Mechanisms of modulation of AMPA-induced Na\(^+\)-activated K\(^+\) current by mGluR1

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Na\textsuperscript{+}-activated K\textsuperscript{+} (K\textsubscript{Na}) channels can be activated by Na\textsuperscript{+} influx via ionotropic receptors and play a role in shaping synaptic transmission. In expression systems, K\textsubscript{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 if K\textsubscript{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\textsubscript{Na} current. However, when intracellular Ca\textsuperscript{2+} was chelated with BAPTA the K\textsubscript{Na} current was enhanced by mGluR1. Activation of protein kinase C (PKC) mimicked the inhibitory effect of mGluR1 on the K\textsubscript{Na} current. Blockade of PKC prevented the mGluR1-induced inhibition of the K\textsubscript{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\textsubscript{Na} current in a Ca\textsuperscript{2+}- and PKC-dependent manner.
INTRODUCTION

Na⁺-activated K⁺ (KNa) 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. 2006). 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 KNa 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 KNa channels are subject to modulation by G-protein coupled receptors (Santi et al. 2006). Activation of muscarinic receptors, metabotropic glutamate receptor 1 (mGluR1) or PKC inhibits Slick channels while 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 if native KNa channels are also subject to modulation by G-protein coupled receptors.

In this study, we examined whether mGluRs can modulate KNa 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; El Manira et al. 2008; Kettunen et al. 2003; Krieger et al. 2000). We now show that mGluR1 also modulates AMPA-induced KNa channels in a
differential manner depending on the intracellular Ca\(^{2+}\) and PKC activation. This mGluR1-mediated modulation of AMPA-coupled K\(_{\text{Na}}\) 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 anaesthetized with MS 222 (100 mg/L, Sigma Aldrich), eviscerated and the spinal cord 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 µg/ml), and penicillin-streptomycin (2 µl/ml) and the osmolarity 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 then distributed in 20 Petri dishes and incubated at 10–12\(^\circ\)C for 1–2 days. The dissociated neurons include motoneurons and crossing interneurons (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): NaCl 124; KCl 2; MgCl\(_2\) 1.2; CaCl\(_2\) 2; glucose 10; Hepes 10 (pH = 7.60). The intracellular solution contained (in mM): KCl 110; MgCl\(_2\) 1.2; glucose 10; Hepes 10; Na-GTP 0.3 (pH = 7.59). The solutions were applied through a gravity-driven microperfusion system with the tip placed close to the recorded cell. (RS)-a-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid hydrobromide
(AMPA, Sigma-Aldrich) was applied for 40 s at a concentration of 100 µM. (RS)-3,5-Dihydroxyphenylglycine (DHPG, 100 µM) and 7-(Hydroxyimino)cyclopropa[b]chromen-1-carboxylate ethyl ester (CPCCOEt, 100 µM, Tocris-Cookson) were used to activate and block mGluR1, respectively. Phorbol 12,13-Dibutyrate (PDBu, 1 µM, Sigma-Aldrich) or Phorbol 12-myristate 13-acetate (PMA, 1 µM, Research Biochemicals International) and 2-[1-(3-Dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X, 5 µM, 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.

Data were recorded using pClamp 8.2 software (Axon Instruments) and the analysis was performed by using Clampfit and Origin (Microcal Software). Data are expressed as mean ± SEM and “n” indicates the number of neurons tested. 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 if 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 $51.8 \pm 3.0$ pA/pF to $23.1 \pm 4.4$ pA/pF by DHPG ($P < 0.001; n = 18$; Fig. 1A, 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 $1.47 \pm 0.06$ to $1.29 \pm 0.04$ in DHPG ($P < 0.001$; Fig. 1C). Since mGluRs can induce release of $Ca^{2+}$ from internal stores, we then
examined if 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 pA/pF to 83.5 ± 3.6 pA/pF in DHPG (P < 0.0001; n = 10, Fig. 1D, E), while 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\(^{2+}\).

The modulation of the AMPA-induced K\(_{Na}\) current is mediated by mGluR1

To determine the receptor subtype mediating the modulation of K\(_{Na}\) channels, a specific mGluR1 antagonist was tested. The mGluR1 antagonist CPCCOEt blocked both the inhibition and enhancement of K\(_{Na}\) induced by DHPG (Fig. 2A). In CPCCOEt, the current density of the AMPA-induced K\(_{Na}\) was 48.4 ± 1.7 pA/pF and remained unchanged after application of DHPG (49.2 ± 2.5 pA/pF), (P > 0.05; n = 10, Fig. 2B). CPCCOEt alone did not significantly affect the amplitude of the K\(_{Na}\) current. Similar results were obtained when intracellular Ca\(^{2+}\) was chelated with BAPTA. The current density of the AMPA-induced current was not affected by DHPG in the presence of CPCCOEt (Fig. 2C). The current density of K\(_{Na}\) in CPCCOEt was 49.5 ± 3.3 pA/pF and in DHPG 50.7 ± 3.8 pA/pF (P > 0.05; n = 11; Fig. 2D). These results suggest that both the inhibition and potentiation of K\(_{Na}\) current are mediated by activation of mGluR1.

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 KNa current induced by AMPA from 51.3 ± 2.0 pA/pF to 25.9 ± 3.1pA/pF (P < 0.0001; n = 16; Fig. 3A, 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 KNa current from 51.0 ± 3.0 pA/pF to 24.4 ± 3.3pA/pF (P < 0.0001; n = 9). To further test if mGluR1 is using PKC pathway to inhibit KNa current we tested DHPG in the presence of PDBu. All neurons were pre-treated with PDBu (1 µM, 30min) and further application of AMPA in the presence of PDBu resulted in an average density of AMPA-induced KNa 24.7 ± 2.6 pA/pF (n = 7; Fig. 3C, D). Addition of 100 µM DHPG did not significantly change the density of the KNa current (25.5 ± 2.8 pA/pF; P > 0.05; n = 7; Fig. 3C, D).

To confirm PKC involvement in the modulation of the KNa current, the specific PKC inhibitor GF109203X was used. GF109203X (5 µM) blocked the inhibition of KNa current by DHPG. The density of the AMPA-induced KNa 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. 4A, B). However, the mGluR1-mediated potentiation of KNa current induced in the presence of BAPTA was not significantly blocked by inhibiting PKC. In the presence of GF109203X, DHPG increased the KNa current density in neurons dialyzed with BAPTA from 51.2 ± 0.8 pA/pF to 78.7 ± 4.9 pA/pF (P < 0.0001; n = 10; Fig. 4C, D). Together these results show that mGluR1 uses a PKC-dependent mechanism to inhibit the AMPA-induced KNa current, while it potentiates KNa current via a PKC-independent signaling pathway when intracellular Ca^{2+} is chelated with BAPTA.
In this study we show 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 been also shown to be in close proximity with mGluR1 in the dissociated hippocampal neurons. Co-expressing K_{Na} channels with mGluR1 or muscarinic receptors in Xenopus oocytes revealed that the two Slo2 channels (Slick and Slack) are differentially modulated by both G-protein coupled receptors. Slick (Slo2.1) channels were inhibited while 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). While 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 co-expressed with G-protein coupled receptors in a mammalian expression system both channels were inhibited by activation of 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 displays 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 Ca²⁺ is necessary for activation of PKC. Chelating Ca²⁺ may interfere with the mGluR1-induced activation of PKC and another signaling pathway may then be activated that will result in a potentiation of the K₉Na current. The signaling mechanism responsible for the potentiation of the K₉Na current by mGluR1 in the absence of Ca²⁺ 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 NMDA current (Nanou et al. 2009). In addition, activation of mGluR1 also induces release of endocannabinoids and nitric oxide that underlie modulation of synaptic transmission and the activity of the locomotor network (Kyriakatos and El Manira 2007). The modulation of K₉Na 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 (see Kettunen et al. 2003). At normal intracellular Ca²⁺ levels mGluR1 will inhibit AMPA-coupled K₉Na current and result in a slower decay of AMPA-induced EPSCs and larger EPSPs (see Nanou et al. 2008). By modulating these K₉Na 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 K₉Na current by substituting Na⁺ with Li⁺ or by quinidine (Nanou et al. 2007; Wallén et al. 2007) completely disrupted the rhythmic pattern. A direct demonstration of the role of K₉Na channels in a network requires the discovery of specific activators and blockers for these channels.
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FIGURE LEGENDS

Fig 1. Activation of group I mGluRs modulates the AMPA-induced K$_{Na}$ current. A: application of AMPA induced an inward current followed by an outward K$_{Na}$ current. The group I mGluR agonist DHPG decreased the amplitude of the K$_{Na}$ current that recovered after washout of DHPG. B: mean density of the AMPA-induced K$_{Na}$ current in control, DHPG and wash. C: ratio of the mean amplitude of the current at the start ($I_{on}$) to that at the end ($I_{off}$) of the AMPA application. D: AMPA-induced K$_{Na}$ current in the presence of the Ca$^{2+}$ chelator BAPTA that was increased in amplitude by DHPG. E: mean density of the AMPA-induced K$_{Na}$ current in control, DHPG and wash in the presence of BAPTA. F: ratio of the mean amplitude of the current at the start ($I_{on}$) and at the end ($I_{off}$) of the AMPA application in control and DHPG in the presence of BAPTA.

Fig. 2. 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.

Fig. 3. PKC activation inhibits the AMPA-induced K$_{Na}$ current. A: activation of PKC by 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 pre-incubated 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.
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 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.
Fig. 2
Fig. 3
Fig. 4

A. Control
GF109203X + DHPG

B. Current density (pA/pF)
GF109203X + DHPG

C. BAPTA
GF109203X + DHPG

D. Current density (pA/pF)
GF109203X + DHPG

Fig. 4