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Research report

# Group I metabotropic glutamate receptor actions in oriens/alveus interneurons of rat hippocampal CA1 region

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**Abstract**

Group I metabotropic glutamate receptors (mGluRs) are important for hippocampal interneuron function. We used whole-cell recording and confocal imaging to characterize group I mGluR actions in CA1 oriens/alveus interneurons in slices. In tetrodotoxin and ionotropic glutamate receptor antagonists, the group I mGluR specific agonist DHPG increased intradendritic $Ca^{2+}$ levels and depolarized interneurons, whereas the group II mGluR specific agonist DCG-IV and the group III mGluR specific agonist L-AP4 did not. DHPG-induced depolarizing and $Ca^{2+}$ responses were antagonized by the group I mGluR antagonist 4CPG, but only $Ca^{2+}$ responses were significantly inhibited by the mGluR1 antagonist CPCCOEt. DHPG-induced depolarizing responses were not blocked by the inositol-1,4,5-trisphosphate ($IP_3$) receptor inhibitor heparin, the protein kinase C (PKC) antagonists GF-109203X, or the inhibitor of phospholipase C (PLC) U73122. Thus, these responses to DHPG may not be transduced by the PLC → $IP_3$/diacylglycerol (DAG) pathway classically linked to group I mGluRs. DHPG-induced depolarizations were not blocked by intracellular GDPβS or bath-application of *N*-ethylmaleimide (NEM), suggesting the involvement of a G protein-independent pathway. Our findings indicate that group I mGluRs induce a depolarization of oriens/alveus interneurons via a G protein-independent mechanism different from their classic signalling pathway. Since depolarizations are associated with intracellular $Ca^{2+}$ rises, these actions may be important for their synaptic plasticity and vulnerability to excitotoxicity.


*Theme:* Neurotransmitters, modulators, transporters, and receptors
*Topic:* Excitatory amino acid receptors: physiology, pharmacology and modulation

*Keywords:* Confocal microscopy; $Ca^{2+}$ imaging; Whole-cell recording; Second messenger; $mGluR1_a$; Inhibitory interneuron

## 1. Introduction

Metabotropic glutamate receptors (mGluRs) are members of the heptahelical G protein-coupled family of receptors (reviewed in Ref. [37]). To date, eight mGluRs have been cloned (mGluR1–8) which have been divided into three groups based on their amino acid sequences, as well as their pharmacological and signalling characteristics (reviewed by Ref. [12]). Group I consists of mGluR1 and mGluR5 which are coupled to phosphoinositide hydrolysis and mobilize $Ca^{2+}$ from intracellular stores sensitive to inositol-1,4,5-trisphosphate ($IP_3$). Neuronal excitability may be enhanced by group I mGluR activation of a transient receptor potential-like non-selective cationic conductance [7,11,13,19,21,23,38], a decrease in potassium conductance [6,8,20], activation of a $Na^+/Ca^{2+}$ exchanger [24,26,40] or amplification of $Ca^{2+}$ signals [9,32]. Group I mGluRs are localized perisynaptically on the soma and dendrites of hippocampal neurons [27] and play a key role in modulating synaptic connections [12]. In addition, blocking activation of group I mGluRs can be neuroprotective following seizures or ischaemic insults [33].

Interneurons play an important role in the hippocampal network by controlling the activity of principal cells [17]. Immunohistochemical studies show that the $mGluR1_a$ splice variant is preferentially expressed in interneurons of the oriens/alveus layers (OA) of the CA1 region of the hippocampus, whereas mGluR5 immunoreactivity is high in principal cells and scattered interneurons of both stratum oriens and stratum radiatum of CA1 [27]. Analysis by single cell reverse transcriptase polymerase chain reaction showed

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that OA interneurons express mGluR1 and/or mGluR5 [42]. Previous investigations have shown that, in the CA1 region, application of the group I/II mGluR agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) increases intracellular $Ca^{2+}$ and depolarizes OA interneurons, but has little effect on interneurons of stratum radiatum/lacunosum moleculare [5,44]. Interestingly, OA interneurons in CA1 are preferentially lost in the kainate model of epilepsy whereas interneurons of stratum radiatum/lacunosum moleculare remain [3,30]. Antagonism of group I mGluRs reduces interneuron loss following kainic acid injections [39]. In addition, excitatory synapses of CA1 OA interneurons undergo an $mGluR1_a$-dependent form of long-term potentiation, which is absent in CA1 stratum radiatum/lacunosum moleculare interneurons [34,36].

In view of the importance of mGluRs in modulating the excitability, the strength of synaptic connections, and the vulnerability of OA interneurons, the aim of the present study was to further characterize the actions of group I mGluRs in hippocampal OA interneurons. More specifically, we sought to examine which subtypes of mGluRs are involved in the intracellular $Ca^{2+}$ rise and depolarization of OA interneurons, and to determine the signalling pathway that mediates these responses.

## 2. Materials and methods

### 2.1. Slice preparation

Transverse hippocampal slices were prepared from young (13–19 days) male Sprague–Dawley rats [44]. All animal experiments were carried out in accordance with the Canadian Institutes of Health Research, as well as the US National Institutes of Health, guidelines for the care and use of laboratory animals. Briefly, rats were anaesthetized with halothane, decapitated and the brains rapidly dissected in cold (5 °C), oxygenated (95% $O_2$/5% $CO_2$) artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 2.5 KCl, 2 $CaCl_2$, 26 $NaHCO_3$, 1.25 $NaH_2PO_4$, 2 $MgSO_4$, 10 glucose, pH 7.35–7.4, ~ 305 mosM. Blocks of brain containing the hippocampus were affixed with cyanoacrylate to a vibratome stage and cut into 300 μm thick slices. Slices were allowed to recover in aCSF at room temperature (22–24 °C) for at least one h before use.

### 2.2. Electrophysiology

Slices were transferred to a recording chamber that was perfused with oxygenated aCSF (18–21 °C, 1–3 ml/min). Patch pipettes were pulled from borosilicate glass (1 mm O.D., A-M Systems, Everett, WA USA) and filled with (in mM) 130 K-methylsulfate, 1 $MgCl_2$, 8 NaCl, 2 Tris–ATP, 0.4 Tris–GTP, 10 HEPES, 0.1 EGTA, 10 $Na_2$-phosphocreatine, 0.15% biocytin and 10–20 μM oregon green BAPTA-I (Molecular Probes, Eugene OR USA), titrated with KOH to pH 7.2–7.25, and adjusted to 280–290 mosM (electrode resistance 4–6 MΩ). Except when indicated, 0.5 μM tetrodotoxin (TTX), 100 μM (±)-2-amino-5-phosphopentanoic acid (APV) and 10 μM 6-cyano-7-nitroquinoxaline-2,3,-dione (CNQX) were present to block action potentials and ionotropic glutamate receptor activation. Whole-cell patch clamp recordings were obtained under visual control using a 40× long-range water-immersion objective from CA1 interneurons with somata located near the oriens/alveus border (OA), as previously described [44,18]. Changes in membrane voltage were monitored using an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA, USA) in bridge mode. Signals were digitized at 22 kHz and recorded on videotape. In addition, signals were filtered at 1 kHz, digitized at 2 kHz (TL-1, Axon Instruments), stored on a PC and analysed using pClamp software (Axon Instruments). The bridge balance was monitored and adjusted using the bridge circuit. Recordings were discontinued if series resistance was more than 20 MΩ at the beginning of the experiment or if it increased to more than 30 MΩ during experiments. Cells with stable resting membrane potential, overshooting action potentials and an input resistance greater than 160 MΩ (before application of TTX, APV and CNQX) were accepted.

### 2.3. Calcium imaging

$Ca^{2+}$ imaging was performed using a laser scanning confocal microscope, as described previously [44,18]. After obtaining the whole-cell configuration, at least 20 min were allowed for intracellular diffusion of the fluorophore. The fluorophore was excited using a 488-nm argon laser. Emission was detected through a high-pass filter (cutoff 515 nm) and recorded to a PC using the MPL software (BioRad) with the confocal aperture opened fully. Time-lapse images were collected at 0.133 Hz. The images were analyzed off-line using Cfocal and Bfocal software (provided by M. Charlton, University of Toronto, Toronto, ON, Canada). The fluorescence intensity ($F$) was averaged for a delimited region of interest of a dendrite for each frame. Changes in fluorescence were calculated for each frame relative to the averaged baseline fluorescence ($F_{rest}$) and expressed as:

$$\%\Delta F/F = [(F - F_{rest})/F_{rest}] \times 100$$

### 2.4. Materials

All metabotropic glutamate receptor agonists (*S*)-3, 5-dihydroxyphenylglycine (DHPG), (2*S*,2′*R*,3′*R*)-2-(2′,3-dicarboxycyclopropyl)glycine (DCG-IV), L(+)-2-amino-4-phosphonobutyric acid (L-AP4) and antagonists (*S*)-4-carboxyphenylglycine (4CPG), (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), 7-(hydroxyimino)cyclopropa[b]chromen-1*a*-carboxylate ethyl ester (CPCCOEt) were purchased from Tocris Cookson (Ballwin, MO, USA). DL-2-amino-5-phosphonopentanoic acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were purchased

from RBI (Natick, MA, USA) or Tocris Cookson. 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U73122), 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidinedione (U73343) and *N*-ethylmaleimide (NEM) were purchased from Calbiochem (La Jolla, CA, USA). 2-[1-(3-dimethylaminopropyl)indo-3-yl]-3-(indo-3-yl)maleimide (GF-109203X) was obtained from Tocris Cookson. K-methylsulphate was purchased from ICN (Costa Mesa, CA, USA). Oregon green BAPTA-1 was from Molecular Probes (Eugene, OR, USA). Other chemicals were purchased from Sigma (Oakville, ON, Canada).

### 2.5. Histology

After recording, the slices containing biocytin-filled cells were transferred to a freshly prepared solution of 4% paraformaldehyde in 0.1 M phosphate buffer and fixed overnight at 4 °C. Slices were washed, stored in 0.1 M phosphate buffer for up to two weeks and processed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) followed by 3,3′-diaminobenzidine with nickel-intensification as previously described [40]. Sections were mounted in DPX mounting medium and examined under a light microscope.

### 2.6. Statistics

Unless otherwise indicated, data are expressed as mean ± S.E.M. Statistical analysis was performed using appropriate tests, as indicated in results and figure legends, and the statistical software SigmaPlot or SPSS. Significance level was set at $p < 0.05$.

## 3. Results

### 3.1. Actions of mGluR agonists in OA interneurons

Whole-cell current-clamp recordings were obtained from OA interneurons in the hippocampal CA1 region. All slices were processed after electrophysiological recordings to reveal biocytin labelling. Biocytin labelling indicated unambiguously that the vast majority of OA cells (65 of 73 cells) were interneurons [17,44]. One OA cell had a triangular-shaped and horizontally oriented soma, dendrites entering stratum radiatum, but the axon was not sufficiently labelled to make a clear identification. For the remaining seven cells, the non-pyramidal nature could not be verified.

Bath application of the specific agonist of group I/II mGluRs ACPD increases somatic as well as dendritic $Ca^{2+}$ levels, and depolarizes OA interneurons [44,18]. To determine which subtype of mGluRs mediates these effects, selective agonists for group I, II and III mGluRs were bath-applied. Peak depolarizing and $Ca^{2+}$ responses were quantified, as these were found to be the most stable measures with repeated applications (data not shown). Fig. 1A shows the projected image of 25 optical sections through an OA interneuron filled with the $Ca^{2+}$ indicator oregon green BAPTA-1. In the presence of TTX, APV and CNQX, following application of the specific group I mGluR agonist DHPG (10 μM), the fluorescence increased in the region of interest, indicating a rise in dendritic $Ca^{2+}$ levels (Fig. 1B$_1$ and C, mean 40.2 ± 9.5% $\Delta F/F$; $n = 12$), and the neurons depolarized (Fig. 1B$_1$ and C, mean 7.2 ± 1.6 mV; resting membrane potential (RMP) −55.6 ± 2.0 mV; $n = 12$). In the presence of TTX, APV and CNQX, neither the specific agonist for group II mGluRs DCG-IV (up to 20 μM), nor the specific agonist for group III mGluRs L-AP4 (up to 1 mM), caused any depolarization or increase in dendritic $Ca^{2+}$ levels in OA interneurons (Fig. 1B$_2$,B$_3$,C; DCG-IV: $Ca^{2+}$ response 0.7 ± 0.3% $\Delta F/F$, depolarization 0.7 ± 0.3 mV, RMP −60.4 ± 0.9 mV, $n = 6$; L-AP4: $Ca^{2+}$ response 0.0 ± 0.5% $\Delta F/F$, depolarization 0.2 ± 0.4 mV, RMP −60.4 ± 2.2 mV, $n = 3$). These data suggest that $Ca^{2+}$ responses and depolarization of OA interneurons are mediated by activation of group I, but not group II and III mGluRs.

### 3.2. Actions of group I mGluR antagonists on DHPG responses in OA interneurons

To confirm that the responses to DHPG were mediated by group I mGluRs, we applied DHPG (5–10 μM) in the presence of various group I mGluR antagonists and compared these $Ca^{2+}$ responses and depolarizations to those induced by DHPG (Fig. 2, control responses are reproduced here from Fig. 1). Fifteen minute pre-incubation of the competitive group I mGluR antagonist 4CPG (500 μM) significantly reduced both the DHPG-induced $Ca^{2+}$ response and membrane depolarization (Fig. 2A$_2$ and B; $Ca^{2+}$ response 1.6 ± 1.2% $\Delta F/F$, depolarization 0.2 ±0.2 mV, in 4CPG (RMP −57.3 ± 2.5 mV); $n = 6$; one-way ANOVA, Dunnett's test, $p = 0.013$ and $p = 0.001$, respectively).

In the presence of the competitive mGluR1 antagonist AIDA (100–200 μM), responses to 5–10 μM DHPG tended to be reduced but did not reach statistical significance (Fig. 2A$_3$ and B; sample traces show greater than average block; $Ca^{2+}$ responses 15.7 ± 4.9% $\Delta F/F$, depolarization 4.8 ± 0.9 mV in AIDA (RMP −62.7 ± 0.9 mV); $n = 8$; one-way ANOVA, Dunnett's test, $p = 0.20$ and $p = 0.6$, respectively). The non-competitive mGluR1 antagonist CPCCOEt (200 μM) significantly reduced the $Ca^{2+}$ response, but not depolarization, induced by DHPG (Fig. 2A$_4$ and B; $Ca^{2+}$ response 1.0 ± 1.0% $\Delta F/F$, depolarization 2.4 ± 1.2 mV in CPCCOEt (RMP −58.4 ± 1.2 mV); $n = 6$; one-way ANOVA, Dunnett's test, $p = 0.014$ and $p = 0.10$, respectively). At the concentrations used, 4CPG inhibits both mGluR1 and mGluR5 whereas AIDA and CPCCOEt should be selective for mGluR1 over mGluR5. The effects of AIDA and CPCCOEt were variable across cells, strongly inhibiting DHPG responses in some cells, but with overall significant effects only for CPCCOEt antagonism of $Ca^{2+}$ responses. Our data

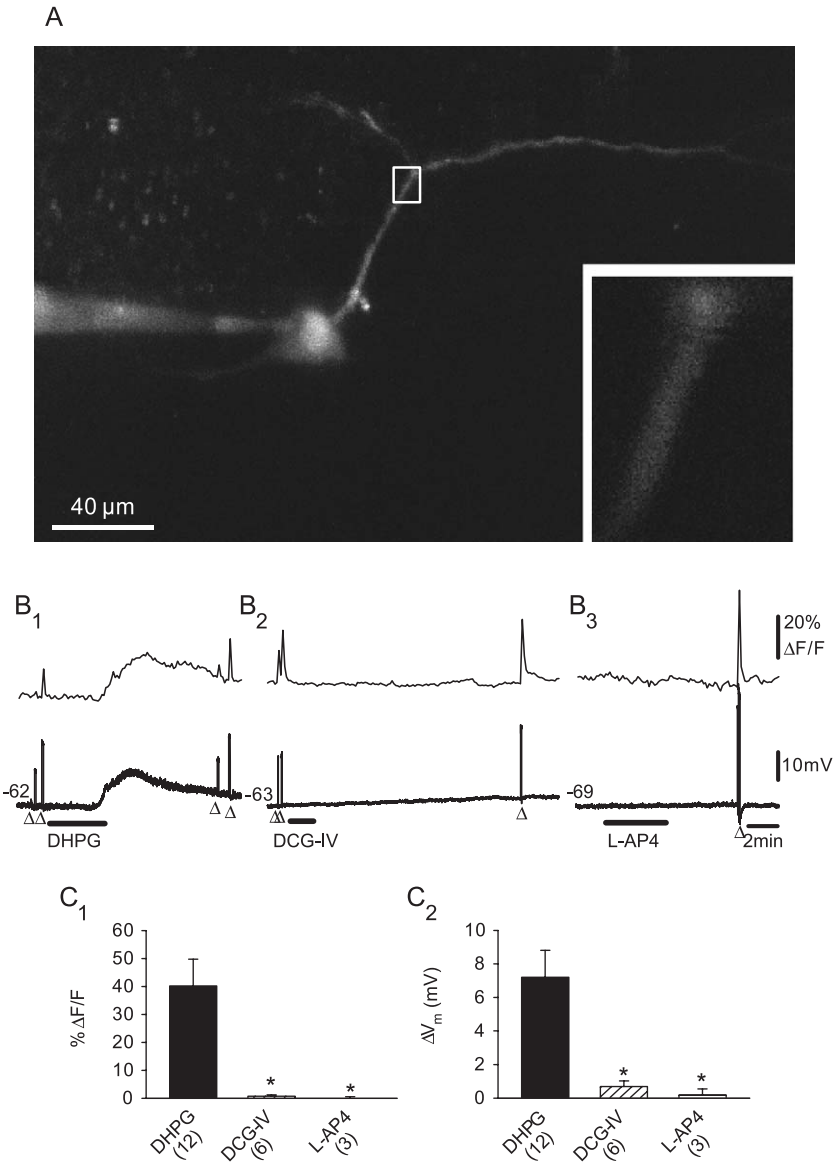


Fig. 1. The group I mGluR specific agonist DHPG increased intradendritic $Ca^{2+}$ levels and depolarized OA interneurons. (A) Projected Z-series of an OA interneuron with the region of interest indicated by the white box and shown at higher magnification in the inset. ($B_1$) Intradendritic $Ca^{2+}$ response (top) and depolarization (bottom) in response to 10 μM DHPG from the neuron shown in A. ($B_2$, $B_3$) Lack of responses, in different cells, to the group II mGluR specific agonist 10 μM DCG-IV and the group III specific agonist 1 mM L-AP4. (C) Histograms summarizing effects of 5–10 μM DHPG, 10–20 μM DCG-IV and 0.5–1 mM L-AP4 on intradendritic $Ca^{2+}$ levels ($C_1$) and membrane potential ($C_2$) in OA interneurons. Asterisks indicate significant differences from responses to DHPG ($p < 0.05$, one-way ANOVA, Dunnett's test). In this and other figures, triangles under membrane potential traces indicate application of depolarizing current pulses (~ 5–7 s duration, 100–300 pA intensity). These stimulations were used to elicit voltage-dependent $Ca^{2+}$ influx and to verify that an absence of drug-evoked response was not due to a non-specific loss of $Ca^{2+}$ responsiveness.

therefore suggest that both mGluR1 and mGluR5 may mediate responses in OA interneurons. If we used higher concentrations of DHPG, or lower concentrations of antagonists, we found it difficult to antagonize DHPG responses (data not shown).

### *3.3. Intracellular signalling pathways associated with DHPG responses*

We next examined the intracellular signalling pathways associated with DHPG-induced depolarization of OA interneurons. The usual signalling pathway for group I mGluRs involves G protein activation of phospholipase C (PLC) leading to the production of $IP_3$ and diacylglycerol (DAG). In the OA region of the hippocampus, there is high expression of group I mGluRs, but $IP_3$ receptors appear to be absent [16]. In a previous study, we demonstrated that ryanodine-sensitive intracellular stores are required for ACPD-induced responses in OA interneurons [44] but did not rule out involvement of $IP_3$ receptor-dependent stores. We therefore tested whether DHPG-evoked responses in OA interneurons were affect-

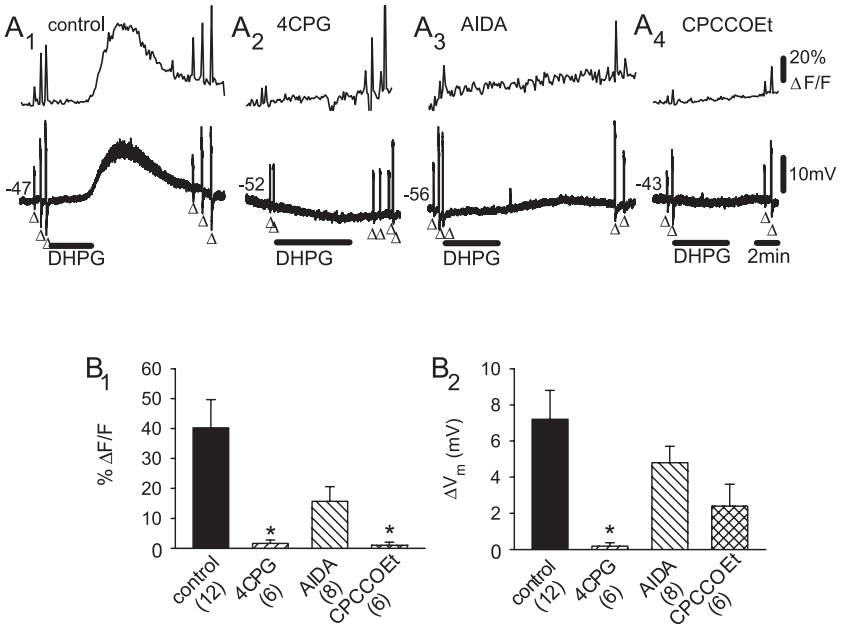


Fig. 2. Responses to DHPG were antagonized by group I mGluR specific antagonists. (A) Intradendritic $Ca^{2+}$ responses (top) and depolarizations (bottom) in response to 5 μM DHPG, in control conditions ($A_1$), in the presence of the competitive antagonists 500 μM 4CPG ($A_2$) and 200 μM AIDA ($A_3$), or the non-competitive antagonist 200 μM CPCCOEt ($A_4$). Antagonists were tested in different cells. (B) Histograms summarizing responses to 5–10 μM DHPG alone or in the presence of one of the antagonists (400–500 μM 4CPG; 100–200 μM AIDA; 100–200 μM CPCCOEt). Asterisks indicate significant differences from control responses with DHPG alone (one-way ANOVA, Dunnett's test).

ed by intracellular application of heparin, which has been shown to block $IP_3$-mediated $Ca^{2+}$ release at concentrations of 100 μg/ml (e.g. Ref. [15]). Inclusion of up to 5–7.5 mg/ml of low molecular weight heparin in the intracellular solution did not block the depolarization induced by 5–10 μM DHPG (Fig. 3). The mean DHPG response immediately after obtaining the whole-cell configuration was 20.3 ± 4.7 mV (RMP −57.9 ± 2.1 mV), whereas after dialysis (RMP −60.7 ± 2.5 mV) it was 18.1 ± 5.2 mV ($p = 0.63$, paired $t$-test; $n = 5$ cells in each group). These results suggest that depolarization of OA interneurons induced by group I mGluRs does not necessitate the activation of $IP_3$ receptors.

Protein kinase C (PKC), which could be activated by DAG and intracellular $Ca^{2+}$ following PLC activation, did not appear necessary either, as responses to 10 μM DHPG were not antagonized by bath application of the PKC inhibitor GF109203X at concentrations ranging from 1 to 50 μM (Fig. 4A; pooled data from cells tested with 10 and 50 μM GF109203X, $n = 4$; DHPG responses 19.5 ± 1.3 mV in control (RMP −54.5 ± 1.9 mV) vs. 18.6 ± 2.5 mV in GF109203X (RMP −57.5 ± 3.3 mV); $p = 0.8$, paired $t$-test). In two cells, 50 μM GF109203X was applied for 50 min and 80 min, respectively, before DHPG responses were tested. Application of vehicle solution alone (0.5% DMSO) did not affect responses to 10 μM DHPG (Fig. 4B; DHPG responses 7.2 ± 3.8 mV in control (RMP −59.5 ± 5.3 mV) vs. 9.5 ± 4.8 mV in DMSO (RMP −63.8 ± 4.9 mV); $n = 3$). GF109203X prevents the mGluR5-mediated potentiation of NMDA responses in CA3 pyramidal neurons [2] without affecting the underlying inward current (Benquet and Gee,

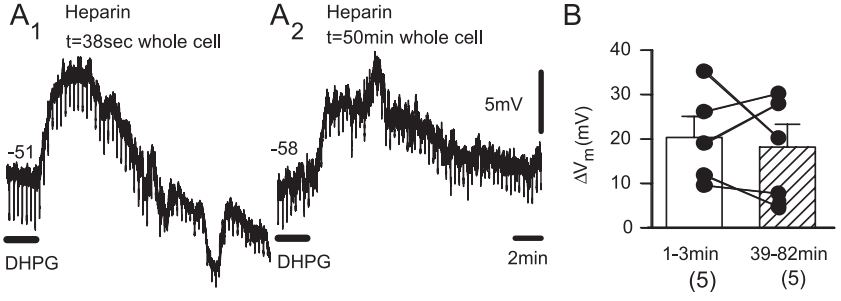


Fig. 3. Blocking $IP_3$ receptors with intracellular heparin did not antagonize responses to DHPG. (A) Sample responses to 10 μM DHPG just after gaining whole-cell access ($A_1$) and after 50 min of recording ($A_2$) with an intracellular solution containing 5 mg/ml low molecular weight heparin. (B) Summary of responses to 5–10 μM DHPG within 3 min of gaining whole-cell access and after 39–82 min of recording for all cells tested (paired $t$-test). In this and following figures, data points joined by a line in graphs indicate repeated measures in a given cell.

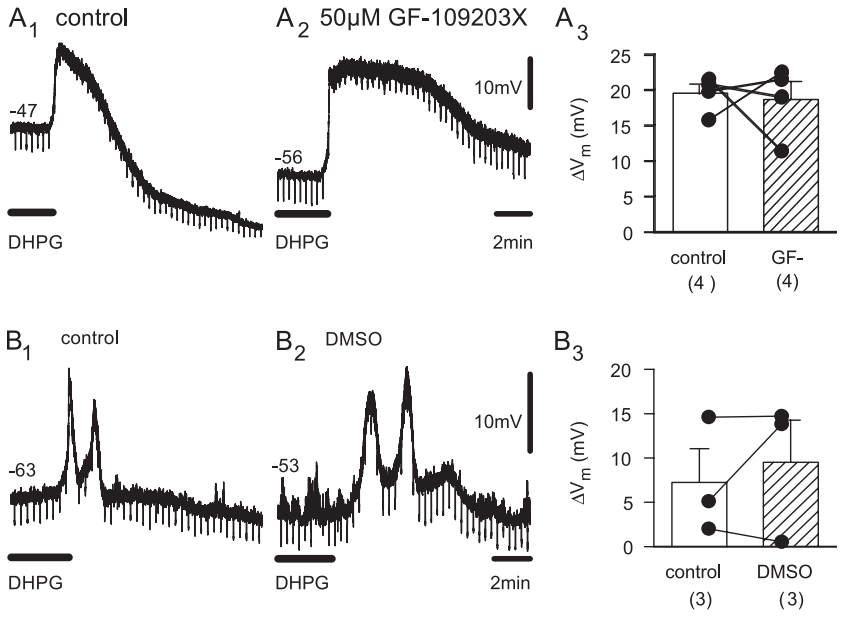


Fig. 4. The inhibitor of PKC, GF-109203X, did not affect DHPG responses. (A) Sample responses to 10 μM DHPG in a cell before ($A_1$) and 50 min after application of 50 μM GF-109203X ($A_2$). Following the first application of DHPG, an atypically large and fast hyperpolarization followed the depolarization in this cell, which partially recovered before the next application of DHPG. Mean responses for all cells tested before and after bath application of 10 or 50 μM GF-109203X were not significantly different ($A_3$; paired *t*-test). (B) Responses to 10 μM DHPG in the absence ($B_1$) and presence ($B_2$) of the vehicle solution alone, 0.5% DMSO, were not significantly different ($B_3$).

unpublished observations). These results suggest that PKC activation is not required for DHPG-evoked responses in OA interneurons.

Inhibiting PLC lipase activity with U73122 did not affect responses of OA interneurons to 5–10 μM DHPG (Fig. 5A and C). Mean DHPG responses were similar in control ($13.0 \pm 2.6$ mV, RMP $-52.6 \pm 2.0$ mV) and in U73122 ($10.3 \pm 2.3$ mV, RMP $-58.4 \pm 1.9$ mV; paired *t*-tests, $p = 0.8$, $n = 7$). Similarly, the inactive analogue U73343 did not affect DHPG responses (Fig. 5B and D; DHPG responses $19.0 \pm 2.1$ mV in control (RMP $-57.6 \pm 2.1$ mV) vs. $19.3 \pm 1.6$ mV in U73343 (RMP $-59.6 \pm 1.6$ mV);

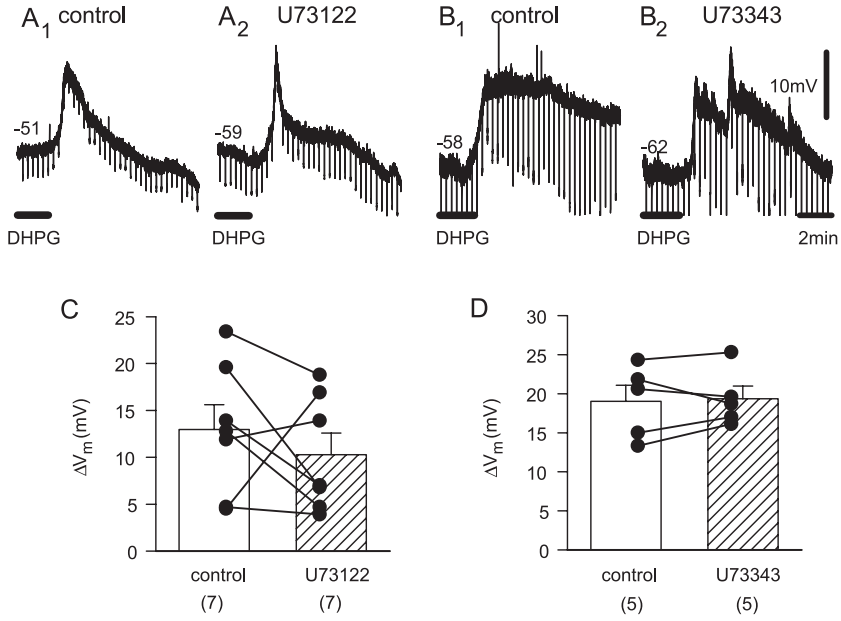


Fig. 5. The inhibitor of PLC, U73122, did not antagonize DHPG responses in OA interneurons. (A) Example of responses to 10 μM DHPG before and after bath application of the inhibitor of PLC, 10 μM U73122. (B) Responses to 10 μM DHPG, in another cell, in the presence and absence of the inactive analogue, 10 μM U73343. (C, D) Mean responses to DHPG for all cells tested, showing no significant effect of the PLC inhibitor (C; U73122) or its inactive analogue (D; U73343) (paired *t*-tests).

paired *t*-tests, $p = 0.9$, $n = 5$). In spinal cord slices, U73122 is effective at blocking the long-term depression of synaptic transmission, without blocking the acute synaptic inhibition, induced by group I mGluRs, thus blocking some but not all actions of group I mGluRs [10]. These data suggest that lipase activity of PLC may not be required for DHPG-induced responses in OA interneurons.

### *3.4. G protein-independent signalling pathway associated with DHPG responses*

We next evaluated whether DHPG responses in OA interneurons involve a G protein-dependent mechanism. GDPβS (up to 1 mM) was included in the patch pipette solution to block G protein activation. Depolarizations were still evoked by DHPG up to one h after obtaining whole-cell access in cells recorded with GDPβS containing solution (Fig. 6B$_3$ and C$_2$; $8.3 \pm 2.1$ mV in five cells recorded with GDPβS (RMP $-53.2 \pm 2.5$ mV) vs. $9.8 \pm 2.9$ mV in six control cells recorded with GTP (RMP $-57.0 \pm 2.7$ mV), unpaired *t*-test, $p = 0.7$). In the same cells, as a positive control, we applied 40 μM baclofen to evoke G protein-mediated $GABA_B$ responses, first immediately after gaining whole-cell access and then at later time points. In the control cells with GTP in the intracellular solution, baclofen evoked hyperpolarizing responses (Fig. 6A$_1$,A$_2$,C$_1$; $-4.5 \pm 0.6$ mV at first application vs. $-3.0 \pm 1.1$ mV at later application, $n = 6$ cells, paired *t*-test, $p = 0.32$). In the cells with GDPβS, the hyperpolarizing responses to baclofen were present initially, but were blocked at later time points (Fig. 6B1,B2,C1; $-4.1 \pm 0.8$ mV at first application vs. $-0.1 \pm 0.1$ mV at later application, $n = 5$ cells, paired *t*-test, $p = 0.001$). These results indicate that intracellular GDPβS treatment was effective in blocking G protein activation in our conditions, but that this treatment did not prevent DHPG responses.

In seven additional cells recorded with solution containing 1 mM GDPβS, we examined responses to 10 μM DHPG and 20 μM carbachol at early and late time points after gaining whole-cell access. In these cells, there was no

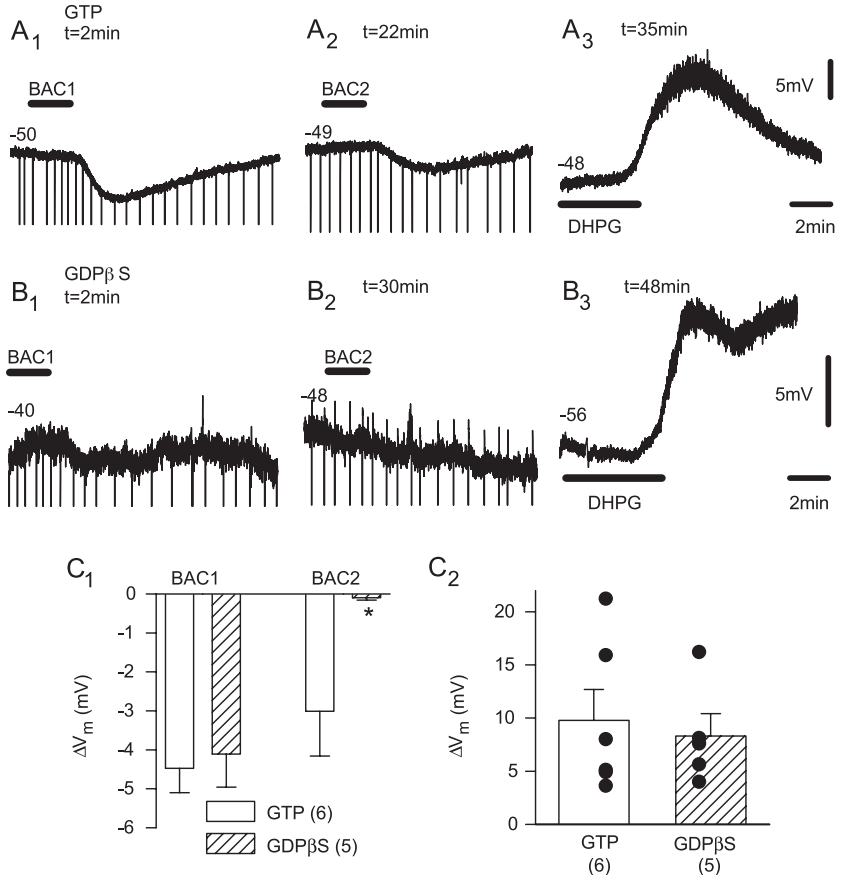


Fig. 6. Inhibition of G protein activation by intracellular GDPβS did not block DHPG responses in OA interneurons. (A) In a control cell recorded with an intracellular solution containing 0.4 mM GTP, 40 μM baclofen evoked hyperpolarizing responses within the first few minutes after gaining whole-cell access (A$_1$, BAC1) and 22 min later (A$_2$, BAC2), whereas DHPG evoked a depolarizing response at a later time point (35 min). (B) In a cell recorded with solution containing 1 mM GDPβS, baclofen evoked a response immediately after gaining whole-cell access (B$_1$; 2 min) but not 30 min later (B$_2$,), whereas a DHPG response was recorded at a later time (B$_3$; 48 min). (C) Summary, for all cells tested with intracellular GTP ($n = 6$) or GDPβS ($n = 5$), of mean responses evoked by baclofen at early (< 5 min; BAC1) and later (>5 min; BAC2) time points (C$_1$; paired *t*-tests), and of responses elicited by 5–10 μM DHPG at the later time points (>30 min; C$_2$; unpaired *t*-test), indicating a block of baclofen responses, but not of DHPG responses, by GDPβS.

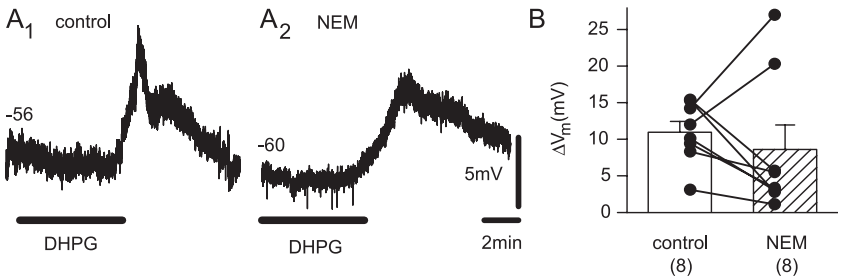


Fig. 7. The inhibitor of $G_i/G_o$, *N*-ethylmaleimide (NEM), did not block DHPG responses. (A) Examples, in the same cell, of responses to DHPG before ($A_1$) and after ($A_2$) bath application of 300–500 μM NEM. (B) Summary for all cells tested, showing that DHPG responses were not significantly changed in the presence of NEM (paired *t*-test).

significant difference between DHPG responses evoked 1–5 min after gaining whole-cell access and 35–80 min later ($15.2 \pm 5.3$ vs. $12.7 \pm 3.0$ mV, respectively; $n = 7$, paired *t*-test, $p = 0.7$, data not shown). Interestingly, responses to carbachol also were not significantly reduced in these cells ($13.0 \pm 2.2$ vs. $9.7 \pm 3.4$ mV, respectively; $n = 7$, paired *t*-test, $p = 0.4$, data not shown). These results indicate that interfering with G protein activation did not affect DHPG responses, suggesting that a G protein-independent signalling pathway may be involved in these responses.

Bath application of *N*-ethylmaleimide (NEM, 300–500 μM), an inhibitor of $G_i/G_o$ [1], did not inhibit responses to DHPG (Fig. 7; DHPG responses $11.0 \pm 3.2$ mV in control (RMP $-58.4 \pm 2.6$ mV) vs. $8.9 \pm 3.4$ mV in NEM (RMP $-60.9 \pm 1.6$ mV); $n = 8$, paired *t*-test, $p = 0.5$). Responses to carbachol were also not significantly reduced by NEM (carbachol responses $11.0 \pm 3.2$ mV in control vs. $5.7 \pm 1.0$ mV in NEM, $n = 8$, paired *t*-test, $p = 0.11$; data not shown). This suggests that depolarization of OA interneurons by activation of mGluR or muscarinic cholinergic receptors is achieved by pathways not requiring coupling to $G_i/G_o$ containing heterotrimeric G proteins. In the absence of APV and CNQX, we observed an increase in 'synaptic noise' with 300 μM NEM ($n = 2$, data not shown), indicating that NEM was active at presynaptic terminals in our preparation [31]. Collectively, these results suggest that responses to the group I mGluR agonist DHPG may occur by a mechanism independent of G protein activation in OA interneurons.

## 4. Discussion

The principal findings of the present study are that the group I mGluR specific agonist DHPG increased intradendritic $Ca^{2+}$ levels and depolarized OA interneurons in rat hippocampal slices, whereas selective agonists of group II and III mGluRs did not. In addition, group I mGluR antagonists reduced responses to the group I specific agonist DHPG. Furthermore, the signalling pathway mediating the depolarization of OA interneurons following activation of group I mGluRs by DHPG appears to be independent of the activation of G proteins, lipase activity of PLC, PKC and $IP_3$ receptors since their inhibitors were unable to prevent the depolarization induced by DHPG in OA interneurons. These results strongly suggest that an alternative intracellular signalling pathway may exist to mediate depolarizing responses to group I mGluR activation in OA interneurons. It remains to be determined, however, whether the same signalling pathways are involved in DHPG-induced $Ca^{2+}$ responses.

### 4.1. Group I mGluR subtypes

We suspected that DHPG responses in OA interneurons would be largely mediated by $mGluR1_a$, since this receptor is highly expressed in CA1 OA layers [27] and is required for long-term potentiation in OA interneurons [25,36]. However, 4CPG, at a concentration that inhibits both mGluR1 and mGluR5, produced a more robust inhibition of both the calcium rise and depolarization induced by DHPG, than antagonists with greater selectivity for mGluR1 over mGluR5 (AIDA, CPCCOEt). This suggests that DHPG responses may be mediated by activation of both mGluR1 and mGluR5 in CA1 OA interneurons. Both mGluR1 and mGluR5 act synergistically to induce a slow EPSC in CA3 interneurons [29] and to mediate responses to DHPG in CA3 pyramidal cells [19]. CPCCOEt was more effective in blocking the calcium rise than the depolarization induced by DHPG, suggesting some divergence in the roles of mGluR1 and mGluR5 in OA interneurons. Thus, mGluR1 may be preferentially coupled to calcium rises in OA interneurons, as in CA1 pyramidal neurons [28]. mGluR1 and mGluR5 have also been shown to couple to different signalling pathways in single CA3 pyramidal neurons [2]. Further pharmacological experiments with more selective ligands will be necessary to clarify the respective roles of mGluR5 vs. $mGluR1_a$ in group I mGluR actions in OA interneurons.

### 4.2. Group I mGluR signalling pathway in OA interneurons

Our data suggest that mGluRs depolarize OA interneurons via an intracellular signalling pathway independent of PLC lipase activity and G protein activation. While group I mGluRs have been shown to couple to this pathway [12], several studies have reported that mGluRs may also couple

to alternative G protein-independent pathways in neurons [2,19,21,23]. GDPβS blocked the hyperpolarizing response to the $GABA_B$ agonist baclofen, suggesting that the inhibition of G proteins was effective. The signalling pathway mediating the group I mGluR responses in OA interneurons remains to be determined, but in recent years several of the heptahelical receptors have been shown to signal through G protein-independent mechanisms (reviewed by Ref. [22]). mGluRs are associated with a variety of other receptors and proteins in the postsynaptic density, some of which could be involved in the G protein-independent signalling (e.g. Ref. [43]). Transient receptor potential (TRP) channels, which are postulated to underlie mGluR- and muscarinic cholinergic receptor-mediated inward currents in CA3 pyramidal cells [19,41], can be opened in response to agonists by lipase-independent activity of PLC [35]. As U73122 only interferes with lipase activity of PLC, PLC may well be involved in coupling mGluRs to neuronal depolarization. Another candidate for mediating group I mGluR signalling is the Homer family of proteins, which bind the carboxyl termini of $mGluR1_a$ and mGluR5 [4], as well as the ryanodine receptor [14]. Thus, Homer proteins could be in a position to mediate the $Ca^{2+}$ release from ryanodine-sensitive stores in OA interneurons [44]. Further experiments aimed at identifying the G protein-independent signalling pathway involved in group I mGluR actions will be important to clarify the actions linked to these receptors and their role in OA interneuron function.

### 4.3. Functional implications

Group I mGluRs have been shown to be important in OA interneuron function. First, OA interneurons are selectively vulnerable to excitotoxicity in the kainate (KA) model of epilepsy [30]. Although the exact mechanism of this selective vulnerability remains to be determined, recent evidence suggests that treatment with a group I mGluR antagonist reduced the OA interneuron loss in KA-treated rats [39]. Thus, activation of group I mGluRs may play a role in the vulnerability of these interneurons to glutamate excitotoxicity during seizures. In addition, the $mGluR1_a$ subtype of group I mGluRs is necessary for the induction of long-term potentiation of glutamatergic synapses onto OA interneurons [25,36]. The selective vulnerability of OA cells to excitotoxicity in the KA model [30] and the presence of long-term potentiation selectively in OA interneurons [36] is consistent with the finding that group I mGluR activation results in a coupling between $Ca^{2+}$ influx via voltage-dependent $Ca^{2+}$ channels and release from ryanodine-sensitive intracellular stores, selectively in OA interneurons, and not in interneurons of stratum radiatum and lacunosum-moleculare [44]. The present findings further suggest that a signalling pathway independent from G proteins, PLC, DAG, PKC and $IP_3$, may be involved in linking group I mGluR activation to intracellular depolarizations. Since these depolarizations are associated with intracellular $Ca^{2+}$ rises, such cell-specific actions and their signalling mechanisms may be important for synaptic plasticity and the selective vulnerability of these interneurons.

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