Supplemental Materials: Voltage-dependent Gating Rearrangements in the Intracellular T1–T1 Interface of a K^+ Channel

Guangyu Wang and Manuel Covarrubias

A computer-controlled rapid solution switching system (LSS-3100, EXFO-Burleigh Instruments) was employed to produce MTSET concentration jumps as described previously (Shahidullah et al., 2003; Wang et al., 2005). Control and test solutions were delivered by gravity from a θ capillary tube (diameter, 150 μm, and septum, 8 μm; Hilgenberg GmbH). The resulting solution streams (flow rate ~4 μl/s) are separated by a sharp interface, which is moved by a piezoelectric translator controlling the position of the θ capillary tube. The static patch pipette was adjacent to the solution interface and~100 μm from the opening of the θ capillary tube. The patch pipette position was optimized to obtain fast and reliable solution switching/exchange times; and slight positive pressure was applied to minimize omega-shaped membrane patches. The solution switching time was determined by measuring the speed of the current response evoked by rapidly changing the liquid-junction potential of an open patch electrode filled

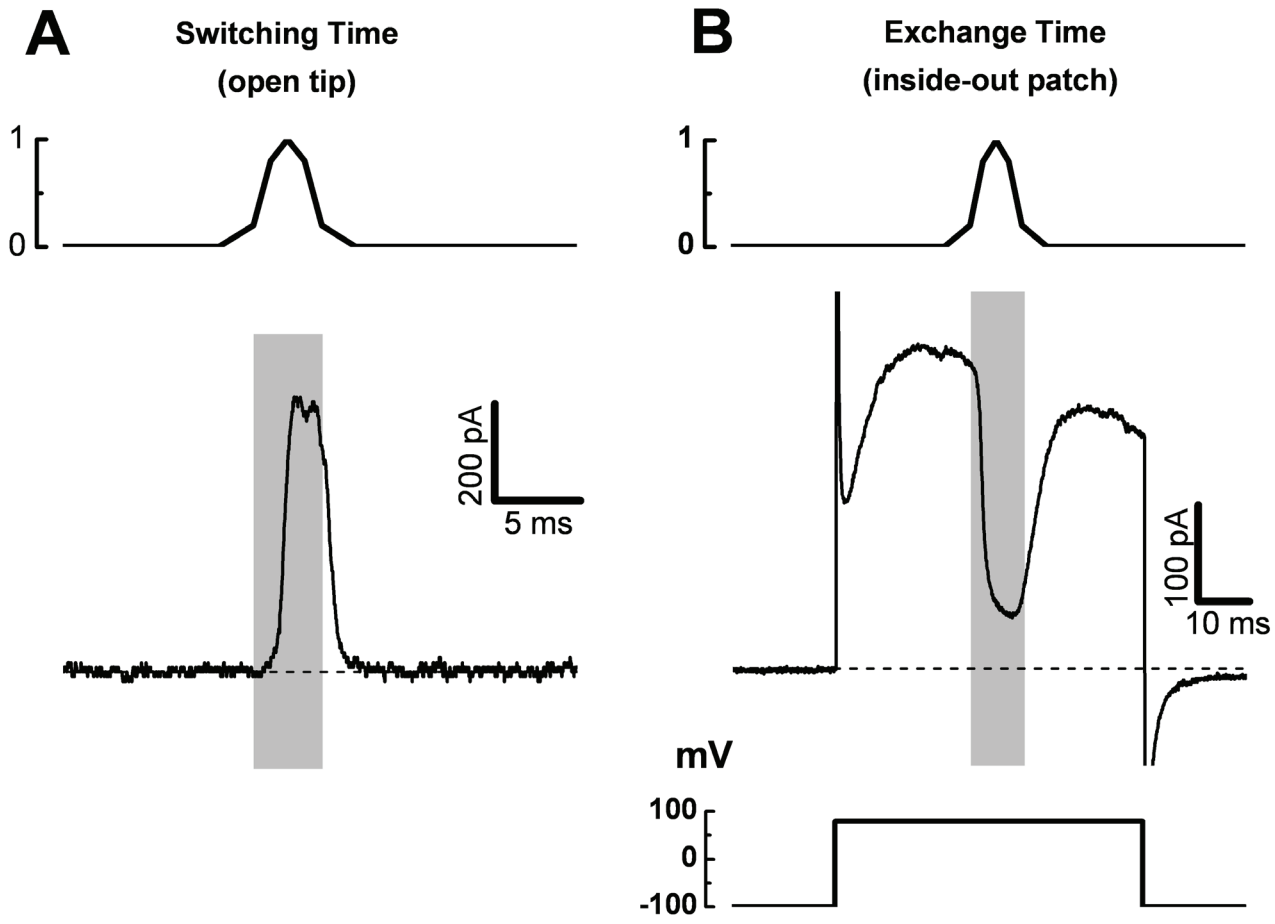

Figure S1. Performance of the rapid solution switching system. (A) Open-tip current response evoked by a KCl concentration jump (in mM): 98→ 9.8→98. The trace above the recorded current represents the normalized piezo displacement profile. This profile minimizes signal ringing (LSS-3100 User's Manual). The actual crossing of the solution interface occurs between 20 and 80% of the total displacement (the steepest segment of the profile). The shaded area under the current trace corresponds to the region of the fastest concentration change. (B) Solution exchange time at the cytoplasmic side of an inside-out patch expressing slowly inactivating rKv2.1 channels. The indicated step depolarization (−100 to +80 mV) evoked the depicted outward current (bottom and center traces, respectively). This current is shown without leak subtraction and capacitive transient cancellation. The piezo displacement profile is as explained above. A KCl concentration jump from 98 mM (control) to 50 mM (test) shifted the K^+ equilibrium potential and induced a rapid suppression of the K^+ current. The suppression is reversible upon return to the control intracellular solution. The shaded area is explained above.

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with 98 mM KCl. The control and test solutions in the θ capillary tube were (in mM) 98 KCl and 9.8 KCl, respectively. The response delay was ≤0.5 ms and the 10–90% current changes yielded switching times on the order of 0.58 ± 0.06 ms (on phase) and 0.84 ± 0.12 ms (off phase) (n = 8; Fig. 5 A). To determine the actual solution exchange time, the cytoplasmic side of inside-out patches expressing slowly inactivating rKv2.1 channels were exposed to K^+ concentration jumps (98→50→98 mM KCl). The 10–90% current changes generated by the shift in reversal potential yielded solution exchange times of the order of 2.8 ± 0.2 ms (on phase) and 6.7 ± 0.3 ms (off phase) (n = 4; Fig. 5 B). The slower exchange times probably resulted from the relatively slow diffusion of solutes caused by the geometry of the inside-out patch, unstirred layers, and cellular debris at the cytoplasmic side of the patch (Jonas, 1995).

REFERENCES

Wang, G., M. Shahidullah, C.A. Rocha, C. Strang, P.J. Pfaffinger, and M. Covarrubias. 2005. Functionally active t1–t1 interfaces revealed by the accessibility of intracellular thiolate groups in kv4 channels. *J. Gen. Physiol.* 126:55–69.

Shahidullah, M., T. Harris, M.W. Germann, and M. Covarrubias. 2003. Molecular features of an alcohol binding site in a neuronal potassium channel. *Biochemistry.* 42:11243–11252.

Jonas, P. 1995. Single Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 231–243.