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INHIBITION OF THE CLONED DELAYED RECTIFIER K^+ CHANNELS, Kv1.5 AND Kv3.1, BY RILUZOLE

H. S. AHN,[a] J.-S. CHOI,[a] B. H. CHOI,[a] M.-J. KIM,[a] D.-J. RHIE,[a] S.-H. YOON,[a] Y.-H. JO,[a] M.-S. KIM,[a] K.-W. SUNG[b] AND S. J. HAHN[a]*

[a]*Department of Physiology, Medical Research Center, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea*

[b]*Department of Pharmacology, Medical Research Center, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea*

Abstract—The action of riluzole, a neuroprotective drug, on cloned delayed rectifier K^+ channels (Kv1.5 and Kv3.1) was examined using the whole-cell patch-clamp technique. Riluzole reversibly inhibited Kv1.5 currents in a concentration-dependent manner with an IC_{50} of 39.69±2.37 μM. G-protein inhibitors (pertussis toxin and GDPβS) did not prevent this inhibition of riluzole on Kv1.5. No voltage-dependent inhibition by riluzole was found over the voltage range in which channels are fully activated. Riluzole shifted the steady-state inactivation curves of Kv1.5 in a hyperpolarizing direction in a concentration-dependent manner. It accelerated the deactivation kinetics of Kv1.5 in a concentration dependent-manner, but had no effect on the steady-state activation curve. Riluzole exhibited a use-independent inhibition of Kv1.5. The effects of riluzole on Kv3.1, the *Shaw*-type K^+ channel were also examined. Riluzole caused a concentration-dependent inhibition of Kv3.1 currents with an IC_{50} of 120.98±9.74 μM and also shifted the steady-state inactivation curve of Kv3.1 in the hyperpolarizing direction. Thus, riluzole inhibits both Kv1.5 and Kv3.1 currents in a concentration-dependent manner and interacts directly with Kv1.5 by preferentially binding to the inactivated and to the closed states of the channel.
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Key words: riluzole, Kv1.5, Kv3.1, G-proteins.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) is a neuroprotective drug that exerts several other pharmacological actions: analgesic, anesthetic, anticonvulsant, anti-ischemic, sedative and antiapoptotic properties (Mantz et al., 1992; Pratt et al., 1992; Doble, 1996; MacIver et al., 1996; Ettaiche et al., 1999; Lang-Lazdunski et al., 1999; Siniscalchi et al., 1999; Wahl and Stutzmann, 1999). It is currently used clinically in the treatment of amyotrophic lateral sclerosis (Bensimon et al., 1994) and can be useful for treating neurodegenerative diseases in animal models of Parkinson's disease and Huntington's disease (Mary et al., 1995; Barnéoud et al., 1996). The cellular and molecular mechanisms associated with the beneficial neuroprotective effects of riluzole are currently under investigations, although the action of the drug has been attributed to the blockage of the presynaptic release of glutamate in the brain and spinal cord (Doble, 1996). However, riluzole has been reported to have multiple effects on a variety of ion channels. For example, it inhibits voltage-gated Na^+ channels and high voltage-activated (HVA) Ca^{2+} channels by shifting the steady-state inactivation curve toward more negative potentials (Huang et al., 1997b; Song et al., 1997; Zona et al., 1998). Furthermore, cellular signaling mechanisms, such as G-proteins and protein phosphorylation, are involved in the inhibition of the HVA Ca^{2+} current by riluzole (Huang et al., 1997a). Although voltage-gated K^+ channels are encoded by the same supergene family as voltage-gated Na^+ channels and Ca^{2+} channels, the effects of riluzole on K^+ channels vary, depending on the K^+ channel subtype. Riluzole reduces the steady-state outward K^+ currents in cultured rat cortical neurons (Zona et al., 1998). By contrast, it increases voltage-gated K^+ channels in GT1 cells (Beltran-Parrazal and Charles, 2003). In addition to these effects, riluzole activates structurally different K^+ channels including large conductance Ca^{2+}-activated K^+ channels, small-conductance Ca^{2+}-activated K^+ channels and the two-pore domain background K^+ channels (TREK-1 and TRAAK) (Wu and Li, 1999; Duprat et al., 2000; Grunnet et al., 2001; Cao et al., 2002). Riluzole also modulates Kv1.4 inactivation via the oxidation of a cysteine residue in the N-terminal inactivation ball (Xu et al., 2001). Thus, the mechanism of action of riluzole on ion channels appears to be complex. However, unlike the extensive studies of its effect on these channels, its mode of action on voltage-gated, delayed rectifier K^+ channels is currently unclear. We report herein on a study of the effect of riluzole on two cloned delayed rectifier K^+ channels, Kv1.5 and Kv3.1. In addition, the mechanism of inhibition of these channels was compared with those for voltage-gated Na^+ channels and Ca^{2+} channels.

EXPERIMENTAL PROCEDURES

Cell culture

Chinese hamster ovary (CHO) cells (ATCC, Rockville, MD, USA) were maintained in Iscove's modified Dulbecco's medium (IMDM; Invitrogen Corporation, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 0.1 mM hypoxanthine and 0.01 mM thymidine in a humidified 5% CO_2 incubator at 37 °C. The CHO cells used were stably expressed Kv1.5 or Kv3.1b channels as previously described (Choi et al., 2001, 2002). The transfected cells were exchanged with fresh IMDM containing 0.3 mg/ml of G418 and passed at 2–3 day intervals using a brief trypsin–EDTA treatment. The cells were seeded onto glass coverslips (diameter: 12 mm, Fisher Scien-

*Corresponding author. Tel: +82-2-590-1170; fax: +82-2-532-9575.
E-mail address: sjhahn@catholic.ac.kr (S. J. Hahn).
Abbreviations: CHO, Chinese hamster ovary; HVA, high voltage-activated; IMDM, Iscove's modified Dulbecco's medium; I–V, current–voltage; PTX, pertussis toxin.

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tific, Pittsburgh, PA, USA) in a 35 mm dish 1 day before use and were incubated in a 5% CO_2 incubator at 37 °C. For the electrophysiological experiments, coverslips with attached cells were transferred to a continually perfused recording chamber (RC-13, Warner Instrument Corporation, Hamden, CT, USA).

Electrophysiological recordings

Voltage-clamp recordings were performed using the whole-cell configuration of the patch-clamp technique at room temperature (22–24 °C) with an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA). Micropipettes were fabricated from PG10165-4 glass capillary tubing (World Precision Instruments, Sarasota, FL, USA) using a puller (Model P-97, Sutter Instrument Co., Novato, CA, USA) and had resistances of 2–3 MΩ when filled with internal pipette solution. The micropipettes were gently lowered onto the cells and giga ohm seal formation was achieved by suction. Following pipette capacitance compensation, the cells were ruptured by a brief application of additional suction. Series resistances were approximately 4–8 MΩ. Whole-cell capacitive currents were compensated with analog compensation. Series resistance compensation (80%) was employed if the current exceeded 1 nA. The currents were low-pass filtered at 2 kHz (four-pole Bessel filter) and sampled at 5 kHz before being digitized. Data acquisition and analysis were performed with an IBM pentium computer, using pClamp 9.01 software (Axon Instruments).

Solutions and drugs

The bath solution contained: 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES and was adjusted to pH 7.3 with NaOH. The internal pipette solution contained: 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM EGTA and was adjusted to pH 7.3 with KOH. The measured osmolarity of the solutions was 300–340 mOsm. Riluzole (Tocris Cookson, Bristol, UK) was dissolved in dimethyl sulfoxide to make 100 mM stock solutions and then appropriately diluted in bath solution. GDPβS (Sigma, St. Louis, MO, USA) was dissolved in pipette solution to give stock solutions of 10 mM and directly added to the internal pipette solution. Pertussis toxin (PTX, Calbiochem, San Diego, CA, USA) was dissolved in distilled water to give 100 μg/ml stock solutions.

Data analysis

Origin 7.0 software (Microcal Software, Inc., Northampton, MA, USA) was used for the analysis. The concentration-response data were fitted with the following logistic equation:

$$y = 1/\{1 + ([D]/IC_{50})^n\}$$

where IC_{50} is the concentration of riluzole required to produce a 50% inhibition, [*D*] the riluzole concentration and *n* the Hill coefficient. Activation curves were obtained by normalizing the tail currents measured at −40 mV after the application of 100 ms depolarizing pulses at potentials between −60 mV and +60 mV in 10 mV increments every 10 s from a holding potential of −80 mV and fitted to the Boltzmann equation:

$$y = 1/\{1 + \exp(-(V - V_{1/2})/k)\} \quad (1)$$

where *k* represents the slope factor, *V* the test potential and $V_{1/2}$ the potential at which the conductance was half-maximal. The voltage dependence of steady-state inactivation was investigated by using a two-pulse voltage protocol; currents were measured by 250 ms depolarizing pulses to +50 mV while 20 s preconditioning pulses were varied from −60 mV to +20 mV stepped by 10 mV in the absence and presence of drugs. The resulting steady-state inactivation data were fitted to the Boltzmann equation:

$$(I - I_c)/(I_{max} - I_c) = 1/\{1 + \exp(V - V_{1/2})/k\}$$

in which I_{max} represents the current measured at the most hyperpolarized preconditioning pulse and I_c represents a non-zero current which was not inactivated at the most depolarized 20 s preconditioning pulse. We eliminated this non-zero residual current by subtracting it from the actual value. The tail currents upon repolarization for the analysis of the deactivation kinetics were fitted with a single exponential function. The data are expressed as the mean±S.E. Student's *t*-test and an analysis of variance were used for statistical analysis. Statistical significance was considered at $P<0.05$.

RESULTS

Concentration dependence of Kv1.5 inhibition

Fig. 1A shows representative recordings of the Kv1.5 current expressed in CHO cells under control conditions and in the presence of riluzole. Under control conditions, Kv1.5 currents were characterized by rapid activation followed by a slow inactivation during a 250 ms depolarizing pulse. After measuring the controls, cells were exposed to different concentrations of riluzole (3, 10, 30 and 100 μM). At 3 μM, riluzole produced a small inhibition of the peak Kv1.5 current. At 10 μM or higher concentrations, riluzole decreased the peak current and enhanced the rate of Kv1.5 current decay with a more marked effect on the current recorded at the end of the pulse than on the peak. This concentration-response relationship for the inhibition of Kv1.5 is shown in Fig. 1B. A nonlinear least-squares fit of the logistic equation to the concentration-response data yielded an IC_{50} of 39.69±2.37 μM and a Hill coefficient of 1.61±0.11 ($n=8$).

Reversible inhibition of Kv1.5

A time course for changes in steady-state currents induced by addition of 30 μM riluzole and by subsequent washout of the drug is shown in Fig. 2. Riluzole inhibited the steady-state current of Kv1.5 at the end of a depolarizing pulse of +50 mV to 58.44±1.79% ($n=4$) of the control value (Fig. 2A). Kv1.5 inhibition appeared within 20 s of drug application and reached a steady state within 2 min (Fig. 2B). After washout, the current completely recovered (98.54±1.55% of control value, $n=4$) with a similar time course. Thus, the effect of riluzole on Kv1.5 currents is reversible.

Effects of G protein inhibitors on the inhibition of Kv1.5 by riluzole

To elucidate whether the inhibitory effects of riluzole on Kv1.5 currents were mediated by G proteins, we investigated the effects of G protein inhibitors using PTX in the bath solution and GDPβS in the pipette solution (Fig. 3A). After preincubation with PTX (200 ng/ml) for 24 h, 30 μM riluzole inhibited Kv1.5, measured at the end of a depolarizing pulse of +50 mV, to 57.42±2.72% of the control ($n=6$), which was not significantly different from the inhibition induced by 30 μM riluzole in the absence of PTX (control; 58.44±1.79%, $n=4$), as shown in Fig. 3B. Thus,

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