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Does Sumoylation Control K2P1/TWIK1 Background K^+ Channels?

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SUMMARY

A novel model for the regulation of cell excitability has recently been proposed. It originates from the observation that the background K^+ channel K2P1 (TWIK1) may be silenced by sumoylation in *Xenopus* oocytes and that inactivation of the putative sumoylation site (mutation K274E) gives rise to robust current expression in transfected COS-7 cells. Here, we show that only the mutation K274E, and not K274R, is associated with an increase of K2P1 current density, suggesting a charge effect of K274E. Furthermore, we failed to observe any band shift by western blot analysis that would confirm an eventual sumoylation of K2P1 in COS-7 cells and oocytes.

INTRODUCTION

K2P1 (or TWIK1) has been cloned from a human kidney cDNA library (Lesage et al., 1996a). Sequence analysis predicted a unique membrane topology with four transmembrane segments and two pore-forming domains (Lesage et al., 1996a). Following the identification of K2P1, homologous K2P proteins were rapidly isolated from *Drosophila* and mammals (Goldstein et al., 2005; Kim, 2005; Lesage and Lazdunski, 2000; Patel and Honore, 2001; Talley et al., 2003). When compared to other K2P channels, K2P1 displays a couple of unique features. Like its closest homolog TWIK2 (K2P6) (Patel et al., 2000), K2P1 produces currents with a rapidly inactivating component. Because of this inactivation, their steady-state current voltage relationships are much more similar to that of the weak inwardly rectifying ROMK1 current (Lesage et al., 1996a) than those of the other K2P currents that follow the Goldman Hodgkin Katz equation (Duprat et al., 1997; Fink et al., 1996). Another unique feature of K2P1 is the difficulty to record currents from transfected cells and the fact that no native currents corresponding to K2P1 have yet been reported. However, mice deficient for K2P1 have impaired regulation of phosphate transport in the proximal tubule and of water transport in the medullary collecting duct, strongly suggesting that K2P1 is functional and contributes to membrane trafficking/expression of transport molecules in the kidney (Nie et al., 2005). We have shown that K2P1 is mainly localized in the recycling endosomal compartment located at the apical side of transfected kidney cells and native proximal tubule cells (Decressac et al., 2004). In a variety of nonpolarized cells, K2P1 immunoreactivity was detected almost exclusively in the pericentriolar recycling compartment (Decressac et al., 2004). The mechanism that controls surface expression/retrieving of K2P1 is not yet characterized but may be under the dependency of the small G protein ARF6 and its nucleotide exchange factor EFA6 that interacts with K2P1 (Decressac et al., 2004).

Recently, it has been suggested that K2P1 is addressed to the cell surface when expressed in *Xenopus* oocytes and that addition of a small ubiquitin modifier (SUMO) peptide to lysine 274 (K274) is responsible for a block of channel activity (Rajan et al., 2005). From these results, the authors of the study proposed that K2P1 is a plasma membrane channel and that its silencing by sumoylation is the major mechanism explaining the loss of active channel expression in transfected and native cells. This work has gained considerable interest not only because it identifies a novel mechanism of ion channel regulation but also for its general implication in cell biology (Wilson and Rosas-Acosta, 2005). Sumoylation is the posttranscriptional modification of lysine residues in target proteins by covalent attachment of a SUMO peptide moiety at the consensus site ψKxE/D (where ψ is a hydrophobic residue and x is any amino acid) (reviewed in Dohmen, 2004). It is primarily a nucleocytoplasmic phenomenon that mediates protein-protein interactions, nucleocytoplasmic trafficking, and activity of transcription factors, but sumoylation is also known to take place in the cytoplasm. Regulation of membrane excitability by sumoylation of background K^+ channels active at rest would provide a novel level of crosstalk between membrane signaling and some nuclear events through coordination of sumoylated

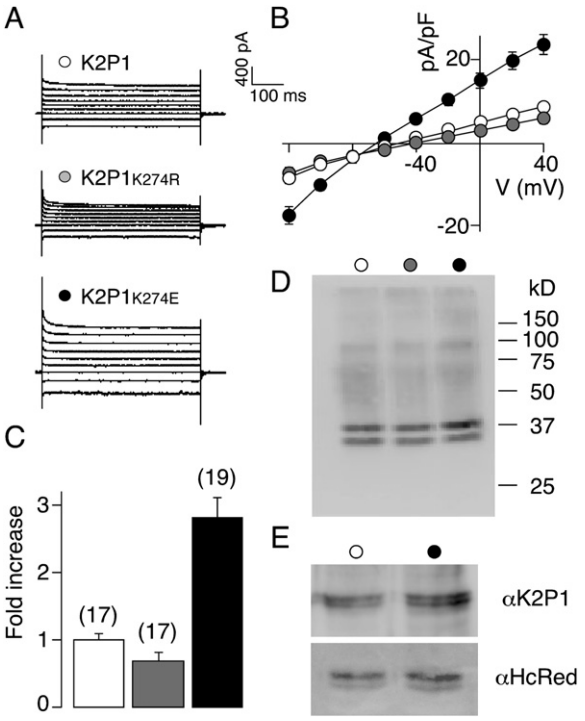

Figure 1. Electrophysiological and Biochemical Characterization of K2P1 and Mutant K2P1K274R and K2P1K274E Channels in COS-7 Cells

(A) Whole-cell current traces from COS-7 cells expressing K2P1, K2P1K274R, and K2P1K274E channels fused to HcRed. Currents were elicited by voltage pulses ranging from −120 mV to 40 mV in 20 mV steps, from a holding potential of −80 mV.

(B) Current density was determined at all test potentials from the steady-state current and whole-cell capacitance. Each value represents the mean ± SEM, n cells for K2P1 (n = 17), K2P1K274R (n = 17), and K2P1K274E (n = 19).

(C) Current density variation for K2P1K274R and K2P1K274E was normalized to the K2P1 value obtained at +40 mV test potential. Values are mean ± SEM.

(D) Western blot analysis of K2P1, K2P1K274R, and K2P1K274E.

(E) Western blot analysis of HcRedK2P1 and HcRedK2P1K274E using anti-K2P1 antibody (as in D) or anti-HcRed antibody. Analysis was carried out as described in Rajan et al. (2005) (lysis buffer containing NEM, an inhibitor of SUMO isopeptidases).

states. However, several aspects of K2P1 sumoylation are highly intriguing. First, the K274 residue in K2P1 does not belong to a classical consensus site for sumoylation ($LK^{274}KF$). Second, if K2P1 is a silenced plasma membrane channel, then it must be kept almost exclusively sumoylated in many different cell types that do not exhibit a robust current following K2P1 expression. This situation has no other example to our knowledge.

Here, we demonstrate that K2P1 is not quantitatively sumoylated in COS-7 cells and *Xenopus* oocytes. In the same expression conditions, the K274E mutation is associated with an increase of K2P1 current density as previously described (Rajan et al., 2005). However, this increase is not observed with the conservative K274R mutation. Taken together these data demonstrate that the increase of current associated with K274E, and absent in K274R, can probably be attributed to a charge effect and that SUMO modification at lysine 274 is not the proposed mechanism for K2P1 silencing.

RESULTS

Expression of K2P1 in COS-7 Cells

In COS-7 cells, K2P1 does not produce macroscopic currents in the majority of the tested cells (not shown). It has been reported that alteration of lysine 274 to glutamate (K274E) leads to functional expression of K2P1 (Rajan et al., 2005). However, we failed to observe any significant current upon expression of K2P1K274E in our batch of COS-7 cells (not shown). We have previously shown that the fusion of the *Heteractis crispa* red (HcRed) fluorescent protein to the amino terminus of K2P1 is associated with a partial expression of the resulting fusion protein at the cell surface of proliferating MDCK cells. In these particular conditions, it was possible to measure K2P1 currents at the plasma membrane (Decressac et al., 2004). The same effect was seen in COS-7 cells where HcRedK2P1 and HcRedK2P1K274E reach the cell surface (not shown) and produce macroscopic currents (Figure 1A). An HcRedK2P1K274E fusion protein produced 2.9 times more current than HcRedK2P1 (Figures 1B and 1C). The current displayed a very fast inactivation component that was previously observed for K2P1 expression in oocytes (Lesage et al., 1996a) and thoroughly characterized for the closely related channel TWIK2 (Patel et al., 2000). The kinetics of K2P1 inactivation was extremely fast and overlapped the membrane capacitive discharge associated with the voltage pulse. However, the fast inactivating peak current was clearly not a stimulation artifact and constitutes a hallmark of the TWIK currents.

We tested another mutant of K2P1, HcRedK2P1K274R, in which lysine 274 is replaced by an arginine residue. This mutation is more conservative than K274E because the positive charge at position 274 is conserved and not substituted by a negative charge. Surprisingly, HcRedK2P1K274R produced almost the same level of current as HcRedK2P1 (Figures 1A–1C). The mutation K274R, unlike K274E, was not associated with an increase of the HcRedK2P1 current. Even though both substitutions are expected to equally prevent the sumoylation of K2P1, these results demonstrate that altering lysine 274 by glutamate or arginine does not have the same effect. Figure 1D shows that K2P1, K2P1K274E, and K2P1K274R transiently expressed in COS-7 cells ran at the same apparent molecular weight (MW) when analyzed by western blot. The 20 kDa shift expected for a protein being covalently bound to a SUMO moiety is not observed for K2P1 (SUMO is only 11 kDa, but it migrates aberrantly at around 20 kDa, even in free form). The apparent MW of the upper band (37–38 kDa) corresponds to the calculated MW of K2P1 and fits the MW of K2P1 previously