Olfactory Nerve Stimulation-Evoked mGluR1 Slow Potentials, Oscillations, and Calcium Signaling in Mouse Olfactory Bulb Mitral Cells

Q. Yuan and T. Knöpfel

Laboratory for Neuronal Circuit Dynamics, RIKEN Brain Science Institute, Saitama, Japan

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Yuan, Q. and T. Knöpfel. Olfactory nerve stimulation-evoked mGluR1 slow potentials, oscillations, and calcium signaling in mouse olfactory bulb mitral cells. J Neurophysiol 95: 3097–3104, 2006. First published February 8, 2006; doi:10.1152/jn.00001.2006. Fast synaptic transmission between olfactory receptor neurons and mitral cells (MCs) is mediated through AMPA and NMDA ionotropic glutamate receptors. MCs also express high levels of metabotropic glutamate receptor 1 (mGluR1) whose functional significance is less understood. Here we characterized a slow mGluR1-mediated potential that was evoked by high-frequency (100-Hz) olfactory nerve (ON) stimulation in the presence of NBQX and d-APV, blockers of ionotropic glutamate receptors, and that was associated with a local Ca2+ transient in the MC dendritic tuft. High-frequency ON stimulation in the presence of NBQX and d-APV also evoked a slow, nearly 2-Hz oscillation of MC membrane potential that was abolished by the mGluR1 antagonist LY367385 (50 μM). Both mGluR slow potential and slow oscillation persisted in the presence of gabazine (10 μM), a GABA_A receptor antagonist, and intracellular QX-314 (10 mM), a Na+ channel blocker. In contrast to a slow mGluR1 potential in cerebellar Purkinje neurons, the MC mGluR1 potential was not depressed by SKF96365 (≤250 μM) and thus is likely not mediated by TRPC1 cation channels. Imaging with the Na+ indicator SBFI revealed a Na+ transient in the MC dendrite accompanying the mGluR1 slow potential. We conclude that the MC mGluR1 potential triggered by glutamate released from the ON supports oscillations and synchronizations of MCs associated within one glomerulus.

INTRODUCTION

Mitral cells (MCs) of the olfactory bulb (OB) express high levels of the metabotropic glutamate receptor 1 (mGluR1) (Sahara et al. 2001; Shigemoto et al. 1992; Van den Pol 1995). In principle these receptors can be activated by glutamate that is released from either olfactory nerve (ON) terminals or MC dendrites (Aroniadou-Anderjaska et al. 1999b; Ennis et al. 1996; Isaacsen 1999; Isaacsen and Strowbridge 1998; Mutoh et al. 2005; Schoppa and Westbrook 2001; Tremblay and Westbrook 1990). The physiological conditions under which MC mGluR1s are activated are only partially understood, as are the physiological responses and mechanisms caused by mGluR1 activity in MCs.

Previous studies indicated that single-shock ON stimulation causes only a modest activation of MC mGluR1s (De Saint Jan and Westbrook 2005; Ennis et al. 2006; Yuan and Knöpfel 2005). However, ON stimulation can cause a slow mGluR1 potential when glutamate transporters are pharmacologically blocked (De Saint Jan and Westbrook 2005; Ennis et al. 2006). It is also known that mGluR1 activation under physiological conditions (i.e., with intact glutamate transporter activity) exerts a tonic increase of mitral cell excitability (Heinbockel et al. 2004) and is involved in ON–MC long-term depression (Mutoh et al. 2005).

Slow excitatory postsynaptic potentials (EPSPs) that are mediated by mGluR1 are extensively characterized in cerebellar Purkinje neurons (PNs) where they can be induced by brief tetanic stimulation of parallel fibers (Batchelor and Garthwaite 1997; Batchelor et al. 1994, 1997; Kim et al. 2003; Reichelt and Knöpfel 2002; Staub et al. 1992; Tempia et al. 1998, 2001). Induction of the PN mGluR1 EPSP is facilitated by blockers of glutamate transporters (Brasno and Otis 2001; Reichelt and Knöpfel 2002) and elevation of intracellular Ca2+ ([Ca2+]i) concentration (Batchelor and Garthwaite 1997). Furthermore, it has been reported that the PN mGluR1 EPSP is depressed by SKF96365 and mediated by TRPC1 cation channels (Kim et al. 2003). It is not known whether the MC mGluR1 potential is also mediated by SKF96365-sensitive cation channels and/or potentiated by elevated [Ca2+]i.

The present experiments were designed to address these issues. We found that, in the presence of ionotropic glutamate receptor blockers, brief tetanic stimulation of the ON induced an mGluR1 EPSP and a Ca2+ transient that was confined to a portion of the MC dendrite. We discovered that the MC mGluR1 EPSP could trigger slow (~2 Hz) oscillations of the MC membrane potential in the presence of ionotropic glutamate receptor blockers. The MC mGluR1 EPSP and the slow oscillations were not affected by the γ-aminobutyric acid type A (GABA_A) receptor antagonist gabazine or by blocking intracellular QX-314-sensitive Na+ channels. The mGluR1-mediated responses in MCs differ from the PN mGluR1 EPSP in their lack of sensitivity to SKF96365 and in their lack of potentiation by elevated [Ca2+]i.

METHODS

Slice preparation and electrophysiology

Horizontal slices (300 μm) of olfactory bulbs were obtained from 18- to 25-day-old ICR mice of both sexes. Slices were cut with a Vibroslicer (VT 1000S, Leica) in ice-cold artificial cerebrospinal fluid (ACSF) then recovered at 32–35°C for 0.5 h and afterward at room temperature (23–25°C). Slices were then transferred into a recording chamber and perfused with ACSF containing (in mM): 118 NaCl, 25 NaHCO3, 1 NaH2PO4, 3 KCl, 1 MgCl2, 2 CaCl2, and 10 glucose, equilibrated with 95% O2-5% CO2. Experiments were performed at room temperature (23–25°C) or 33–37°C. For electrophysiological preparations, slices were placed on a glass chopping dish and the ON was stimulated by current pulses (300 μA, 0.1 ms, 3 Hz) presented to an area of 200–300 μm2 around the MC body using a tungsten electrode (20–30 MΩ).

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and fluorescence recordings, slices were placed in an immersion-type perfusion chamber mounted on the stage of an upright microscope (Nikon, E600FN) and visualized using Nikon 63 × water immersion lenses (NA = 0.9). The procedures had approval from the Animal Experiments Committee of the RIKEN Brain Science Institute and were done in accordance with National Institutes of Health guidelines.

Patch-clamp recordings were carried out in whole cell configuration. Glass pipettes (resistance: 3.5–5 MΩ) were pulled from borosilicate glass using a two-stage vertical puller (Narishige, Tokyo, Japan). Pipettes contained in (mM): 0.2 Oregon Green BAPTA-1 or 1 SBFI (tetraammonium salt), 120 K-glucuronate, 3.48 MgCl₂, 9 KCl, 10 KOH, 4 NaCl, 10 HEPES, 17.5 sucrose, 0.4 Na₂ATP, and 0.4 Na₂GTP, pH 7.25. Some cells were patched with internal solution containing N-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314, 10 mM). Whole cell patch-clamp recordings were made from the somata of MCs using an Axonpatch 200B apparatus (Axon Instruments, Sunnyvale, CA). MCs were identified and selected under differential interference contrast visual guide. Seals were routinely monitored by differential interference contrast imaging with a 10× objective. Seals were considered adequately formed when 300-mV differences were sustained for at least 5 min. Cells that had a resting membrane potential more negative than −45 mV at zero holding current and without correction for junction potentials were selected for recording. A holding current of ≤400 pA was used to hold the membrane potential at rest between −60 and −65 mV. Backpropagating action potentials (APs) were generated by current injection (0.7–2 nA, 2 ms for single or 4 APs at 40 Hz; 0.5–1.2 nA for 500-ms trains of APs) through the patch pipette. AP size and shape were compared to confirm a stable state of cells throughout experiments. The EPSPs were elicited by extracellular stimulation of the olfactory nerve using a glass pipette (0.8–1 MΩ) containing normal ACSF solution. The electrode was positioned, under visual control, on a bundle of presynaptic axons just outside the imaged glomerulus.

Drugs applied in the bath were made from aqueous stock solutions. To isolate mGluR responses, the ACSF contained 50 mM d-amino-phosphonovaleric acid [d-APV, an N-methyl-d-aspartate (NMDA) receptor antagonist] and 40 mM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo(f)quinolinoxaline-7-sulfonamide [NBQX, an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist]. The mGluR antagonists methyl-4-carboxyphenylglycine (MCPG, 1 mM, a group I/II mGluR antagonist) and LY367385 (50 or 100 μM, a specific mGluR1 antagonist), a GABAᵦ receptor antagonist SR 96631 hydrobromide (gabazine, 10 μM), a nonspecific glutamate transporter blocker d-threo-benzoyloxyaspartate (TBOA, 50 μM), and a nonselective antagonist of receptor-operated cation channels SKF96365 (50–250 μM) were bath applied as indicated. All drugs were purchased from Tocris Cookson (Bristol, UK) except gabazine (Sigma-Aldrich).

Ca²⁺ and Na⁺ imaging

Fluorescence of Oregon Green BAPTA-1 (excitation: 488 nm) or SBFI (excitation: 390 nm) was elicited by whole-field epi-illumination with light supplied by a monochromator (Polychrome IV; Till Photonics, Gräfelfing, Germany), and detected by a cooled CCD camera (PCO Sensicam, PCO Imaging, Kelheim, Germany) with spatial resolution of 0.226 μm/pixel (63 × objective, 520 × 680 pixels, 2 × 2 binning), and operated at a frame rate of 20 Hz under the control of ImagePro software (Media Cybernetics, Silver Spring, MD). Optical filters for whole-field epifluorescence consisted of a dichroic beam splitter (Oregon Green BAPTA-1: DCLP 505 LP; SBFI: DCLP 410 LP; Chroma Technology, Brattleboro, VT) and an emission filter (Oregon Green BAPTA-1: 535 ± 25 nm; SBFI: 480–700 nm). Fluorescence from intracellularly loaded dye equilibrated throughout the cell within 20–30 min of commencing the whole cell configuration. Changes in [Ca²⁺]ᵢ and intracellular Na⁺ ([Na⁺]ᵢ) were measured as relative fluorescence changes (ΔF/F, where F is the baseline fluorescence before a stimulus and ΔF is the evoked change in fluorescence). Fluorescence values were determined by subtracting pixel values averaged over regions outside the stained cell from each pixel of the image series (i.e., fluorescence values are “background subtracted”). The ΔF/F images were spatially low pass filtered with a Gaussian kernel of half-width 0.5–5 μm. Color-coded maps of ΔF/F were obtained using custom-made macros in ImagePro Plus. Color-coded images show the maps of the peak Ca²⁺ or Na⁺ transient if not otherwise stated.

RESULTS

High-frequency ON stimulation induced a slow potential and an associated Ca²⁺ transient in the MC dendritic tuft

We previously found that group I mGluRs do not significantly contribute to the electrophysiological and [Ca²⁺]ᵢ responses to single-shock ON stimulation in the MC apical dendritic tuft (Yuan and Knöpfel 2005). In the present experiments we used antagonists of AMPA and NMDA receptors and applied trains of ON stimuli—conditions under which synaptic responses mediated by group I mGluRs are more likely to be expressed (Batchelor et al. 1994; Reichelt and Knöpfel 2002; Tempia et al. 1998, 2001). In control ACSF, single-shock stimulation of bundles of ON axons at an intensity that evoked a subthreshold EPSP also evoked a local Ca²⁺ transient in the distal MC apical dendrite (Fig. 1, A1 and B1). Application of the NMDA receptor antagonist D-APV (50 μM) and the AMPA receptor antagonist NBQX (40 μM) virtually abolished the ON stimulation–induced EPSPs and the associated Ca²⁺ transients in most (16 of 22) cells (Fig. 1A2). Only in a subset of cells (6 of 22) could a small residual local Ca²⁺ transient be resolved with single ON stimuli in the presence of D-APV and NBQX (Fig. 1B2). In contrast, a train of high-frequency (10 pulses at 100 Hz) ON stimulation robustly induced a slow potential lasting 500 ms to several seconds (Fig. 1B3) that was accompanied by a locally confined Ca²⁺ transient in the glomerular dendritic tuft (Fig. 1, A3 and B3). The localization of this Ca²⁺ transient (yellow arrow in Fig. 1A3) corresponded to that of the D-APV–sensitive calcium transient observed in control ACSF (Fig. 1A1), indicating that it occurred at sites where the density of activated ON–MC synapses was largest (Yuan and Knöpfel 2005). Next, we characterized the stimulation frequency dependency of this slow synaptic potential and its associated Ca²⁺ transient. Figure 1C shows responses evoked by 10 stimulation pulses delivered at different frequencies (100, 50, 20, and 10 Hz). The synaptic potential and the associated Ca²⁺ signal in dendritic tufts were induced most efficiently with 100-Hz stimulation and their magnitudes decreased steeply with decreasing stimulation frequency (Fig. 1, C–E).

These slow synaptic responses recorded in the presence of NMDA and AMPA receptor blockers likely correspond to those previously reported in a subset of rat MCs (De Saint Jan and Westbrook 2005). Consistent with this expectation, both the slow synaptic potential and the Ca²⁺ transient evoked by high-frequency ON stimulation were abolished or largely reduced by the group I/II mGluR antagonists MCPG (1 mM) and the specific mGluR1 antagonist LY367385 (50 μM) (Fig. 2). The responses that were blocked by MCPG were partially restored after MCPG washout (n = 2; Fig. 2A). Summary plots demonstrated that both MCPG (n = 3) and LY367385 (n = 3) consistently antagonized the slow synaptic potential (Fig. 2B) as well as the associated Ca²⁺ transient (Fig. 2, C–E). To take into account the rundown of the Ca²⁺ signal during the time required to washin drugs, recordings of synaptic potentials
were interleaved with recordings of single backpropagating action potentials (bAPs; Yuan and Knöpfel 2005). We confirmed that MCPG specifically reduced synaptic stimulation-induced Ca\(^{2+}\) transients by comparing the synaptically induced Ca\(^{2+}\) transients before and after wash in of MCPG (3.60 ± 0.14%, mean ± SE before MCPG, vs. 0.69 ± 0.26% in the presence of MCPG; P < 0.001, n = 3, paired t-test) with bAP-induced Ca\(^{2+}\) transients before and after wash in of MCPG (control, 5.39 ± 1.56%, mean ± SE, vs. 5.12 ± 0.80% in the presence of MCPG; P > 0.7, n = 3, paired t-test) (Fig. 2C). Similar results were obtained with LY367385 (control, 7.62 ± 2.14%, mean ± SE, vs. 1.44 ± 0.61% in the presence of LY367385; P < 0.05, n = 3, paired t-test; Fig. 2D). Finally, the ratio of synaptically induced Ca\(^{2+}\) signals after and before the application of the mGluR antagonists (0.18 ± 0.04, mean ± SE) significantly differed from that of bAP-induced signals (0.90 ± 0.09, mean ± SE, P < 0.001, n = 6, paired t-test; Fig. 2E). These data unequivocally demonstrate that the 10-pulse/100-Hz ON stimulation–induced slow potential and the associated localized Ca\(^{2+}\) signal measured in the presence of d-APV and NBQX is mediated by mGluR1. We term this potential MC mGluR1 slow synaptic potential.
mGluR1 slow synaptic potential is enhanced by blocking glutamate transporter activity

mGluR1-mediated EPSPs induced by single ON shocks in MCs and by tetanic stimulation of parallel fibers in PNs are enhanced when glutamate uptake is blocked (Brasnjo and Otis 2001; De Saint Jan and Westbrook 2005; Reichelt and Knöpfel 2002). We used a nonspecific glutamate transporter blocker, D-threo-beta-benzyloxyaspartate (TBOA, 50 μM), and found that it not only enhanced and prolonged the mGluR1 slow synaptic potential (peak amplitude increased to 266 ± 57% of control, duration increased to 410 ± 139% of control; P < 0.05, n = 5, paired t-test; Fig. 3A4), but also enhanced the associated local Ca²⁺ transient (ΔF/F increased to 230 ± 97% of control, n = 5; Fig. 3A, A3 and A5). The slow synaptic potential recorded in the presence of TBOA was significantly reduced by 50 to 100 μM LY367385 (the area of the slow potential reduced to 27.3 ± 2.24% of control; n = 3, P < 0.05; paired t-test; Fig. 3, B1 and B2).

mGluR1 slow synaptic potential can trigger slow (≈2-Hz) oscillations of MC membrane potential

In a subset of MCs (n = 20 of 50 cells), 10 pulses of 100-Hz ON stimulation in the presence of NBQX and p-APV triggered a slow (≈2-Hz) oscillatory fluctuation of the MC membrane potential (Fig. 4A). The incidence and duration of these slow oscillations were enhanced by TBOA or by increasing stimulation intensity, as described previously (Schoppa and Westbrook 2001; Fig. 4, B and C). The slow oscillations induced in the presence of NBQX and p-APV were depressed by LY367385 (not shown). Interestingly, the associated Ca²⁺ transients (acquired at 20 Hz) did not exhibit any signs of oscillations that correlated with the nearly 2-Hz fluctuations in MC membrane potential (Fig. 4C). Furthermore, the slow oscillation, as well as the mGluR slow EPSP, were affected neither by the GABAA receptor antagonist gabazine (10 μM, n = 4) nor by the internal Na⁺ channel blocker QX-314 (10 mM, n = 4, Fig. 4D).

mGluR1 slow potential is neither enhanced by a preceding [Ca²⁺] transient nor blocked by the TRPC1 blocker SKF96365

The characteristics of the MC mGluR1 EPSP described so far are reminiscent of the mGluR1 EPSP induced by parallel fiber stimulation in PNs (induction by high-frequency stimulation and facilitation by glutamate transporter blockade). We therefore examined whether the MC mGluR1 slow synaptic potential could be enhanced by elevated [Ca²⁺] level as seen
in PNs (Batchelor and Garthwaite 1997). In a first set of experiments, we induced four action potentials (APs, at 40 Hz) that gave rise to a large, fast-rising Ca\(^{2+}\) transient. We then evoked the mGluR1 slow potential either in isolation or 125 ms after the train of the 4 APs (Fig. 5A1). The mGluR1 Ca\(^{2+}\) transient summed with the AP-induced Ca\(^{2+}\) transient (Fig. 5A2). However, the 4 AP-induced elevation of [Ca\(^{2+}\)]\(_i\) did not enhance the mGluR1 potential (Fig. 5A1 and B), nor was there a supralinear Ca\(^{2+}\) response (Fig. 5A2). To exclude the possibility that the Ca\(^{2+}\) transient induced by 4 APs was too small to affect the mGluR1 potential, we also injected a long-lasting (500 ms, 0.5 to 1.2 nA) depolarizing current into the soma of the MCs. This depolarization evoked a train of APs (8–25) and a large rise of [Ca\(^{2+}\)]\(_i\) (ΔF/F > 100%, n = 5, data not shown). The mGluR1 slow synaptic potential was also not significantly affected by a preceding massive elevation of [Ca\(^{2+}\)]\(_i\) (Fig. 5C).

Finally, we examined whether the mGluR1 slow potential was mediated through an SKF96365-sensitive nonselective cation channel such as TRPC1 (Kim et al. 2003; but also see Tempia et al. 2001). After recording baseline mGluR1 synaptic potentials, we added SKF96365 (50 μM, n = 10; or 100 μM, n = 3, or 250 μM, n = 2) into the bath solution. Baseline responses to synaptic stimulations were recorded every 3 min to minimize the rundown of responses. In the presence of SKF96365, the mGluR potential did not decline beyond the rate of the rundown observed during baseline (Fig. 5, D1 and D2; data were pooled in Fig. 5D2 after we confirmed that the action of the drug did not differ within the range of concentrations tested), indicating that the MC mGluR1 potential is not mediated by SKF96365-sensitive channels.

mGluR1 slow potential is accompanied by an increase in intracellular Na\(^+\) concentration

Slow potentials caused by the activation of mGluR1 (or the related mGluR5) have been characterized in many cell types. The mechanisms that generate these potentials differ between cell types and several mechanisms may exist within one cell (Coutinho and Kno¨pfel 2002). In principle, slow mGluR1-mediated potentials in Purkinje cells and dopamine cells are associated with an increase in [Na\(^+\)]\(_i\) level (Guita¨eo et al. 1999;
mGluR1 synaptic responses can trigger slow (~2 Hz) oscillations of MC membrane potential. A: 3 sequential recordings from a MC in which 10 pulses, 100-Hz ON stimulation induced a ~2-Hz slow oscillation in the presence of NBQX and d-APV. B: response to ON stimulation recorded under control condition (NBQX 40 μM + d-APV 50 μM) and in the presence of TBOA. C: Ca$^{2+}$ signals associated with the oscillatory membrane potential fluctuations. Responses to 10-pulse/100-Hz ON stimulation (black square) recorded under control condition and in the presence of TBOA. Note in B and C a single membrane fluctuation event under control condition (asterisk) that developed into a ~2-Hz oscillatory response after enhancement of the mGluR1 synaptic potential in the presence of TBOA. D: response to ON stimulation recorded under control condition and in the presence of the γ-aminobutyric acid type A (GABA$_A$) receptor antagonist gabazine (10 μM) with QX-314 in the internal solution.

Knöpfel et al. 2000, demonstrating activation of an inward current. We conducted corresponding experiments and found that the MC slow mGluR potential was accompanied by a locally confined [Na$^+$] transient in the glomerular dendritic tuft (Fig. 6, n = 6).

**DISCUSSION**

In the present study we described and characterized a slow mGluR1-mediated potential that was induced in MCs by a brief high-frequency stimulation of the ON. This mGluR1 potential was associated with a [Ca$^{2+}$] transient that was confined to a portion of the MC dendritic tuft. Both the slow potential and the Ca$^{2+}$ signal were enhanced after blocking glutamate uptake. High-frequency synaptic stimulation could also trigger a slow (~2 Hz) oscillation of the MC membrane potential, which was abolished by LY367385, but not affected by gabazine or intracellular QX-314. The mGluR synaptic potential was not enhanced by a preceding increase in [Ca$^{2+}$], nor was it blocked by SKF96365.

Previously we reported that single ON stimulation induced a Ca$^{2+}$ transient in the MC dendritic tuft that was mediated through NMDA receptors. These NMDA-receptor–dependent Ca$^{2+}$ transients occurred at discrete portions of the MC dendritic tuft (“hot spots”) that likely represented sites where ON synapses were activated at a high density (Yuan and Knöpfel 2005). In the present study we blocked AMPA and NMDA receptors and under this condition high-frequency stimulation pulses (10 pulses at 100 Hz) induced a slow mGluR1-mediated potential in MCs. This result differs from a concurrent study in rat MCs where a slow mGluR1 potential could be robustly activated only after blockade of glutamate transporter (Ennis et al. 2006). The difference in species (rat vs. mouse) and experimental protocols (e.g., different stimulation electrodes and positions; recording conditions) may account for the discrepancy between the studies. We demonstrated that the mGluR1 slow synaptic potentials are associated with Ca$^{2+}$ and Na$^+$ transients that are confined to the same discrete portions of the MC dendritic tuft as the NMDA-receptor–dependent hot spots (Figs. 1, A1 and A3, and 6). This suggests that both slow potentials are largest at sites where synaptically released glutamate can pool as the result of a high density of activated synapses. In contrast to the NMDA-receptor–dependent slow potential, induction of the mGluR1 slow synaptic potential was facilitated by the accumulation of glutamate during repetitive high-frequency stimulation or by blockade of glutamate transporters (Figs. 1–3). Because the slow mGluR1 synaptic potentials were recorded with NMDA receptors blocked, they unlikely mediate dendritic glutamate release but instead are, at
least initially, activated by glutamate released from the ON terminals. The mGluR1 Ca\(^{2+}\) transient might have triggered subsequent dendritic release of glutamate. However, this is unlikely because the mGluR1 slow potential had already declined during the peak of the associated Ca\(^{2+}\) transient (Fig. 5A2).

Slow potentials that are mediated by mGlur1 have been extensively characterized in cerebellar PNs (Batchelor et al. 1994; Brasnjo and Otis 2001; Canepari et al. 2001; Kim et al. 2003; Reichelt and Knöpfel 2002; Staub et al. 1992; Tempia et al. 1998, 2001). The mGluR1 potential induced by tetanic parallel fiber stimulation in PNs is, like the MC mGluR1 potential, facilitated by blockers of glutamate transporter (Brasnjo and Otis 2001; Reichelt and Knöpfel 2002). However, even with transporters blocked, single stimuli are usually not sufficient to induce a slow mGluR1 potential in PNs, whereas in a fraction of MCs they are (Fig. 1B; De Saint Jan and Westbrook 2005). The reason for this difference may lie in the much lower release probability of parallel fiber PN synapses compared with ON synapses because the low release probability limits the pooling of glutamate released by neighboring synapses. Consistent with this idea, another study (Matsukawa et al. 2003) showed that mGluR1 slow synaptic potentials are induced with fewer parallel fiber stimuli when the release probability at parallel fiber PN synapses was increased by ablation of presynaptic delayed rectifier potassium channels. The PN mGluR1 slow potential is enhanced by a priming [Ca\(^{2+}\)] transient (Batchelor and Garthwaite 1997) and is mediated by SKF96365-sensitive TRPC1 cation channels (Kim et al. 2003; but see Tempia et al. 2001). Our present data suggest that the MC mGluR1 EPSP is distinct in this aspect because it is not affected by SKF96365 (Fig. 5, D1 and D2) and is not enhanced by a preceding Ca\(^{2+}\) transient (Fig. 5, A–C). Therefore it is likely that the transduction pathway and effector of the mGluR1 slow potentials differ between these two cell types. However, as in PNs (Knöpfel et al. 2000), the MC slow mGluR1 EPSP was associated with a Na\(^{+}\) transient demonstrating activation of a Na\(^{+}\) inward current.

A somewhat surprising finding was that the MC mGluR1 potential could trigger slow (2-Hz) oscillations of the MC membrane potential in the presence of AMPA and NMDA receptor blockers. These oscillations resemble those elicited by odors in vivo (Adrian 1950; Chaput and Holley 1980, 1985; Kay and Laurent 1999; Meredith 1986; Onoda and Mori 1980) and those previously described in vitro (Schoppa and Westbrook 2001). The oscillations described by Schoppa and Westbrook (2001) were blocked by AMPA receptor blockers, leading these authors to conclude that they are mediated by NMDA and AMPA autoreceptors and that they were caused by regenerative glutamate release. The AMPA and NMDA receptors were blocked in our experiments and the oscillatory potentials were clearly faster than the mGluR1 potential and were not associated with oscillations in [Ca\(^{2+}\)] (Fig. 4). We therefore propose that these oscillations represent regenerative Na\(^{+}\) currents that can be activated by the slow mGluR EPSP as well as by the slow NMDA EPSP [in the experimental setting of Schoppa and Westbrook (2001)] of MCs. The identity of this putative Na\(^{+}\) current is not known but our experiments with QX-314 exclude the involvement of several voltage-gated Na\(^{+}\) channel subtypes. In agreement with Schoppa and Westbrook (2001), the slow oscillations were not abolished by blocking GABA\(_A\) receptors.

The MC mGluR1 slow synaptic potential complements the large repertoire of mechanisms that support oscillations and synchronization of the MCs that project into the same glomerulus (Christie et al. 2005; Didier et al. 2001; Friedman and Strowbridge 2000; Hayar et al. 2005; Isaacson 1999; Schoppa and Westbrook 2001, 2002).

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