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Sulfhydryl Oxidation Regulates Cloned Mechanosensitive Two-Pore K^+ Channel Expressed in Mammalian Cell Lines

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Oxidative stress has been considered as a major cause of inducing cell damage, but it is recently recognized that mild oxidative stress or receptor-mediated production of ROS contributes to the regulation of various cellular functions. Several ion channels, such as L-type Ca^{2+} channels and Ca^{2+}-activated K^+ channels, have been shown to be regulated by oxidation of thiol group in their structure, and are suggested to be involved in ROS-sensitive cellular signaling. In the present study, we have investigated the effect of oxidizing agents on two-pore and four transmembrane possessing K^+ channels.

TREKs (TWIK-RElated K^+ channel) and TRAAK (TWIK-Related Arachidonic acid Activated K^+ channel) were expressed in CHO cells, and the channel activities were recorded from inside-out membrane patches voltage clamped at -40 mV in symmetrical 140 mM K^+ solutions. Intracellular application of oxidizing agent, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, 2 mM), markedly decreased the activity of TREK-2 channels (90 ± 9.7% (n=30)) and this inhibiting action persisted even after washout. These effects were reversed by the reducing agent dithiothreitol (5 mM). The channel activity of TREK-2 was also decreased by hydrogen peroxide (0.1%), 2,2'-dithio-bis(5-nitropyridine) (DTBNP, 50 μM) and oxidized glutathione by 50 ± 10.2% (n=3), 68 ± 16.5% (n=4), and 82 ± 15.2% (n=3), respectively. On the contrary, all oxidizing agents did not affect the activities of TRAAK or TREK-1. Since TREK-2 is distinguished from the others by characteristic long C-terminus, we examined the possibility that target sites for sulfhydryl oxidant are located in the C-terminus. We made point mutations at two cysteine residues (C494G and C507G) and two chimeras by the replacement of portions of the C-terminus of TREK-2 with that of TASK-3 (TREK-2(1-383)/TASK-3C and TREK-2(1-353)/TASK-3C). The sensitivity to oxidation was not changed in mutated TREK-2 channels and TREK-2(1-383)/TASK-3C chimera (n=7), but abolished in TREK-2(1-353)/TASK-3C chimera (n=11).

These results indicate that TREK-2 channels are modulated by sulfhydryl oxidation, and that the target site may possibly be located between 353-383 amino acid residues in C-terminus.