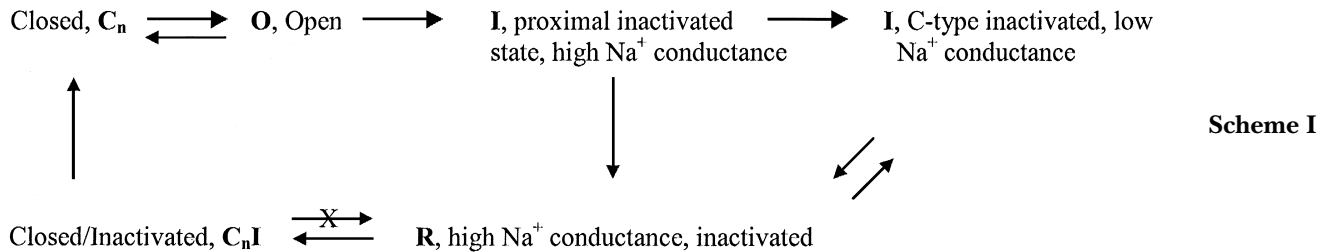


Our present understanding of the pathway by which Kv1.5 and a number of other channels, including Kv2.1, inactivate and recover from inactivation has been established by previous work from our laboratory (Wang, Z.R., J.C. Hesketh, and D. Fedida. 2000. *Biophys. J.* 79:2416–2433; Wang, Z., and D. Fedida. 2001. *Biophys. J.* 81:2614–2627) and others (Kiss, L., J. LoTurco, and S.J. Korn. 1999. *Biophys. J.* 76:253–263), and is shown diagrammatically below. Only the open state (O) is potassium conducting. Inactivated channels generally recover through the higher Na<sup>+</sup> conductance state (R), to close in the inactivated form, and return back to resting closed states via a slow vertical transition.



The proximal inactivated state is not detected during the onset of inactivation as it is traversed too rapidly, but its presence can be revealed by adding 1–2 mM concentrations of internal K<sup>+</sup> (Kiss, L., J. LoTurco, and S.J. Korn. 1999. *Biophys. J.* 76:253–263; Wang, Z.R., J.C. Hesketh, and D. Fedida. 2000. *Biophys. J.* 79:2416–2433). Consequently, the two inactivated states (perhaps P- and C-type) are conflated and are considered as such in our experimental analysis here and in the modeling (see Fig. 8); however, it should be noted that this does not materially affect the conclusions. If acid pH stabilizes channels in closed-inactivated states, depolarization should activate these channels if the forward transition (labelled X) is allowed, and give outward slowly relaxing currents that reflect the high Na<sup>+</sup> conduction of the R state (Wang, Z.R., J.C. Hesketh, and D. Fedida. 2000. *Biophys. J.* 79:2416–2433). However, no substantial outward currents are seen (see Figs. 2 and 5) at pH 6.4. We also considered the possibility that channels could bypass the Na<sup>+</sup>-permeable R state during “activation” from closed-inactivated states (Scheme II). This formulation of the model fails in numerous ways to accurately reproduce experimental data, but is especially problematic in the reproduction of slowly decaying test pulse currents after brief repolarizations (see Fig. 7). Specifically, the model cannot be formulated such that channels bypass R during activation from CI states, but subsequently activate to R after brief repolarizations. Based on this outcome, we concluded that the channel states that give rise to the Na<sup>+</sup> tail and the supernormal Na<sup>+</sup> current during the second pulse can only be accessed along the activation pathway (top row), and not from closed-inactivated states.

