Metabotropic Glutamate Receptor-Mediated Depression of the Slow Afterhyperpolarization Is Gated by Tyrosine Phosphatases in Hippocampal CA1 Pyramidal Neurons

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Ireland, David R., Diane Guevremont, Joanna M. Williams, and Wickliffe C. Abraham. Metabotropic glutamate receptor-mediated depression of the slow afterhyperpolarization is gated by tyrosine phosphatases in hippocampal CA1 pyramidal neurons. J Neurophysiol 92: 2811–2819, 2004. First published July 7, 2004; 10.1152/jn.01236.2003. Group I metabotropic glutamate receptor (mGluR) agonists increase the excitability of hippocampal CA1 pyramidal neurons via depression of the postspike afterhyperpolarization. In adult rats, this is mediated by both mGluR1 and -5, but the signal transduction processes involved are unknown. In this study, we investigated whether altered levels of tyrosine phosphorylation of proteins are involved in the depression of the slow-duration afterhyperpolarization (sAHP) by the Group I mGluR agonist (RS)-3,5-dihydroxyphenylylglycerine (DHPG) in CA1 pyramidal neurons of rat hippocampal slices. Preincubation with the tyrosine kinase inhibitors lavendustin A or genestein, or the Src-specific inhibitor 3-(4-chlorophenyl) 1-(1,1-dimethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (PP2), did not inhibit the DHPG-mediated depression of the sAHP. However, preincubation with the tyrosine phosphatase inhibitor orthovanadate reduced the effects of DHPG. This effect of orthovanadate was prevented by simultaneous inhibition of tyrosine kinases with lavendustin A. Selective activation of either mGluR1 or -5 by application of DHPG plus either the mGluR5 antagonist 2-methyl-6-(phenylethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (PP2), did not inhibit the DHPG-mediated depression of the sAHP. However, preincubation with the tyrosine phosphatase inhibitor orthovanadate reduced the effects of DHPG. This effect of orthovanadate was prevented by simultaneous inhibition of tyrosine kinases with lavendustin A. Selective activation of either mGluR1 or -5 by application of DHPG plus either the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) or the mGluR1 antagonist (S)-(+)-(+)α-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385) demonstrated that the effect of inhibiting tyrosine phosphatases is not specific to either subtype of mGluR. These results suggest that the depression of the sAHP induced by activation of mGluR1 and -5 is gated by a balance between tyrosine phosphorylation and dephosphorylation.

INTRODUCTION

Modulation of intrinsic neuronal excitability is widely believed to be a key factor in the regulation of synaptic efficacy (Linden 1999; Schrader et al. 2002) and hence would be expected to play an important role in learning and memory. Indeed, postlearning changes in cell excitability have been demonstrated (e.g., Coulter et al. 1989). Changes in excitability can be effected by modulation of the spike afterhyperpolarization, in particular the slow afterhyperpolarization (sAHP) that is a principal determinant of firing frequency and spike frequency adaptation in hippocampal pyramidal neurons (Storm 1990). In these cells, activation of a variety of neurotransmitter receptors including metabotropic glutamate receptors (mGluRs) strongly depresses the sAHP (Pedarzani and Storm 1993) and as a result increases cell excitability. In CA1 pyramidal neurons of adult rats, the mGluR-induced depression of the sAHP and accompanying increase in excitability is dependent on both subtypes of Group I mGluR, mGluR1 and -5 (Ireland and Abraham 2002; Mannaioni et al. 1999, 2001). Typically, metabotropic actions of receptors are transduced by intracellular signaling pathways, but previous attempts to elucidate the signaling pathway(s) involved in the mGluR-induced depression of the sAHP in hippocampal neurons of the CA1 region in rat hippocampus have failed to identify the enzymes in question. Nevertheless, these studies have demonstrated that although Group I mGluRs classically couple to phosphoinositide hydrolysis (Abe et al. 1992; Aramori and Nakanishi 1992), none of phospholipase C (PLC), protein kinase C, and inositol 1,4,5-trisphosphate (IP3)-sensitive Ca2+ stores are involved (Ireland and Abraham 2002; Nouranifar et al. 1998). In addition, the depression of the sAHP current is not dependent on protein kinase A or Ca2+/calmodulin-dependent kinase II (Pedarzani and Storm 1996). These findings suggest that Group I mGluRs regulate the sAHP by a nonclassical pathway. In dentate gyrus granule cells, the mGluR-dependent suppression of the current underlying the sAHP has been shown to be dependent on tyrosine kinase activity (Abdul-Ghani et al. 1996), but the role that tyrosine phosphorylation plays in mGluR depression of the sAHP has not been previously examined in CA1 neurons. The present study therefore aimed to determine the role, if any, of tyrosine phosphorylation in the mGluR-mediated depression of the sAHP in CA1. Our results demonstrate that both the mGluR1- and mGluR5-triggered depression of the sAHP in CA1 neurons are promoted by tyrosine dephosphorylation of a regulatory protein. Portions of these data have been reported in preliminary form (Ireland and Abraham 2003).

METHODS

Tissue preparation

Transverse hippocampal slices (400 μm) were prepared from young adult male Sprague-Dawley rats (6–8 wks). All procedures were performed in accordance with New Zealand animal welfare legislation, and the experiments and procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Rats were anesthetized with ketamine (100 mg/kg ip) and decapitated, and the brain was quickly removed and cooled with ice-cold artificial cerebrospinal fluid (ACSF). The hippocampi were dissected free, and area CA3 was removed with a manual knife-cut to reduce potential hyperexcitability and/or slow-
onset potentiation (Bortolotto and Collingridge 1993). Slices were transferred to a humidified recording chamber and superfused (2 ml/min) with ACSF of the following composition (in mM): 124 NaCl, 3.2 KCl, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 2.5 CaCl$_2$, 1.3 MgCl$_2$, and 10 glucose (equilibrated with 95% O$_2$-5% CO$_2$). Before recording, slices were allowed to equilibrate for 2 h while the temperature was increased slowly to 32.5°C.

**Intracellular recording**

Intracellular recording microelectrodes were pulled from borosilicate glass and filled with 2 M potassium acetate (resistances: 70–130 MΩ). Current-clamp recordings were made from CA1 pyramidal neurons using an Axoclamp 2A amplifier (Axon instruments) and pCLAMP 8.0 software (Axon instruments), and data were stored on a computer for off-line analysis using pCLAMP 8.0 software. The membrane potential of the impaled neuron was held constant throughout the experiment at −65 mV by manually adjusting the holding current. The sAHP was induced once every 2 min by a train of four action potentials, each elicited by a separate depolarizing current pulse (2 ms, 3 nA, 5-ms interpulse interval). sAHP amplitude was measured at peak amplitude and expressed as a percentage change from baseline values. In some cells, the inhibition of the sAHP revealed a small afterdepolarization, and in these cases, the percentage change in the amplitude of the afterhyperpolarization was capped at −100%. The number of action potentials fired by a depolarizing current pulse (0.5 nA, 250 ms) was recorded once every 2 min as a measure of cell excitability and is expressed as the difference in the number of spikes from baseline values. Only one cell was studied per slice.

Drugs were applied by bath perfusion, and all inhibitors were applied for ≥20 min prior to addition of (RS)-3,5-dihydroxyphenylglycine (DHPG), which was applied for 10 min. Doses of drugs were chosen based on both experience in our laboratory and on previous studies in hippocampal slices. The effects of DHPG on the sAHP peak amplitude and excitability were quantified by averaging the last 2 data points (i.e., the last 4 min) of drug application. Statistical significance of drug effects was determined by performing unpaired Student’s t-test between drug groups at the P < 0.05 significance level, and data are presented as means ± SE.

**Western blot procedure**

**PROTEIN EXTRACTS.** Hippocampal slices were incubated in 35-mm tissue culture wells containing 1 ml ACSF in a humidified and oxygenated atmosphere for 2 h while the temperature was slowly raised to 32.5°C. Once this temperature was reached, four slices were exposed to each of the four different conditions (no drug, sodium orthovanadate, lavendustin A, lavendustin A plus orthovanadate). In the lavendustin plus orthovanadate group, lavendustin was added 20 min prior to orthovanadate. Two of the slices in each condition were removed immediately frozen, while the remaining two slices in each condition were rinsed in ACSF and the incubation continued for 1 h. Subsequently, protein cellular extracts were prepared from the frozen slices by sonication in the following buffer (in mM): 20 Tris.Cl (pH 7.6), 1 EDTA, 2 dithiothreitol (DTT), 0.1 phenyl methanesulfonyl fluoride (PMSF), 25 NaF, 10 sodium pyrophosphate (NaPPi), 10 Complete protease inhibitor (Roche), 10 3-(3-cholamidopropyl)dimethylammonio)-1-propane sulfonate (CHAPS), 0.5% SDS, and 1 orthovanadate and 10 μM lavendustin A according to Williams et al. (1998). Protein concentration was estimated using bicinechonic acid (BCA) assay (Smith et al. 1985) with BSA protein standards.

**QUANTITATIVE WESTERN BLOT ANALYSIS.** Equal amounts of protein extracts (25 μg) were separated by SDS-PAGE (9%) and transferred to nitrocellulose membrane (Schleicher and Schuell). Membranes were probed with PY99 monoclonal antibody (Santa Cruz Biotechnology) to detect tyrosine phosphorylated residues. Antibody binding was detected using HRP-conjugated secondary antibodies (DAKO) and enhanced chemiluminescence (Amersham). Autoradiographs were scanned using a BioRad imaging densitometer and quantified using Molecular Analyst software. To ensure that the densitometric analysis was carried out within the linear range of the film, standard curves were established and multiple exposures taken. Only the most prominent band (180 kDa) was quantitated as it was representative of the other visible bands. Results are expressed as the ratio of tyrosine phosphorylation in each drug-treated group to tyrosine phosphorylation in the no-drug control group. Statistical significance was determined by performing paired Student’s t-test at the P < 0.05 significance level.

**Drugs and chemicals**

All salts were obtained from BDH Chemicals (Poole, UK); (RS)-DHPG, lavendustin A, genistein, LY367385, MPEP, and PP2 from Tocris Cookson (Bristol, UK); sodium orthovanadate from Sigma. Drugs were dissolved in H$_2$O (RS-DHPG, MPEP, orthovanadate), 100 mM NaOH (LY367385), or dimethyl sulfoxide (lavendustin A, genistein, PP2) and diluted 1000-fold to their final concentration in ACSF.

**RESULTS**

**mGluR-induced depression of the sAHP is not dependent on tyrosine kinase activation**

In the first experiment, we replicated our previous report (Ireland and Abraham 2002) that the Group I–specific mGluR agonist DHPG produces a rapid-onset and persistent depression of the sAHP in CA1 neurons and a corresponding increase in excitability. Application of DHPG (20 μM) for 10 min acutely reduced the peak amplitude of the sAHP by −91 ± 3% of baseline (n = 14) followed by a long-lasting depression (Fig. 1, A and C). When measured 40 min after washout of agonist, the sAHP had recovered to −56 ± 4% of baseline (n = 10). DHPG also produced an acute increase in excitability of 7.6 ± 0.6 spikes/pulse above baseline (n = 14) that had recovered to 1.7 ± 0.6 spikes/pulse above baseline (n = 10) by 40 min after agonist washout (Fig. 1, B and D).

To assess the possible involvement of tyrosine kinases in the signal transduction pathway activated by mGluR stimulation, we preincubated the slices in the tyrosine kinase inhibitor lavendustin A (10 μM) for ≥20 min prior to application of DHPG. During incubation, lavendustin A had no effect on the basal sAHP or excitability (Fig. 2). Lavendustin did not significantly inhibit either the acute depression of the sAHP by DHPG (−87 ± 5%, n = 4; Fig. 2A) or the depression measured 40 min after agonist washout (−51 ± 8, n = 4; Fig. 2A). Lavendustin also did not significantly affect the increase in excitability induced by DHPG either during agonist application (7.4 ± 1.0 spikes/pulse above baseline, n = 4; Fig. 2B) or after 40 min washout (2.0 ± 1.3 spikes/pulse above baseline, n = 4; Fig. 2B). In separate experiments, we investigated the effect of an alternative tyrosine kinase inhibitor, genistein. Genistein (50 μM) did not affect the basal sAHP or excitability and did not significantly inhibit the acute depression of the sAHP by DHPG (−62 ± 17%, n = 4; Fig. 2A). However, genistein did significantly reduce the increase in excitability in response to DHPG (2.8 ± 1.0 spikes/pulse above baseline, n = 4, P < 0.05; Fig. 2B). Because genistein may exert significant nonspecific effects (Geissler et al. 1990), we employed a third
tyrosine kinase inhibitor, PP2, which is a highly potent and specific inhibitor of the Src-family tyrosine kinases (Hanke et al. 1996). Src tyrosine kinases have been previously reported to mediate the signal transduction of mGluRs in hippocampal neurons (Heuss et al. 1999). PP2 (10\(^{-6}\)M) did not affect the basal sAHP or excitability and also did not inhibit either the response of the sAHP to DHPG (sAHP, \(-99 \pm 0.5\%\), \(n = 4\); Fig. 2A) or the response of excitability to DHPG (10 \pm 0.9 spikes/pulse above baseline, \(n = 4\); Fig. 2B). The inhibitory effect of genistein on the mGluR-induced excitability increase may therefore have been due to a nonspecific effect of genistein, such as inhibition of ser/thr kinases, which it has been reported to also inhibit (Geissler et al. 1990). Taken together, these data indicate that the mGluR suppression of the sAHP and increase in excitability in CA1 pyramidal cells does not require the activation of tyrosine kinases that are sensitive to lavendustin A or PP2.

mGluR-induced depression of the sAHP is tyrosine phosphatase dependent

We next investigated whether tyrosine phosphatases are involved in the mGluR-mediated suppression of the sAHP because they have been reported to contribute to the DHPG-induced long-term depression of evoked synaptic potentials in CA1 (Moult et al. 2002). To achieve this, slices were preincubated for \(\geq 20\) min in 1 mM orthovanadate (Coussens et al. 2000; Gordon 1991; Moult et al. 2002). This treatment did not significantly affect the amplitude of the sAHP, although in some cells it caused an increase in sAHP width (data not shown) and on average caused a small but significant reduction in excitability (\(P < 0.05\); Fig. 3B). In the presence of orthovanadate, the DHPG-induced depression of the sAHP was on average significantly reduced compared with that in the absence of orthovanadate (\(-53 \pm 9\%\), \(n = 16\), \(P < 0.005\); Fig. 3A) as was the DHPG-induced increase in excitability (4.6 \pm 1.1 spikes/pulse above baseline, \(n = 16\), \(P < 0.05\); Fig. 3B). Forty minutes after agonist washout both the sAHP (\(-51 \pm 5\%\), \(n = 12\)) and excitability (2.6 \pm 0.8 spikes/pulse above baseline, \(n = 12\)) had recovered to levels not different than those seen in experiments performed with DHPG alone. Interestingly, the sAHP in 8 of 16 cells appeared to be noticeably less sensitive to orthovanadate, and in general there was also less of an effect of orthovanadate on the mGluR-induced increase in excitability in those cells (Fig. 3E). The acute depression of the sAHP in these cells was only \(-18 \pm 4\%\) (\(n = 8\)) during DHPG application and \(-43 \pm 12\%\) (\(n = 4\)) after 40 min of agonist washout (Fig. 3C). In the same cells, excitability increased by only 1.1 \pm 0.5 spikes/pulse above baseline (\(n = 8\)) during DHPG application and was 0.4 \pm 0.7 spikes/pulse above baseline (\(n = 4\)) after 40 min of agonist washout (Fig. 3D).
The effects of orthovanadate that we have observed are mediated via changes in tyrosine phosphorylation rather than by nonspecific actions. Surprisingly, the increase in excitability in response to DHPG (4.9 ± 1.5 spikes/pulse above baseline, n = 10; Fig. 4B) was not significantly different to that in the presence of orthovanadate only, suggesting that other factors (e.g., other conductances) contributing to the excitability change may not be regulated in the same way.

Tyrosine phosphorylation is increased by inhibition of tyrosine phosphatase

The preceding data suggest that inhibition of tyrosine phosphatases with orthovanadate leads to an increase in tyrosine kinase activity. To investigate whether orthovanadate treatment directly affects tyrosine phosphorylation levels under our experimental conditions, we incubated hippocampal slices with either orthovanadate, lavendustin A, or both, for 20 min and carried out Western Blot analyses using an antibody that specifically recognizes phosphotyrosine residues. Orthovanadate caused a generalized increase in tyrosine phosphorylation after 20 min exposure compared with non-drug-treated controls (Fig. 5A). Densitometric analysis of the most prominent phosphotyrosine band (180 kDa) revealed a significant increase in tyrosine phosphorylation after 20 min orthovanadate exposure, relative to the untreated controls (Fig. 5B; 2.01 ± 0.39, n = 5, P < 0.05). This was reversed after 60 min of washing (1.02 ± 0.10, n = 5, data not shown). In contrast, lavendustin A did not significantly affect general tyrosine phosphorylation (Fig. 5A) or phosphorylation of the 180-kDa band (Fig. 5B; 0.89 ± 0.10, n = 5). In the presence of lavendustin A, orthovanadate appeared to cause a reduced effect on general tyrosine phosphorylation (Fig. 5A) and the 180-kDa band (1.68 ± 0.19, n = 5), but the increase was still significant (P < 0.05). This suggests that the tyrosine kinases blocked by lavendustin A are relatively minor contributors to the increase in generalized hippocampal tyrosine phosphorylation occurring in response to orthovanadate treatment.

Effects of inhibiting tyrosine phosphatase are not specific to either mGluR1 or mGluR5

We have previously shown that both mGluR1 and -5 contribute to the DHPG-induced depression of the sAHP and increase in excitability (Ireland and Abraham 2002). The possibility therefore exists that the bimodal effects of orthovanadate could be explained by a selective action on either the mGluR1- or mGluR5-signaling pathway. Because selective full agonists of mGluR1 and -5 are not available at present, we therefore used a combination of DHPG and specific antagonists to investigate this possibility. To assess the effects of orthovanadate on the mGluR1-mediated signaling pathway, we applied DHPG (20 μM) after prior incubation with orthovanadate plus the mGluR5-specific antagonist MPEP (10 μM). Under these conditions, DHPG depressed the sAHP by −27 ± 7% (n = 7; Fig. 6A). Consistent with orthovanadate having some inhibitory effect on the mGluR1-mediated component, this appeared to be a lesser degree of depression of the sAHP than seen in the absence of orthovanadate (−45 ± 14%, n = 7; Fig. 6A) (data taken from Ireland and Abraham 2002), although the difference was not significant. Conversely, to assess the effects of orthovanadate on mGluR5-mediated sig-

**FIG. 2.** mGluR-mediated depression of the sAHP is not dependent on activation of tyrosine kinases. A: percent change in peak amplitude of the sAHP in cells exposed to 20 μM DHPG for 10 min in the presence of either 10 μM lavendustin A (●), 50 μM genistein (○), or PP2 (□). B: change in number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in the same cells as in A. ••••, period of exposure to antagonists.
naling pathways, we applied DHPG after prior incubation in orthovanadate plus the mGluR1-specific antagonist, LY367385 (100 μM). Under these conditions, DHPG depressed the sAHP by −34 ± 10% (n = 8; Fig. 6C), which like MPEP produced a nonsignificantly lesser depression than in the absence of orthovanadate (−57 ± 10%, n = 7; Fig. 6C), consistent with an inhibitory effect on the mGluR5-mediated component of the sAHP depression. These results suggest that orthovanadate does not selectively inhibit sAHP-depression by one mGluR subtype over the other.

In the presence of MPEP plus orthovanadate, DHPG increased the excitability by 1.3 ± 0.5 spikes/pulse above baseline (n = 7; Fig. 6B); this was not significantly different to that in the absence of orthovanadate (1.8 ± 0.8 spikes/pulse above baseline, n = 6; Fig. 6B). However, in the presence of LY367385 plus orthovanadate, DHPG increased the excitability by 1.1 ± 0.8 spikes/pulse above baseline (n = 8; Fig. 6D); this was significantly less than that in the absence of orthovanadate (3.4 ± 0.5 spikes/pulse above baseline, n = 7, P < 0.05; Fig. 6D). These data suggest that the increase in excitability mediated by mGluR5 may be somewhat preferentially inhibited by orthovanadate, presumably due to effects on a factor other than the sAHP that also contributes to the DHPG-induced excitability increase.

**DISCUSSION**

We have demonstrated that the suppression of the sAHP by a Group I mGluR agonist does not occur via activation of tyrosine kinases in CA1 pyramidal neurons. Instead, our results suggest that the suppression of the sAHP by mGluR1 and -5 is gated by the degree of tyrosine phosphorylation of a target protein, such that relatively lower tyrosine phosphorylation permits, and higher tyrosine phosphorylation prevents, suppression of the sAHP. The conclusions of the present study are supported by recent work on mGluR-dependent long-term depression (LTD) of evoked synaptic responses in CA1 that demonstrated that mGluR-LTD is dependent on tyrosine phosphatase activity (Moul et al. 2002). However, the dependence of the mGluR-mediated suppression of the sAHP on tyrosine dephosphorylation is somewhat surprising given the evidence for the involvement of serine/threonine kinases in the suppres-
sion of the sAHP by monoaminergic, kainic, and muscarinic agonists (Krause and Pedarzani 2000; Melyan et al. 2002; Pedarzani and Storm 1993, 1995, 1996), most probably by phosphorylation of the underlying Ca\(^{2+}\)-activated K\(^+\) channels (\(K_{\text{sAHP}}\)) and a consequent reduction in their open probability (Sah and Isaacson 1995). Furthermore, previous work in dentate gyrus granule cells found that the suppression of the sAHP is dependent on tyrosine kinases (Abdul-Ghani et al. 1996).

In the present study, we have revealed a role for a tyrosine phosphatase in promoting the mGluR-depression of the sAHP, but it appears that the importance of that role may vary across cells. This potentially could be explained by differences between mGluR1 and -5 in their sensitivity to orthovanadate and their relative abundance between cells. However, because we have shown that this is not an mGluR subtype-specific effect, an alternative explanation is that more than one signaling pathway, not all dependent on tyrosine dephosphorylation, could mediate the mGluR depression of the sAHP and that their relative importance or the level of redundancy between them could differ between neurons. Redundancy of mGluR signaling pathways has been previously demonstrated (Morikawa et al. 2003).

**mGluR-suppression of the sAHP is gated by tyrosine dephosphorylation**

One possible role of tyrosine phosphatases is to act as an essential component of the mGluR-triggered signaling pathway to depress the sAHP in the same way that monoamine transmitters are thought to depress the sAHP by activation of serine/threonine protein kinases that phosphorylate \(K_{\text{sAHP}}\) and consequently alter channel opening (Pedarzani and Storm 1993; Sah and Isaacson 1995). However, dual lines of evidence argue against this interpretation. First activation of mGluRs has been shown to increase tyrosine phosphorylation (Siciliano et
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increases tyrosine phosphorylation thus preventing sAHP depression.

inhibition of tyrosine phosphatases, the action of tyrosine kinases (TK) allowing depression of the sAHP by mGluR activation. However, during phosphorylation via kinase inhibition. Instead, our results show that blocking tyrosine kinases does not mimic mGluR depression of the sAHP yet can rescue it from inhibition by tyrosine phosphatase blockade. A more likely role for the tyrosine phosphatase therefore seems to be a permissive one that “gates” the mGluR-activated transduction pathway(s) that are responsible for suppression of the sAHP as has been suggested for the serine/threonine phosphatase-dependent muscarinic depression of the sAHP (Krause and Pedarzani 2000). In this scenario (Fig. 7), a balance exists between tyrosine phosphorylation and dephosphorylation of a substrate protein(s). The basal level of this balance is weighted toward the nonphosphorylated state, which permits inhibition of the sAHP by mGluR activation. A disruption of this balance by inhibition of the tyrosine phosphatase leads to a consequent increase in substrate phosphorylation by tyrosine kinases. When this increase in phosphorylation is prevented by simultaneous inhibition of the tyrosine kinases, the relatively nonphosphorylated state and ability of DHPG to suppress the sAHP are maintained.

One possible site of tyrosine dephosphorylation is an enzyme within the mGluR-activated transduction pathway. However, the identity of this pathway has remained elusive in CA1 pyramidal neurons with several candidate enzymes [protein kinase A, protein kinase C, Ca2+/calmodulin-dependent kinase II, tyrosine kinase, phospholipase C, and inositol 1,4,5-trisphosphate (IP3)-sensitive Ca2+ stores] having been ruled out (Ireland and Abraham 2002; Nouranifar et al. 1998; Pedarzani and Storm 1996; present study). An alternative possibility would be tyrosine dephosphorylation of either KaHP itself or of the Ca2+ channels that supply Ca2+ to activate KaHP, such that a relatively dephosphorylated state allows them to be inhibited by mGluR-activated signaling pathways. Both Ca2+-activated K+ channels and voltage-dependent Ca2+ channels can be regulated by tyrosine phosphorylation (Ling et al. 2000; Potier and Rovira 1999) although transmitter-suppression of the sAHP does not appear to be associated with a measurable change in the cytosolic Ca2+ transient (Charpak et al. 1990; Knopfel et al. 1990). A further possibility is tyrosine dephosphorylation of the mGluRs themselves or of a closely associated protein because mGluRs have been shown to be subject to tonic tyrosine phosphorylation and dephosphorylation, although it is not clear what effect tyrosine phosphorylation state has on receptor function (Orlando et al. 2002). In addition, based on the location of the tyrosine residues on mGluR5 (Minakami et al. 1997) it has been suggested that the tyrosine phosphorylation state of the receptor may not influence the activation of the phosphoinositide hydrolysis transduction pathway but might instead affect coupling of the

FIG. 6. Orthovanadate affects both mGluR1- and mGluR5-mediated responses to DHPG. A: percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG in the presence of 10 µM MPEP (○) or 10 µM MPEP plus 1 mM orthovanadate (●). B: change in the number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in the same cells as in A. C: percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG in the presence of 100 µM LY367385 (○) or 100 µM LY367385 plus 1 mM orthovanadate (●). D: change in the number of action potentials in a train evoked by a 250-ms, 0.5-nA depolarizing pulse in the same cells as in C. • • •, period of exposure to antagonists.

FIG. 7. Proposed mechanism for gating of mGluR-mediated depression of the sAHP by tyrosine phosphorylation state. Candidate tyrosine phosphoproteins include mGluRs, signaling enzymes, and the Ca2+-activated K+ channels that underlie I

al. 1994), which is not consistent with activation of a tyrosine phosphatase. Second, if DHPG was suppressing the sAHP by decreasing tyrosine phosphorylation, then we would have expected to mimic the effect of DHPG by decreasing tyrosine phosphorylation via kinase inhibition. Instead, our results show that blocking tyrosine kinases does not mimic mGluR depression of the sAHP yet can rescue it from inhibition by tyrosine phosphatase blockade. A more likely role for the tyrosine phosphatase therefore seems to be a permissive one that “gates” the mGluR-activated transduction pathway(s) that are responsible for suppression of the sAHP as has been suggested for the serine/threonine phosphatase-dependent muscarinic depression of the sAHP (Krause and Pedarzani 2000). In this scenario (Fig. 7), a balance exists between tyrosine phosphorylation and dephosphorylation of a substrate protein(s). The basal level of this balance is weighted toward the nonphosphorylated state, which permits inhibition of the sAHP by mGluR activation. A disruption of this balance by inhibition of the tyrosine phosphatase leads to a consequent increase in substrate phosphorylation by tyrosine kinases. When this increase in phosphorylation is prevented by simultaneous inhibition of the tyrosine kinases, the relatively nonphosphorylated state and ability of DHPG to suppress the sAHP are maintained.

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receptor to non-PLC pathways (Orlando et al. 2002). This would be consistent with our previous conclusion that the mGluR depression of the sAHP is independent of PLC activity (Ireland and Abraham 2002). Tyrosine dephosphorylation could also regulate the function of an mGluR-associated protein such as Homer, which binds selectively to Group I mGluRs (Brakeman et al. 1997) and regulates their activity (Ango et al. 2001; Minami et al. 2003), localization (Ango et al. 2000; Serge et al. 2002), and interactions with other proteins (Kammermeier et al. 2000; Tu et al. 1998; Xiao et al. 1998).

Functional implications

The afterhyperpolarization and cell excitability are increasingly thought to be important determinants of synaptic plasticity (Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996), and it has been recently demonstrated that the mGluR-induced persistent reduction in the afterhyperpolarization results in improved precision and reliability of cell firing (Sourdet et al. 2003). Therefore the regulation of the mGluR-dependent suppression of the sAHP by tyrosine phosphorylation state raises the issue of what influence tyrosine dephosphorylation has on synaptic function and information transfer through a neuron. Forms of plasticity such as long-term potentiation (Cavus and Teyle 1996; Huang and Hsu 1999; O’Dell et al. 1991), paired-pulse facilitation (Moresco et al. 2003), and mGluR-dependent LTD (Camodeca et al. 1999) have been shown previously to be dependent on tyrosine kinase activity. Recently, tyrosine phosphatases have also been demonstrated to play a role in synaptic function and neuroplasticity, for example, in the regulation of mGluR-induced slow excitatory synaptic potentials in cerebellar Purkinje neurons (Canepari and Ogden 2003), in mGluR-dependent LTD in CA1 (Moul et al. 2002), and in the regulation of spatial learning (Skelton et al. 2003). An important question is what role does tyrosine phosphatase-dependent regulation of the sAHP play in other forms of synaptic plasticity, such as the mGluR-dependent “priming” of long-term potentiation (Cohen et al. 1999). Tyrosine dephosphorylation could represent an important means of regulating how a neuron reacts to incoming synaptic stimuli through a mechanism that gates the activity of signaling pathways.

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