Na⁺ Entry Through AMPA Receptors Results in Voltage-Gated K⁺ Channel Blockade in Cultured Rat Spinal Cord Motoneurons

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Glutamate is the main excitatory neurotransmitter in the CNS. At the postsynaptic membrane, glutamate acts on two classes of receptors: ionotropic and metabotropic glutamate receptors (for review, see Ozawa et al. 1998). Among the ionotropic glutamate receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is mainly responsible for fast synaptic transmission. Under certain conditions, excessive activation of AMPA receptors can lead to excitotoxicity (Choi 1992; Shaw and Ince 1997; Westbrook 1993). The excitatory ion flux through AMPA receptors has been shown to modulate other membrane conductances, mainly K⁺ conductances. Ca²⁺ influx through Ca²⁺-permeable AMPA receptors results in an outward Ca²⁺-activated K⁺ current in cortical neurons, midbrain dopaminergic neurons, and in hilar granule precursor cells, thereby counteracting the membrane depolarization during excessive AMPA receptor stimulation (Backus et al. 1998; Mercuri et al. 1996; Omura et al. 1993). On the contrary, Na⁺ or Ca²⁺ influx through the AMPA receptor has been reported to block K⁺ currents in different cell types in the CNS. In Bergman glial cells, AMPA receptor agonists have been found to block a resting K⁺ conductance (Muller et al. 1992). AMPA receptor stimulation with kainate has been shown to inhibit delayed rectifier K⁺ currents (Kᵥ) in oligodendrocyte precursor cells (Borges et al. 1994; Borges and Kettenmann 1995; Gallo et al. 1996), glial cells of hippocampal slices (Jabs et al. 1994), cultured cortical astrocytes (Robert and Magistretti 1997), embryonic chick telencephalic neurons (Mike et al. 1996), and cerebellar granule neurons (Jones et al. 2000). In Bergmann glial cells and chick telencephalic neurons and cerebellar granule cells, the K⁺ current inhibition was caused by Ca²⁺ influx through AMPA receptors, whereas in stellate astrocytes and oligodendrocyte precursor cells, Na⁺ influx appeared to be the trigger mechanism.

Inhibition of K⁺ currents on AMPA receptor stimulation can interfere with the determination of properties of AMPA receptors. Inward rectification is one of the properties of Ca²⁺-permeable AMPA receptors, which are thought to be involved in the vulnerability of motoneurons to excitotoxicity (Carriedo et al. 1996; Greig et al. 2000; Van Den Bosch et al. 2000).

In this study, we show that AMPA receptor stimulation leads also to K⁺ current inhibition in cultured rat spinal cord motoneurons. We used the perforated patch-clamp technique in combination with Fluo-4-based microfluorometry to explore the possibility that this inhibition is induced by Ca²⁺ influx through Ca²⁺-permeable AMPA receptors. Our data further demonstrate that K⁺ current inhibition seriously hampers the analysis of AMPA receptor-mediated currents. Finally, our data suggest that K⁺ current inhibition does not play a crucial role in the high vulnerability of motoneurons to AMPA receptor agonists.
**METHODS**

**Cell cultures**

Motoneurons were cultured as previously described (Van den Berghe et al. 1998). In brief, ventral spinal cords were dissected from 14-day-old Wistar rat embryos in Hanks’ balanced salt solution (HBSS), cut into pieces of ~1 mm, and digested for 15 min in 0.05% trypsin in HBSS at 37°C. After treatment with DNase, the tissue was further dissociated by trituration. A motoneuron-enriched neuronal population was purified from the ventral spinal cord by centrifugation on a 6.5% metrizamide cushion and was cultured on a glial feeder layer that had been preestablished on 18-mm round glass coverslips coated with poly-L-ornithine and laminin. The culture medium consisted of L15 supplemented with sodium bicarbonate (0.2%), glucose (3.6 mg/ml), progesterone (20 nM), insulin (5 µg/ml), putrescine (0.1 mg/ml), sodium selenite (30 nM), penicillin (100 IU/ml), streptomycin (100 mM), conalbumin (0.1 mg/ml), sodium pyruvate (3.6 mg/ml), progesterone (20 nM), insulin (5 µg/ml), putrescine (0.1 mg/ml), sodium selenite (30 nM), penicillin (100 IU/ml), streptomycin (100 µg/ml), and horse serum (2%). The cultures were kept in a 7% CO₂-humidified incubator at 37°C. The neurons were used for experiments between days 7 and 13. Dorsal horn neurons were dissociated from the dorsal spinal cord with the same protocol, except that the metrizamide gradient centrifugation was omitted.

**Immunocytochemistry**

To evaluate the purity of the motoneuron cultures, immunostainings for the motoneuron marker peripherin (Escrutat et al. 1990) were performed. Cultures were fixed for 20 min in 4% paraformaldehyde-PBS on day 8 in culture, permeabilized with methanol for 10 min, incubated overnight at 4°C with rabbit anti-peripherin (1:1,000; Chemicon International), then incubated with biotinylated swine anti-rabbit antibodies (1:500; Dako) for 1 h. Diaminobenzidine was used to develop the stain. A high purity of the motoneuron cultures could be obtained, because 80% of the cells (80.3 ± 5.1%; n = 9) stained positive for peripherin (Fig. 1).

**Electrophysiology**

The gramicidin perforated-patch-clamp technique (Kyrozis and Reichling 1995) was used for the electrophysiological recordings. Pipettes were backfilled with pipette solution containing 50–75 µg/ml of gramicidin after tip filling with gramicidin-free solution. Gramicidin was dissolved in DMSO (1 mg/20 µl) before each experiment. The pipettes had a resistance of 2–4 MΩ when filled with intracellular solution. Motoneurons were identified according to previously defined morphological criteria (Vandenberge et al. 2000). After seal formation, the progress of perforation was followed by evaluation of the decrease in series resistance. Cells were accepted for study if series resistance dropped below 30 MΩ and remained stable during the experiment. Cells were held at a membrane potential of ~60 mV, and current-voltage (I-V) relationships were generated with voltage ramps from +100 to +50 mV or vice versa. Signals were recorded with an amplifier (L/IM-EPIC7; List Medical), filtered at 3 kHz, sampled at 2 kHz, and analyzed off-line (Digidata 1200, pClamp8; Axon Instruments). To compare current amplitudes between different cells of different sizes, current densities were obtained by dividing the current amplitude by the cell capacitance. The rectification of kainate-induced currents was quantified with the equation: rectification index (RI) = [I_{up}(40 \rightarrow E_{rest})]/[I_{down}(−60 \rightarrow E_{rest})] (Ozawa et al. 1991). All recordings were performed at room temperature.

The normal pipette solution consisted of 30 mM KCl, 95 mM K-ascetate, 1.2 mM MgCl₂, 10 mM HEPES, 2 mM Na₂-ATP, and 1 mM EGTA, pH adjusted to 7.3 with KOH. The standard extracellular solution contained (in mM) 129.1 NaCl, 5.9 KCl, 3.2 CaCl₂, 1.2 MgCl₂, 11.6 HEPES, 11.5 glucose, pH adjusted to 7.3 with NaOH. A Na+ -free solution was obtained by substituting Na+ with equimolar amounts of N-methyl-D-glucamine or LiCl; the Ca++ -free solution was a nominal Ca++ -free solution supplemented with 2 mM EGTA. Kainate (100 µM) and LY 300164 (50 µM) were used as the agonist and selective antagonist, respectively, of AMPA receptors. All experiments were carried out in the presence of 500 nM tetrodotoxin (TTX) and 10 µM MK-801 to block voltage-gated Na+ channels and N-methyl-D-aspartate (NMDA) receptors, respectively. Cd++ (100 µM) was used to block voltage-operating Ca++ channels as indicated. In some experiments pertussis toxin (100 ng/ml for 24 h) was used to inhibit inhibitory G proteins. (RS)-α-methylserine-O-phosphate monophenyl ester (MSOPPE, 200 µM) and E4CPG (200 µM) were used as metabotropic glutamate receptor antagonists. To obtain an alternative route for Na+ influx, the Na+ ionophore monensin (10 µM) (Lichtstein et al. 1979) was applied.

**Intracellular Ca++ imaging**

For Ca++ imaging combined with electrophysiology, neurons were loaded with Fluo-4 by incubating the cells in culture medium containing Fluo-4 AM for 20 min at 37°C. Fluo-4 was dissolved in DMSO (50 µg/25 µl) and used in a final concentration of 5 µM with 0.02% pluronic acid. Neurons were illuminated at 488 nm, and the emitted fluorescence was collected at wavelengths >515 nm with a photomultiplier tube. Fluorescence signals collected from regions covering the cell soma and proximal neurites were filtered at 100 Hz, sampled at 2 kHz, and analyzed off-line (Digidata 1200, pClamp8; Axon Instruments). Intracellular Ca++ signals were shown as ΔF/F, i.e., fluorescence increase divided by prestimulus fluorescence.

**Materials**

Media and additives were obtained from Gibco BRL (Grand Island, NY); TTX was from Calbiochem (San Diego, CA); MK-801, MSOPPE, and E4CPG were from Tocris Cookson (Bristol, UK); and Fluo-4 and pluronic acid were from Molecular Probes. LY 300164 (50 µM) was kindly provided by Dr. J. D. Leander (Eli Lilly, Lilly Corporate Center, Indianapolis, IN). All other chemicals were from Sigma (St. Louis, MO).

**RESULTS**

Kainate-induced currents display an apparent inward rectification due to concomitant inhibition of an outward current

Application of 100 µM kainate in the presence of the NMDA receptor blocker MK-801 produced a large inward
current at negative membrane potentials in motoneurons (Fig. 2, inset) and dorsal horn neurons. With the use of normal external and pipette solutions, I-V curves of the kainate-induced current were obtained by subtracting a control ramp from the ramp during kainate application. As indicated for a motoneuron in Fig. 2, the I-V curve of the kainate-induced current displayed an apparent strong inward rectification with no clear reversal. A similar shape of the I-V curve was observed in dorsal horn neurons (data not shown). Because no clear reversal potential was observed, we considered whether the strong inward rectification could be related to a concomitant change of a current component at positive potentials.

To avoid fluctuations in intracellular Ca\(^{2+}\) concentration during kainate application or ramping from \(-50\) to \(+100\) mV, we used ramps from \(+50\) to \(-100\) mV after the cells were held at \(+50\) mV for 1.5 s. This resulted in a stably elevated intracellular Ca\(^{2+}\) level during ramps (Fig. 3A). To identify overlapping current components, control I-V ramps before (Fig. 3B, trace 1) and immediately after (Fig. 3B, trace 3) kainate washout were compared.

As shown in Fig. 3C, the difference between the ramp before and after washout of kainate yields an outwardly rectifying current component (trace 1–3) activated at potentials positive to \(-40.5 \pm 1.3\) mV (n = 23). The mean current density at \(+45\) mV amounted to \(9.6 \pm 1.1\) pA/pF (n = 23). When the control ramp immediately after kainate washout (trace 3 in Fig. 3B) was subtracted from the ramp during kainate application, the I-V curve for the kainate-induced current (trace 2–3 in Fig. 3C) was nearly linear (RI = \(0.91 \pm 0.08\); n = 23), with a clear reversal around \(+7.5 \pm 1.7\) mV (n = 23). Thus the apparent strong inward rectification of the kainate-induced current shown in Fig. 2 was not a genuine property of the AMPA receptor current but reflects an overlapping outward current component inhibited by kainate.

We also checked the presence of this kainate-induced inhibition in dorsal horn neurons, which are known to be resistant to kainate-induced cell death. However, there was no differ-

![FIG. 2. Inward rectification of kainate-induced current in motoneurons.](http://jn.physiology.org/)

![FIG. 3. Inhibition of outward current by kainate.](http://jn.physiology.org/)

ence between motoneurons and dorsal horn neurons with respect to this inhibition (current density of the kainate-inhibited current in dorsal horn neurons was \(9.4 \pm 3.0\) pA/pF, \(P = 0.9\); n = 4).
The kainate-inhibited current is a voltage-gated outwardly rectifying $K^+$ current

The kainate-inhibited outward current was insensitive to 10 mM external Ba$^{2+}$ (data not shown) but could be blocked by 30 mM external tetraethylammonium (TEA$^+$; 7.7 ± 1.2 pA/pF at +45 mV without TEA$^+$, 0.9 ± 0.6 pA/pF at +45 mV in the presence of TEA$^+$, $P = 0.02$; $n = 3$) when applied together with 100 $\mu$M Cd$^{2+}$ to suppress voltage-operated Ca$^{2+}$ channels (Fig. 4A). At 50 mM external K$^+$ concentration, $I$-$V$ relationships of the kainate-inhibited current shifted to more positive potentials, and a clear reversal potential was apparent at −25 mV, a value close to the predicted K$^+$ equilibrium potential (Fig. 4B). Thus the kainate-inhibited current could be identified as an outwardly rectifying TEA$^+$-sensitive K$^+$ current activated at potentials positive to −40.5 mV. It was also apparent that in the presence of 30 mM external TEA$^+$ and 100 $\mu$M Cd$^{2+}$, $I$-$V$ relationships for the kainate-induced current obtained by subtracting either a control ramp or a ramp after kainate washout almost fully matched (Fig. 4C). However, the RI determined in 30 mM external TEA$^+$ was lower (0.76 ± 0.04; $n = 28$) than the RI determined by subtraction of a ramp after kainate application (0.91 ± 0.08; $P = 0.06$; $n = 23$).

Time course of $K^+$ current inhibition

To study the time course of the K$^+$ current inhibition, ramps were repetitively applied at a frequency of 0.1 Hz before, during, and after kainate application. Current densities were measured at −80 and +45 mV (Fig. 5A). As illustrated by the $I$-$V$ relationships taken before and immediately after washout of kainate (Fig. 5B), the currents measured at +45 mV merely reflected the time course of the K$^+$ current inhibition. Currents measured at −80 mV, on the other hand, reflected the genuine time course of AMPA receptor activation by kainate.

The time course of the K$^+$ current inhibition could be approximated with a single exponential with a time constant of $\tau = 76.0 ± 8.1$ s (data not shown) but could be blocked by 10.2 ± 0.3 $\mu$M isoxazolepropionic acid (AMPA) receptor current, whereas the current at +45 mV reflects the gradual decrease in K$^+$ conductance. The kainate-inhibited outward current was insensitive to 10 mM external Ba$^{2+}$ (data not shown) but could be blocked by 30 mM external tetraethylammonium (TEA$^+$; 7.7 ± 1.2 pA/pF at +45 mV without TEA$^+$, 0.9 ± 0.6 pA/pF at +45 mV in the presence of TEA$^+$, $P = 0.02$; $n = 3$) when applied together with 100 $\mu$M Cd$^{2+}$ to suppress voltage-operated Ca$^{2+}$ channels (Fig. 4A). At 50 mM external K$^+$ concentration, $I$-$V$ relationships of the kainate-inhibited current shifted to more positive potentials, and a clear reversal potential was apparent at −25 mV, a value close to the predicted K$^+$ equilibrium potential (Fig. 4B). Thus the kainate-inhibited current could be identified as an outwardly rectifying TEA$^+$-sensitive K$^+$ current activated at potentials positive to −40.5 mV. It was also apparent that in the presence of 30 mM external TEA$^+$ and 100 $\mu$M Cd$^{2+}$, $I$-$V$ relationships for the kainate-induced current obtained by subtracting either a control ramp or a ramp after kainate washout almost fully matched (Fig. 4C). However, the RI determined in 30 mM external TEA$^+$ was lower (0.76 ± 0.04; $n = 28$) than the RI determined by subtraction of a ramp after kainate application (0.91 ± 0.08; $P = 0.06$; $n = 23$).

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10.2 ± 2.2 s at +45 mV (n = 7). The time constant for recovery from inhibition amounted to 33.5 ± 8.8 s (n = 7) at +45 mV.

Na\textsuperscript{+} influx through the AMPA receptor causes the K\textsuperscript{+} current inhibition

Further experiments to elucidate the mechanism of the inhibitory action of kainate indicated that the inhibited K\textsuperscript{+} current was not affected by preincubation with pertussis toxin or application of the metabotropic glutamate receptor antagonists MSOPPE and EC4PG (data not shown). On the other hand, the selective AMPA receptor antagonist LY 300164 blocked both the excitatory and inhibitory action of kainate (Fig. 6), suggesting that ion flux through the AMPA receptor is mandatory for K\textsuperscript{+} channel inhibition.

Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+}-permeable AMPA receptors was not involved because removing extracellular Ca\textsuperscript{2+} did not suppress the inhibitory action of kainate (data not shown). Replacing extracellular Na\textsuperscript{+} with N-methyl-D-glucamine (13.6 ± 4.3 pA/pF in 140 mM external Na\textsuperscript{+}, 2.4 ± 2.3 pA/pF in 0 mM external Na\textsuperscript{+}, P = 0.028; n = 8) or clamping the membrane close to the reversal potential for Na\textsuperscript{+} during kainate application (8.5 ± 1.5 pA/pF in control, 0.8 ± 0.6 pA/pF when clamped at +80 mV during kainate application, P = 0.01; n = 5), however, clearly attenuated the inhibition of the K\textsuperscript{+} current (Fig. 7, A and B). Removing extracellular Na\textsuperscript{+} had a dual effect on K\textsuperscript{+} conductances. Initially, the kainate-induced K\textsuperscript{+} current inhibition was abolished, revealing the AMPA receptor outward current at +45 mV (Fig. 7C). However, in the continued absence of extracellular Na\textsuperscript{+}, an irreversible decrease of outward currents occurred. Replacing NaCl with LiCl did not affect the kainate-inhibited K\textsuperscript{+} current (data not shown). Finally, raising the intracellular Na\textsuperscript{+} concentration by using the Na\textsuperscript{+} ionophore monensin in a normal Na\textsuperscript{+}-containing solution mimicked the inhibitory effect of kainate on outward currents at positive potentials (10.0 ± 1.0% inhibition, P = 0.04; n = 5; Fig. 7D). Taken together, these results indicate that Na\textsuperscript{+} influx through AMPA receptors leads to a substantial inhibition of a TEA\textsuperscript{2+}-sensitive voltage-gated K\textsuperscript{+} conductance.

Both K\textsubscript{V} and K\textsubscript{A} are blocked by kainate

Several types of K\textsuperscript{+} conductances have been described in motoneurons (McLarson et al. 1995; Viana et al. 1993). To determine the nature of the K\textsuperscript{+} conductance affected by kainate, suitable voltage protocols were used to elicit sustained (K\textsubscript{V}) and transient (K\textsubscript{A}) voltage-gated K\textsuperscript{+} currents (Jones et al. 2000). As illustrated in Fig. 8A, stepping from −30 mV (at which K\textsubscript{A} inactivates) to +30 mV activated the delayed rectifier K\textsuperscript{+} current, K\textsubscript{V}. In the presence of 100 μM kainate, K\textsubscript{V} was almost completely blocked. This result is compatible with the outward current inhibition during voltage ramps. We further looked at the effect of kainate on the transient outward K\textsuperscript{+} current, K\textsubscript{A}. K\textsubscript{A} was activated by stepping from −120 to +50 mV and isolated by subtracting the K\textsubscript{V} component activated by a step from −50 to +50 mV (Fig. 8B). In the presence of kainate, the K\textsubscript{A} component was also clearly abolished (Fig. 7C).

DISCUSSION

Activation of AMPA receptors at resting membrane potentials evokes an inward current that leads to a postsynaptic membrane depolarization. Apart from this primary action, Na\textsuperscript{+} or Ca\textsuperscript{2+} influx through AMPA receptors also appears to modulate K\textsuperscript{+} conductances. In this study, we demonstrate that AMPA receptor activation causes inhibition of voltage-gated outward K\textsuperscript{+} currents in cultured rat spinal motoneurons. The inhibitory action of kainate is linked to ion flux through the AMPA receptor, because no inhibition is observed in the presence of the selective AMPA receptor antagonist LY 300164. Antagonists of metabotropic glutamate receptors or preincubation with pertussis toxin did not affect the inhibitory action of kainate.

The kainate-induced K\textsuperscript{+} current inhibition seriously hampers the analysis of AMPA receptor-mediated currents. Particularly, the genuine rectification of AMPA receptor currents is obscured by this K\textsuperscript{+} channel block. In this study, two methods were used to overcome this problem. A voltage ramp given 1.5 s after kainate application was subtracted from one given during kainate application or voltage-gated K\textsuperscript{+} currents were blocked by 30 mM external TEA\textsuperscript{+}. Using the subtraction method, the RI of AMPA receptor currents amounted to 0.91 and was higher than the value of 0.76 obtained with the TEA\textsuperscript{+} method (P = 0.06). The higher value obtained with the subtraction method most likely reflects different levels of K\textsuperscript{+} current inhibition during the two separate voltage ramps. Therefore, the TEA\textsuperscript{+} method seems more appropriate for studying AMPA receptor current properties.

Inhibition of voltage-gated K\textsuperscript{+} currents induced by AMPA receptor activation has been observed in a number of neuronal and glial cell preparations (Borges and Kettenmann 1995; Borges et al. 1994; Gallo et al. 1996; Jabs et al. 1994; Jones et
al. 2000; Mike et al. 1996; Muller et al. 1992; Robert and Magistretti 1997). However, important differences appear to exist between different cell types.

First, in Bergmann glial cells (Muller et al. 1992), chick telencephalic neurons (Mike et al. 1996), and cerebellar granule cells (Jones et al. 2000), K⁺ current inhibition appears to depend on Ca²⁺ influx through AMPA receptors. We found that in motoneurons removal of extracellular Ca²⁺ or holding the intracellular Ca²⁺ at high levels did not prevent the inhibition. Similar to observations in stellate astrocytes (Robert and Magistretti 1997) and oligodendrocyte precursor cells (Borges and Kettenmann 1995), we found that K⁺ current inhibition in motoneurons depends largely on Na⁺ influx through AMPA receptors. Removal of extracellular Na⁺ or clamping the cell close to the Na⁺ equilibrium potential during kainate application abolished the K⁺ current inhibition, while increasing intracellular Na⁺ concentra-

FIG. 7. Inhibition of K⁺ conductance depends on Na⁺ influx through the AMPA receptor. A: I-V relationship of KA-inhibited current in the presence (1-3.140Na⁺) and absence (1-3.0Na⁺) of extracellular Na⁺ ions, obtained with the experimental protocol in Fig. 2. B: I-V relationship of the KA-inhibited current obtained with protocol in Fig. 3, but while holding the cell at −60 (1-3.60) or +80 mV (1-3.80) during KA application. C: voltage ramps from −100 to +50 mV were given every 5 s before, during, and after KA application in a Na⁺-free external solution, and current densities at −80 and +45 mV are plotted as a function of time. Application of KA initially produced an outward current at +45 mV (reflecting the outward AMPA receptor current at +45 mV without any inhibition of K⁺ current (cf. Figure 5A) followed by a sustained and irreversible inward current shift. D: current density at +35 mV was calculated for a motoneuron exposed to 10 μM monensin and plotted as a function of time.
tions with monensin induced inhibition. These data are consistent with intracellular Na$^+$ ions directly interacting with K$^+$ permeation, a mechanism previously suggested for inhibition of voltage-gated K$^+$ channels in squid axon (Bezanilla and Armstrong 1972).

Second, the sensitivity to K$^+$ channel blockers varies between different cell types. In cerebellar granule cells (Jones et al. 2000) and hippocampal glial cells (Jabs et al. 1994), the current was sensitive to Ba$^{2+}$; in oligodendrocyte precursor cells to 4-aminopyridine [mouse, (Borges et al. 1994)] or TEA [rat, (Gallo et al. 1996)], whereas in motoneurons, the K$^+$ current was blocked by 30 mM TEA but was insensitive to 10 mM Ba$^{2+}$.

A third difference concerns the nature of the outwardly rectifying K$^+$ current. In oligodendrocyte precursor cells (Borges et al. 1994) and cerebellar granule cells (Jones et al. 2000), only the delayed rectifier K$^+$ current K$_V$ was blocked, whereas in hippocampal glial cells (Jabs et al. 1994), mainly the transient K$^+$ current K$_A$ was affected. Similar to our findings in cultured spinal cord motoneurons, both K$_V$ and K$_A$ were sensitive to kainate in cortical astrocytes. This diversity in blocking intracellular ion, sensitivity to external K$^+$ channel blockers and the nature of the affected outwardly rectifying K$^+$ current between the various cell types suggests that, at the molecular level, different K$^+$ channels with different modulation are involved.

Gallo et al. (1996) showed that inhibition of K$_V$ by kainate application diminished proliferation of preoligodendrocytes. In motoneurons, the inhibition of outward K$^+$ currents on AMPA receptor stimulation may enhance the membrane depolarization evoked by glutamate. This could facilitate synaptic transmission by lowering the amount of glutamate needed to reach the threshold for voltage-operated Na$^+$ or Ca$^{2+}$ channels or could result in activation of NMDA receptors. However, during physiological synaptic transmission, it is possible that the K$^+$ current inhibition does not occur for two reasons: the inhibition of K$^+$ currents have a slow time course (time constant 10.2 s), and the Na$^+$ influx during synaptic transmission is much smaller than the Na$^+$ influx during a voltage-clamp experiment at −60 mV when the agonist is applied for several seconds. Under excitotoxic or ischemic conditions, when the extracellular glutamate concentration is raised, the K$^+$ current inhibition is more likely to occur. This inhibition during chronic AMPA receptor stimulation may enhance excitotoxicity by loss of repolarization or, on the contrary, may provide protection by limiting Ca$^{2+}$ influx or excessive loss of intracellular K$^+$ ions. However, the ability of kainate to inhibit the outward K$^+$ currents was similar in dorsal horn neurons, which were previously shown to be resistant to AMPA receptor agonist mediated excitotoxicity (Van Den Bosch et al. 2000). These findings therefore suggest that K$^+$ current inhibition induced by AMPA receptor stimulation is rather ubiquitous and probably does not play a cell-specific role in vulnerability to or in protection against excitotoxic stress.

FIG. 8. KA inhibits both K$_V$ and K$_A$. A: K$_V$ currents in the absence and presence of 100 µM KA. K$_V$ was elicited by stepping from −30 to +30 mV, as indicated by voltage protocol. Inset, K$_V$ currents at enlarged scale (scale bar, 0.1 nA and 5 ms). B: voltage protocol used to isolate K$_A$. Both K$_V$ and K$_A$ were activated by stepping the cell from −120 to +50 mV (trace at top). K$_V$ was isolated by subtracting K$_V$ activated by stepping the cell from −50 to +50 mV (trace at bottom). C: K$_A$ current elicited at +50 mV in the absence (Control) and presence of 100 µM KA.
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