Mechanisms of Modulation of AMPA-Induced Na\(^+\)-Activated K\(^+\) Current by mGluR1

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talk of an increase in the current mediated by mGluR1 and a possible relationship to the glutamate receptor, but a role in shaping synaptic transmission. In expression systems, K\(_{Na}\) channels are modulated by G protein–coupled receptors, but such a modulation has not been shown for the native channels. In this study, we examined whether K\(_{Na}\) channels coupled to AMPA receptors are modulated by metabotropic glutamate receptors (mGluRs) in lamprey spinal cord neurons. Activation of mGluR1 strongly inhibited the AMPA-induced K\(_{Na}\) current. However, when intracellular Ca\(^{2+}\) was chelated with 1,2-bis(2-aminophenoxy)ethane-N,N',N''-N'''-tetraacetic acid (BAPTA), the K\(_{Na}\) current was enhanced by mGluR1. Activation of protein kinase C (PKC) mimicked the inhibitory effect of mGluR1 on the K\(_{Na}\) current. Blockade of PKC prevented the mGluR1-induced inhibition of the K\(_{Na}\) current, but did not affect the enhancement of the current seen in BAPTA. Together these results suggest that mGluR1 can differentially modulate AMPA-induced K\(_{Na}\) current in a Ca\(^{2+}\)- and PKC-dependent manner.

INTRODUCTION

Na\(^+\)-activated K\(^+\) (K\(_{Na}\)) channels are encoded by the Slack (Slo2.2; sequence like a calcium-activated K\(^+\) channel) and Slick (Slo2.1; sequence like an intermediate conductance K\(^+\) channel) genes (Bhattacharjee et al. 2003; Yuan et al. 2003). These channels are activated by Na\(^+\) influx via voltage-dependent and leak channels and control the action potential duration (Bader et al. 1985; Dryer et al. 1989; Hess et al. 2007) and timing (Yang et al. 2007). They have also been shown to contribute to the resting membrane potential (Haimann et al. 1992), the adaptation of the firing rate, and the slow afterhyperpolarization (Franceschetti et al. 2003; Kim and McCormick 1998; Sanchez-Vives et al. 2000). Recently we showed that K\(_{Na}\) channels can be activated by Na\(^+\) influx via ionotropic glutamate receptors and act as a negative feedback to control the excitatory synaptic response (Nanou and El Manira 2007; Nanou et al. 2008).

In expression systems, K\(_{Na}\) channels are subject to modulation by G protein–coupled receptors (Santi et al. 2006). Activation of muscarinic receptors, metabotropic glutamate receptor 1 (mGluR1), or protein kinase C (PKC) inhibits Slick channels, whereas it strongly enhances the current mediated by Slack (Slo2.2) channels. Using immunohistochemistry, Slick (Slo2.1) channels have been shown to colocalize with mGluR1 in hippocampal neurons in culture (Santi et al. 2006).

However, it is not known whether native K\(_{Na}\) channels are also subject to modulation by G protein–coupled receptors.

In this study, we examined whether mGluR1 can modulate K\(_{Na}\) channels activated by Na\(^+\) influx via AMPA receptors in lamprey spinal cord neurons. Lamprey spinal cord neurons express Slack (Slo2.2) channels (Wallen et al. 2007), and mGluR1 activation has been shown to modulate different cellular targets and potentiate the activity of the spinal locomotor network (El Manira et al. 2002, 2008; Kettunen et al. 2003; Krieger et al. 2000). We now show that mGluR1 also modulates AMPA-induced K\(_{Na}\) channels in a differential manner depending on the intracellular Ca\(^{2+}\) and PKC activation. This mGluR1-mediated modulation of AMPA-coupled K\(_{Na}\) channels can result in changes in synaptic transmission and thereby contribute to the potentiation of the activity of the locomotor network.

METHODS

Experiments were performed on isolated spinal cord neurons of larval lampreys. All protocols were approved by the Animal Research Ethical Committee, Stockholm. Lampreys were anesthetized with MS 222 (100 mg/liter, Sigma Aldrich) and eviscerated, and the spinal cord was dissected out. For cell culture, the spinal cord from larval lampreys was dissociated in Leibovitz’s L-15 culture medium (Sigma) supplemented with gentamicin (1 \(\mu\)g/ml) and penicillin-streptomycin (2 \(\mu\)l/ml), and the osmolarity was adjusted to 271 mOsm (El Manira and Bussieres 1997). The spinal cord was treated with collagenase (1 mg/ml; 30 min; Sigma Aldrich) and then in protease (2 mg/ml, 45 min; Sigma Aldrich). The tissue was subsequently washed with the culture medium and triturated through a sterilized glass pipette. The dissociated neurons were distributed in 20 petri dishes and incubated at 10–12°C for 1–2 days. The dissociated neurons include motoneurons and crossinnereurons (Hess and El Manira 2001).

Whole cell recordings were made from the dissociated spinal cord neurons at room temperature, and their membrane potential was held at −60 mV. The neurons were continuously perfused with an extracellular solution containing (in mM) 124 NaCl, 2 KCl, 1.2 MgCl\(_2\), 2 CaCl\(_2\), 10 glucose, and 10 HEPES (pH = 7.60). The intracellular solution contained (in mM) 110 KCl, 1.2 MgCl\(_2\), 10 glucose, 10 HEPEs, and 0.3 Na-GTP (pH = 7.59). The solutions were applied through a gravity-driven microperfusion system with the tip placed close to the recorded cell. AMPA (Sigma-Aldrich) was applied for 40 s at a concentration of 100 \(\mu\)M. (RS)-3,5-dihydroxyphenylglycine (DHPG, 100 \(\mu\)M) and 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylic ethyl ester (CPCCOEt, 100 \(\mu\)M, Tocris-Cookson) were used to activate and block mGluR1, respectively. Phorbol 12,13-dibutyrate (PDBu, 1 \(\mu\)M, Sigma-Aldrich) or phorbol 12-myristate 13-acetate (PMA, 1 \(\mu\)M, Research Biochemicals International) and 2-[1-(3-dimethylaminopropyl)-3-yl] (3-iodo-3-yl) maleimide (GF109203X, 5 \(\mu\)M, Tocris-Cookson) were used to activate and inhibit PKC, respectively. We also used 1,2-bis(2-aminophenoxy)ethane-N,N',N''-N'''-tetraacetic acid (BAPTA, 10 mM, Invitrogen) to chelate calcium.

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Data were recorded using pClamp 8.2 software (Axon Instruments), and the analysis was performed using Clampfit and Origin (Microcal Software). Data are expressed as means ± SE, and $n$ indicates the number of neurons tested. A paired t-test was used for statistical comparison of data.

RESULTS

Modulation of the AMPA-induced $K_{Na}$ current by group I mGluRs

To test whether AMPA-induced $K_{Na}$ channels are modulated by activation of mGluR1, whole cell recordings were made from dissociated lamprey spinal neurons. Application of AMPA induced an inward current followed by an outward $K_{Na}$ current (Nanou and El Manira 2007). Application of the group I mGluRs agonist DHPG decreased the amplitude of the $K_{Na}$ current and slowed down the decay of the AMPA-induced inward current. The mean current density of $K_{Na}$ current was decreased from 51.8 ± 3.0 to 23.1 ± 4.4 pA/pF by DHPG ($P < 0.001$; $n = 18$; Fig. 1, A and B). The ratio of the current amplitude at the start ($I_{on}$) to that at the end ($I_{off}$) of the AMPA application decreased from 1.47 ± 0.06 to 1.29 ± 0.04 in DHPG ($P < 0.001$; Fig. 1C). Because mGluRs can induce release of Ca$^{2+}$ from internal stores, we examined whether intracellular Ca$^{2+}$ is involved in the modulation of $K_{Na}$ by dialyzing neurons with the chelator BAPTA. Surprisingly, in the presence of BAPTA, DHPG increased the amplitude of the $K_{Na}$ current and increased the decay of the inward current. The $K_{Na}$ current increased from 51.7 ± 4.0 to 83.5 ± 3.6 pA/pF in DHPG ($P < 0.0001$; $n = 10$; Fig. 1, D and E), whereas the ratio of the amplitude of the current at the start ($I_{on}$) to that at the end ($I_{off}$) of the AMPA application increased from 1.47 ± 0.07 to 1.71 ± 0.08 in DHPG ($P < 0.05$; Fig. 1F). These results suggest that DHPG modulates the AMPA-induced $K_{Na}$ in opposite ways dependent on the level of intracellular Ca$^{2+}$.

PKC inhibits AMPA-induced $K_{Na}$ current

To examine whether PKC is involved in mediating the modulation of AMPA-induced $K_{Na}$ by mGluR1, we first tested whether activation of PKC by PDBu affects the $K_{Na}$ current. Indeed, application of PDBu (1 μM) decreased the amplitude of the $K_{Na}$ current induced by AMPA from 51.3 ± 2.0 to 25.9 ± 3.1 pA/pF ($P < 0.0001$; $n = 16$; Fig. 3, A and B). The ratio of the amplitude of the current at the start ($I_{on}$) to that at the end ($I_{off}$) of AMPA application decreased from 1.57 ± 0.07 to 1.29 ± 0.09 in PDBu ($P < 0.05$). Similar results were obtained when another PKC activator, PMA, was used. Application of PMA (1 μM) decreased the amplitude of the AMPA-induced $K_{Na}$ current from 51.0 ± 3.0 to 24.4 ± 3.3 pA/pF ($P < 0.0001$; $n = 9$). To further test whether mGluR1 is using the
Addition of 100 μM DHPG did not significantly change the density of the K_{Na} current (25.5 ± 2.8 pA/pF; \( P > 0.05 \); \( n = 7 \); Fig. 3, C and D).

To confirm PKC involvement in the modulation of the K_{Na} current, the specific PKC inhibitor GF109203X was used. GF109203X (5 μM) blocked the inhibition of K_{Na} current by DHPG. The density of the AMPA-induced K_{Na} current was 49.6 ± 2.8 pA/pF in GF109203X and was not significantly affected in DHPG (48.7 ± 3.3 pA/pF, \( P > 0.05 \); \( n = 6 \); Fig. 4, A and B). However, the mGLuR1-mediated potentiation of K_{Na} current induced in the presence of BAPTA was not significantly blocked by inhibiting PKC. In the presence of GF109203X, DHPG increased the K_{Na} current density in neurons dialyzed with BAPTA from 51.2 ± 0.8 to 78.7 ± 4.9 pA/pF (\( P < 0.0001 \); \( n = 10 \); Fig. 4, C and D). Together these results show that mGluR1 uses a PKC-dependent mechanism to inhibit the AMPA-induced K_{Na} current, whereas it potentiates K_{Na} current via a PKC-independent signaling pathway when intracellular Ca^{2+} is chelated with BAPTA.

D I S C U S S I O N

In this study, we showed that activation of mGluR1 differentially modulates native K_{Na} channels activated by Na^{+} influx via AMPA receptors. The mGluR1 activation induces inhibition of the K_{Na} current that requires PKC. However, chelating intracellular Ca^{2+} results in a potentiation of the K_{Na} current by mGluR1.

K_{Na} channels have been shown to colocalize with muscarinic receptors in rat brain sections and in hippocampal neurons in culture (Santi et al. 2006). Slick (Slo2.1) channels have also been shown to be in close proximity with mGluR1 in the

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**FIG. 2.** 7-(Hydroxymimino)cyclopenta[b]chroomen-1a-carboxylate ethyl ester (CPCCOEt) blocks the DHPG-mediated modulation of the AMPA-induced K_{Na} current. A: DHPG-induced decrease in the amplitude of the AMPA-induced K_{Na} current was blocked by CPCCOEt. B: mean density of the AMPA-induced K_{Na} current in CPCCOEt alone and together with DHPG. C: CPCCOEt also blocked the DHPG-induced increase in the AMPA-induced K_{Na} current seen in the presence of BAPTA. D: mean density of the AMPA-induced K_{Na} current in CPCCOEt alone and together with DHPG in the presence of BAPTA.

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**FIG. 3.** Protein kinase C (PKC) activation inhibits the AMPA-induced K_{Na} current. A: activation of PKC by phorbol 12,13-dibutyrate (PDBu) decreased the amplitude of the K_{Na} current. B: mean density of the AMPA-induced K_{Na} current in control and PDBu. C: in neurons pretreated with the PKC activator PDBu, application of DHPG failed to decrease the amplitude of the K_{Na} current. D: mean density of the AMPA-induced K_{Na} current in PDBu alone and together with DHPG.

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**FIG. 4.** Inhibition of PKC blocks the mGluR1-mediated inhibition of AMPA-induced K_{Na} current. A: AMPA-induced K_{Na} current in the presence of the PKC antagonist 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X) that remained unchanged in DHPG. B: mean density of the AMPA-induced K_{Na} current in GF109203X alone and together with DHPG. C: AMPA-induced K_{Na} current in GF109203X and when DHPG was added in the presence of BAPTA. D: mean density of the AMPA-induced K_{Na} current in GF109203X alone and together with DHPG in the presence of BAPTA.
dissociated hippocampal neurons. Coexpressing $K_{Na}$ channels with mGluR1 or muscarinic receptors in *Xenopus* oocytes showed that the two Slo2 channels (Slick and Slack) are differentially modulated by both G protein–coupled receptors. Slick (Slo2.1) channels were inhibited, whereas Slack (Slo2.2) channels were activated, and both these effects were mimicked by activation of PKC. Similar modulation of $K_{Na}$ channels by activation of PKC has been shown in a mammalian expression system (Santi et al. 2006). It has also been proposed that PKC regulation of Slack/Slick heteromeric potassium channels is distinct from that of homomeric channels (Chen et al. 2009). Although Slack channels are augmented by PKC, heteromeric Slack/Slick channels are inhibited by PKC activation. However, another report showed that, when $K_{Na}$ channels were coexpressed with G protein–coupled receptors in a mammalian expression system, both channels were inhibited by activation of thyrotropin-releasing hormone (TRH) receptors (Berg et al. 2007). Similarly, Slick (Slo2.1) channels were inhibited by activation of both mGluR1 and mGluR5 receptors.

In this study, the AMPA-induced $K_{Na}$ current was facilitated by activation of mGluR1 when intracellular Ca$^{2+}$ was chelated by BAPTA, and this effect was not dependent on PKC. The $K_{Na}$ current activated by Na$^{+}$ influx via AMPA receptors in lamprey spinal cord neurons shows properties similar to the cloned Slack channels because it is not modulated by increased intracellular Cl$^{-}$ or ATP (Nanou and El Manira 2007). Slack channels have been shown to be inhibited by raises in intracellular calcium (Joiner et al. 1998). It is possible that increased intracellular Ca$^{2+}$ is necessary for activation of PKC. Chelating Ca$^{2+}$ may interfere with the mGluR1-induced activation of PKC, and another signaling pathway may be activated that will result in a potentiation of the $K_{Na}$ current. The signaling mechanism responsible for the potentiation of the $K_{Na}$ current by mGluR1 in the absence of Ca$^{2+}$ is not yet known and needs to further be examined.

All three groups of mGluRs (mGluRI, II, III) have been shown to exist in the lamprey spinal cord (Krieger et al. 1996). mGluR1 is located postsynaptically where it inhibits leak channels and potentiates N-methyl-D-aspartate (NMDA) current (Nanou et al. 2009). In addition, activation of mGluR1 also induces release of endocannabinoids and NO that underlie modulation of synaptic transmission and the activity of the locomotor network (Kyrjakatos and El Manira 2007). The modulation of $K_{Na}$ current is not a consequence of the inhibition of leak current by mGluR1, because the membrane potential of neurons was held at −60 mV, which corresponds to the reversal potential of the leak current (Kettunen et al. 2003). At normal intracellular Ca$^{2+}$ levels, mGluR1 will inhibit AMPA-coupled $K_{Na}$ current and result in a slower decay of AMPA-induced EPSCs and larger excitory postsynaptic potentials (EPSPs) (Nanou et al. 2008). By modulating these $K_{Na}$ channels, mGluR1 would enhance excitatory synaptic transmission and thereby the activity of the spinal locomotor network. It has not been possible to test directly the contribution of this modulation in shaping the locomotor activity because blocking $K_{Na}$ current by substituting Na$^{+}$ with Li$^{+}$ or by quinidine (Nanou et al. 2008; Wallen et al. 2007) completely disrupted the rhythmic pattern. A direct demonstration of the role of $K_{Na}$ channels in a network requires the discovery of specific activators and blockers for these channels.

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**References**


