Enhancement of substrate-gated Cl\(^-\) currents via rat glutamate transporter EAAT4 by PMA

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5. Enhancement of substrate-gated Cl\(^-\) currents via rat glutamate transporter EAAT4 by PMA. Am J Physiol Cell Physiol 289: C1334–C1340, 2006; doi:10.1152/ajpcell.00443.2005.—Glutamate transporters (also called excitatory amino acid transporters, EAAT) are important in regulating extracellular levels of glutamate, a major excitatory neurotransmitter. The five EAATs are neuronally expressed in the central nervous system. The transporter function is revealed by our study. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

GluTAT transporters, also called excitatory amino acid transporters (EAAT), are important in regulating extracellular concentrations of glutamate (10), a major excitatory neurotransmitter in the central nervous system. By transporting glutamate from the extracellular to intracellular space under physiological conditions, EAATs prevent extracellular glutamate accumulation and regulate glutamate neurotransmission. Five EAATs have been identified (10): in rats, EAAT1 and EAAT2 are found in glial cells and EAAT3 and EAAT4 are mainly expressed in neurons, whereas EAAT5 is located in neurons and glial cells of the retina (1, 28, 36). All five EAATs are Na\(^+\)-dependent. They use the transmembrane gradient of Na\(^+\), K\(^+\), and H\(^+\) as a driving force (5, 10). They cotransport two or three Na\(^+\) and probably one H\(^+\) with one negatively charged glutamate molecule into the cell in exchange for one K\(^+\) and possibly one OH\(^-\)/HCO\(_3^-\). Thus the transport of glutamate is electrogenic (5, 10).

EAAT4 is expressed predominantly in the cerebellum. Its proteins have been found on Purkinje cell bodies and dendrites, particularly in the extrajunctional spaces of climbing fiber and mossy fiber synapses (19, 32, 47). These transporters were found to play a role in modulating the decay of postsynaptic responses (3, 39). In addition to its glutamate transport function, EAAT4 has been demonstrated to have substrate-gated Cl\(^-\) channel properties (17, 29), which may also contribute to the regulation of neurotransmission.

Protein kinase C (PKC), by phosphorylating serine/threonine residues in the substrate proteins, has been demonstrated to modify multiple protein functions and diverse cell activities (13, 20). Various responses of the activity of EAATs to PKC activation, including inhibition of EAAT1 and EAAT2 activity (8, 18) and increase of EAAT2 and EAAT3 activity (6, 14), have been reported. However, the PKC regulation of EAAT4 is unknown. In the present study, we found that glutamate-induced currents in EAAT4-expressing oocytes were enhanced by phorbol 12-myristate 13-acetate (PMA), a PKC activator (7). These enhanced EAAT4 currents were consistent with Cl\(^-\) conductance and partially inhibited by PKC inhibitors. However, these enhanced currents were not coupled to an increased transport function. Thus a novel pattern of PKC regulation of EAATs is revealed by our study.

MATERIALS AND METHODS

The animal care protocol was approved by the Institutional Animal Care and Use Committee at the University of Virginia. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, Revised 1996). All efforts were made to minimize the number of animals used and to minimize the animals’ pain and suffering.

Chemicals. All agents, unless specified below, were obtained from Sigma (St. Louis, MO). PMA, 4α-phorbol-12,13-didecanoate (4αPDD), calphostin C, Ro-31-8425 (Calbiochem, San Diego, CA) and staurosporine were initially dissolved in 0.1% DMSO and then diluted into their final concentrations in the recording solution. The final concentrations of DMSO in the recording solution were 0.025% or less. Preliminary study showed that 0.025% DMSO did not affect glutamate-induced current responses in EAAT4-expressing oocytes. All other chemicals were water soluble and were dissolved in the recording solution.

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EAAT4 cloning. EAAT4 cDNA was generated by RT-PCR using total RNA samples from the cerebellum of Sprague-Dawley rats. The polymerases used in the reaction were Superscript II RT and Taq DNA ( Gibco-BRL, Gaithersburg, MD). The 5'-end primer sequence was 5'-TCTGAATTCGGCACGAGCGCAGACACAGAG-3' and the 3'-end primer sequence was 5'-TCTAGATGTCGGCCACAGACGG-3', corresponding to positions 1 and 1930 of the full length of rat EAAT4 cDNA (GenBank accession no. U89608) (29). The generated cDNA was ligated into pcDNA3.1 cloning vector (Invitrogen, Paisley, UK) using the EcoRI and XbaI sites. The full length of the cDNA was sequenced in both directions.

The amino acid sequence predicted from our cDNA sequence was similar to that reported before (29), except for changes in three amino acids (see RESULTS). To verify our EAAT4 cDNA sequence, RT-PCR was performed on total cerebellar RNA samples of six rats (each rat provided one sample for one reaction) using primers to reversibly transcribe the segments that included those three changes. These segments of DNA were also sequenced in both directions.

cRNA preparation. The EAAT4 cDNA in pcDNA3.1 was linearized with restriction enzyme NotI and the capped cRNAs were transcribed using commercial T7 polymerase (Ambion, Austin, TX). The oocytes were surgically removed from the frog, the oocytes were defolliculated with Xenopus laevis from adult female frogs (Xenopus I, Dexter, MI) 1 day before cRNA injection, stages V and VI oocytes were isolated that contained (in mM) 82.5 NaCl, 2.0 KCl, 1.0 MgCl2, and 5.0 CaCl2. These oocytes were injected (Nanoject; Drummond Scientific, Broomall, PA) with a 1 ml microdispenser (model DAS-8; Keithley-Metrabyte, Taunton, MA) on a peristaltic pump. The flow was 5 ml/min, and the pump was pulled from capillary glass (10-μm diameter). These procedures were performed as described before (14, 18).

Electrophysiological recordings. These procedures were performed at room temperature as we described before (14, 18). Oocytes with or without injection of rat EAAT4 cRNA were superfused by gravity flow with ND96, containing (in mM) 96 NaCl, 2 KCl, 1.8 CaCl2, and 10 HEPES (pH adjusted to 7.4) for 2 h at room temperature (22°C). The oocytes were injected (Nanoinject; Drummond Scientific, Broomall, PA) with 40 ng of cRNA of EAAT4. Oocytes were then incubated at 16°C in modified Barth’s solution that contained (in mM) 82.5 NaCl, 2.0 KCl, 1.0 MgCl2, and 5.0 CaCl2 for 1 day before cRNA injection and the capped cRNAs were transcribed using commercial T7 polymerase (Ambion, Austin, TX). Oocyte preparation and injection. As we described before (14, 18), 1 day before cRNA injection, stages V and VI oocytes were isolated from adult female Xenopus laevis frogs (Xenopus I, Dexter, MI) anesthetized with 0.2% 3-aminobenzoic acid ethyl ester. After being surgically removed from the frog, the oocytes were defolliculated with 20 mg of collagenase (type 1a) in 20 ml of Ca2+-free OR2 solution that contained (in mM) 82.5 NaCl, 2.0 KCl, 1.0 MgCl2, and 5.0 HEPES (pH adjusted to 7.4) for 2 h at room temperature (22°C). Oocytes were injected (Nanoinject; Drummond Scientific, Broomall, PA) with 40 ng of cRNA of EAAT4. Oocytes were then incubated at 16°C in modified Barth’s solution that contained (in mM) 88 NaCl, 1 KCl, 2.4 NaHCO3, 0.41 CaCl2, 0.82 MgSO4, 0.3 Ca(NO3)2, 0.1 gentamicin, and 15 HEPES, pH adjusted to 7.4, for 4–5 days before they were used for glutamate uptake or voltage-clamping experiments.

Glutamate uptake assay. Oocytes injected with or without EAAT4 cRNA previously were washed twice with wash buffer (in mM: 10 HEPES, 140 NaCl, 5 Tris base, 2.5 KCl, 1.2 CaCl2, 1.2 K3HPO4, and 10 dextrose, pH 7.2). They were then incubated with 10 μM [3H]-L-glutamate (specific activity 56 Ci/mM; Amersham Biosciences, Piscataway, NJ) in the wash buffer for 10 min at room temperature. Incubation was terminated by removing the incubation buffer and by washing the oocytes three times with ice-cold wash buffer. Oocytes were lysed in 0.2 ml of 2% SDS, and radioactivity was measured in a liquid scintillation counter.

RESULTS

Electrophysiological recordings. These procedures were performed at room temperature as we described before (14, 18). Oocytes with or without injection of rat EAAT4 cRNA were superfused by gravity flow with ND96, containing (in mM) 96 NaCl, 2 KCl, 1.8 CaCl2, and 10 HEPES (pH adjusted to 7.4). The flow was ~5 ml/min, and the oocyte chamber volume was ~1 ml. Clamping microelectrodes were pulled from capillary glass (10-μM Microdispenser; Drummond Scientific) and were broken at the tip (~10 μm diameter). These electrodes had resistance of 3 MΩ when filled with 3 M KCl. Agar bridges were used as ground electrodes to reduce junction potential resulting from buffer changes. Oocytes were voltage clamped using a two-electrode voltage-clamp amplifier (model OC725A; Warner, New Haven, CT), which was connected to an analog-to-digital conversion board (model DAS-8; Keithley-Metabyte, Taunton, MA) on a personal computer. Data acquisition and analysis were performed using the OoClamp program (15). Currents were measured for 60 s (25-s application of glutamate, 35-s recovery with a glutamate-free superfusate) at a holding potential of ~60 mV.

The current-voltage relationships for glutamate-induced EAAT4 currents were determined using a two-electrode voltage-clamp technique with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) and a personal computer equipped with pCLAMP6 software (Axon Instruments) for data acquisition. The glutamate-induced currents were calculated by subtracting steady-state currents in the absence of glutamate from the corresponding currents in the presence of 10 μM glutamate during 100-ms voltage pulses from potential −100 to +40 mV in steps of 10 mV.

Application of experimental chemicals. In some experiments, Na+ in the bath solution was replaced by Li+ to determine the Na+ dependence of glutamate-induced currents. The PMA-induced and substrate-gated Cl− conductance of EAAT4 was identified by using ND96 solutions containing different Cl− concentrations (104, 56, and 26 mM) replaced by equimolal gluconate. The responses of EAAT4 to activation of PKC were studied by preincubating oocytes with 100 nM PMA in ND96 for 10 min before voltage clamping or glutamate uptake experiments were performed. In some experiments, oocytes were preincubated with 9 μM staurosporine, 50 μM chelerythrine, 9 μM calphostin C, 50 nM or 1 μM Ro-31-8245 for 1 h before the incubation of the PKC inhibitors plus 100 nM PMA for 10 min. Four millimoles of BAPTA (Calbiochem, San Diego, CA), a Ca2+ chelating agent, were injected intracellularly 1 h before the incubation with PMA. The inactive analog of PMA, 100 nM 4ePDD, was applied to oocytes for 10 min before voltage clamping was performed.

Statistical analysis. Because of the variation in the expression level of EAAT4 proteins in oocytes of different batches, glutamate-induced response under various treatment conditions was normalized to the response of the oocytes to glutamate alone. Results are means ± SD. Statistical analysis was performed by unpaired t-test. A value of P < 0.05 was accepted as significant. EC50 or Km, and Imax were derived by analyzing data with Prism 3.0 (GraphPad Software, San Diego, CA).

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dose dependent and saturable at high glutamate concentrations with a $K_m$ 0.97 ± 0.40 µM for glutamate (Fig. 3), similar to that reported for EAAT4 in the literature (35).

Increase of glutamate-gated currents via EAAT4 by PMA. Glutamate-induced currents in oocytes expressing EAAT4 were significantly increased after a 10-min incubation with 100 nM PMA, a PKC activator (Figs. 2–4). However, neither PMA incubation alone nor PMA incubation and glutamate application induced a current in oocytes uninjected with EAAT4 cRNA (data not shown), suggesting that the PMA enhancement of glutamate-induced currents in oocytes is EAAT4 specific. This enhancement is due to the increased $I_{\text{max}}$ of EAAT4 to glutamate, whereas PMA incubation did not change the affinity of EAAT4 for glutamate (Fig. 3). This enhancement was PMA dose dependent and saturable at PMA concentrations >300 nM. The EC$_{50}$ for PMA to enhance glutamate-induced EAAT4 currents was 74.5 ± 28.6 nM (Fig. 4). Thus 100 nM PMA was used in other experiments.

PMA enhancement of glutamate-gated Cl$^-$ conductance via EAAT4. A previous study (17) reported that Cl$^-$ conductance represented ~95% of the L-aspartate-evoked currents in oocytes expressing human EAAT4 at −60 mV. The substrate-induced EAAT4 currents reversed at about −20 mV (17), in contrast to the reversal potential of above −40 mV for the substrate-induced currents in voltage-clamped oocytes expressing EAAT1–3 that mainly have Na$^+$ conductance (26, 27, 42). Consistent with these previous results, glutamate-induced EAAT4 currents had a reversal potential of −17.3 ± 3.3 at extracellular Cl$^-$ concentration ([Cl$^-$]$_o$) 104 mM (Fig. 5A). The reversal potential had a linear relationship with log[Cl$^-$]$_o$ (Fig. 5D) and was −1.8 ± 4.4 and 20.3 ± 7.7 mV at [Cl$^-$]$_o$ 56 and 26 mM, respectively (Fig. 5). Thus the reversal potential of the glutamate-induced currents shifted by ~62 mV per 10-fold change in [Cl$^-$]. These features suggest that the majority of glutamate-induced currents in oocytes expressing rat EAAT4 are due to Cl$^-$ conductance. When oocytes expressing rat EAAT4 were preincubated with 100 nM PMA for 10 min, glutamate-induced currents were enhanced at various levels of clamping voltages (Fig. 5). The reversal potentials for the glutamate-induced EAAT4 currents in the oocytes preincu-
bated with PMA were $-16.6 \pm 4.8$, $-3.5 \pm 4.1$, and $20.0 \pm 11.5$ mV at $[\text{Cl}^-]_o$, 104, 56, and 26 mM, respectively, with an $\sim 60$-mV shift per 10-fold change in $[\text{Cl}^-]_o$ (Fig. 5). These values are very similar to those measured in the absence of PMA preincubation and suggest that the majority of glutamate-induced currents in EAAT4 oocytes after incubation with PMA are also Cl$^-$ currents.

Involvement of PKC in PMA enhancement of glutamate-gated Cl$^-$ currents via EAAT4. Consistent with the idea that PKC is involved in the effects of PMA on EAAT4, 4apoPDD, an inactive analog of PMA to activate PKC (45), failed to increase glutamate-induced currents in oocytes expressing EAAT4 (Fig. 6). In addition, three PKC inhibitors, staurosporine, chelerythrine, and calphostin C, partially inhibited PMA enhancement of glutamate-induced currents in oocytes expressing EAAT4 (Fig. 6), whereas these inhibitors did not affect glutamate-induced currents in oocytes without PMA incubation (Table 1). Furthermore, Ro-31-8425, a PKC inhibitor that inhibits conventional PKC isozymes at low concentrations (nM level) and inhibits other PKC isozymes as well at high concentrations (31, 46) partially inhibited PMA enhancement of glutamate-induced currents only at a high concentration (1 $\mu$M) (Fig. 6). BAPTA, a Ca$^{2+}$-chelating agent, did not affect PMA enhancement of glutamate-induced currents (Fig. 6), suggesting that intracellular Ca$^{2+}$ is not involved in the effects of PMA on EAAT4.

No effects of PMA on transport function of EAAT4. To determine whether PMA affects the transport function of EAAT4, uptake of [3H]-L-glutamate by oocytes was measured. Oocytes expressing EAAT4 had 10-fold higher glutamate uptake than oocytes without injection of EAAT4 cRNA (Fig. 7A). However, in contrast to the results that PMA enhanced glutamate-induced currents in oocytes expressing EAAT4, PMA did not increase glutamate uptake in these oocytes (Fig. 7). The PKC inhibitors staurosporine, chelerythrine, calphostin C, and Ro-31-8425 did not affect glutamate uptake activity of EAAT4 in the presence or absence of PMA (Fig. 7B).

DISCUSSION

In this study, we isolated an EAAT4 clone from rat cerebellum. This EAAT4 has a $K_m \sim 1$ $\mu$M for glutamate, which is...
Thus our results suggest that PMA increases glutamate transport in human and rat EAAT4 when L-aspartate was used as the substrate (17). Our glutamate transport has been reported to exist in human and rat EAAT4. The uncoupling between Cl⁻ conductance and glutamate transport, because PMA did not increase the uptake of glutamate by oocytes expressing EAAT4 during direct effects of PMA on EAAT4, because coapplication of selective channel activity of this glutamate transporter.

A major finding of our study is that PMA dose-dependently enhanced glutamate-induced EAAT4 currents. This enhancement was the result of increased I_{max}. PMA had no effects on the affinity of EAAT4 for glutamate. The reversal potential of glutamate-induced EAAT4 currents in oocytes incubated with or without PMA reversed at −16.6 and −17.3 mV, respectively, at 104 mM [Cl⁻]o. These values are close to the reported reversal potential (−24 mV) for Cl⁻ conductance (4) and are different from that for sodium (+60 to +80 mV) (9, 11), Ca²⁺ (+40 mV) (11), protons (+10 mV) (12), or potassium (−95 mV) (9) conductance in Xenopus oocytes. Thus our results suggest that PMA increases glutamate-gated Cl⁻ currents. These increased currents are not thermodynamically coupled to glutamate transport, because PMA did not increase the uptake of glutamate by oocytes expressing EAAT4. The uncoupling between Cl⁻ currents and glutamate transport has been reported to exist in human EAAT4 when L-aspartate was used as the substrate (17). Our results suggest that the two important EAAT4 functions, substrate transport and Cl⁻ channel-like activity, can be regulated separately. Interestingly, previous studies (16, 35, 41) have shown that arachidonic acid and niflumic acid activate substrate-gated proton currents that are not coupled to substrate transport in human and rat EAAT4. Taken together, these results suggest the complexity of the ion permeation pathways associated with EAAT4 and the potential for regulation of selective channel activity of this glutamate transporter.

The observed PMA effects on EAAT4 may not be due to the direct effects of PMA on EAAT4, because coapplication of PMA with glutamate to oocytes expressing EAAT4 during current recording did not induce currents bigger than those induced by glutamate alone (data not shown). PMA is a PKC activator (7). Thus the PMA effects on EAAT4 may involve PKC. To support this idea, 4αPDD, a PKC analog that does not activate PKC, did not affect glutamate-induced currents in oocytes expressing EAAT4. In addition, the three PKC inhibitors, staurosporine, calphostin C, and chelerythrine, partially inhibited the increased glutamate-gated currents by PMA. At least 11 PKC isozymes have been identified. They are classified into three groups: conventional PKCs (cPKC) (α, βI, βII, and γ), novel PKCs (nPKC) (δ, ε, η, and θ), and atypical PKCs (aPKC) (ζ and ηA) (44). The activity of both cPKC and nPKC isozymes is regulated by phorbol esters such as PMA. The cPKC isozymes require Ca²⁺ for activity, whereas nPKC and aPKC isozymes are Ca²⁺ independent (33). In our study, Ro-31-8425, a PKC inhibitor that inhibits cPKC isozymes at low concentrations (nM level) (31, 46), and intracellular application of BAPTA, a Ca²⁺-chelating agent, did not inhibit the PMA enhancement of glutamate-gated currents. Thus our results suggest that PKC isozymes such as nPKCs may play a role in the effects of PMA on EAAT4.

PKC regulation of EAATs other than EAAT4 has been reported. PKC has been shown to phosphorylate EAAT1 and reduce its activity (8). Multiple studies (14, 21, 25) have demonstrated that the activity of EAAT3 increases after PKC activation. PKC has also been implicated in the regulation of EAAT2 activity (18). However, various responses to PKC stimulation, including increase (6), decrease (18), and no change (40) in EAAT2 activity, have been reported. One of the

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<tr>
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<tr>
<td>Calphostin C (9 μM)</td>
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Values are means ± SD; n = 6 animals. Glutamate (1 μM)-induced currents in oocytes expressing rat excitatory amino acid transporters were measured after the oocytes were incubated with PKC inhibitors for 1 h. Units used to quantify responses are relative (fold) changes over the control values (currents induced by glutamate in the absence of PKC inhibitors) from the same oocytes with the control value set at 1. No statistical significance (paired t-test) was found in glutamate-induced currents after addition of the PKC inhibitor incubation compared with controls.

~10-fold lower than that of EAAT1–3 for glutamate (2). This EAAT4 also has Na⁺-dependent and substrate-gated activation of a Cl⁻ conductance by glutamate. These features are consistent with those for human and rat EAAT4 reported before (17, 29).

Table 1. Results of control experiments with PKC inhibitors

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Fig. 7. A: glutamate uptake measured by 10 μM [³H]-L-glutamate in oocytes with (EAAT4⁺) or without (EAAT4⁻) EAAT4 mRNA. Oocytes were preincubated with or without 100 nM PMA for 10 min before the uptake measurements were performed. Results are means ± SD (n = 4). *p < 0.05 compared with corresponding values from EAAT4⁻ oocytes. B: glutamate uptake measured by 10 μM [³H]-L-glutamate in oocytes injected with EAAT4 mRNA. The concentrations of the agents used were the following: 100 nM PMA, 50 μM chelerythrine, 9 μM calphostin C, 1 μM Ro-31-8425, and 9 μM staurosporine. Results are means ± SD (n = 4).
important mechanisms for the changed EAAT activity after acute activation of PKC is the alteration of the amount of EAATs in the plasma membrane, the functional site of EAATs. This mechanism is especially important for EAAT3, because unlike EAAT1, -2, and -4, which are mainly in the plasma membrane, a significant amount of EAAT3 proteins is intracellular (10). For example, many studies have shown that increased EAAT3 activity is associated with redistribution of EAAT3 to the plasma membrane within minutes of PKC activation (21, 25). Because substrate-induced currents of EAAT1–3 have been shown to be coupled to their substrate transport (5, 48), substrate-induced currents or the amount of substrates transported have been used to represent the activity of EAAT1–3 in the previous studies regarding PKC regulation of these EAATs. In this study, we showed that PKC may regulate only the substrate-gated EAAT4 Cl\(^{-}\) currents but not EAAT4 transport function. Signaling molecules other than PKC may also be involved in the observed PMA effects on EAAT4 in our study. It has been demonstrated that other signaling molecules, such as Ca\(^{2+}\)-ATPase, can be activated by PKC, which is independent of PKC activation (34). In addition, staurosporine, calphostin C, chelerythrine, and Ro-31-75, at the concentrations that are 250, 500, and 1000 nM, respectively, which is independent of PKC activation (34). In addition, staurosporine, calphostin C, chelerythrine, and Ro-31-PMA, which is independent of PKC activation (34). In addition, staurosporine, calphostin C, chelerythrine, and Ro-31-8425, at the concentrations that are >70-fold of their IC\(_{50}\) for PKC (20) and that reversed PMA effects on EAAT2 expressed also in oocytes (18), only partially inhibited the PMA effects on EAAT4.

Our findings may be physiologically relevant. EAAT4 is densely expressed postsynaptically in the extrajunctional structure of climbing fiber- and parallel fiber-Purkinje cell synapses (19, 32, 47). Purkinje cells are GABAergic neurons. Evidence has suggested that neuronal EAATs contribute to neurotransmitter GABA synthesis and inhibitory synaptic strength via their functions: uptake of glutamate that is used as substrate for GABA synthesis in these neurons (30, 38). In addition, unlike EAAT1–3, EAAT4 has large substrate-gated Cl\(^{-}\) currents that are not coupled to substrate transport (17). Thus there are a few mechanisms by which activation of EAAT4 might modulate neuronal excitability: the uptake of glutamate to terminate glutamate neurotransmission to Purkinje cells, supplying glutamate to these cells to enhance inhibitory synaptic strength, and dampening of neuronal excitability with its substrate-gated anion conductance such as Cl\(^{-}\) conductance. Consistent with these mechanisms, previous studies have demonstrated that inhibition of EAATs at climbing fiber- and parallel fiber-Purkinje cell synapses prolongs the decay time and reduces the amplitude of the excitatory postsynaptic currents (3, 39). Although we do not yet know the contribution of each of the mechanisms to the modulation of neurotransmission by EAATs, PKC regulation of the substrate-gated Cl\(^{-}\) conductance via EAAT4, as demonstrated in our study, may be part of mechanisms that finely adjust the neurotransmission through climbing fiber- and parallel fiber-Purkinje cell synapses. Recent studies (24, 43) have shown a low level of EAAT4 expression in astrocytes of the rat cortex, spinal cord, and retina. Thus our findings may have implications for understanding the functions of these astrocytes and their surrounding neurons.

In summary, we have shown that PMA enhances glutamate-gated Cl\(^{-}\) currents that are not thermodynamically coupled to glutamate transport via rat EAAT4. PKC isoforms other than cPKC may be involved in these PMA effects.

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