Original Paper

Sigma Receptor 1 Is Preferentially Involved in Modulation of N-Methyl-D-Aspartate Receptor-Mediated Light-Evoked Excitatory Postsynaptic Currents in Rat Retinal Ganglion Cells

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Abstract

Using patch-clamp whole-cell recording, we investigated how activation of the sigma receptor 1 (σR1) modulates light-evoked excitatory postsynaptic currents (eEPSCs) of ganglion cells (GCs) in rat retinal slice preparations. Bath application of the σR1 agonist SKF10047 (SKF) suppressed N-methyl-D-aspartate (NMDA) receptor-mediated eEPSCs at different holding potentials in ON, OFF and ON-OFF GCs, and the effects were blocked when the preparations were pre-incubated with the σR1 antagonist BD1047. In contrast, SKF had no effects on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated eEPSCs of these GCs. Furthermore, application of SKF did not affect AMPA receptor-mediated miniature EPSCs of GCs, suggesting that activation of σR1 did not change the release of glutamate from bipolar cells. These results suggest that σR1 may be involved in the regulation of output signaling of GCs by preferentially modulating NMDA receptor-mediated eEPSCs of these retinal neurons.

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Introduction

Sigma receptor (σR) is a distinct pharmacological entity, representing unique nonopiate, nonphencyclidine binding sites in mammalian nervous systems [1]. σR was originally classified into three subtypes (σR1, σR2 and σR3) [2, 3], but σR3 may be a subclass of the brain histamine H_1 receptor [4]. σR1 has been implicated in various physiological and pathological processes in the central nervous system [for a review, see 5]. In the mammalian retina, σR1 mRNA and protein are expressed in neural elements [6, 7]. Specifically, in the rat retina it was previously shown that σR1 is present in horizontal cells, several subtypes of amacrine cells and almost all ganglion cells (GCs) [6]. Much work has demonstrated the efficacy of σR1 ligands in the protection of retinal neurons [8–12]. For instance, the σR1 agonist Pre-084 inhibits the expression of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which mediates the toxic effect of amyloid β [11], and σR1 agonists antagonize the excitotoxicity induced by glutamine/N-methyl-D-aspartate (NMDA) in GCs [8–10, 12]. Furthermore, our recent study suggests a neuromodulatory role of σR1 in the retina by showing that activation of this receptor suppresses NMDA receptor-mediated currents of pharmacological-

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ly isolated rat GCs [13]. Nevertheless, whether and how σR1 is involved in signal processing of GCs is still largely unknown.

In the present work, using patch-clamp techniques, we found that the σR1 agonist SKF10047 (SKF) suppressed NMDA receptor-, but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-, mediated light-evoked excitatory postsynaptic currents (eEPSCs) of ON, OFF and ON-OFF GCs. Furthermore, SKF did not influence miniature EPSCs (mEPSCs) of GCs, suggesting that the effect of SKF was mediated by a direct action of this agonist on σR1 expressed on rat GCs.

Materials and Methods

Retinal Slice Preparation and Whole Cell Patch-Clamp Recording

Retinas were prepared from Sprague-Dawley rats ranging in age from P14 to P20, with the day of birth denoted as P0. All efforts were made to minimize the number of animals used and their pain and discomfort in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines of Fudan University on the ethical use of animals. Retinal slices were prepared following the procedures reported previously [14] with minor modifications. The eyes were enucleated following deep anesthesia with 0.1–0.2 ml urethane (25 mg/ml). The posterior eyecups were placed in ice-cold Ringer's for about 1 min and retinas were then isolated from the sclera. 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, then viewed through a fixed-stage upright microscope (BX51WI; Olympus, Japan) equipped with a 60× water-immersion ceramic objective and DIC optics. In light stimulation experiments, rats were dark-adapted for 3 h prior to an experiment and retinal slices were prepared under dim red illumination. Other experiments were conducted under room illumination.

Unless otherwise declared, 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$_2$PO$_4$ 1.25, NaHCO$_3$ 25, glucose 15, and was warmed up to 32°C by an inline heater (TC-324B; Warner, USA).

Whole-cell membrane currents of GCs were recorded with pipettes of 8–10 MΩ resistance in voltage-clamp modes filled with a solution containing (in mM) CsCH$_3$SO$_3$ 120, TEA-Cl 10, CaCl$_2$ 0.1, MgCl$_2$ 4, EGTA 1, HEPES 10, ATP 3, GTP 0.5, and creatine 12, pH 7.2 adjusted with CsOH. Drug-containing Ringer's was administrated in bath medium through an inlet by gravity. Pipettes were mounted on a motor-driven micromanipulator (MP-285; Sutter, USA), and connected to an EPC10 patch-clamp amplifier (Heka, Germany) for light stimulation experiments or an Axon 700B amplifier (Axon, USA) for recording mEPSCs. Data were acquired at a sampling rate of 5 kHz, filtered at 2 kHz and then stored for further analysis. mEPSCs were detected by pClamp

10 (Axon, USA) and analyzed by Mini Analysis Program (Synaptosoft, USA). Events detection was done automatically and occurred when the recorded current showed a rapid excursion from the baseline and reached a preset current threshold. The events recorded during a selected period of 120 s were averaged for subsequent quantitative analyses.

Light Stimulation

As previously described [15], voltage steps were used to illuminate an 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 of 1 μW/cm^2 were given for 3 s at 30-second intervals. eEPSCs of GCs were examined at holding potentials between –80 and +40 mV in 20-mV steps.

Identification of GC Subtypes

Subtypes of GCs were identified based on their responses to light steps [16, 17]. An ON GC responded only to light onset with an inward current, whereas an OFF GC produced an inward current only in response to light offset, and an ON-OFF GC responded to both light onset and offset.

Chemicals

All chemicals were obtained from Sigma except D-AP5, SKF, NBQX (Tocris). The drugs were stored in frozen stock solution and dissolved in the solution before an experiment. All the lipophilic drugs, such as NBQX, were first dissolved in dimethyl sulfoxide (DMSO) and then added to Ringer's. The final concentration of DMSO was <0.1% with no effect on the currents of GCs obtained by whole cell recording.
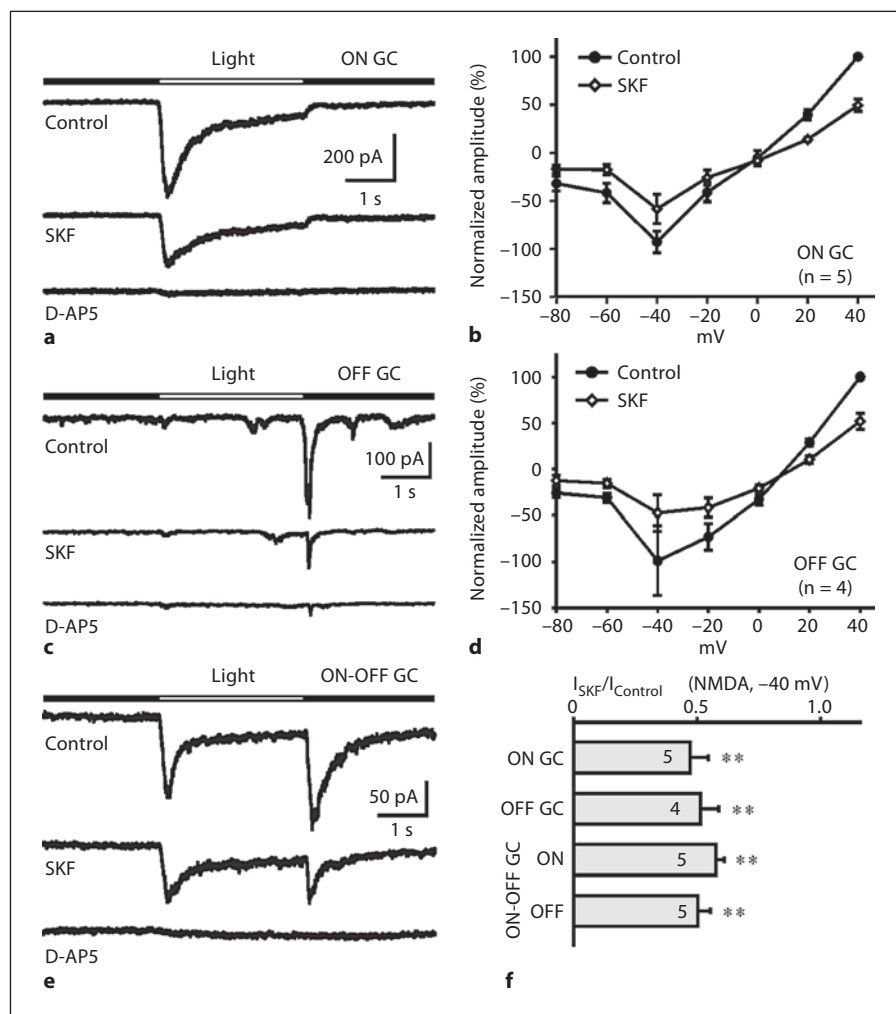
Statistical Analysis

All data, obtained in cells which were mostly from different retinas, were pooled and presented as mean ± SEM. Paired Student's t test was used, with values of p < 0.05 being considered statistically significant.

Results

SKF Suppresses NMDA Receptor-Mediated eEPSCs of GCs

NMDA receptor-mediated eEPSCs of GCs were pharmacologically isolated by adding the AMPA receptor antagonist NBQX (10 μM), antagonists of GABA$_A$ and glycine receptors bicuculline (10 μM), strychnine (10 μM), and the sodium channel blocker TTX (0.5 μM) to bath Ringer's. In the presence of these antagonists, eEPSCs recorded from ON (a), OFF (c) and ON-OFF (e) GCs, all clamped at –40 mV, are respectively shown in figure 1a, c, and e. The ON GC responded to the light onset, but not to the offset, with an inward current, and the current, as expected, was almost completely suppressed by the NMDA receptor antagonist D-AP5 (50 μM) (fig. 1a). Bath application of 1 μM SKF significantly reduced the eEPSC

Fig. 1. Activation of σR1 suppresses NMDA receptor-mediated eEPSCs in different types of GCs. **a**, **c**, **e** EPSCs (upper traces) elicited by a 3-second, full-field light stimulus at an intensity of 1 μW/μm² from an ON GC (**a**), OFF GC (**c**) and ON-OFF GC (**e**), all clamped at −40 mV, were suppressed by 1 μM SKF (middle traces). All the currents were completely suppressed by 50 μM D-AP5 (lower traces). **b**, **d** eEPSCs from ON GCs (**b**) and OFF GCs (**d**) exhibited the J-shaped current-voltage (I–V) relationships that were characteristic of NMDA currents in normal Ringer's (control). SKF of 1 μM reduced the eEPSCs at all holding potentials tested, as shown by the I–V curves of eEPSCs obtained in the presence of 1 μM SKF. Data points for each cell were normalized to the control eEPSC recorded at +40 mV and then averaged. **f** Bar chart summarizing the effects of SKF (1 μM) on NMDA receptor-mediated eEPSCs from ON GCs, OFF GCs and ON-OFF GCs clamped at −40 mV. Cell numbers are indicated inside the bars. ** p < 0.01.

amplitude (from −407.5 to −206.3 pA). Similar reductions were observed in four other ON GCs. On average, following the perfusion of 1 μM SKF, the eEPSCs were reduced to 47.28 ± 7.26% of control (p < 0.01) when the cells were clamped at −40 mV. The relationships of the eEPSCs (I) versus holding potentials (V) (I–V) of ON GCs (n = 5) obtained before and during SKF application are shown in figure 1b, and both of them showed a prominent J-shape, indicating the voltage-dependent block of NMDA receptor channels by external Mg²⁺. It was clear from figure 1b that reductions in eEPSC amplitudes were observed at all holding potentials tested, ranging from −80 to +40 mV.

The effect of SKF on eEPSCs of OFF GCs, which produced an inward current only in response to light offset, was similar. Figure 1c and d show the change in the eEPSC of an OFF GC and that in the average I–V relationship of GC eEPSCs (n = 4) after SKF application, respec-

tively. SKF of 1 μM produced considerable decreases in eEPSC amplitudes at different holding potentials. At −40 mV, the eEPSCs were reduced to 48.69 ± 7.35% of control (p < 0.01) by SKF perfusion.

Similarly, application of 1 μM SKF greatly suppressed both ON and OFF components of the ON-OFF GC eEPSC (fig. 1e), with an average suppression of 42.43 ± 3.28% for ON components and of 49.60 ± 4.83% for OFF components respectively (n = 5, p < 0.01, V_{hold} = −40 mV). The reductions of eEPSCs obtained at −40 mV in the ON, OFF and ON-OFF GCs due to application of 1 μM SKF are shown in figure 1f for comparison.

SKF-induced suppression of eEPSCs could be blocked by BD1047, a σR1 antagonist. We first perfused the preparations with 10 μM BD1047 for 8 min and then observed effects of 1 μM SKF on NMDA receptor-mediated eEPSCs of ON, OFF and ON-OFF GCs clamped at −40

Fig. 2. BD1047 blocks the suppression effects of SKF on NMDA receptor-mediated eEPSCs of GCs, clamped at –40 mV. With pre-incubation of 10 µM BD1047, 1 µM SKF failed to suppress the eEPSCs of an ON (**a**), OFF (**b**) and ON-OFF GC (**c**).
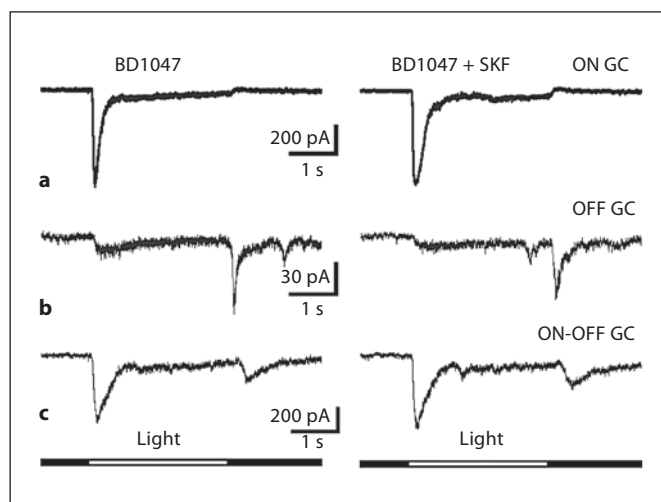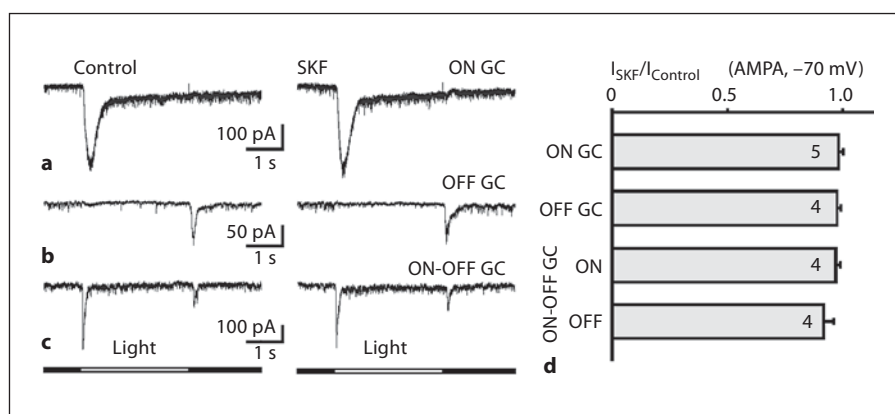

Fig. 3. SKF does not change AMPA receptor-mediated eEPSCs in different types of GCs clamped at –70 mV. **a–c** 1 µM SKF had no effects on the eEPSCs of an ON (**a**), OFF (**b**) and ON-OFF GC (**c**). **d** Bar chart summarizing the effects of SKF (1 µM) on AMPA receptor-mediated eEPSCs from ON GCs, OFF GCs, and ON-OFF GCs. Cell numbers are indicated inside the bars.

mV. As shown in figure 2a–c, following pre-incubation with 10 µM BD1047, perfusion of 1 µM SKF failed to suppress the eEPSCs, and the eEPSCs recorded in the presence of 1 µM SKF were 98.89 ± 4.67% of control (n = 10, p > 0.05) for ON components and 93.10 ± 4.41% of control (n = 5, p > 0.05) for OFF components. Here, data from ON, OFF and ON-OFF GCs were pooled for statistical analysis.

SKF Has No Effects on AMPA Receptor-Mediated GC eEPSCs

There is evidence that AMPA receptors mainly mediate non-NMDA excitatory synaptic responses of both ON and OFF GCs [16, 18]. Effects of SKF on AMPA receptor-mediated eEPSCs of GCs were further tested. AMPA receptor-mediated eEPSCs of GCs were pharmacologically isolated by adding D-AP5 (50 µM), bicuculline (10 µM), strychnine (10 µM), and TTX (0.5 µM) to bath Ringer's. In the presence of these antagonists eEPSCs recorded

from ON, OFF and ON-OFF GCs, clamped at –70 mV, are respectively shown in the left panel of figure 3a–c. Unlike NMDA receptor-mediated eEPSCs, the I–V curves of AMPA receptor-mediated eEPSCs became linear [16] (data not shown). As shown in the right panel of figure 3a–c, bath application of 1 µM SKF hardly changed the AMPA receptor-mediated eEPSCs of these cells. The relative eEPSC amplitudes were 98.20 ± 2.15% of control (n = 5, p > 0.05) for ON GCs; 97.53 ± 1.79% of control (n = 4, p > 0.05) for OFF GCs; 97.09 ± 1.92% of control (n = 4, p > 0.05) for ON components, and 91.77 ± 4.44% of control (n = 4, p > 0.05) for OFF components of ON-OFF GCs, respectively (fig. 3d).

mEPSCs of GCs Were Not Affected by SKF

To test whether the suppression effect of SKF might be a consequence of possible changes in glutamate release of bipolar cells, which are presynaptic to GCs, we studied effects of SKF on the mEPSC of GCs that is a reflection
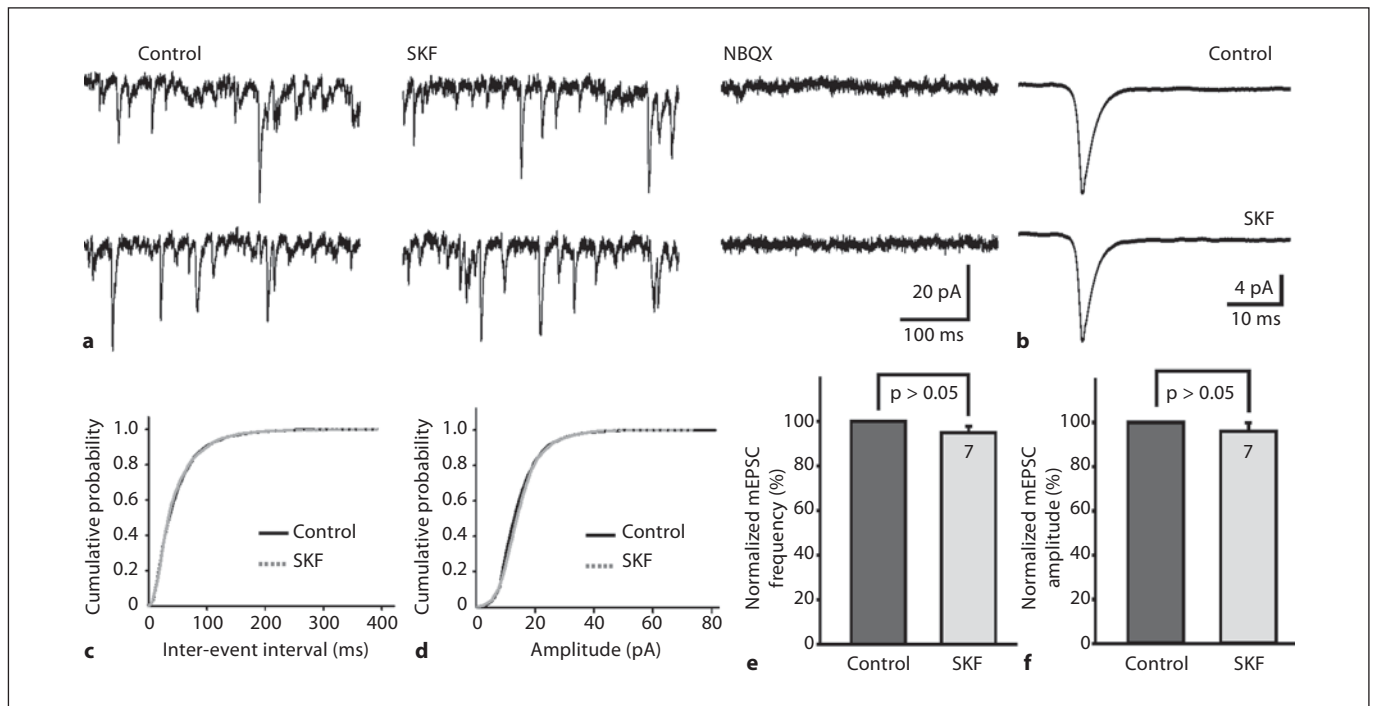
Fig. 4. mEPSCs of GCs are not affected by SKF. **a** Representative mEPSC recordings of a GC in normal Ringer's (left two traces), in the presence of 1 μM SKF (middle two traces) or 10 μM NBQX (right two traces), respectively. Note that the mEPSCs were blocked by NBQX and SKF had no effect on the mEPSCs. **b** Averaged mEPSCs waveforms derived from events recorded during 120 s in normal Ringer's (control) and in the presence of 1 μM SKF from the same cell as shown in **a**, demonstrating no change in kinetics of mEPSCs following SKF application. **c, d** Cumulative distributions for inter-event interval (**c**) and amplitude (**d**) of mEPSCs obtained from the cell shown in **a**. No changes in these curves were found. **e, f** Bar graphs summarizing the effects of 1 μM SKF on frequencies (**e**) and amplitudes (**f**) of GC mEPSCs. All data for each cell were normalized to control and then averaged.

of the spontaneous release of glutamate from bipolar cells. For these experiments, bicuculline (10 μM), strychnine (10 μM) and TTX (0.5 μM) were added to Ringer's to isolate mEPSCs of GCs. Figure 4a shows the representative mEPSC recording from a GC voltage-clamped at –70 mV (left two traces). The currents were completely blocked by application of 10 μM NBQX (right two traces), indicating that they were solely mediated by AMPA receptors. Application of SKF (1 μM) did not significantly change the mEPSCs (fig. 4a, middle two traces). Additionally, as shown in figure 4b, the average mEPSC derived from the events recorded during 120 s in 1 μM SKF-containing Ringer's (lower trace) was unchanged in kinetics after SKF perfusion, as compared to the control response (upper trace). Furthermore, the cumulative probability distribution curves of inter-event intervals and amplitudes of these events recorded in the presence of 1 μM SKF did not show any shift, as compared to those in normal Ringer's (control), indicating little change in frequency and amplitude (fig. 4c, d). Similar results were

obtained in six other GCs. Neither average frequency nor amplitude of mEPSCs were significantly changed in the presence of SKF (98.36 ± 4.17% of control for frequency and 105.54 ± 5.01% of control for amplitude, n = 5, p > 0.05) (fig. 4e, f).

Discussion

Three types of ionotropic glutamate receptor subtypes, NMDA, AMPA and kainite receptors, are all expressed on GCs [19–24]. Since NMDA receptors may be located extrasynaptically and activated only by the concomitant release of many synaptic vesicles [25], they are thought to contribute, along with AMPA receptors, mainly to light-/electrically evoked EPSCs of these cells [16, 18], but much less to spontaneous EPSCs [25]. Meanwhile, kainite receptors contribute little direct synaptic current to eEPSCs [18]. In this work we demonstrated that SKF preferentially suppressed NMDA receptor-, but not

AMPA receptor-, mediated eEPSCs of rat ON, OFF and ON-OFF GCs. This is similar to that observed in mouse cultured hippocampal pyramidal neurons, in which σ binding site ligands attenuated NMDA, but not AMPA, evoked currents [26]. This effect of SKF could be blocked by application of BD-1047, suggesting the involvement of σR1. It is likely that σR1 is indeed involved in photic signaling of rat GCs. It is of interest to note that in primate GCs the blockade of NMDA receptors tends to result in a more transient light response of these cells [27], which could be mainly mediated by AMPA receptors [16]. It therefore implies that activation of σR1 may change the kinetics of light responses of rat GCs by preferentially suppressing NMDA receptor-mediated eEPSCs.

Under our experimental conditions, mEPSCs of rat GCs that are mainly produced by spontaneous glutamate release from bipolar cells could be completely suppressed by NBQX (fig. 4a), suggesting that these events were exclusively mediated by AMPA receptors. It has been documented that spontaneous EPSCs of GCs are largely contributed by AMPA receptors [28–30]. While it has been reported that the activation of σR1 increases the frequency of mEPSCs of immature hippocampal neurons [31, 32] and of prelimbic cortical neurons [33], the present work showed that application of SKF affected neither frequency nor amplitude of the mEPSC of rat GCs, meaning no effects of SKF on spontaneous glutamate release of bipolar cells. This may be due to the fact that all rat bipolar cells do not express σR1, as revealed by a recent immunocytochemical study [6]. It seems likely that the SKF-induced suppression of NMDA receptor-mediated eEPSCs may be a consequence of SKF-induced activation of σR1 in GCs. Actually, in pharmacologically isolated rat GCs, we recently reported that activation of σR1 by SKF or PRE-084 suppressed NMDA receptor-mediated current responses [13].

Although endogenous ligands for σR1 are not yet clearly identified, it is generally thought that neurosteroids may be the most plausible candidates [34, 35]. In the retina, neurosteroids are synthesized by specific types of retinal cells, and metabolized via the blood-circulating system [36]. Moreover, there are several studies reporting that neurosteroids protect retinal tissue against ischemia/reperfusion damage, in which σR1 may be involved [37, 38]. It has also been reported that some neurosteroids significantly affect NMDA receptor-mediated responses in GCs [39] and modulate retinal GABAergic neurotransmission and electroretinographic activity [40]. What types of retinal neurons may secrete neurosteroids and how these steroids act on σR1 expressed on GCs may be worthwhile exploring further.

Acknowledgements

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