Abstract # 6254

Expression and Characterization of TWIK-2 in CHO cells

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Abstract

We have recently demonstrated that TWIK-2, a two-pore domain potassium channel, is highly expressed in rat middle cerebral artery (MCA) (AJP 291:H770, 2006). In this study, we cloned TWIK-2 from rat MCA and expressed it heterologously as a fusion protein to GFP for characterization. Direct fluorescence analysis by deconvolution microscopy in stably transfected CHO cells revealed that GFP-rTWIK-2 localized to the plasma membrane in a punctate pattern by 72 hours after passaging. Stable transfectants of GFP-rTWIK-2 were analyzed by whole-cell electrophysiology. Whole cell currents were 30-fold greater than the nontransfected control group, were non-rectifying in physiological K^+, but were inwardly rectifying in symmetrical K^+. The observed reversal potentials in different concentrations of extracellular K^+ were similar to the theoretical reversal potentials. In physiological K^+, membrane potential was -81 ± 0.5 mV (n=13) for transfected and -51 ± 2.5 mV (n=16) for control cells. 1 mM $BaCl_2$ inhibited currents by 90 ± 1% with a calculated IC_{50} of 86 μM (n=5). The TWIK-2 currents were minimally affected by 10 mM TEA, 3 mM 4-AP, and 10 μM glibenclamide (n=3). Arachidonic acid (100 μM) increased the currents 88 ± 15% (n=6). PKA inhibition with 100 nM H-89, a competitive PKA inhibitor, decreased baseline currents by 49 ± 11% (n=3). For PKC, activation with 400 nM PMA resulted in a 44 ± 20% (n=3) decrease in whole cell currents. Since vascular smooth muscle of the rat MCA expresses a functional TWIK-2 channel, TWIK-2 has the potential of being an important regulator of vascular tone.

Introduction

Potassium (K) channels on vascular smooth muscle are important regulators of cerebral blood flow. When activated they elicit membrane hyperpolarization which dilates vessels by closing the voltage-sensitive calcium channels. K channels represent a therapeutic target for regulating blood flow.

A novel family of K channels, two-pore domain K channels (K_{2P}), has recently been described in the vascular system. TWIK-2, a K_{2P}, is highly expressed in cerebral vascular smooth muscle. The physiological function of TWIK-2 is not known.

Questions:

1. **What are the characteristics of rTWIK-2 currents?**
2. **Is rTWIK-2 sensitive to protein kinase activation and inhibition?**

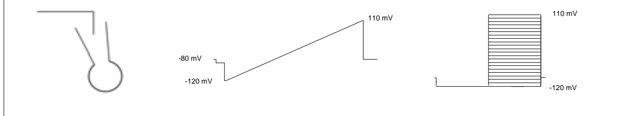
Methods

Molecular Cloning and Expression

TWIK-2 was cloned from rat middle cerebral arteries. Stable transfectants were selected for in CHO cells expressing rTWIK-2 as an n-terminal fusion protein to Ac-GFP.

Electrophysiology

TWIK-2 currents were measured using the whole cell configuration of the patch clamp method. Pipette buffer consisted of (in mM): 110 gluconate (K salt), 30 KCl, 1 $MgCl_2$, 2.2 $CaCl_2$, 3 EGTA, pH 7.2. Bath buffer consisted of (in mM): 140 NaCl, 4.2 KCl, 3 $NaHCO_3$, 1.2 KH_2PO_4, 2 $MgCl_2$, 0.1 $CaCl_2$, pH 7.4.



Results

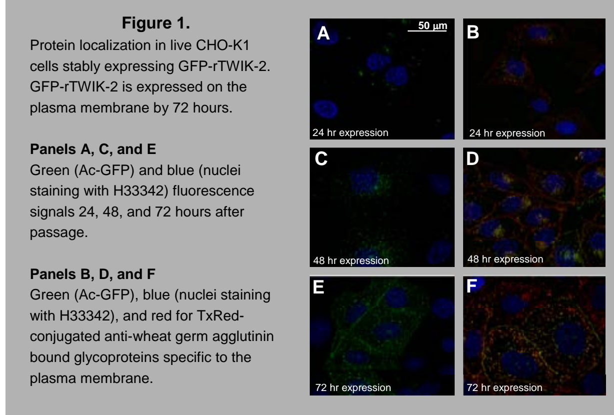


Figure 1.
Protein localization in live CHO-K1 cells stably expressing GFP-rTWIK-2. GFP-rTWIK-2 is expressed on the plasma membrane by 72 hours.

Panels A, C, and E
Green (Ac-GFP) and blue (nuclei staining with H33342) fluorescence signals 24, 48, and 72 hours after passage.

Panels B, D, and F
Green (Ac-GFP), blue (nuclei staining with H33342), and red for TxRed-conjugated anti-wheat germ agglutinin bound glycoproteins specific to the plasma membrane.

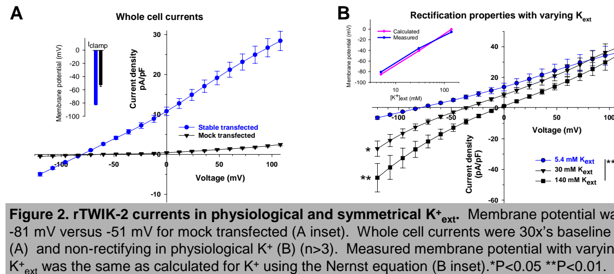


Figure 2. rTWIK-2 currents in physiological and symmetrical K^+_{ext}. Membrane potential was -81 mV versus -51 mV for mock transfected (A inset). Whole cell currents were 30x's baseline (A) and non-rectifying in physiological K^+ (B) (n>3). Measured membrane potential with varying K^+_{ext} was the same as calculated for K^+ using the Nernst equation (B inset).*P<0.05 **P<0.01.

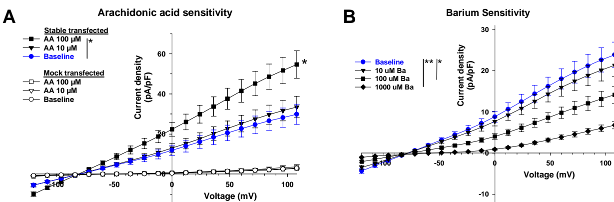


Figure 3. Known rTWIK-2 activator and inhibitor. rTWIK-2 is activated by arachidonic acid (EC_{50} = 50 μM) (A) and inhibited by barium (IC_{50} = 86 μM) (n=6) (B). **P<0.05, **P<0.01.*

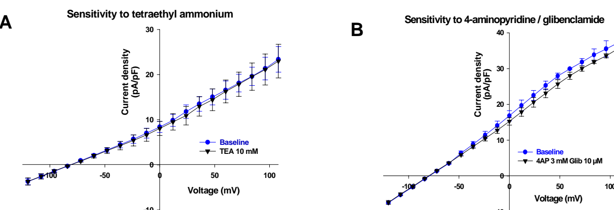


Figure 4. rTWIK-2 sensitivity to classical K channel blockers. GFP-rTWIK-2 is insensitive to TEA (10 mM) (A), 4AP (3 mM) and glibenclamide (10 μM) (B) (n=3).

Figure 5. Putative phosphorylation sites of rTWIK-2 (4A) and change in rTWIK-2 current with kinase effectors (5B-5C). PKA inhibition with 100 nM H-89 (5B), a competitive PKA inhibitor; PKC activation with 400 nM PMA (5C), a selective PKC activator (n=3). **P<0.05.*

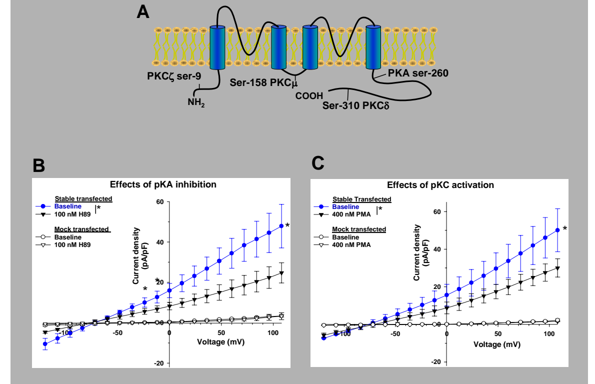
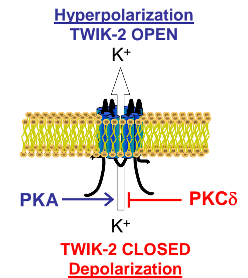


Figure 6. Proposed model for rTWIK-2 regulation.
We hypothesize that PKA and PKC reciprocally activate and inhibit rTWIK-2 by phosphorylation of intracellular serine residues.

Channel states:

- •OPEN –maintain or increase in baseline current
- •CLOSED –decrease in baseline current



Conclusions

1. GFP-rTWIK-2 localized to the plasma membrane in a punctate pattern 72 hours after passaging.
2. rTWIK-2 K^+ currents reverse at -81 mV and are:
   a. 30 times baseline
   b. Non-rectifying in physiological K^+
   c. Activated by arachidonic acid
   d. Inhibited by Ba^{2+} but not affected by TEA, 4AP, or glibenclamide
3. rTWIK-2 contains 1 PKA consensus site and 3 PKC consensus sites.
4. rTWIK-2 is activated by PKA and inhibited by PKC.
5. TWIK-2 may be an important regulator of vascular tone in cerebral arteries and arterioles.

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