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One Sumo is Sufficient to Silence the Dimeric Background Potassium Channel K2P1

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SUMO, a 101 residue peptide well-known to regulate nucleocytoplasmic trafficking and function of transcription factors, was recently shown to reversibly regulate the activity of K2P1 channels in cell surface membranes (Rajan et al, Cell **121**; 2005). Thus, K2P1 channels are silenced by interaction of SUMO with lysine at position 274 (K274) and activated by SUMO-specific protease (SENP1). As such, channels with K274 altered to glutamine (K274Q) are constitutively active and insensitive to SUMO and SENP1. Here we report that, like wild-type K2P1 channels (WT), channels formed by two subunits linked in tandem (WT-WT) are silent at baseline and activated by exposure to SENP1 when studied in CHO cells by patch-clamp recording. Suggesting that channel silencing requires only one SUMO, channels bearing one wild-type subunit (WT-K274Q and K274Q-WT) behave like WT. To test this hypothesis, GFP-labeled subunits were studied using total internal reflection microscopy and stepwise decreases in fluorescence due to single-particle photobleaching (SPPB) to count the number of fluorophores per channel. Validating the method, two bleaching steps are recorded with GFP fused to WT or K274Q subunits because K2P channels are dimeric (Lopes et al., 2001. JBC **276**:24449-52; Kollewe et al., 2009. JGP **34:**53-68) and four steps seen with GFP on Kv2.1 subunits that form tetrameric channels. Next, GFP-SUMO was observed in discrete plasma membrane particles when expressed with WT but not K274Q subunits. Finally, SPPB was used to identify two GFP-SUMO with each WT or WT-WT channel but only one with WT-K274Q or K274Q-WT channels. The data show K2P1 channels to assemble with two SUMO subunits but a single SUMO to be sufficient for silencing.