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

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ABSTRACT

Group I metabotropic glutamate receptor (mGluR) agonists increase the excitability of hippocampal CA1 pyramidal neurons via depression of the post-spike afterhyperpolarization. In adult rats this is mediated by both mGluR1 and mGluR5, but the signal transduction processes involved are unknown. In this study, we investigated whether altered levels of tyrosine phosphorylation of proteins is involved in the depression of the slow-duration afterhyperpolarization (sAHP) by the Group I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) in CA1 pyramidal neurons of rat hippocampal slices. Preincubation with the tyrosine kinase inhibitors lavendustin A or genistein, or the Src-specific inhibitor 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 mGluR5 by application of DHPG plus either the mGluR5 antagonist MPEP or the mGluR1 antagonist 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 mGluR5 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, post-learning 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 mGluR5 (Ireland and Abraham 2002, Mannaioni et al. 1999, 2001). Typically, metabotropic actions of receptors are transduced by intracellular signalling pathways, but previous attempts to elucidate the signalling pathway(s) involved in the mGluR-induced depression of the sAHP in pyramidal 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), neither phospholipase C (PLC), protein kinase C nor inositol 1,4,5-trisphosphate (IP$_3$)-sensitive Ca$^{2+}$ 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 Ca$^{2+}$/calmodulin-dependent kinase II (Pedarzani and Storm 1996). These findings suggest that Group I mGluRs regulate the sAHP by a
non-classical 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 anaesthetized with ketamine (100 mg/kg, i.p.), 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 (mM): 124 NaCl, 3.2 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2.5 CaCl2, 1.3 MgCl2, 10 glucose (equilibrated with 95% O2: 5% CO2). Before recording, slices were allowed to equilibrate for 2 hr 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 at least 20 min prior to addition of DHPG, which was applied for 10 min. Doses of drugs were chosen based both on 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-tests between drug groups at the $P<0.05$ significance level, and data are presented as mean ± SEM.

*Western Blot procedure*

**Protein extracts**

Hippocampal slices were incubated in 35 mm tissue culture wells containing 1 ml ASCF in a humidified and oxygenated atmosphere for 2 hr 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 and immediately frozen, while the remaining two slices in each condition were rinsed in ACSF and the incubation continued for 1 hr.

Subsequently, protein cellular extracts were prepared from the frozen slices by sonication in the following buffer (mM): 20 Tris.Cl (pH 7.6), 1 EDTA, 2 DTT, 0.1 PMSF, 25 NaF, 10 NaPPi, 10 Complete protease inhibitor (Roche), 10 CHAPS, 0.5% SDS, 1 orthovanadate and 10 µM lavendustin A) according to Williams et al.(1998).

Protein concentration was estimated using 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, Inc) 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-tests 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 H₂O (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. 1A, 1C). When measured 40 min after wash-out 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) which had recovered to 1.7 ± 0.6 spikes/pulse above baseline (n=10) by 40 min after agonist wash-out (Fig. 1B, 1D).

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 at least 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 wash-out (–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 wash-out (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 non-specific 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 µM) did not affect the basal sAHP or excitability and also did not inhibit either the response of the sAHP to DHPG (sAHP, -99 ± 0.5%, n=4; Fig. 2A) or the response of excitability to DHPG (10 ± 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 non-specific 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, since 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 at least 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 to that in the absence of orthovanadate (−53 ± 9%, n=16, P < 0.005; Fig. 3A) as was the DHPG-induced increase in excitability (4.6 ± 1.1 spikes/pulse above baseline, n=16, P < 0.05; Fig. 3B). Forty min after agonist wash-out both the sAHP (-51 ± 5%, n=12) and excitability (2.6 ± 0.8 spikes/pulse above baseline, n=12) had recovered to levels not different to those seen in experiments performed with DHPG alone. Interestingly, the sAHP in 8 out 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 ± 4% (n=8) during DHPG application and −43 ± 12% (n=4) after 40 min of agonist wash-out (Fig. 3C). In the same cells, excitability increased by only 1.1 ± 0.5 spikes/pulse above baseline (n=8) during DHPG application and was 0.4 ± 0.7 spikes/pulse above baseline (n=4) after 40 min of agonist wash-out (Fig. 3D). In the remainder of cells the mean depression of the sAHP was −89 ± 4% (n=8) during DHPG application and −55 ± 4% (n=8) after 40 min of agonist wash-out (Fig. 3C). In these cells excitability increased by 8.1 ± 1.0 spikes/pulse above baseline (n=8) during DHPG application and was 4.0 ± 1.0 spikes/pulse above baseline after 40 min of agonist wash-out (n=8) (Fig. 3D). These data demonstrate that, in approximately 50% of CA1 neurons, tyrosine dephosphorylation plays an important role in the mGluR-dependent depression of the sAHP and the corresponding increase in excitability.

Inhibition of DHPG-effects is prevented by prior blockade of tyrosine kinases

Since inhibition of tyrosine phosphatases could lead to an increase in tyrosine phosphorylation by tyrosine kinases, we next addressed whether increased tyrosine
phosphorylation is required for inhibition of the mGluR effect by orthovanadate. Slices were preincubated in lavendustin A for at least 20 min prior to and during application of orthovanadate in order to inhibit tyrosine kinases and tyrosine phosphatases simultaneously. Under these conditions, DHPG produced an acute depression of the sAHP (-82 ± 5%, n=10) which was significantly different to that in the presence of orthovanadate (P < 0.05), but was not significantly different to that with DHPG alone (Fig. 4A). These data suggest that the effects of orthovanadate on the mGluR-dependent depression of the sAHP are dependent on an associated increase in tyrosine kinase activity. They also confirm that the effects of orthovanadate that we have observed are mediated via changes in tyrosine phosphorylation rather than by non-specific 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 above 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 which specifically recognizes phosphotyrosine residues. Orthovanadate caused a generalized increase in tyrosine phosphorylation after 20 min exposure compared to 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 mGluR5 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-signalling pathway. Since selective full agonists of mGluR1 and mGluR5 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 signalling pathway, we applied DHPG (20 \(\mu\)M) after prior incubation with orthovanadate plus the mGluR5-specific antagonist MPEP (10 \(\mu\)M). 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 signalling 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 non-significantly 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) which 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) which 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 mGluR5 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 which demonstrated that mGluR-LTD is dependent on tyrosine phosphatase activity (Moult 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 suppression 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_{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 mGluR5 in their sensitivity to orthovanadate and their relative abundance between cells. However, since we have shown that this is not an mGluR subtype-specific effect, an alternative explanation is that more than one signalling 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 signalling 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 signalling 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 which 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. Firstly, activation of mGluRs has been shown to increase tyrosine phosphorylation (Siciliano et al. 1994), which is not consistent with activation of a tyrosine phosphatase. Secondly, if DHPG were 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 non-phosphorylated 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 non-phosphorylated 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, Ca\(^{2+}\)/calmodulin-dependent kinase II, tyrosine kinase, phospholipase C, and inositol 1,4,5-trisphosphate (IP\(_3\))-sensitive Ca\(^{2+}\) 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 \(K_{sAHP}\) itself or of the Ca\(^{2+}\) channels that supply Ca\(^{2+}\) to activate \(K_{sAHP}\), such that a relatively dephosphorylated state allows them to be inhibited by mGluR-activated signalling pathways. Both Ca\(^{2+}\)-activated K\(^{+}\) channels and voltage-dependent Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 since 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 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 their 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 Teyler 1996; Huang and Hsu 1999; O’Dell et al. 1991), paired-pulse facilitation (Moresco et al. 2003) and mGluR-dependent long-term depression (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 long-term depression in CA1
(Moult 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 LTP (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 signalling pathways.
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FIGURE LEGENDS

Fig 1  Activation of Group I mGluRs depresses the sAHP and increases excitability.  
A, Single experiment showing percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG for 10 min.  Inset waveforms are single responses taken at the times indicated.  Action potentials have been truncated.  B, change in number of action potentials in a train evoked by a 250 ms, 0.5 nA depolarizing pulse in the same experiment as in A.  Inset waveforms are single responses taken from the same experiment and at the same times as in A.  C, Average of experiments (n=14) such as that shown in A.  D, Average of experiments (n=14) such as that shown in B.

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 (filled circles), 50 µM genistein (open circles), or PP2 (filled triangles).  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.  Thick dashed line indicates period of exposure to antagonists.

Fig 3  mGluR-mediated depression of the sAHP is dependent on tyrosine dephosphorylation.  
A, percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG for 10 min in the presence of 1 mM orthovanadate.  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.  C, percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG in the presence of 1 mM orthovanadate in orthovanadate-insensitive cells (filled circles) and orthovanadate-sensitive cells (open circles).
circles). D, change in number of action potentials in a train evoked by a 250 ms, 0.5 nA depolarizing pulse in the same groups of cells as in C. E, plot showing percent change in peak amplitude of the sAHP versus change in number of action potentials in a train in cells exposed to 20 µM DHPG (open circles) or DHPG in the presence of 1 mM orthovanadate (filled circles). Each point represents an individual experiment.

Fig 4 Effects of blocking tyrosine dephosphorylation depend on increased tyrosine phosphorylation. A, percent change in peak amplitude of the sAHP in cells exposed to 20 µM DHPG for 10 min in the presence of both lavendustin A and orthovanadate. 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.

Fig 5 Blocking tyrosine phosphatases causes increased tyrosine phosphorylation. A, Representative Western Blot showing tyrosine phosphorylated proteins extracted from hippocampal slices exposed to different experimental conditions (as labeled). Arrow indicates a prominent 180 kDa band. Lines indicate position of molecular weight markers (200, 116, 97, 66, 45 kDa). B, Summary histogram of Western Blot experiments (n=5) in which each column represents the average band density in relative absorbance units (drug/no drug) for the 180 kDa band. Error bars indicate SEM. Asterisks indicate a significant difference from no-drug group. C, Summary histogram in which each column represents the average percent depression of the sAHP under the conditions labeled. Error bars indicate SEM. Asterisk indicates a significant difference from DHPG group.
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 (open circles) or 10 µM MPEP plus 1mM orthovanadate (filled circles). 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 (open circles) or 100 µM LY367385 plus 1mM orthovanadate (filled circles). 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. Thick dashed line indicates 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 Ca^{2+}-activated K^+ channels that underlie sAHP. Under basal conditions phosphorylation of the tyrosine phosphoprotein is relatively low due to a dominant tyrosine phosphatase (TP), allowing depression of the sAHP by mGluR activation. However during inhibition of tyrosine phosphatases the action of tyrosine kinases (TK) increases tyrosine phosphorylation thus preventing sAHP depression.
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