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# Calcium Dependence of Native Metabotropic Glutamate Receptor Signaling in Central Neurons

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## Abstract

Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors that are distributed throughout the brain and play important roles in regulation of synaptic efficacy. Some studies report that mGluRs heterologously expressed in nonneuronal cells are sensitive not only to glutamate but also to extracellular $Ca^{2+}$ ($Ca^{2+}_o$). We studied the $Ca^{2+}_o$-sensitivity of native mGluRs in mammalian central neurons. In cerebellar Purkinje cells that naturally express type-1 mGluR (mGluR1), physiological levels of $Ca^{2+}_o$ (around 2 m*M*) activate mGluR1-mediated intracellular $Ca^{2+}$ mobilization. The activation of the native mGluR1 response to $Ca^{2+}_o$ appears to be slower than that to glutamate. $Ca^{2+}_o$ (2 m*M*) also augments glutamate analog-evoked, native mGluR1-mediated inward cation current and intracellular $Ca^{2+}_o$ mobilization. Detailed analysis of this effect suggests that $Ca^{2+}_o$ modulates the glutamate responsiveness of native and heterologously expressed mGluR1s in different manners. These findings suggest that $Ca^{2+}_o$ may enhance the basal level and glutamate responsiveness of neuronal mGluR signaling in vivo.

**Index Entries:** metabotropic glutamate receptor; calcium; central nervous system; neuron; synapse.

## Introduction

Metabotropic glutamate receptors (mGluRs) are a family of G protein-coupled receptors (GPCRs) for the major excitatory neurotransmitter glutamate *(1–4)*. mGluRs are distributed throughout the brain *(1–4)* and play important roles in the induction of slow excitatory postsynaptic potentials (EPSPs) *(5,6)*, intracellular $Ca^{2+}$ mobilization *(7–9)*, synaptic plasticity *(10–15)*, and the developmental refinement of immature synapses *(12,16)*. mGluRs form a gene superfamily (family C) with $Ca^{2+}$-sensing receptor (CaR) *(17)* and B-type γ-aminobutyric

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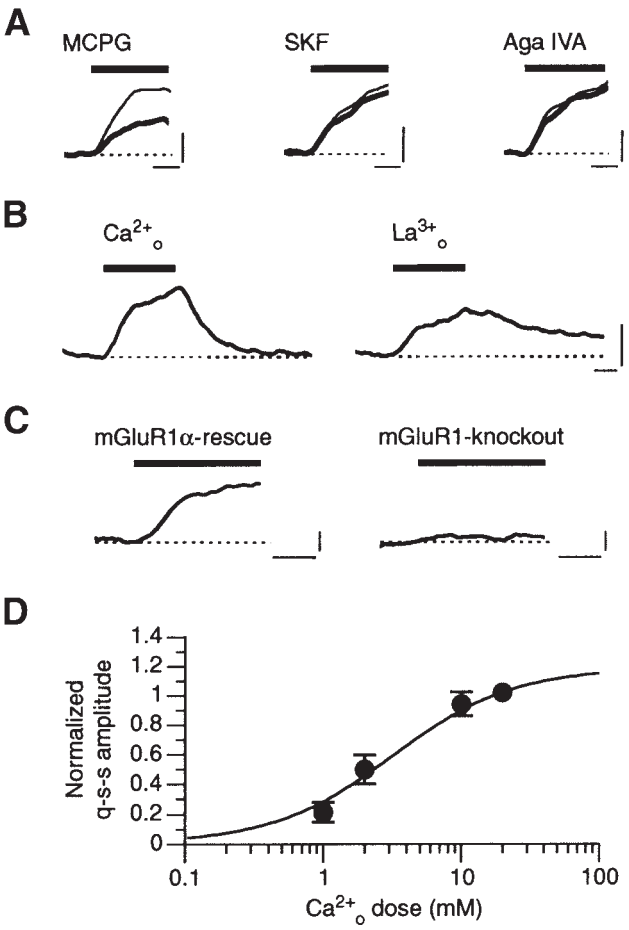


Fig. 1. Activation of native mGluR1 by $Ca^{2+}_o$. **(A)** $Ca^{2+}_o$ (10 m*M*)-evoked $[Ca^{2+}]_i$ increases measured before (thin traces) and after (thick traces) treatment with MCPG (0.5 m*M*), SKF-96365 (25 μ*M*, "SKF"), or ω-agatoxin IVA (50 n*M*, "Aga IVA"). Thick bars indicate $Ca^{2+}_o$ application periods. Three sets of the superimposed records are taken from different Purkinje cells derived from wild-type mice. Calibration bars = 10 s and 10 n*M*. In this and the following figures, the $[Ca^{2+}]_i$ in Purkinje cells loaded with fura-2 (a $Ca^{2+}$ indicator) was estimated from the ratio of fluorescence signals excited at 340 and 380 nm. **(B)** $[Ca^{2+}]_i$ increases in response to 10 m*M* $Ca^{2+}$ and 5 m*M* $La^{3+}$ measured in the same Purkinje cell derived from a wild-type mouse. Calibration bars = 10 s and 10 n*M*. **(C)** $Ca^{2+}_o$ (5 m*M*)-evoked $[Ca^{2+}]_i$ increases measured in a Purkinje cell derived from the mGluR1α-rescue mouse and in a cell derived from the mGluR1-knockout mouse. Calibration bars = 10 s and 10 n*M*. **(D)** Mean dose-response relation of the $Ca^{2+}_o$-evoked $[Ca^{2+}]_i$ increase. *Y*-axis: the quasi-steady-state level at the end of a 30-s $Ca^{2+}_o$ application. Error bars: ±SEM. For each point, data are taken from 5–10 Purkinje cells derived from wild-type mice. Sigmoid curve: a Hill function with apparent $K_d$ of 3.1 m*M*. (Modified from ref. *22*.)

acid receptor ($GABA_BR$) *(18)*. These GPCRs possess large ligand-binding extracellular domains with simliar amino acid sequences, suggesting their cross-reactivity for ligands. Kubo and colleagues were the first to describe that millimolar levels of extracellular $Ca^{2+}$ ($Ca^{2+}_o$) activate type-1, 2, 3, and 5 mGluRs heterologously expressed in nonneuronal cells *(19,20)*.

In mammalian central neurons, native mGluRs are exposed to a millimolar level of $Ca^{2+}_o$ contained in the cerebrospinal fluid under physiological conditions. Thus, $Ca^{2+}_o$ could influence their function. We examined this possibility, using type-1 mGluR (mGluR1) naturally occurring in cultured mouse cerebellar Purkinje cells *(21–23)*. In Purkinje cells, mGluR1 outnumbers other mGluR subtypes *(1)*. Besides, the activity of native mGluR1 is readily monitored by two classes of well-characterized cellular responses. One is an inward current through nonselective cation channels that are coupled to mGluR1 via a signaling cascade involving $G_q$ protein *(24)* but not inositoltriphosphate ($IP_3$) or protein kinase C *(6,25)*. The other response is a $Ca^{2+}$ release from the intracellular stores, which is coupled to mGluR1 via a signaling cascade consisting of $G_q$ protein, phospholipase C, and $IP_3$ receptor *(7–9,26)*. In this review, we compare in detail the physiological properties of native and heterologously expressed mGluR1s in terms of their $Ca^{2+}_o$ dependence. We then discuss the possible underlying mechanisms and the physiological

significance of the $Ca^{2+}_o$ dependence of native mGluR signaling in central neurons.

## Activation of Native mGluR by $Ca^{2+}_o$

We tested whether $Ca^{2+}_o$ activates native mGluR1 by rapidly applying $Ca^{2+}_o$ to Purkinje cells *(21,22).* In this experiment, we fluorometrically measured an increase in the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) during $Ca^{2+}_o$ application (Fig. 1). This $[Ca^{2+}]_i$ increase is thought to reflect the mGluR1-mediated $Ca^{2+}$ release from the intracellular stores (*see* Introduction) and its amplitude can represent mGluR1 activity. This notion is supported by the following observations. First, (R,S)-α-methyl-4-carboxyphenylglycine (MCPG), a general mGluR antagonist, reduces the $[Ca^{2+}]_i$ increases (Fig. 1A). Second, blockade of Purkinje cells' main $Ca^{2+}$ influx pathways with SKF-96365 (a general antagonist against receptor-operated $Ca^{2+}$ channels) and ω-agatoxin IVA (a selective antagonist against P-type $Ca^{2+}$ channel) affects the $[Ca^{2+}]_i$ increases very little (Fig. 1A). Third, extracellular $La^{3+}$ ($La^{3+}_o$), another polyvalent cation agonist potent for heterologously expressed mGluR1 *(20),* evoked $[Ca^{2+}]_i$ increases with a similar time-course (Fig. 1B). Last, the $[Ca^{2+}]_i$ increases are observed in Purkinje cells derived from the mGluR1α-rescue mice *(12)* but not in cells derived from the mGluR1-knockout mice *(27,28)* (Fig. 1C). The mGluR1α-rescue mice are the transgenic mice that express full-length mGluR1 (mGluR1α) only in Purkinje cells in their cerebelli *(12).*

Physiological levels (≥1 m*M*) of $Ca^{2+}_o$ evoke detectable $[Ca^{2+}]_i$ increases (Fig. 1D). The amplitude of the $[Ca^{2+}]_i$ increases changes smoothly with $Ca^{2+}_o$ dose in a range of 1–10 m*M* and saturated at ~20 m*M* (apparent dissociation constant [$K_d$] = 3.1 m*M*) (Fig. 1D). A similar dose-response relation is observed for heterologously expressed mGluR1 ($EC_{50}$ = 4.7 m*M*) *(20).* Native and heterologously expressed mGluR1 may have similar relative sensitivities to $Ca^{2+}_o$ over glutamate. In Purkinje cells, 2–10 m*M* $Ca^{2+}_o$ and 5 μ*M* R,S-3,5-dihydroxyphenylglycine (DHPG, a type-1/5 mGluR-selective glutamate analog) evoke the $[Ca^{2+}]_i$ increases with comparable peak amplitudes (a few tens of n*M*) *(22).* In *Xenopus* oocytes, 5 m*M* $Ca^{2+}_o$ and 5 μ*M* glutamate increase the heterologously expressed mGluR1-mediated potentiation of a $Cl^-$ current by comparable extents (~10 μA) *(20).*

In Purkinje cells, the $[Ca^{2+}]_i$ increases gradually with time and approaches the steady-state ~30 s after the onset of $Ca^{2+}_o$ application (Fig. 1A). This slow activation is thought to be due to slow interaction between $Ca^{2+}_o$ and native mGluR1 because glutamate analogs can evoke much faster $[Ca^{2+}]_i$ increases, which reach the peak levels within 5–10 s of application onset. By contrast, in *Xenopus* oocytes, both $Ca^{2+}_o$- and glutamate-evoked, heterologously expressed mGluR1-mediated responses reach the peak levels within 5–10 s of application onset *(20).* The kinetic difference between native and heterologously expressed mGluR1s indicates that the cellular environment may modulate $Ca^{2+}_o$-mGluR1 interaction.

## Enhancement of Glutamate Sensitivity of Native mGluR by $Ca^{2+}_o$

We examined whether $Ca^{2+}_o$ influences native mGluR1's sensitivity to glutamate analogs in Purkinje cells *(21,22).* In this series of experiments, we monitored mGluR1 activity using both the mGluR1-mediated inward current (Fig. 2A) and $[Ca^{2+}]_i$ transient (Fig. 3A) (*see* Introduction). The inward current was measured under voltage clamp in a perforated-patch, whole-cell mode. In the absence of $Ca^{2+}_o$, both types of responses are evoked by ≥5 μ*M* DHPG and their peak amplitudes are saturated by ≥500 μ*M* DHPG (for the inward current, apparent $K_d$ = 12 μ*M*) (Figs. 2B and 3B, open symbols). A similar steepness of DHPG dose-response relation is observed for heterologously expressed mGluR1 *(29).* In contrast, in the presence of a physiological level (2 m*M*) of $Ca^{2+}_o$, the threshold DHPG doses of both the inward currents and $[Ca^{2+}]_i$ transients are lowered by 2–3 log units (Figs. 2B and 3B, closed

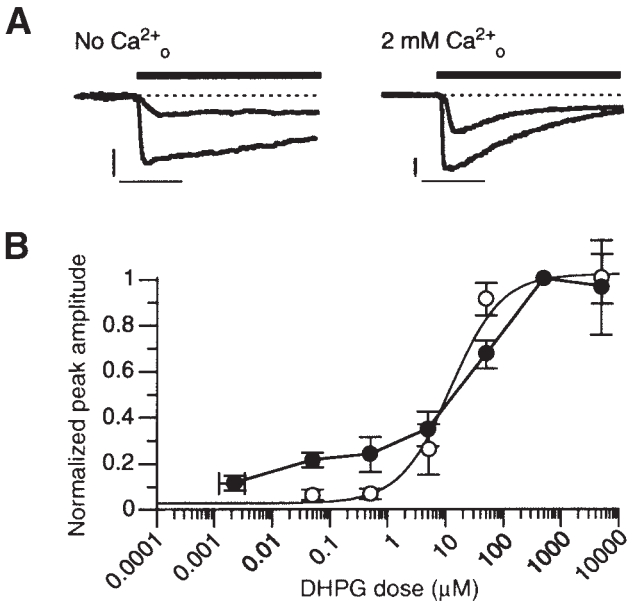


Fig. 2. Enhancement of glutamate analog-evoked, native mGluR1-mediated inward currents by $Ca^{2+}_o$. **(A)** DHPG-evoked inward currents in the absence or presence of 2 m*M* $Ca^{2+}_o$. Thick bars indicate DHPG application periods. Calibration bars = 10 s and 50 pA. Two sets of the superimposed records indicate responses to 5 and 500 μ*M* DHPG taken from different Purkinje cells. In this and the following figures, Purkinje cells were derived from wild-type mice, and the current was measured under voltage clamp (holding potential = –70 mV). **(B)** Mean dose-response relation of the DHPG-evoked inward current in the absence (open symbols) or presence (closed symbols) of 2 m*M* $Ca^{2+}_o$. Error bars: ±SEM. *N* = 4–15 for each point. (Modified from ref. *22*.)

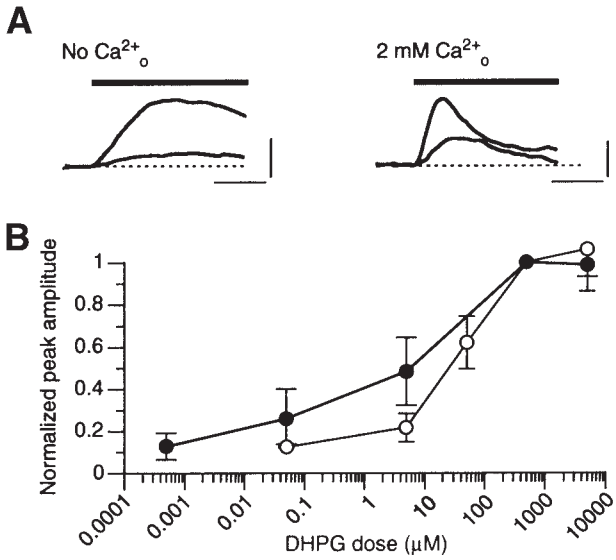


Fig. 3. Enhancement of glutamate analog-evoked, native mGluR1-mediated $[Ca^{2+}]_i$ transients by $Ca^{2+}_o$. **(A)** DHPG-evoked $[Ca^{2+}]_i$ transients in the absence or presence of 2 m*M* $Ca^{2+}_o$. Thick bars indicate DHPG application periods. Calibration bars = 10 s and 50 n*M*. Two sets of the superimposed records indicates responses to 5 and 500 μ*M* DHPG taken from different Purkinje cells. **(B)** Mean dose-response relation of the DHPG-evoked $[Ca^{2+}]_i$ transients in the absence (open symbols) or presence (closed symbols) of 2 m*M* $Ca^{2+}_o$. Error bars: ±SEM. *N* = 4–10 for each point. (Modified from ref. *22*.)

symbols). Interestingly, $Ca^{2+}_o$ selectively enhances the relative amplitudes at lower doses of DHPG and does not shift the saturated DHPG dose. As a result, the dynamic range, in which native mGluR1 efficiently encodes the agonist dose by response amplitude, is broadened. This effect is not specific to DHPG but also occurs for (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, another mGluR agonist *(22).* The dynamic range is most effectively broadened with the physiological level of $Ca^{2+}_o$. When the $Ca^{2+}_o$ level is elevated to an abnormally high level (10 m*M*), the saturated DHPG dose is lowered and thus, the dynamic range shrinks (Fig. 4) *(21).* $Ca^{2+}_o$ (2 m*M*) enhances heterologously expressed mGluR1-mediated responses to lower doses of glutamate *(30).* However, the dynamic range is not broadened because $Ca^{2+}_o$ lowers the saturated glutamate dose.

In Figs. 2–4, the amplitudes of the responses are normalized to the value at a reference DHPG dose (500 μ*M*) for comparing the overall profiles of the dose–response relations. When compared in the absolute value, the average amplitude of $[Ca^{2+}]_i$ transients evoked by 500 μ*M* DHPG is

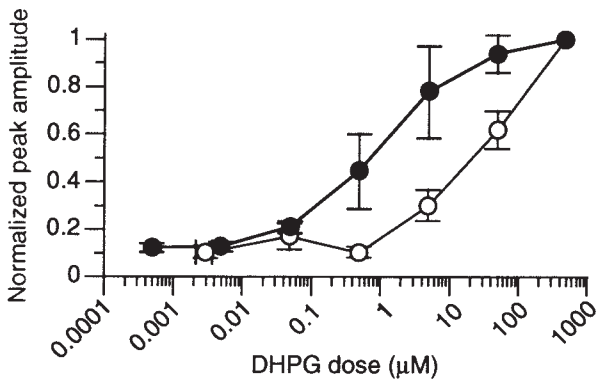


Fig. 4. $Ca^{2+}_o$ broadens the dynamic range of native mGluR1 only at the physiological level. Mean dose-response relation of the DHPG-evoked inward current in the presence of 2 m*M* (open symbols) or 10 m*M* (closed symbols) $Ca^{2+}_o$. Only in this experiment, $HCO^-_3$ contained in the standard saline was removed to secure the dissolution of a high concentration of $Ca^{2+}_o$. Error bars: ±SEM. *N* = 4–14 for each point.

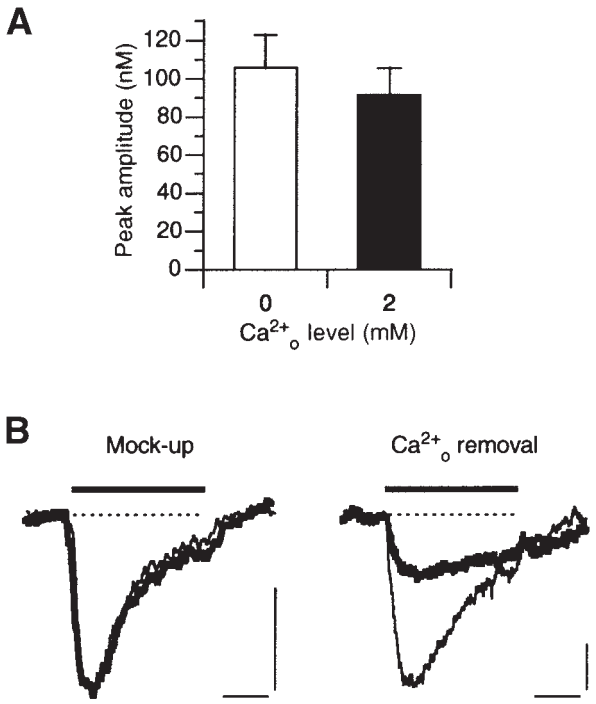


Fig. 5. Effect of $Ca^{2+}_o$ on the absolute amplitudes of glutamate analog-evoked, native mGluR1-mediated responses. **(A)** Mean peak amplitudes of $[Ca^{2+}]_i$ transients evoked by 500 μ*M* DHPG (*see* Fig. 3) in the absence (*n* = 12) or presence (*n* = 5) of 2 m*M* $Ca^{2+}_o$. Error bars: ±SEM. **(B)** 50 μ*M* DHPG-evoked inward currents recorded from individual Purkinje cells before (thin traces) and after (thick traces) continuous perfusion with a 2 m*M* $Ca^{2+}_o$-containing external saline ("mock-up" manipulation) or removal of $Ca^{2+}_o$ from the saline. Thick bars indicate DHPG application periods. Calibration bars = 10 s and 10 pA. (Panel B is modified from ref. *22*.)

not different between Purkinje cells perfused with a $Ca^{2+}_o$-free saline and ones perfused with a 2 m*M* $Ca^{2+}_o$-containing saline (Fig. 5A). Thus, $Ca^{2+}_o$ (2 m*M*) is thought to augment the absolute amplitudes of $[Ca^{2+}]_i$ transients evoked by lower doses of DHPG. In the case of the inward currents, it was difficult to compare the absolute amplitudes between collective data because of a wide cell-to-cell deviation *(22)*. Instead, we compared the amplitudes of the inward currents before and after $Ca^{2+}_o$ removal in individual Purkinje cells (Fig. 5B). $Ca^{2+}_o$ removal always reduces the inward currents evoked by 50 μ*M* DHPG, indicating that $Ca^{2+}_o$ (2 m*M*) augments the absolute amplitude of the inward currents even with a high dose of DHPG.

## Kinetic Change of Glutamate Analog-Evoked Native mGluR Responses by $Ca^{2+}_o$

$Ca^{2+}_o$ may accelerate the desensitization of native mGluR1. In Purkinje cells, the inward current reaches the peak level typically within 5–10 s of 500 μ*M* DHPG application onset regardless of the $Ca^{2+}_o$ level (Fig. 2A). In the absence of $Ca^{2+}_o$, the inward current is inactivated by 41% from the peak level at the end of a 30-s DHPG application (Fig. 2A). In the presence of 2 m*M* $Ca^{2+}_o$, the inward current is inactivated by as much as 66% (Fig. 2A). Similar $Ca^{2+}_o$-induced facilitation of inactivation is observed for the DHPG-evoked, native mGluR1-mediated $[Ca^{2+}]_i$ transients in Purkinje cells (Fig. 3A) but not for glutamate-

evoked, heterologously expressed mGluR1-mediated responses in the nonneuronal cells *(20,30).*

## Possible Mechanisms Underlying Effects of $Ca^{2+}_o$ on Native mGluR

MCPG (0.5 m*M*), which acts on the glutamate-binding site of mGluRs, partially inhibits $Ca^{2+}_o$-evoked responses of heterologously expressed mGluR1 *(20).* Point mutation of an amino acid residue near the glutamate-binding site greatly reduces the $Ca^{2+}_o$-sensitivity of the heterologously expressed mGluR1 *(20,30,31).* These observations suggest that $Ca^{2+}_o$ directly activates heterologously expressed mGluR1 although there is a report against this possibility *(32).* $Ca^{2+}_o$ does not necessarily bind tightly to the amino acid residue. An X-ray crystallography analysis *(33)* does not clearly show bound $Ca^{2+}$ at the residue, although the same analysis was able to detect a bound divalent metal ion at another residue. The dose-response relation of heterologously expressed mGluR1-mediated responses predicts a relatively low affinity for $Ca^{2+}_o$ ($EC_{50}$ = 4.7 m*M*) *(20).* Thus, $Ca^{2+}_o$ could activate mGluR1 by loosely interacting with the proximity of the glutamate-binding site *(31).* In Purkinje cells, the $Ca^{2+}_o$-evoked, native mGluR1-mediated response displays susceptibility (~50%) to 0.5 m*M* MCPG (Fig. 1A), comparable sensitivities to $Ca^{2+}_o$ and $La^{3+}_o$ (Fig. 1B), and a low apparent $K_d$ (3.1 m*M*, Fig. 1D). Similarities in these properties to heterologously expressed mGluR1 *(20)* indicate that the loose $Ca^{2+}_o$-mGluR1 interaction may underlie the activation of native mGluR1.

In Purkinje cells, the $Ca^{2+}_o$-induced enhancement of the relative amplitudes to lower doses of the glutamate analogs is commonly observed for the two classes of native mGluR1 responses carried by the different signaling cascades (*see* Introduction) (Figs. 2 and 3). Thus, this effect is thought to reflect facilitation of ligand-receptor interaction rather than upregulation of the signaling/effector molecules. The physiological level of $Ca^{2+}_o$ exerts the enhancement without lowering the saturated agonist dose (Figs. 2B and 3B). A possibility is that $Ca^{2+}_o$ at this level modulates only a certain fraction of the total mGluR1 population; a considerable fraction remaining as low-sensitivity forms may maintain the saturated agonist dose to a high level *(21).* As expected from this hypothesis, a very high level of $Ca^{2+}_o$, which may modulate a much larger fraction of the mGluR1 population, lowers the saturated agonist dose (Fig. 4).

$Ca^{2+}_o$ may enhance the glutamate-analog sensitivities of native and heterologously expressed mGluR1s in different manners. The degree of a $Ca^{2+}_o$-induced lowering in the threshold glutamate-analog dose is much greater for native mGluR1 (Figs. 2 and 3) than for heterologously expressed mGluR1 *(30).* $Ca^{2+}_o$ (~2 m*M*) shifts the saturated glutamate-analog dose of heterologously expressed mGluR1 *(30)* but not that of native mGluR1 (Figs. 2 and 3). $Ca^{2+}_o$ is reported to enhance the ligand sensitivity of heterologously expressed $GABA_BR$ in a similar manner to that of heterologously expressed mGluR1 *(34,35).* In the case of heterologously expressed $GABA_BR$, the direct action of $Ca^{2+}_o$ on the proximity of the ligand-binding site may be important for the enhancement *(34,35).* $Ca^{2+}_o$ might modulate native mGluR1 through a mechanism different from such direct interaction. There is increasing evidence that various GPCRs form complexes in which the GPCRs modulate one another *(36).* CaR is one of the candidates to form such complexes with mGluRs *(37).* Thus, a possibility to be tested in future is that $Ca^{2+}_o$ modulates native mGluRs indirectly via a mGluR-associated molecule(s).

In Purkinje cells, $Ca^{2+}_o$ augments the absolute amplitude of inward currents evoked by a nearly saturated dose of DHPG (Fig. 5B). Similar augmentation was not seen for $[Ca^{2+}]_i$ transients evoked by a nearly saturated dose of DHPG (Fig. 5A). Thus, $Ca^{2+}_o$ may not only facilitate the ligand-receptor interaction but also upregulate the cation channels or their gating molecules. Supporting this notion, con-

tinuous presence of $Ca^{2+}_o$ elevates the basal level of intracellular $Ca^{2+}$ *(22)*, which is shown to potentiate the inward current *(6)*.

The $Ca^{2+}_o$-induced acceleration of inactivation is commonly observed for the glutamate analog-evoked, native mGluR1-mediated responses carried by the different signaling cascades (Figs. 2A and 3A). Thus, this effect is thought to reflect the modulation of the receptor itself. Such effect is not obvious for heterologously expressed mGluR1 *(20,30)*, suggesting that it may depend on the cellular environment. $Ca^{2+}_o$ could exert this effect by acting on an intracellular target(s) after entering the cytoplasm because the intracellularly applied $Ca^{2+}_o$ chelator slows down the inactivation of the DHPG-evoked inward current (*see* ref. *22*).

## Possible Physiological Significance of Effects of $Ca^{2+}_o$ on Native mGluRs

Abrupt changes in the $Ca^{2+}_o$ level can repetitively activate heterologously expressed mGluR1 *(20)*. Thus, it was formerly thought that a sub-second fluctuation in the $Ca^{2+}_o$ level in the synaptic cleft during synaptic transmission *(38)* could activate native mGluRs. However, native mGluRs often localize where they may not experience such a fluctuation. Type-1 and 5 mGluRs are concentrated at the annuli of the dendritic spines (i.e., the perisynaptic area of the postsynaptic membrane) *(39–42)*. Group II mGluRs are scattered throughout the presynaptic membrane without close association with synaptic specializations *(43,44)*. Moreover, native mGluR1 should be activated only slightly during a sub-second period because of its slow activation kinetics (Fig. 1A,B).

Native mGluRs outside the center of the synapses should be always exposed to a relatively constant level (~2 m*M*) of $Ca^{2+}_o$ contained in the cerebrospinal fluid. Thus, one of the physiological effects of $Ca^{2+}_o$ may be continuous activation of mGluRs. The $Ca^{2+}_o$-evoked, native mGluR1-mediated response is not inactivated for at least 30 s (Fig. 1A,B). A previous study in the heterologous system *(20)* suggests that $Ca^{2+}_o$ evokes a mGluR1-mediated cellular response, such as cellular morphology transformation, that takes tens of hours. Continuous activation of native mGluR1 signaling may affect the morphological development of dendrites *(45)* and neuronal death from excitotoxicity *(46)*.

Another physiological effect of $Ca^{2+}_o$ may be continuous enhancement of the glutamate sensitivity of native mGluRs (Figs. 2 and 3). In cerebellar slices, repetitive stimulation of parallel fibers elicits mGluR1-mediated slow EPSPs in Purkinje cells. The amplitude of the slow EPSPs reaches the maximal level only at a very high stimulation frequency *(5,6)*. A majority of the postsynaptic mGluR1 population localizes to the perisynaptic area, where the extracellular glutamate concentration should be lower than that in the synaptic cleft. Perisynaptic glutamate transporters may further limit the extracellular glutamate concentration around native mGluR1 *(47)*. These observations indicate that native mGluR1 rarely experiences the saturated dose of glutamate under physiological conditions. $Ca^{2+}_o$ may enhance postsynaptic mGluR1 signaling by facilitating the receptor's sensitivity to low doses of glutamate (Figs. 2, 3, & 5). mGluR-mediated slow EPSPs that are not readily saturated under physiological conditions *(4,48)* and perisynaptically concentrated mGluRs (see above) are seen outside the cerebellum. Thus, $Ca^{2+}_o$ may exert similar enhancement in various brain regions. Postsynaptic mGluR signaling is essential for the induction of cerebellar long-term depression *(11–15,28)* and other forms of synaptic plasticity outside the cerebellum *(3,4,49)*. The $Ca^{2+}_o$-dependent enhancement of postsynaptic mGluR signaling may secure the induction of these forms of synaptic plasticity.

Moreover, continuous action of $Ca^{2+}_o$ may enable the unique function of the mGluR-mediated slow EPSPs in some central neurons, including cerebellar Purkinje cells *(5,6,48)*. The slow EPSPs display a graded change in amplitude according to the amount of released glutamate over a wide range and thereby serve as

efficient detectors of the presynaptic activities *(5,6)*. A previous study *(50)* ascribed the wide dynamic range of the slow EPSP in Purkinje cells to the coexistence of mGluR1 splice variants with different glutamate sensitivities *(51,52)*. However, a steep dose-response relation seen in the absence of $Ca^{2+}_o$ (Figs. 2B and 3B, open symbols) indicates functional predominance of a single mGluR1 variant (probably mGluR1α) in Purkinje cells. A more recent study *(47)* suggests the contribution of perisynaptic glutamate transporters. However, this cannot fully explain the broad dynamic range of the slow EPSP because pharmacological blockade of the transporters does not completely abolish it. The broad dynamic range of native mGluR1 itself seen in the presence of $Ca^{2+}_o$ (Figs. 2 and 3) may contribute to the unique property of the slow EPSP and may confer central neurons a powerful computation tool for integrating presynaptic information.

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