmeister et al., 2010; Koeberle et al., 2010; Lipton and Tauck, 1987). Neuronal K^+ channels are key factors in determining the resting membrane potential and modulating the cell excitability (Hille, 2001). D1 receptors are extensively expressed in the CNS and activation of these receptors could modulate voltage-gated K^+ currents to alter neuronal excitability and synaptic integration, thus regulating many functions including locomotion, reward related behaviors, and working memory (Bissière et al., 2003; Gulledge and Jaffe, 1998; Paspalas and Goldman-Rakic, 2005; Penit-Soria et al., 1987; Yang and Seamans, 1996). However, there is little evidence concerning effect of DA receptors on the outward K^+ channels in RGCs. In the present work, we aimed to explore whether and how activation of D1 receptors may modulate the outward K^+ currents in acutely dissociated rat RGCs using patch-clamp techniques. Our results showed that D1 receptor agonist SKF81297 suppressed outward K^+ currents of the cells, which could be reversed by D1 receptor antagonist SCH23390. We further demonstrated that SKF81297 mainly suppressed the 4-aminopyridine (4-AP)- and glybenclamide (Gb)-sensitive K^+ current components through the intracellular protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase II (CaMKII) signaling pathways.

2. Results

2.1. *Suppression of outward K^+ currents by activating D1 receptors*

We first examined whether activation of D1 receptors may modulate outward K^+ currents in acutely isolated rat RGCs. Fig. 1A shows a typical dissociated RGC that was retrogradely labeled by rhodamine-B-isothiocyanate (green). Outward K^+ currents were induced by a series of 400 ms depolarizing voltage pulses from a holding potential of −70 mV to +30 mV in increments of 10 mV. As shown in Fig. 1B, a series of outward K^+ currents was recorded in a RGC. Perfusion of SKF81297 (10 μM), a selective D1 receptor agonist, significantly and reversibly suppressed the currents. For example, at +30 mV test potential the steady-state current amplitude was reduced to 59.6±5.7% of control by 2 min SKF81297 perfusion (n=12, P=0.003), and washout with the normal external solution for 3 min brought the current amplitude to control level (94.9±3.9% of control, n=12, P=0.215) (Fig. 1B). Fig. 1C shows current–voltage (I–V) curves before and after SKF81297 application, revealing that SKF81297 voltage-dependently suppressed the current amplitudes. We then examined whether the SKF81297 effect is mediated by D1 receptor. A representative result is shown in Fig. 1D (left panel). In these experiments, K^+ currents were evoked by a depolarizing voltage pulse from −70 mV to +30 mV. In the presence of SCH23390 (10 μM), a selective D1 receptor antagonist, addition of 10 μM SKF81297 failed to suppress the K^+ current (Fig. 1D). The average current amplitude at +30 mV