Neuroscience 150 (2007) 299–308

HYPERPOLARIZATION-ACTIVATED CATION CURRENT IS INVOLVED IN MODULATION OF THE EXCITABILITY OF RAT RETINAL GANGLION CELLS BY DOPAMINE

L. CHEN AND X.-L. YANG*

Institute of Neurobiology, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China

Abstract—Modulation of membrane properties and excitability of retinal ganglion cells (RGCs) by dopamine was investigated in rat retinal slices, using whole cell patch clamp techniques. Application of dopamine (10 μM) caused a small depolarization of the membrane potential, a reduction of the input resistance and a decrease in the number of current-evoked action potentials of RGCs, and these effects were blocked by a D1 antagonist (SCH23390, 10 μM), but not by a D2 antagonist (sulpiride, 10 μM). SKF38393 (10 μM), a D1 agonist, but not quinpirole (10 μM), a D2 agonist, mimicked the effects of dopamine on RGCs. Like dopamine, 8-Br-cAMP, a membrane-permeable analog of cAMP, produced similar changes in the membrane properties and the excitability of RGCs. All these results suggest that these effects of dopamine are likely mediated by D1 receptors. Pre-application of KT5720, an inhibitor of protein kinase A (PKA), blocked the dopamine effects, indicating that the effects were PKA-dependent.

Possible involvement of hyperpolarization-activated cation currents (I_h) in the dopamine effects was tested. Inward currents were induced by voltage steps, with an activation threshold of around −70 mV, in the presence of TTX, Cd^{2+}, TEA-Cl and 4-AP. These currents, with a reversal potential of −33.2 mV, displayed inward rectification and were blocked by ZD7288, a specific I_h channel blocker. These results are indicative of the presence of I_h in rat RGCs. Dopamine increased the amplitude of I_h and shifted the activation curve of I_h to a range of more positive potentials. SKF38393 and 8-Br-cAMP increased the amplitude of I_h, which was blocked by KT5720. The dopamine effects were abolished when the preparations were pre-incubated by ZD7288. These data strongly suggest that the dopamine effects on rat RGCs may be, at least in part, mediated by modulation of I_h through the cAMP- and PKA-dependent pathway.

Key words: D1 receptor, membrane properties, retinal slice, protein kinase A.

Dopamine (DA), an important neuroactive substance, is involved in diverse functions of the CNS, by binding to specific receptors. Five DA receptor types (D1–D5) belong to two families: D1 and D2 receptors. These receptors are coupled to adenylate cyclase and their activation changes intracellular cAMP levels (Kebabian and Calne, 1979; Missale et al., 1998). In the retina, DA released by dopaminergic amacrine cells and interplexiform cells, as a chemical messenger, may be involved in adaptation-dependent signal transfer, in addition to regulating trophic functions of the retina (see Witkovsky, 2004 for review). It is generally thought that DA release is higher in light, especially in flickering light than in darkness (Kramer, 1971; Bauer et al., 1980; Dearry and Burnside, 1989; Kirsch and Wagner, 1989; Umino et al., 1991). However, there is evidence suggesting that DA release could be also induced in prolonged darkness (Mangel and Dowling, 1985; Yang et al., 1988, 1994; Weiler et al., 1997; Xin and Bloomfield, 1999). In mammalian retinal ganglion cells (RGCs), DA increases the maintained discharge of OFF center cells, but decreases that of ON center cells (Jensen and Daw, 1984, 1986). DA also inhibits spikes of RGCs elicited by light or glutamate (Straschill and Perwein, 1969; Thier and Alder, 1984). Moreover, DA is found to regulate the surrounding of the RGC receptive field under fully light-adaptation conditions through mediation of the D1 receptor (Jensen, 1989, 1991). These effects on mammalian RGCs are supposed to be owing to a DA-induced regulation of the retinal network formed by bipolar cells, amacrine cells and RGCs (Witkovsky, 2004). On the other hand, direct actions of DA on RGCs have been also reported. Using dissociated cell preparations, it was reported in turtle (Liu and Lasater, 1994) and goldfish (Vaquero et al., 2001) that DA could regulate the excitability of RGCs by inhibiting discharges of these cells. The DA effect was interpreted by changes in conductance of voltage-gated Ca^{2+} channels (in turtle) (Liu and Lasater, 1994) or it could be related to voltage-gated Na^{+} channels (in goldfish) (Hayashida and Ishida, 2004).

Both D1 and D2 receptors are widely expressed in rat retina (Tran and Dickman, 1992; Wagner et al., 1993; Vuvan et al., 1993; Behrens and Wagner, 1995; Veruki and Wässle, 1996; Bjelke et al., 1996; Veruki, 1997; Nguyen-Legros et al., 1997, 1999; Derouiche and Asan, 1999; Koulen, 1999). Specifically, there are lots of cells in the ganglion cell layer that exhibit staining of both D1 and D2 receptors (Tran and Dickman, 1992; Wagner et al., 1993; Veruki and Wässle, 1996; Bjelke et al., 1996; Veruki, 1997; Nguyen-Legros et al., 1997; Koulen, 1999), and these cells may be ganglion cells or displaced amacrine cells.

In the present work, we tested effects of DA on membrane properties and excitability of rat RGCs in retinal slice preparations, using whole cell patch clamp techniques. Our results suggest that hyperpolarization-activated cation current (I_h), which has been shown to regulate firing of neurons

*Corresponding author. Tel: +86-21-6422-1975; fax: +86-21-5423-7738.
E-mail address: xlyang@fudan.edu.cn (X.-L. Yang).

Abbreviations: DA, dopamine; I_h, hyperpolarization-activated cation current; I_{ins}, instantaneous current; I_{ss}, steady-state current; I_{tail}, tail current; PKA, protein kinase A; RGC, retinal ganglion cell; R_{in}, input resistance.

0306-4522/07$30.00+0.00 © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2007.09.015

and synaptic integration (see Robinson and Siegelbaum, 2003 for review), may be involved in DA effects on rat RGCs.

EXPERIMENTAL PROCEDURES

Retinal slice preparation

Retinal slice preparations were prepared following the procedure described by Euler et al. (1996), with minor modifications. All experiments were performed in accordance with the guidelines of the National Institutes of Health for animal experimentation and those of Fudan University regarding animal use. All efforts were made to minimize the number of animals used and their suffering. Retinas were obtained from Sprague–Dawley rats ranging in age from P18 to P25, with the day of birth denoted as P0. The animals were dark-adapted for at least 4 h before experiments. Following deep anesthesia with pentobarbital sodium, the eyes were enucleated. The posterior eyecups were placed in ice-cold Ringer's for about 1 min and the retinas were then isolated from the pigment epithelium. The isolated retinas were cut into 250-μm-thick slices in ice-cold Ringer's using a manual cutter (ST-20, Narishige, Tokyo, Japan). The slices were first incubated at 34 °C in Ames' solution for 30–45 min, which was bubbled with 95% O_2 and 5% CO_2 to maintain pH at 7.4, and subsequently transferred to a recording chamber, continuously perfused with oxygenated Ringer's (3 ml/min) at 34 °C. All these procedures were performed under dim red illumination.

Whole-cell recording

An Olympus (Tokyo, Japan) BX51WI fixed-stage upright microscope equipped with a 60× water-immersion lens was used to view neurons in the slices under infrared illumination. Recordings were made by using the whole cell configuration of the patch-clamp technique in both current- and voltage-clamp modes with an amplifier (EPC10, Heka Electronics, Lamprecht, Germany). Recording electrodes, pulled from borosilicate glass (BF150-86-10, Sutter Instrument Co., Novato, CA, USA) by using a multi-stage horizontal puller (P97, Sutter Instrument Co.), had a resistance of 6–8 MΩ when filled with the intracellular solution (see below). After seal formation and membrane rupture for whole-cell configuration, the cell being recorded was kept in a stabilized state for at least 8 min before data were collected. Cell membrane potentials were regularly checked throughout the experiment, and only cells with a stable resting potential of −60 mV∼−80 mV were given drug treatments. Sixty percent of the series resistance of the recording electrode was compensated. Fast capacitance current caused by the electrode was always fully cancelled and cell capacitance was partially cancelled by the circuit of the amplifier. Analog signals were filtered at 2 kHz, digitized at 5–10 kHz, and stored on PC hard disk for further analysis. During recordings, the slices were continuously perfused with the oxygen-bubbled extracellular solution at about 34 °C (see below), which was fed in and out of the chamber through inlets by a peristaltic pump (World Precision Instruments, Sarasota, FL, USA) at a rate of 3–5 ml/min.

Solutions and drug application

The extracellular solution with an osmolality of 310 mOsm/kg H_2O contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 15 dextrose, adjusted to pH 7.4 by bubbling with 95% O_2 and 5% CO_2. The intracellular solution for whole-cell recordings consisted of (in mM): 125 K-gluconate, 10 KCl, 10 Hepes, 4 $MgCl_2$, 0.05 $CaCl_2$, 5 EGTA, 4 Na_2-ATP, 0.2 Na-GTP, 8 phosphocreatine, and its osmolarity and pH were adjusted to 285 mOsm/kg H_2O and 7.3 respectively. Chemicals were bath applied in the extracellular solution. Synaptic transmission was blocked by the inclusion of CNQX (10 μM), D-AP-5 (50 μM), bicuculline (10 μM) and strychnine (2 μM) in the extracellular solution. The drugs were stored in frozen stock solution and dissolved in the extracellular or intracellular solution before an experiment. DA and SKF38393 were freshly prepared just before application. When DA solution was made, 0.1% ascorbic acid was added to prevent oxidation. This concentration of ascorbic acid had no effect on physiology of rat RGCs. All DA antagonists were bath applied 8–10 min prior to application of DA agonists. Drugs were all purchased from Sigma Chemicals (St. Louis, MO, USA), unless specified otherwise.

Data processing

Data analysis was performed using Pulsefit 8.52 (Heka), pClampfit 8.0 (Axon Instruments Inc., Foster City, CA, USA), Igor 4.0 (WaveMetrics, Inc., Lake Oswego, USA) and OriginPro 7.0 (OriginLab Corporation, Northampton, USA). Input resistance (R_{in}) of RGCs was determined by the linear fit of the voltage responses to a series of current steps of 400 ms, ranging from −30–30 pA, at a holding potential of −70 mV. To assess I_h, cells were held at approximately −50 mV and hyperpolarized for 2 s in 10 mV increments. I_h was determined as the difference between the steady-state current (I_{ss}) and the instantaneous current (I_{ins}) at each hyperpolarization voltage command. I_{ins} was measured ∼30 ms after the onset of the pulse after the capacitive artifact, whereas I_{ss} was measured at the end of the 2 s step voltage commands.

The steady-state activation curve of I_h was constructed as described in previous reports (Wu and Hablitz, 2005; Vargas and Lucero, 1999). The conductance (g) underlying I_h was determined as $g=I_h/(V-E_{rev})$, where I_h is the difference between I_{ss} and I_{ins} ($I_h=I_{ss}-I_{ins}$) and E_{rev} is the reversal potential of I_h. The conductance for each cell was normalized by G, which was the maximum conductance of I_h at −120 mV, and the data for all cells were then averaged. The averaged g was plotted as a function of voltage, and the data were fitted by a Boltzmann curve of the form: $g/G=[1+\exp((V-V_{1/2})/K)]^{-1}$, where $V_{1/2}$ is the voltage at which g is half-maximum value and K is the slope factor.

All data were expressed as mean±S.E.M. Statistical analysis was performed using paired Student's *t*-test, and $P<0.05$ was considered significant.

RESULTS

Effects of DA on membrane properties and excitability of rat RGCs

Rat RGCs were distinguished from displaced amacrine cells in the ganglion cell layer based on soma diameter and firing characteristics (Protti et al., 1997). The somata of the cells studied, which were located in the proximal border of the ganglion cell layer, were the largest (>15 μm in diameter) in this layer, whereas the somata of displaced amacrine cells in rat are commonly less than 10 μm in diameter (Perry, 1981), having relatively small amplitude action potentials (Boos et al., 1993). To block synaptic transmission, all experiments were performed in the presence of the antagonists for blocking glutamatergic, GABAergic and glycinergic inputs (see Experimental Procedures).

Bath application of 10 μM DA produced a small (2∼3 mV) and reversible depolarization after a 2∼5 min delay in 11 of 14 RGCs tested (Fig. 1A). Addition of TTX (1 μM) to block Na^+ channels and of $CdCl_2$ (200 μM) to block Ca^{2+} channels did not affect the DA-induced depolarization (data not shown), suggesting that this depolarization was independent of Na^+ and Ca^{2+} channels. The average change in membrane potential obtained in the presence of