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SUPPRESSION OF OUTWARD K^+ CURRENTS BY WIN55212-2 IN RAT RETINAL GANGLION CELLS IS INDEPENDENT OF CB1/CB2 RECEPTORS

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Abstract—Cannabinoid CB1 receptor (CB1R) signaling system is extensively distributed in the vertebrate retina. Activation of CB1Rs regulates a variety of functions of retinal neurons through modulating different ion channels. In the present work we studied effects of this receptor signaling on K^+ channels in retinal ganglion cells by patch-clamp techniques. The CB1R agonist WIN55212-2 (WIN) suppressed outward K^+ currents in acutely isolated rat retinal ganglion cells in a dose-dependent manner, with an IC_{50} of 4.7 μM. We further showed that WIN mainly suppressed the tetraethylammonium (TEA)-sensitive K^+ current component. While CB1Rs were expressed in rat retinal ganglion cells, the WIN effect on K^+ currents was not blocked by either AM251/SR141716, specific CB1R antagonists, or AM630, a selective CB2R antagonist. Consistently, cAMP-protein kinase A (PKA) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways were unlikely involved in the WIN-induced suppression of the K^+ currents because both PKA inhibitors H-89/Rp-cAMP and MAPK/ERK1/2 inhibitor U0126 failed to block the WIN effects. WIN-induced suppression of the K^+ currents was not observed when WIN was intracellularly applied. Furthermore, an endogenous ligand of the cannabinoid receptor anandamide, the specific CB1R agonist ACEA and the selective CB2R agonist CB65 also suppressed the K^+ currents, and the effects were not blocked by AM251/SR141716 or AM630 respectively. All these results suggest that the WIN-induced suppression of the outward K^+ currents in rat retinal ganglion cells, thereby regulating the cell excitability, were not through CB1R/CB2R signaling pathways.

Key words: cannabinoid receptor, CB1/CB2 receptor agonist, TEA-sensitive K^+ current, anandamide, patch-clamp.

INTRODUCTION

Endocannabinoid (eCB) signaling is involved in regulating multiple neuronal functions in the central nervous system (CNS), by activating G-protein-coupled CB1 receptors (CB1Rs) (Howlett and Fleming, 1984; Howlett and Mukhopadhyay, 2000; Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001; Chevaleyre et al., 2006; Wang et al., 2006; Galve-Poperh et al., 2007; Hashimotodani et al., 2007). Growing evidence has shown that CB1R signaling is also extensively distributed in the vertebrate retina (Yazulla, 2008; Zabouri et al., 2011). CB1Rs and eCBs, such as anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), as well as the eCB degradative enzyme, fatty acid amide hydrolase (FAAH), are present in a variety of retinal cell populations and in the inner plexiform layer (IPL) (Straiker et al., 1999; Yazulla et al., 1999, 2000; Porcella et al., 2000; Lalonde et al., 2006). These results suggest that eCBs may regulate the functions of retinal neurons, thus being involved in visual information processing conducted by multiple circuits (Straiker et al., 1999; Yazulla et al., 1999, 2000). Activation of CB1Rs modulates various ion channels, particularly voltage-gated K^+ and Ca^{2+} channels in the retina (Yazulla, 2008 for review). In these studies, WIN55212-2 (WIN), a cannabinoid receptor agonist, has been extensively used. Neuronal K^+ channels are key factors in determining the resting membrane potential and modulating the cell excitability (Hille, 2001). In the retina, WIN suppresses K^+ currents in tiger salamander rods and cones (Straiker and Sullivan, 2003), but exhibits a dose-dependent biphasic modulation of K^+ current in goldfish cones (Fan and Yazulla, 2003). It was also reported that WIN inhibited delayed rectifying K^+ channels in goldfish retinal bipolar cells (BCs) (Yazulla et al., 2000). All these effects were mediated by the activation of CB1Rs (Yazulla et al., 2000; Fan and Yazulla, 2003).

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Abbreviations: AC, adenylate cyclase; ACEA, *N*-(2-Chloroethyl)-5*Z*, 8*Z*,11*Z*,14*Z*-eic osatetraenamide; 2-AG, 2-Arachidonoylglycerol; AEA, anandamide; 4-AP, 4-aminopyridine; BC, bipolar cell; CB65, *N*-Cyclohexyl-7-chloro-1-[2-(4-morpholinyl) ethyl]quinolin-4(1*H*)-one-3-carboxamide; CB1R, CB1 receptor; CB2R, CB2 receptor; DMSO, dimethyl sulfoxide; eCB, endocannabinoid; EGTA, ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid; ERK, extracellular signal-regulated kinase; FAAH, fatty acid amide hydrolase; Gb, glybenclamide; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; RITC, rhodamine-B-isothiocyanate; TEA, tetraethylammonium; Δ^9-THC, Δ^9-tetrahydrocannabinol; U0126, 1,4-diamino-2,3-dicyano-1,4-*bis*[2-aminophenylthio]butadiene; WIN, WIN55212-2.

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Retinal ganglion cells, output neurons of the retina, express many types of voltage-gated K^+ channels (Lipton and Tauck, 1987; Ettaiche et al., 2001; Clark et al., 2009; Fohlmeister et al., 2010; Koeberle et al., 2010). While CB1Rs are present in retinal ganglion cells (Straiker et al., 1999; Zabouri et al., 2011), there is no evidence concerning effects of CB1R signaling on the K^+ channels in these cells. In the present work we showed, using whole-cell patch-clamp techniques, that WIN suppressed outward K^+ currents in acutely isolated rat retinal ganglion cells in a dose-dependent manner. We further showed that the WIN effect was not mediated by activating CB1Rs and/or CB2 receptors (CB2Rs).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats, weighing 100–150 g and obtained from SLAC Laboratory Animal Co. Ltd. (Shanghai, China), maintained under a 12-h/12-h light/dark cycle for at least 1 week before they were used for experiments. All experimental procedures dealing with the animals in the present work were in accordance with the National Institute of Health (NIH) guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and the guidelines of the Fudan University on the ethical use of animals. All animal care and procedures in this work were approved by the Institutes of Brain Science, Institute of Neurobiology and the State Key Laboratory of Medical Neurobiology of the Fudan University, Shanghai, China, and all efforts were made to minimize the number of animals used and their suffering.

Retrograde labeling of retinal ganglion cells

Retrograde labeling of retinal ganglion cells was previously described in detail (Zhao et al. 2010; Ji et al., 2011). Briefly, after the rats were deeply anesthetized with 40 mg/ml sodium pentobarbital (0.1 ml/100 g), 4% rhodamine-B-isothiocyanate (RITC) (List Biological Laboratories, Campbell, CA, USA) (2 μl) was injected into the superior colliculus bilaterally (6.0 mm posterior and 2.0 mm lateral to the bregma and 4–5 mm deep from the cortical surface). After a survival period of 5–7 days, retinal ganglion cells were clearly labeled for electrophysiological recording.

Preparation of isolated retinal ganglion cells for electrophysiology

Retinal ganglion cells were acutely dissociated from retinas retrogradely labeled with RITC by enzymatic and mechanical methods as previously described (Zhao et al., 2010; Ji et al., 2011). Isolated retinas were incubated in oxygenated Hank's solution containing the following (in mM): NaCl 137, $NaHCO_3$ 0.5, NaH_2PO_4 1, KCl 3, $CaCl_2$ 2, $MgSO_4$ 1, HEPES 20, sodium pyruvate 1 and glucose 16 adjusted to pH 7.4 with NaOH, and then digested in 1.6 U/ml papain (Worthington Biochemical, Freehold, NJ, USA) containing Hank's solution, supplemented with 0.2 mg/ml L-cysteine and 0.2 mg/ml bovine serum albumin for 30 min at 35.5–36.5 °C. Mechanical dissociation of retinal neurons was conducted using fire-polished Pasteur pipettes and cell suspension was plated onto a culture dish mounted on an inverted microscope (IX 70; Olympus Optical, Tokyo, Japan). RITC-labeled retinal ganglion cells, showing red fluorescence, were chosen for whole-cell patch-clamp recording within 2–3 h after dissociation.

Whole-cell recording

Membrane currents were recorded by whole-cell voltage-clamp techniques (Zhao et al., 2010; Yang et al., 2011; Ji et al., 2012). Patch pipettes used in the present work had a resistance of 2–5 MΩ after filling with an internal solution consisting of (in mM): KCl 140, $NaCl_2$ 9, $MgCl_2$ 1, EGTA 0.2, ATP-Mg 2, GTP-Na 0.25, HEPES 10, and adjusted to pH 7.2 with KOH and to 290–300 mOsm/L. To record voltage-gated K^+ currents, the dissociated cells were superfused continuously with the extracellular solution of the following composition (in mM): NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, and glucose 20, with 0.4 μM tetrodotoxin (TTX) and 100 μM $CdCl_2$, pH adjusted to 7.4 with NaOH and to 290–300 mOsm/L. Whole-cell membrane currents were recorded by a patch amplifier (Axonpatch 200B), Digidata 1322A data acquisition board, and Clampex 8.0 software (Molecular Devices, Foster City, CA, USA) at a sample rate of 10 kHz, filtered at 1 kHz. Fast capacitance was fully canceled and cell capacitance was partially canceled as much as possible by the amplifier circuits. Seventy percent of the series resistance of the recording electrode was compensated. Leakage currents were subtracted on-line using a P/4 subtraction procedure.

Reagents and drug application

WIN, AEA, AM630, AM251, *N*-(2-Chloroethyl)-5*Z*, 8*Z*,11*Z*,14*Z*-eic osatetraenamide (ACEA) and *N*-Cyclohexyl-7-chloro-1-[2-(4-morpholinyl)ethyl]quinolin-4(1*H*)-one-3-carboxamide (CB65) were obtained from Tocris Bioscience (Ellisville, MO, USA), and SR141716 was from Cayman Chemicals (Ann Arbor, Michigan, MI, USA). All the other chemicals were from Sigma (Sigma–Aldrich, Inc., St. Louis, MO, USA). WIN, AEA, AM251, SR141716, ACEA, CB65 and 1,4-diamino-2,3-dicyano-1,4-*bis*[2-aminophenylthio]butadiene (U0126) were first dissolved in dimethyl sulfoxide (DMSO) and then added to the extracellular or internal solution, with the final concentration of DMSO being less than 0.1%. The other chemicals were freshly dissolved in the extracellular solution. Drugs were delivered by a superfusion drug application system (DAD-8VCSP, ALA Scientific Instruments, Westbury, NY, USA), which has eight pressurized 5 ml reservoirs, each with its own control valve to feed fluid through an eight-to-one tubing manifold (500 μm inner diameter; ALA Scientific Instruments). The open/close switch of each valve was manually controlled. Once the valve was open, the solution in the corresponding reservoir was pressure ejected by nitrogen gas along the pipes. With the large