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Modulation of recombinant and native neuronal SK channels by the neuroprotective drug riluzole

Ying-Jun Cao [a], John C. Dreixler [b], Jonathan J. Couey [a], Khaled M. Houamed [a,*]

[a] Department of Anesthesia and Critical Care, University of Chicago, 5841 South Maryland Avenue, MC4028, Chicago, IL 60637, USA
[b] Department of Neurobiology, Pharmacology and Physiology, University of Chicago, Chicago, IL 60637, USA

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Abstract

Small conductance, Ca^{2+}-activated K^+ channels (SK channels) regulate neuronal excitability. We used patch clamp to study the actions of the neuroprotective drug riluzole on recombinant SK2 channels expressed in HEK293 cells and native SK channels underlying the afterhyperpolarization current (I_{AHP}) in cultured hippocampal neurons. External riluzole activated whole-cell SK2 channel currents in HEK293 cells dialyzed with a Ca^{2+}-free intracellular solution. When applied to the intracellular aspect of the membrane of giant inside-out patches, riluzole enhanced the membrane current activated by 100 nM Ca^{2+} in a reversible and concentration-dependent manner; 30 μM riluzole applied to the intracellular aspect of the patches sensitized the channels to activation by Ca^{2+}, resulting in a leftward shift of the Ca^{2+} activation curve. Riluzole also enhanced the I_{AHP} and reduced the spontaneous action potential frequency in chemically stimulated neurons. Modulation of SK channel activity by riluzole may contribute to its cellular, behavioral, and clinical effects.

Keywords: K^+ channel; Ca^{2+}; SK channel; Dequalinium; Afterhyperpolarization; Neuron

1. Introduction

Small conductance Ca^{2+}-activated K^+ channels (SK channels) regulate electrical excitability in neurons (Haylett and Jenkinson, 1990; Vergara et al., 1998); activation of SK channels mediates spike frequency adaptation and underlies the afterhyperpolarization (AHP) observed at the end of a spike train. Neuronal SK channels may also mediate certain types of fast and slow inhibitory synaptic transmission (Fiorillo and Williams, 1998; Oliver et al., 2000), and a membrane response to ischemia (Tanabe et al., 1999). Defects in SK channel function or expression may contribute to such disorders as myotonic muscular dystrophy, bipolar affective disorder, and schizophrenia (Behrens et al., 1994; Chandy et al., 1998). The KCNN family of K^+ channel genes encodes three SK channels (Chandy et al., 1998; Kohler et al., 1996).

Riluzole (2-amino-6-trifluoromethoxy benzothiazole; RP 54274; Rilutek) has analgesic, anesthetic, anticonvulsant, and neuroprotective actions (Doble, 1996; Mantz et al., 1992; Siniscalchi et al., 1999; Wahl and Stutzmann, 1999). It is currently used for treating amyotrophic lateral sclerosis (Ludolph et al., 1999), and has been proposed for amelioration of trauma and stroke (Siniscalchi et al., 1999). It may be useful for treating several neurodegenerative diseases (Doble, 1999; Rosas et al., 1999) or, prophylactically, for reducing the risk of spinal cord injury during high-risk operations on the thoracoabdominal aorta (Lang-Lazdunski et al., 1999). The cellular and molecular mechanism underlying the behavioral and clinical effects of riluzole is unclear. Riluzole may derive its effects from inhibiting release of glutamate (Doble, 1996), block of neuronal voltage-gated Ca^{2+} channels (Huang et al., 1997), Na^+ channels, and voltage-gated K^+ channels (Benoit and Escande, 1991), or activation of large conductance Ca^{2+}-activated K^+ channels (BK channels; Wu and Li, 1999) and the two-pore domain background K^+ channel TRAAK (Fink et al., 1998). Recently, it was shown that riluzole also modulates recombinant SK3 channels (Grunnet et al., 2001).

We investigated the effect of riluzole on recombinant rat SK2 channels (rSK2; rKCNN2) and native SK channels underlying the afterhyperpolarization current (I_{AHP}) in hip-

* Corresponding author. Tel.: +1-773-702-7552; fax: +1-773-702-4791.
E-mail address: Khouamed@midway.uchicago.edu (K.M. Houamed).

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pocampal neurons. The SK2 channel subtype is widely expressed in the brain and likely to form most of the binding sites to the convulsant peptide apamin (Kohler et al., 1996; Stocker and Pedarzani, 2000; Stocker et al., 1999).

2. Materials and methods

2.1. Recombinant SK channels

The cell line expressing rSK2 channels has been previously characterized (Cao et al., 2001; Dreixler et al., 2000a,b); it was generated by transfecting HEK293 cells with a plasmid encoding the rSK2 sequence and a geneticin-resistance gene contained within the mammalian expression vector pcDNA 3.1 (Invitrogen, Carlsbad, CA, USA). Geneticin-resistant colonies were clonally purified, propagated, and tested for channel expression by patch clamp. The cells were grown in minimal essential medium supplemented with 10% fetal calf serum, antibiotics (fungizone, penicillin, and streptomycin), sodium pyruvate, Glutamax, and 1 mg/ml geneticin (Life Technologies, Rockville, MD, USA), maintained at 37 °C in a water-saturated 5% CO_2 atmosphere, and plated on 35-mm plastic Petri dishes 1 to 2 days before experiments.

2.2. Neuron cultures

Neuronal cultures were prepared from hippocampi of 6-day-old rat pups with a modification of published procedures (Brewer, 1997; Shah and Haylett, 2000; Wilding and Huettner, 1997). Briefly, hippocampi were dissected into ice-cold, O_2-saturated saline of the composition (in mM): 128 NaCl, 20 HEPES, 6 $MgCl_2$, 5 KCl, 1 $CaCl_2$, 25 glucose, 0.05 DL-APV, 1 kynurenic acid; pH, 7.2, supplemented with 50 μg/ml gentamicin. The tissue was sectioned into 500-μm-thick horizontal slices using a MacIlwain chopper. The CA1–CA3 fields were then dissected using a surgical microblade, incubated in papain (Worthington, Lakewood, NJ, USA) at 33 °C for 75 min, and gently triturated. The neurons were then plated onto poly-L-lysine-coated glass coverslips in Neurobasal-A medium (Life Technologies) supplemented with 10% fetal calf serum (FCS). Once the neurons adhered, the medium was replaced with one lacking FCS. The neurons were fed twice weekly and used within 3 weeks of culture.

2.3. Electrophysiology

Whole-cell patch clamp recording of rSK2 channel currents was performed as previously described (Cao et al., 2001; Dreixler et al., 2000a,b). Briefly, patch pipettes were pulled from borosilicate glass capillaries to ~ 3 MΩ tip resistance when filled with a nominally Ca^{2+}-free intracellular solution composed of (in mM): 137.5 K $MeSO_4$, 1 $MgCl_2$, 10 K_4BAPTA, 10 HEPES, 3 K_2ATP, 5 glucose; pH, 7.4. The cells were voltage clamped at − 100 mV holding potential and constantly perfused with a modified mammalian Ringer's solution (30 mM K^+ Ringer's) composed of (in mM): 117 NaCl, 30 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 5 glucose, 2 $NaHCO_3$; pH, 7.4. The K^+ reversal potential under these conditions was ~ − 39 mV. Test solutions were applied with a local microperfusion device consisting of a 500-μm pipette connected, via a 10-way manifold, to reservoirs containing test solutions. The outlet of this device was mounted on a micromanipulator and visually maneuvered to within 100 μm of the cell under study. The time constant for solution change of this system was < 1 s.

Giant inside-out membrane patch recordings of rSK2 channel currents were essentially as described by Hilgemann and Lu (1998). Briefly, pipettes were pulled from thick-walled borosilicate glass capillaries; the tip was broken, heat-polished to an inner tip diameter ~ 6 μm, and coated with a viscous mineral oil mixture. The pipettes were filled with 30 mM K^+ Ringer's solution. GigaOhm seals were formed with pipettes while the cell was exposed to an isotonic K^+ Ringer's solution composed of (in mM): 160 K

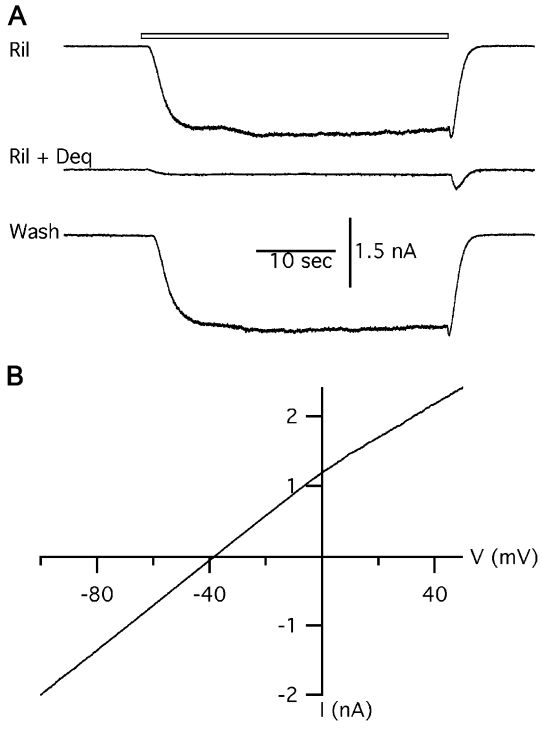

Fig. 1. Extracellular riluzole activated rSK2 channel currents in HEK293 cells. (A) The membrane current traces were recorded with the application of 100 μM riluzole (Ril), 100 μM riluzole with 10 μM dequalinium (Ril + Deq), and 100 μM riluzole again after washing out dequalinium (Wash). Holding potential and currents were − 100 mV and ~ − 210 pA, respectively. The bar indicates the duration of drug application. (B) Current–voltage relation for the current induced by 100 μM riluzole. To generate the current–voltage relation, a 150-mV, 1-s ramp voltage command was applied from a − 100 mV holding potential. In the relation shown, the membrane currents in the absence of riluzole have been digitally subtracted; it reversed at ~ − 39 mV, the calculated K^+ reversal potential.

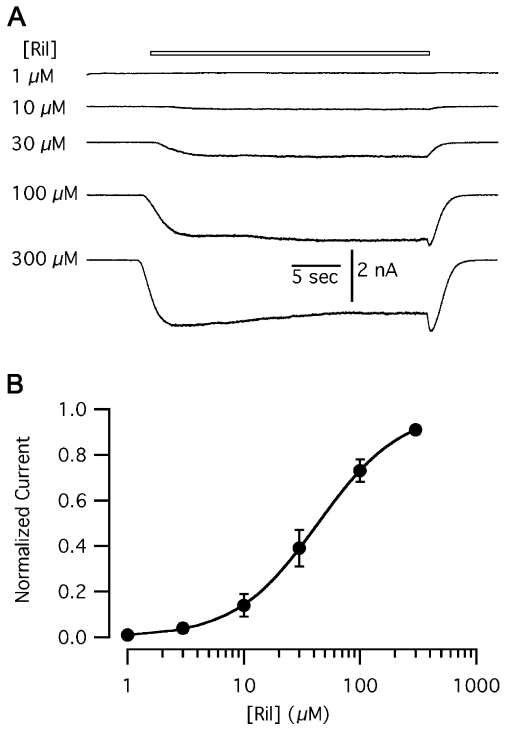

Fig. 2. Extracellular riluzole activated rSK2 currents in a concentration-dependent manner. (A) Traces of rSK2 channel currents activated by increasing riluzole concentrations in the same cell as shown in Fig. 1. The holding potential was − 100 mV. The bar represents riluzole application at the concentration (in μM) indicated at the beginning of each current trace. (B) Concentration-dependent activation of rSK2 currents by riluzole. The peak currents induced by riluzole were normalized to the peak response induced by 1 mM 1-EBIO in the same cell. Each data point represents the average of six to eight independent determinations in different cells. The error bars depict the S.E.M. The solid line is a least-square fit of the equation in Materials and methods to the pooled data.

aspartate, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 2 $NaHCO_3$, 10 HEPES; pH, 7.3. A second pipette was then maneuvered to the tip of the first pipette and the cell was mechanically bisected to generate a giant inside-out membrane patch in a nominally Ca^{2+}-free intracellular solution composed of (in mM): 160 K aspartate, 10 KCl, 10 NaCl, 1 EGTA, 1 $MgCl_2$, 5 HEPES; pH, 7.3. The patch pipette was then placed at the outlet of the microperfusion system to expose the intracellular aspect of the patch alternately to test solutions varying in [Ca^{2+}] from 30 nM to 3 μM and a control, nominally Ca^{2+}-free solution. These solutions were composed of (in mM): 160 K aspartate, 10 chelator, 5 HEPES; pH, 7.3. HEDTA [*N*-(2-hydroxyethyl)ethylene diamine *N*,*N*′,*N*′-triacetic acid] was used as the chelator for [Ca^{2+}] in the range 0.5–3.0 μM; for [Ca^{2+}] in the range 30–200 nM, a mixture of 5 mM HEDTA and 5 mM EDTA [ethylene diamine-teraacetic acid] was used. The amounts of $CaCl_2$ or $MgCl_2$ added to this solution to achieve a particular [Ca^{2+}] or [Mg^{2+}] were determined using published stability constants and software based on the method of Bers et al. (1994) and available from the Web site http://www.stanford.edu/~cpatton/maxc.html. Membrane currents were recorded by an EPC9 patch clamp amplifier (Heka, Lambrecht, Germany). The experiments were filtered at 33 Hz (8 pole Bessel), and sampled at 100 Hz. All experiments on rSK2 channels were performed at room temperature (22 to 25 °C).

Whole-cell recording of hippocampal neuron I_{AHP} and current-clamp recording of spontaneous action potential frequency were as previously described (Pedarzani et al., 2001; Shah and Haylett, 2000; Stocker et al., 1999). Neurons were perfused with warm (~ 32 °C) artificial cerebrospinal fluid (ACSF) composed of (in mM): 125 NaCl, 1.25 KCl, 1.5 $MgCl_2$, 1.25 KH_2PO_4, 25 $NaHCO_3$, 16 glucose, 2.5 $CaCl_2$. ACSF was continuously bubbled with 95% O_2/5% CO_2. All drugs were dissolved in ACSF and applied by bath perfusion. Tetraethylammonium (TEA; 1 mM), and 4-aminopyridine (4AP; 50 μM) were added to the ACSF to enhance and isolate the I_{AHP} (Stocker et al., 1999). At these

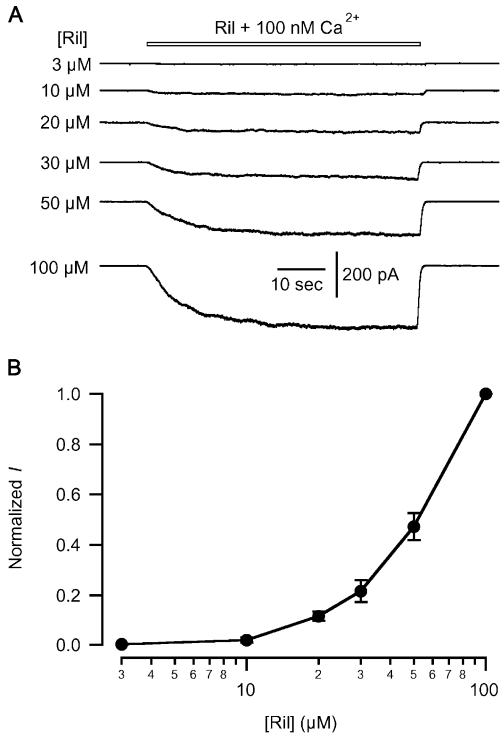

Fig. 3. Intracellular riluzole activated rSK2 currents in giant inside-out patches excised from rSK2 channel-expressing HEK293 cells. (A) Traces of rSK2 channel currents activated by co-application of 100 nM Ca^{2+} and increasing riluzole concentrations in a giant-membrane patch. The holding potential and basal membrane current were − 100 mV and < − 10 pA, respectively. The horizontal bar indicates riluzole and Ca^{2+} application. Riluzole concentration ([Ril]) is indicated at the beginning of each current trace. (B) Concentration-dependence of the activation of rSK2 channels by riluzole. The membrane current response to riluzole plus Ca^{2+} was determined by calculating the mean current during the last 20-s segment of the current trace during riluzole and Ca^{2+} application. After subtraction of the baseline holding current, the current was normalized to the maximal current activated by 100 μM riluzole and 100 nM Ca^{2+} in the same patch. Each data point represents the mean (± S.E.M.) of 5 to 10 independent determinations in separate patches.

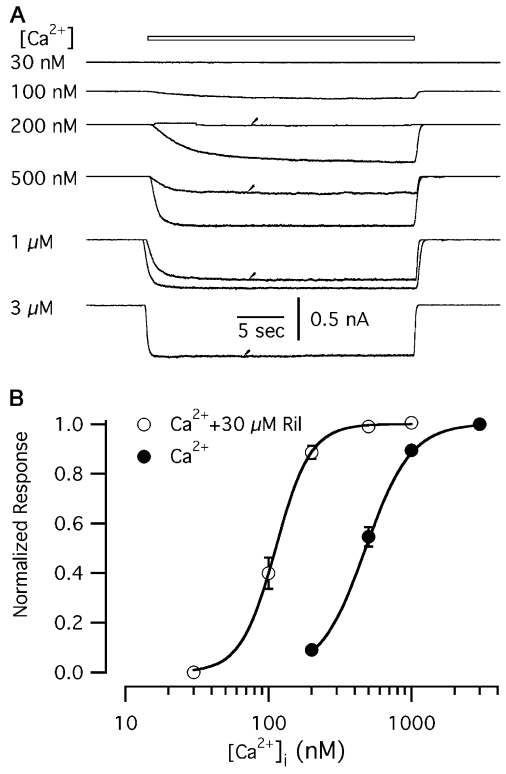

Fig. 4. Riluzole enhanced rSK2 channel sensitivity to Ca^{2+}. (A) Traces of rSK2 channel currents activated by application increasing $[Ca^{2+}]$ in the absence and presence of 30 μM riluzole, recorded in an inside-out giant membrane patch. The holding potential and baseline current were −100 mV and <−7 pA, respectively. The bar represents the application of Ca^{2+} or Ca^{2+} plus 30 μM riluzole. Traces marked by the arrowheads indicate current responses during the application of Ca^{2+} alone. The Ca^{2+} concentration is indicated at the beginning of each trace. (B) Riluzole shifted the $[Ca^{2+}]$-rSK2 channel activation curve to the left. Each point depicts the average membrane current response in the last 5-s segment of the application of Ca^{2+} (filled circles) or Ca^{2+} plus 30 μM riluzole (open circles), corrected for baseline current, and normalized with respect to the response to 3 μM Ca^{2+} in the same patch. Each point on the graph represents the mean (± S.E.M.) of five to nine determinations in separate patches.

concentrations, TEA blocks delayed rectifier and BK channel currents responsible for spike repolarization and the fast AHP (Storm, 1987), whereas 4AP blocks the D-current (Storm, 1988). Spontaneous action potential frequency was measured in ACSF lacking $MgCl_2$ and containing 100 μM picrotoxin. The perforated whole-cell patch recording technique was used to record I_{AHP} and action potential frequency (Rae et al., 1991). Patch pipette tips were filled with an intracellular solution composed of (in mM): 121 K $MeSO_4$, 4.5 KCl, 5 $MgCl_2$, 5 HEPES, 0.1 BAPTA, 3 Na_2ATP, 0.1 Na_2GTP; pipettes were then back filled with this solution containing 0.66 mg/ml amphotericin-B and 1% pluronic F-127. The pulse protocol used to evoke and measure I_{AHP} consisted of holding the membrane at −60 mV and stepping to +20 mV for 500 ms to evoke a robust Ca^{2+} current. The I_{AHP} was observed as a slowly decaying tail current upon repolarization to −40 mV.

2.4. Chemicals and drugs

Amphotericin-B, 4 AP, picrotoxin, and TEA were from Sigma (St. Louis, MO, USA). All other drugs and salts were from Fluka (St. Louis, MO, USA), except K_2ATP, which was from ICN (Costa Mesa, CA, USA), $KMeSO_4$ (Pfaltz and Bauer; Waterbury, CT, USA) and Pluronic F-127 (Molecular Probes; Eugene, OR, USA). 1-EBIO was from Aldrich (St. Louis, MO, USA); it was diluted into recording solutions from a 100-mM frozen stock in DMSO. Riluzole was from Tocris Neuramin (Ballwin, MO, USA); it was diluted from a 50-mM frozen stock in DMSO.

2.5. Data analysis

Data were analyzed offline using Pulse (Heka), Igor (WaveMetrics, Lake Oswego, OR, USA), and Excel (Microsoft, Redmond, WA, USA). Concentration–response data were least-squares fitted with a relation of the form: $I = I_{max}/[1+(EC_{50}/C)^n]$; where I is membrane current, I_{max} is the maximal current, 'EC_{50}' is the concentration for half maximum activation, C is the applied concentration and n the Hill coefficient. Membrane currents evoked by rSK2 channel activation were normalized either to the response to 1

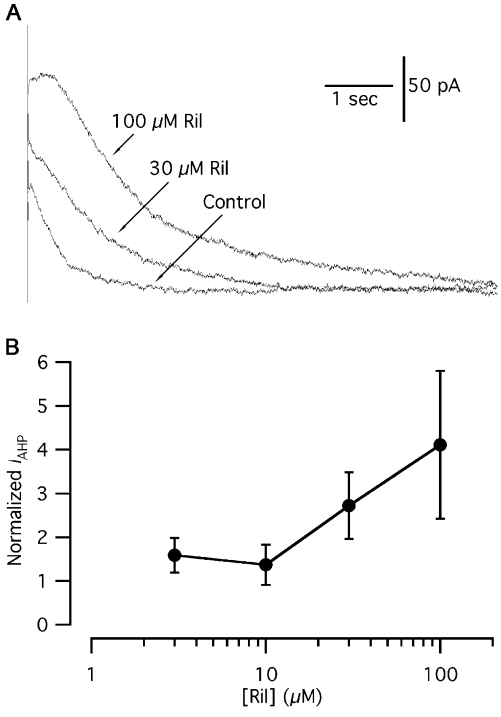

Fig. 5. Riluzole enhanced I_{AHP} in cultured rat hippocampal neurons. (A) A family of I_{AHP}s recorded under control conditions (Control) and during the application of 30 and 100 μM riluzole (Ril). The baseline, recorded in the absence of a prepulse, was digitally subtracted. (B) Concentration-dependent enhancement of I_{AHP} by riluzole. The normalized I_{AHP} enhancement was calculated by integrating the first 5 s of the I_{AHP} in the presence of riluzole and dividing the equivalent charge determined under control conditions. Each point depicts the mean (± S.E.M.) of five to eight determinations in separate neurons.

mM 1-EBIO (whole-cell) or the response to 3 μM Ca^{2+} (giant inside-out patch recording). Drug effects on I_{AHP}s were quantified from the time integral (charge) over the first 5 s. This procedure has been used previously to quantify drug effects on I_{AHP}s in hippocampal slices (Abdul-Ghani et al., 1996). The first 25 ms was blanked from the analysis to eliminate contamination by capacitance transients. The action potential frequency was averaged over 8-s epochs. Data are expressed as means ± S.E.M.

3. Results

3.1. Modulation of rSK2 channel currents by extracellularly applied riluzole

We tested whether extracellularly applied riluzole can activate rSK2 channel currents when applied to rSK2-expressing HEK293 cells, dialyzed with a nominally Ca^{2+}-free intracellular solution. Fig. 1 shows that 100 μM riluzole, applied externally, activated an inward membrane current in an rSK2-expressing HEK293 cell internally dialyzed with nominally Ca^{2+}-free intracellular solution. This current was blocked by the SK2 channel blocker dequalinium (Dreixler et al, 2000a; Fig. 1A) and reversed at ~ −39 mV (Fig. 1B), as expected for a K^+ channel current under our recording conditions (Cao et al., 2001). This riluzole-activated current was not observed in non-transfected HEK293 cells (data not shown).

The activation of whole-cell rSK2 channel currents by riluzole was concentration-dependent (Fig. 2). Higher riluzole concentrations progressively activated larger rSK2 channel currents, and the rise time of activation was more rapid. Membrane currents activated by high riluzole concentrations peaked then declined, and the washout was often accompanied by a rebound current bump (Fig. 2A). The concentration-dependence of rSK2 channel current activation by riluzole is shown in Fig. 2B. Each data point

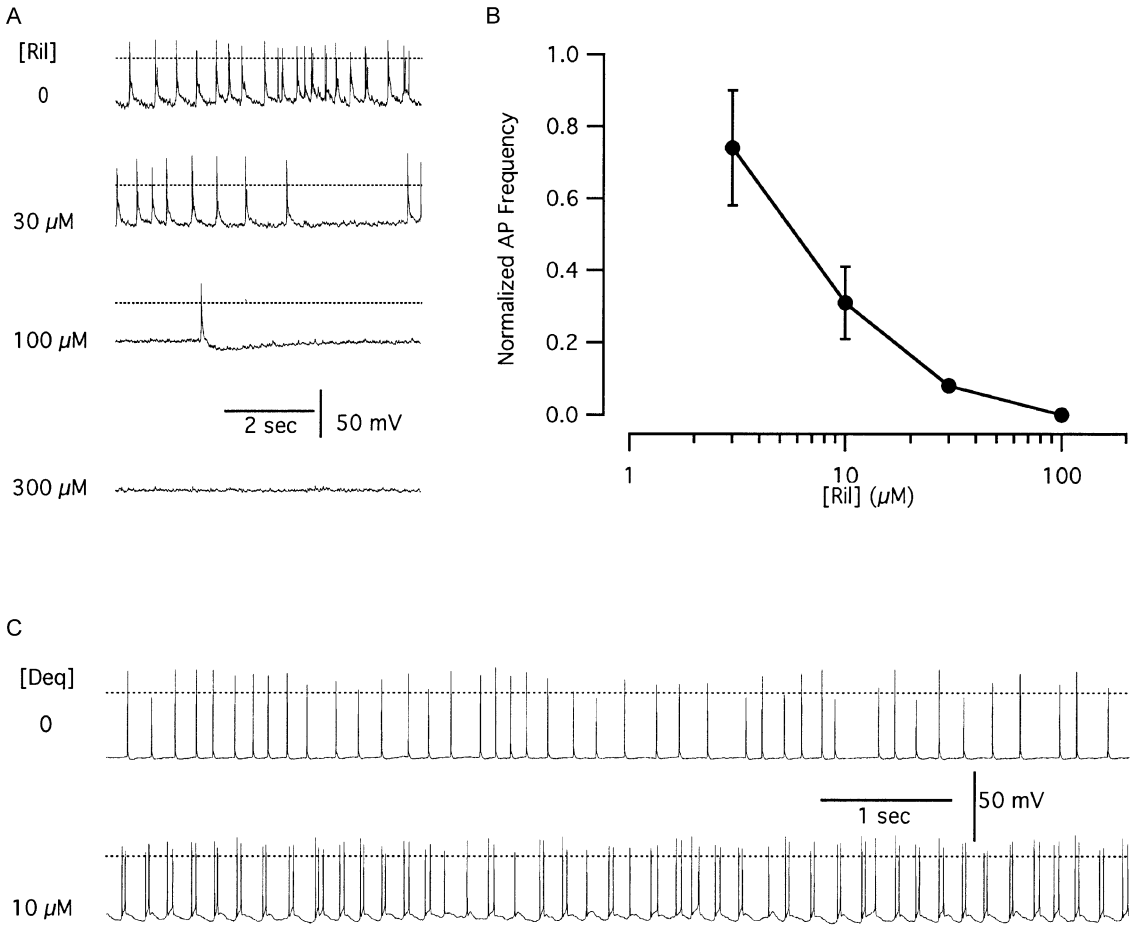

Fig. 6. Riluzole decreased hippocampal neuron excitability. (A) Membrane voltage traces in a chemically excited neuron in the presence of increasing riluzole concentrations. Riluzole concentration [Ril] is indicated at the beginning of each trace. Note the prominent sAHP following the action potential in the presence of 100 μM riluzole (third trace from top). (B) Riluzole decreased hippocampal neuron excitability in a concentration-dependent manner. For each riluzole concentration ([Ril]), the action potential frequency was averaged over an 8-s epoch and normalized with respect to the frequency determined in the absence of the drug. Each point depicts the mean (± S.E.M.) of five to nine determinations in separate neurons. (C) Enhancement of neuron excitability by the SK channel blocker dequalinium. Membrane voltage traces in a chemically excited neuron in the absence and presence of 10 μM dequalinium. Dequalinium concentration ([Deq]) is indicated at the beginning of each trace.

represents the mean (± S.E.M.) of six to eight independent determinations on different cells. Before averaging, the responses were internally normalized to the current activated by 1-ethyl-2-benzimidazolinone (1-EBIO; 1 mM), a potent activator of rSK2 channels (Cao et al., 2001). The continuous curve is the best fit of the data to the equation in Materials and methods; it yielded EC_{50} = 43 μM and n = 1.2.

The activation of rSK2 channel currents by riluzole was further characterized in excised giant inside-out membrane patches (Figs. 3 and 4). Application of 3 μM Ca^{2+} maximally activated rSK2 channel currents, whereas 100 nM Ca^{2+} did not activate a detectable current, in agreement with previous studies (Kohler et al., 1996; Xia et al., 1998). In the same patches, co-application of 100 nM Ca^{2+} with 3 μM to 100 μM riluzole evoked progressively larger rSK2 channel currents (Fig. 3A). The pooled data in Fig. 3B shows that, in the presence of 100 nM intracellular Ca^{2+}, riluzole activated rSK2 channel currents in a concentration-dependent manner.

The effect of a single riluzole concentration on the $[Ca^{2+}]$-rSK2 channel current activation relation was tested in giant inside-out patches. The membrane current traces marked with arrowheads in Fig. 4A depict the activation of rSK2 channels currents by Ca^{2+} applied to the intracellular surface of the patch; the unmarked traces are due to activation of rSK2 channels by increasing amounts of Ca^{2+} in the presence of 30 μM riluzole. The relative enhancement of rSK2 channel currents by riluzole decreased at higher $[Ca^{2+}]$; riluzole did not enhance currents activated by maximal $[Ca^{2+}]$. The pooled data in Fig. 4B show that riluzole shifts the Ca^{2+} activation curve of rSK2 channels leftward, reducing the EC_{50} from 470 to 112 nM.

3.2. Effects of riluzole on SK channel-mediated conductances in hippocampal neurons

The effects of riluzole on I_{AHP}s and spike frequency were tested in pyramidal neurons isolated from the CA1–CA3 fields of 6-day-old postnatal rat hippocampus and cultured for 7–21 days; neurons from nine different cultures were utilized for this study. Riluzole enhanced the I_{AHP}s. Fig. 5A shows a family of I_{AHP}s recorded before (marked 'control') and during application of 30 μM and 100 μM riluzole. Pooled data (five to eight cells per point), plotted in Fig. 5B shows that riluzole enhanced the I_{AHP} in a concentration-dependent manner.

Because native neuronal SK channels play an important role in regulating spike frequency, we also tested the effect of riluzole on spike frequency. Spontaneous spike frequency was boosted by removal of extracellular Mg^{2+} and addition of TEA, 4AP, and picrotoxin. Addition of riluzole to spontaneously firing neurons reduced the spike frequency in a concentration-dependent manner (Fig. 6A,B). In the absence of riluzole, the spike frequency was 3.36 ± 0.73 Hz (S.E.M.; 14 different recordings). In 3 μM riluzole, the frequency was 1.47 ± 0.53 Hz (n = 5); in 10 μM riluzole, the frequency was 0.71 ± 0.23 Hz (n = 9). In 30 μM riluzole, the spike frequency decreased to 0.09 ± 0.04 Hz (n = 8), whereas in 100 μM riluzole the spike frequency decreased to zero (n = 8). From this data, it is calculated that riluzole inhibits spike discharge with an IC_{50} ~ 6 μM. In contrast, the SK channel blocker dequalinium enhanced the spike-firing rate (Fig. 6C). In five cells, 10 μM dequalinium increased the action potential firing frequency (averaged over 8-s epochs) by 61% ± 13% (S.E.M.).

4. Discussion

We have examined the modulatory action of the neuroprotective drug riluzole on recombinant rSK2 channels and the I_{AHP} in hippocampal neurons. We report three novel findings. First, riluzole modulates recombinant rSK2 channels, leading to enhanced current at constant $[Ca^{2+}]_i$. Second, riluzole enhances the I_{AHP} in hippocampal neurons, suggesting that native SK channels are also modulated by this drug. Third, riluzole decreases the electrical excitability of chemically stimulated hippocampal neurons. Consistent with this is the observation that the SK channel blocker dequalinium has the opposite effect. Riluzole modulated rSK2 channels and I_{AHP} with similar potency to its previously observed effects on other ion channels and neurotransmitter release (Benoit and Escande, 1991; Huang et al., 1997; Fink et al., 1998; Lingamaneni and Hemmings, 1999; Wu and Li, 1999).

The present study was motivated by three previous observations. First, the cellular and behavioral effects of riluzole are opposite to those elicited by the SK channel blocker apamin. Thus, the effects of riluzole on brain excitability and sleep patterns are similar to those evoked by classical potassium channel openers and opposite the effects of apamin (Stutzmann et al., 1988, 1991). Second, riluzole activates structurally disparate K^+ channels including BK channels and tandem pore channels (Fink et al., 1998; Wu and Li, 1999), raising the possibility that it may activate other K^+ channel types including SK channels. It has been shown that riluzole enhances the activation of recombinant SK3 channels, in agreement with our study (Grunnet et al., 2001). Third, riluzole has some structural similarity to compounds such as chlorzoxazone, 1-EBIO, and zoxazolamine that modulate native and recombinant SK channels (Cao et al., 2001; Pedarzani et al., 2001).

Our results suggest that riluzole can act from either side of a membrane. The rebound "bump" in membrane current seen upon washout of riluzole may be caused by an additional pore-blocking action of this drug. Similar waveforms were also observed with chlorzoxazone and related compounds (Cao et al., 2001).

Native SK channels underlie a biphasic AHP in hippocampal neurons. The two phases, named medium AHP and slow AHP, are kinetically and pharmacologically distinct. The medium component has pharmacological properties

similar to recombinant SK channels, consistent with the idea that these subunits underlie this component (Stocker et al., 1999); it is unclear how recombinant SK channels are functionally related to the slow AHP component. In examining the modulation of hippocampal AHP by riluzole, we were unable to distinguish whether either phase was enhanced exclusively or preferentially. It appears that both phases were modulated, in agreement with a recent study examining the effects of 1-EBIO on AHPs in hippocampal slices (Pedarzani et al., 2001). However, because of its lack of specificity, our results with riluzole cannot be taken to imply that SK2 channels are the molecular correlate of either of the I_{AHP} phases.

Activation of neuronal SK channels by riluzole is consistent with its neuroprotective effects, because it hyperpolarizes the membrane and attenuates spike firing rates, leading to reduced transmitter release. If transmitter release is pathologically high, such as during ischemia, its attenuation will contribute to the neuroprotective action of riluzole. The multiple actions of riluzole on a variety of neuronal cation channels, including block of Na^+ and Ca^{2+} channels and activation of K^+ channels, would all be expected to contribute to decreased excitability and decreased transmitter release. Thus, the broad specificity of riluzole may underlie its neuroprotective effects. Alternatively, the broad specificity may also underlie its broad pharmacological profile, including some side effects.

In conclusion, we have shown that riluzole enhances the activity of native and recombinant rat brain SK channels. Modulation of native neuronal SK channels may underlie some of the behavioral and pharmacological effects of riluzole, and may be a useful strategy for development of novel neuroprotective drugs or for the treatment of disorders involving neuron hyperexcitability.

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