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ACTIVATION OF THE SIGMA RECEPTOR 1 MODULATES AMPA RECEPTOR-MEDIATED LIGHT-EVOKED EXCITATORY POSTSYNAPTIC CURRENTS IN RAT RETINAL GANGLION CELLS

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Abstract—Sigma receptor (σR), a unique receptor family, is classified into three subtypes: σR1, σR2 and σR3. It was previously shown that σR1 activation induced by 1 μM SKF10047 (SKF) suppressed N-methyl-D-aspartate (NMDA) receptor-mediated responses of rat retinal ganglion cells (GCs) and the suppression was mediated by a distinct Ca^{2+}-dependent phospholipase C (PLC)–protein kinase C (PKC) pathway. In the present work, using whole-cell patch-clamp techniques in rat retinal slice preparations, we further demonstrate that SKF of higher dosage (50 μM) significantly suppressed AMPA receptor (AMPAR)-mediated light-evoked excitatory postsynaptic currents (L-EPSCs) of retinal ON-type GCs (ON GCs), and the effect was reversed by the σR1 antagonist BD1047, suggesting the involvement of σR1. The SKF (50 μM) effect was unlikely due to a change in glutamate release from bipolar cells, as suggested by the unaltered paired-pulse ratio (PPR) of AMPAR-mediated EPSCs of ON GCs. SKF (50 μM) did not change L-EPSCs of ON GCs when the G protein inhibitor GDP-β-S or the protein kinase G (PKG) inhibitor KT5823 was intracellularly infused. Calcium imaging further revealed that SKF (50 μM) did not change intracellular calcium concentration in GCs and persisted to suppress L-EPSCs when intracellular calcium was chelated by BAPTA. The SKF (50 μM) effect was intact when protein kinase A (PKA) and phosphatidylinostiol (PI)-PLC signaling pathways were both blocked. We conclude that the SKF (50 μM) effect is Ca^{2+}-independent, PKG-dependent, but not involving PKA, PI-PLC pathways. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: σR1, AMPA receptor, neurotransmission, excitatory postsynaptic current, ganglion cell, retina.

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INTRODUCTION

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For the sigma receptor (σR), a unique brain receptor family, three subtypes (σR1, σR2 and σR3) have been identified (Quirion et al., 1992; Myers et al., 1994). Among them σR1 is involved in regulating a variety of physiological and pathological processes in the CNS, such as neuronal firing, the activity of various ion channels, neurotransmitter release and neuroprotection (see Maurice and Su, 2009 for a review). Expression of σR1 in the retina has been described (Ola et al., 2001; Liu et al., 2010). Specifically, σR1 is abundantly expressed in ganglion cells (GCs) (Liu et al., 2010). We previously showed that the activation of σR1 by SKF10047 (SKF), a σR1 agonist, at a relatively lower concentration (1 μM) suppressed N-methyl-D-aspartate receptor (NMDAR)-mediated currents and NMDAR-mediated light-evoked excitatory postsynaptic currents (L-EPSCs) in rat GCs through a distinct Ca^{2+}-dependent phospholipase C–protein kinase C (PLC–PKC) pathway, but SKF at this low concentration did not show effects on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated L-EPSCs (Zhang et al., 2011a,b). Recent studies have demonstrated, however, that SKF of higher concentrations are required for σR1-mediated modulation of the activity of several ion channels. In the nucleus tractus solitarius of guinea pig, for instance, 300 μM SKF is needed to significantly inhibit AMPAR-mediated electrically evoked EPSCs (E-EPSCs) by activating σR1 (Ohi et al., 2011). In a variety of cells 100–300 μM SKF is needed to suppress K^+ currents (Wilke et al., 1999a,b; Aydar et al., 2002). It is of interest, therefore, to explore whether SKF of relatively higher concentrations could modulate AMPAR-mediated L-EPSCs of GCs through a pre- and/or post-synaptic actions.

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Abbreviations: AMPAR, a-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; BiP, binding immunoglobulin protein; BisIV, bisindolylmaleimide IV; $[Ca^{2+}]_i$, intracellular calcium concentration; DMSO, dimethyl sulfoxide; E-EPSC, electrically evoked excitatory postsynaptic current; EGTA, ethylene glycol tetraacetic acid; GC, ganglion cell; Gö6976, 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KT5720, (9S,10S,12R)-2,3,9,10,11,12-Hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid hexyl ester; KT5823, (9S,10R,12R)-2,3,9,10,11,12-Hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo [3,4-i][1,6]benzodiazocine-10-carboxylic acid methyl ester; L-EPSC, light-evoked excitatory postsynaptic current; MK-801, dizocilpine; NMDAR, N-methyl-D-aspartate receptor; ON GCs, ON-type ganglion cell; PI-PLC, phosphatidylinositol-specific phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; PPR, paired-pulse ratio; SKF, SKF10047; TTX, tetrodotoxin; U73122, 1-[6-[((17β)-3-Methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione; σR, sigma receptor.

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In the present study, using whole-cell patch-clamp recording techniques we found that SKF (50 μM)-induced activation of σR1 led to a considerable suppression of AMPAR-mediated L-EPSCs of ON-type GCs (ON GCs). We further showed that the effect, unlike the SKF effect on NMDAR-mediated responses, was Ca^{2+}-independent, but protein kinase G (PKG)-dependent.

EXPERIMENTAL PROCEDURES

Male Sprague–Dawley rats (14–20 days of age) were used in this study. The animals were housed in the Fudan University animal facility on a 12:12-h light–dark cycle. Food and water were available *ad libitum.* Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996 and the regulations of Fudan University regarding the ethical use of animals. All efforts were made to minimize the number of animals used and their suffering.

Retinal slice preparations

Retinal slices were prepared following previously described procedures (Zhang et al., 2011a,b). Briefly, after being dark adapted for 3–4 h, rats were deeply anesthetised with 25% urethane (8 ml/kg). Eyes were immediately enucleated under dim red illumination and retinas were removed. The isolated retinas were cut into 200-μm-thick slices in Ringer's using a manual cutter (ST-20, Narishige, Tokyo, Japan). The slices were transferred into a recording chamber with the cut side up and held mechanically in place by a grid of parallel nylon strings glued onto a U-shape frame of platinum wire, and then viewed through a fixed-stage upright microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a 60× water-immersion ceramic objective and DIC optics. For experiments dealing with light responses of GCs, retinal slices were prepared under dim red illumination and kept in the dark. All other experiments were conducted under room illumination. GCs were distinguished from displaced amacrine cells in the ganglion cell layer based on soma diameters (> 15 μm) and physiological criteria (Tian et al., 1998; Chen and Yang, 2007; Zhang et al., 2011a; Liu et al., 2015). Subtypes of GCs were identified based on their responses to light steps (Margolis and Detwiler, 2007; Liu et al., 2013). ON GCs that responded only to the light onset with an inward current were selected for further experiments.

Whole-cell patch-clamp recording

Retinal slices were perfused continuously with oxygenated and carbogen-bubbled Ringer's, which contained (in mM) NaCl (125), KCl (2.5), $CaCl_2$ (2), $MgCl_2$ (1), NaH_2PO_4 (1.25), $NaHCO_3$ (25), glucose (15) and was warmed up to 32 °C by an inline heater (TC-324B, Warner, Hamden, CT, USA). Pipettes of 8–10 MΩ resistance filled with a solution containing (in mM) $CsCH_3SO_3$ (115), $CsCl_2$ (20), HEPES (10), $MgCl_2$ (2.5), EGTA (0.6), MgATP (4), Na_3GTP (0.4) and sodium phosphocreatine (10), pH 7.2 adjusted with CsOH were used for recording whole-cell light-evoked AMPAR-mediated EPSCs of ON GCs. Pipettes, mounted on a motor-driven micromanipulator (MP-285, Sutter, Novato, CA, USA), were connected to an EPC10 patch-clamp amplifier (HEKA, Lambrecht, Germany). During the experiments GCs were clamped at −70 mV and a mixture of synaptic blockers that contained MK-801 (15 μM), bicuculline (10 μM), strychnine (10 μM) and tetrodotoxin (TTX) (0.5 μM) were added to the extracellular solution to block NMDA receptor-, $GABA_A$ receptor- and glycine receptor-mediated components and sodium currents, respectively. Drug-containing Ringer's was administrated in bath medium through an inlet by gravity. Fast capacitance was fully canceled and cell capacitance was partially canceled by the circuits of the amplifier as much as possible. Sixty percent of the series resistance of the recording electrodes was compensated. Data were collected at a sampling rate of 10 kHz, filtered at 2 kHz and then stored for further analysis.

As previously described (Zhang et al., 2011b), light stimuli were generated using an LED (λ = 500 nm). Voltage steps were used to illuminate the LED mounted on the bottom of the condenser in an Olympus BX51WI microscope. Full-field illumination was focused orthogonally to the main axis of the photoreceptors. Light stimuli (7.5×10^{11}–1.4×10^{12} photons cm^{-2} s^{-1}) were given for 3 s at 60 s intervals. For E-EPSC recordings of ON GCs, bipolar cells at the inner nuclear layer (INL) was stimulated by focal extracellular simulation (duration, 0.1 ms; intensity, 2–40 μA) with a glass electrode filled with Ringer's (Wei et al., 2012). Paired-pulse ratio (PPR, ratio of second to first pulse) of E-EPSCs, which was measured at an interpulse interval of 1 s (von Gersdorff et al., 1998; Wei et al., 2012), was used to determine the synaptic responses of bipolar-ganglion cell synapses.

Preparation of isolated GCs

The detailed procedures for making isolated GCs refer to a previous work (Chen et al., 2004) with minor modifications. Retinas were quickly removed and incubated in oxygenated Hanks' solution, containing (in mM): NaCl (137), NaH2PO4 (1), $NaHCO_3$ (0.5), KCl (3), $CaCl_2$ (2), $MgSO_4$ (1), HEPES (20), sodium pyruvate (1) and glucose (16), adjusted to pH 7.4 with NaOH. The retinas were then digested in 5–7 mg/ml papain (Sigma–Aldrich, St. Louis, MO, USA) containing Hanks' solution, supplemented with L-cysteine and bovine serum albumin (0.75 mg/ml for each) for 25–30 min at 33.5–34.5 °C. The solution was continuously bubbled with 100% O_2. After several rinses in Hanks' solution, the retinas were mechanically dissociated by gently triturating with fire-polished Pasteur pipettes and cell suspension was plated onto a culture dish mounted on an inverted microscope (IX 70, Olympus). The cells with large somata (> 15 μm) were selected for calcium-imaging experiments.

UNCORRECTED PROOF

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