Mechanosensitivity of GIRK Channels Depends on Channel-PIP_2 Interactions

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[**Aim**] G protein-activated inwardly rectifying K^+ channel (GIRK) currents are inhibited by mechanical stretch of the cell membrane. The mechanism(s) involved are not understood. We have investigated the role of phosphatidylinositol 4,5-bisphosphate (PIP_2) on the mechanosensitivity of GIRK channels. [**Methods**] Whole-cell currents were recorded using the two-electrode voltage clamp technique from *Xenopus* oocytes heterologously expressing either mechano-sensitive GIRK channels or mechano-insensitive IRK1 channels. [**Results**] Membrane stretch, induced by cell swelling through a 50% reduction of osmotic pressure, caused a prompt reduction of GIRK4 currents by 42.6%, the reduction in current was reversible upon return to isosmotic conditions. In contrast, IRK1 current was unaffected by membrane stretch. The mechanosensitivity of GIRK4 current was abolished by the insertion of the high affinity PIP2 binding domain of IRK1, (K207-L245), into GIRK4. Further characterization of this domain showed that the mechanosensitivity of GIRK4 was also abolished by a single point mutation (I229L). Overexpression of $G_{\beta\gamma}$, which is known to strengthen the channel-PIP_2 interaction, attenuated the mechanosensitivity of GIRK4 channel. [**Conclusion**] These results suggest that the mechanosensitivity of GIRK channels is mediated primarily by a modulation of channel-PIP_2 interaction.

Reduction of the Two Cysteine Residues in Paired Domain by Ref-1 is Essential for the Transcriptional Activity of Pax-8

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[Aim] Pax-8 is a transcription factor regulating the expression of thyroid specific genes such as thyroglobulin (TG). It binds to DNA through paired domain. We previously demonstrated that the DNA-binding activity of Pax-8 is redox regulated: oxidation abolishes DNA-binding while reduction restores it. Furthermore, in thyroid cell thyrotropin was shown to increase the level of reduced Pax-8 and redox factor-1 (Ref-1), a reducing enzyme. These increases were accompanied by stimulation of TG transcription. We thus studied which residues in Pax-8 are sensitive to the redox regulation and whether Ref-1 stimulates transcription activity of Pax-8 through reduction of such residues. **[Methods]** Gel shift assay (GSA), using translated wild type Pax-8 and its mutants with substitution of cysteine with serine, was performed to identify redox-sensitive cysteines for DNA-binding *in vitro*. A luciferase reporter gene driven by TG promoter was used to assess the effect of Ref-1-mediated redox regulation of Pax-8 on its transcriptional activity *in vivo*. **[Results]** GSA showed that oxidation by diamide of either Cys-45 or Cys-57 in paired domain abolished Pax-8 DNA-binding and subsequent reduction by DTT or Ref-1 restored it. The reporter gene assay revealed that overexpression of Ref-1 enhanced transcriptional activities of wild type Pax-8 as well as the mutants with a single substitution of either Cys-45 or Cys-57, while it had no effect on that of the mutant with their double substitutions. **[Conclusion]** Reduction of both Cys-45 and Cys-57 in the paired domain by Ref-1 is critical for the transcriptional activity of Pax-8.