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Coumarsabin hastens C-type inactivation gating of voltage-gated K^+ channels

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ABSTRACT

During prolonged depolarization, voltage-gated K^+ (Kv) channels display C-type inactivation, a process which is due to selectivity filter destabilization and serves to limit K^+ flux. Here we reported that coumarsabin, a coumarin derivative isolated from *Juniperus Sabina*, could hasten C-type inactivation and thus cause block of Kv channels in neuronal NG108-15 cells and Kv1.2 channels heterologously expressed in lung epithelial H1355 cells. In NG108-15 cells, extracellular, but not intracellular, coumarsabin (30 μM) strongly speeded up Kv current decay and caused a left-shift in the steady-state inactivation curve. Coumarsabin inhibited end-of-pulse Kv currents with an IC_{50} of 13.4 μM. The kinetics and voltage-dependence of activation were not affected by coumarsabin. The degree of block by coumarsabin was not enhanced by a reduction in intracellular K^+ concentration. Data reveal that coumarsabin was a closed channel blocker and it displayed a frequency-independent mode of inhibition. Coumarsabin did not accelerate current decay in a Kv1.2 mutant (V370G) defective in C-type inactivation. Taken together, our data suggest that Kv channel inhibition by coumarsabin did not appear to result from a direct obstruction of the outer pore but relied on C-type inactivation.

1. Introduction

In response to depolarization, voltage-gated K^+ (Kv) channels open to permit K^+ efflux, resulting in repolarization of excitable cells. During prolonged depolarization, most Kv channels undergo a process called inactivation to limit the efflux of K^+ (Hille, 2001; Kukuljan et al., 1995). In certain Kv channel members (Kv1.4, Kv3.1 and Kv4.2) a rapid inactivation takes place in which the cytoplasmic N-terminus acts as a "ball-and-chain", occluding the internal vestibule of the opened channel (Kukuljan et al., 1995). A slow inactivation occurs in almost all Kv channels. This slow inactivation (also called C-type inactivation), often spanning seconds, involves destabilization of the outer channel pore surrounding the selectivity filter (Kurata et al., 2001; Andalib et al., 2004; Kurata and Fedida, 2006). Evidence suggests that the interaction between residues in the selectivity filter and the adjacent pore helix via hydrogen bonding is important for the stability of the selectivity filter and thus, the rate and extent of C-type inactivation (Cordero-Morales et al., 2007, 2011).

Since Kv channels are responsible for repolarization, inhibition of the Kv channels would enhance cellular excitability. For instance, Kv channel block by 4-aminopyridine (4-AP) has been proven useful in speeding up conduction of the demyelinated nerves in multiple sclerosis (Judge and Bever, 2006). Apoptotic cells have been known to over-express Kv channels and permit excessive K^+ efflux, thus losing cellular K^+ and allowing caspases to be activated (Yu et al., 1997; Yu, 2003). Inhibition of Kv channels has been demonstrated to suppress neuronal apoptosis (Yu et al., 1997; Yu, 2003; Hu et al., 2006). Pharmacological potential of Kv channel inhibition has recently been reviewed (Leung, 2010). Most of the classical blockers of Kv channels are known to directly occlude the K^+ conduction pathway: tetraethylammonium (TEA) could block by obstructing at the extracellular pore mouth or the internal cavity; 4-AP obstructs at the internal cavity (see Leung, 2012). In recent years a number of compounds, namely, KN-93, HMJ-53A, rhynchophylline and 6β-acetoxy-7α-hydroxyroyleanone (AHR), have been found to inhibit Kv channels not by directly blocking at the outer pore or

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the internal cavity, but by hastening destabilization of the selectivity filter (i.e., accelerating C-type inactivation) (Rezazadeh et al., 2006; Chao et al., 2008; Chou et al., 2009; Leung et al., 2010a). These compounds have the property of strongly suppressing the sustained Kv current while only mildly affecting the peak current; this has the advantage of preventing prolonged K^+ loss while only minimally compromising the fast repolarizing function. The therapeutic potential of these compounds has been reviewed in a recent article (Leung, 2012).

In a further search for novel compounds as modifiers of the C-type inactivation gating, we identified coumarsabin (Fig. 1), a naturally occurring coumarin derivative isolated from the leaves of *Juniperus Sabina* (De Pascual et al., 1981). While coumarsabin shares the core features (namely, acceleration of C-type inactivation without affecting activation) with previously established compounds, it is distinct from them by being a closed channel blocker and exhibiting a frequency-independent mode of block. The pharmacological profile of coumarsabin is discussed.

2. Materials and methods

2.1. Chemicals and cell culture

Coumarsabin was purified from leaves of *J. sabina* as previously described (> 99.0% purity by HPLC) (De Pascual et al., 1981). 6β-acetoxy-7α-hydroxyroyleanone (AHR), a diterpenoid compound isolated from *Taiwania cryptomerioides*, was purified as previously described (> 99.0% purity by HPLC) (Kuo et al., 1979). NG108-15 cells were cultured at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and penicillin–streptomycin (100 units/ml, 100 μg/ml) (Invitrogen). NG108-15 cells were induced to differentiate into more mature neurons by being incubated in the above-mentioned medium with 0.1% fetal bovine serum, 10 μM retinoic acid and 30 μM forskolin for 3 days. Lung epithelial H1355 cells were cultured at 37 °C in 5% CO_2 in Roswell Park Memorial Institute 1640 medium (Gibco) with the same FBS and antibiotics supplements as in NG108-15 cells.

2.2. Mutagenesis and transfection

pcDNA3.1-Kv1.2 was kindly provided by Prof. H. Gaisano (University of Toronto) and pEGFP (as marker) was purchased from Clontech, Palo Alto, CA. Primers used were: forward (5′ to 3′) CTC CTT TGA GTT TCT GGT; reverse (5′ to 3′): ACT CTT ACC AAC CGG ATG. Mutation of pcDNA3.1-Kv1.2 wild type (WT) into V370G was performed according to the protocols of QuikChange® II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The mutation was confirmed by DNA sequencing. For heterologous expression of Kv1.2 WT and mutant channels, the plasmids were transiently transfected into H1355 cells using TurboFect (Invitrogen) according to the manufacturer's instructions.

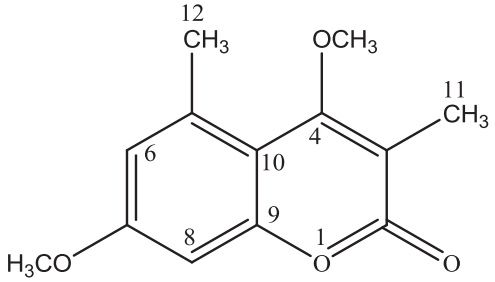

Fig. 1. Chemical structure of coumarsabin.

2.3. Electrophysiology

Electrophysiological experiments were performed according to previous protocols (Leung et al., 2003; Chao et al., 2008). NG108-15 and H1355 cells were voltage-clamped in the whole-cell

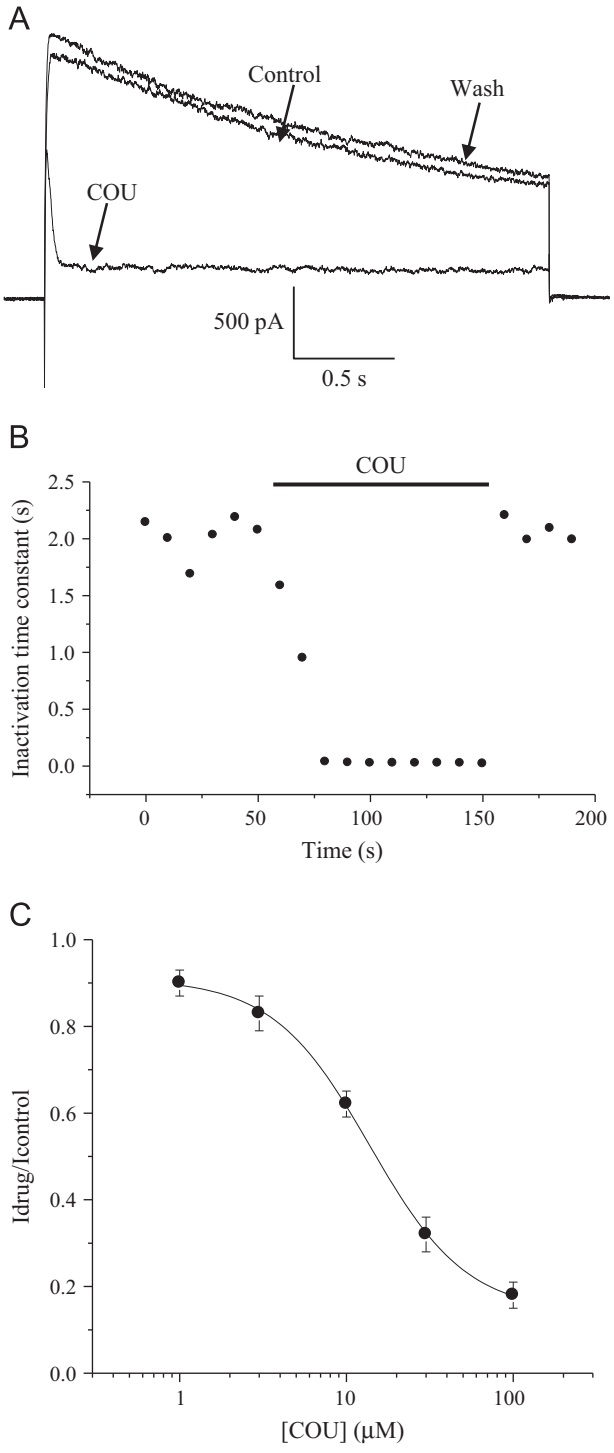

Fig. 2. Coumarsabin hastened C-type inactivation of Kv currents in NG108-15 cells: (A) representative records of outward K^+ currents triggered by +30 mV before coumarsabin addition, after 30 μM coumarsabin treatment, and after drug washout, (B) the inactivation time constants as observed in (A) are plotted against time. Similar results were observed in seven more separate experiments, and (C) with +30 mV stimulations, the end-of-pulse Kv current in the presence of coumarsabin is normalized with the maximum end-of-pulse current (in the absence of drug) and then plotted against coumarsabin concentrations. The Hill equation is used to fit the curve. Results are means ± S.E.M. from 7–10 cells.