# Gating currents from a delayed rectifier $K^+$ channel with altered pore structure and function

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## INTRODUCTION

For voltage-sensitive ion channels, charged elements within the membrane electric field are thought to generate displacement or gating currents (1). Topographical models (2–4) have assigned the charged elements or voltage sensor to a fourth transmembrane α-helix (S4) and the models have received support from mutagenesis experiments (5–6). The pore has been assigned to a different region, and in $K^+$ channels, this appears to be the highly-conserved S5-S6 linker (7–9). To test the hypothesis that the pore and the voltage sensor are structurally distinct, we compared ion conduction and gating currents arising from two delayed rectifier $K^+$ channels, DRK1 (10) and a chimera DRK/NGK L374V (11, 12), whose sequences even though identical in S4, the putative voltage sensor differed at eight of the 21 residues in the S5-S6 linker (6) as shown in the following alignment.

| | | |
|---|---|---|
| DRK1 | 357: | $S_5$ . . FKSIPASFWWATITMTTVGYGDIYPKTLLGKIVGGLC . . $S_6$ |
| DRK/NGK L374V | 357: | $S_5$ . . ----------VV---------M--Q-WS-ML-----. . $S_6$ |

## RESULTS

### Whole-cell and single channel $K^+$ currents expressed by DRK1 and DRK/NGK L374V cRNAs injected in *Xenopus* oocytes

*Xenopus* oocytes injected with DRK1 and DRK/NGK L374V-specific cRNAs expressed $K^+$-selective currents with delayed rectifier properties. Under voltage clamp, depolarizing test potentials from a holding potential of −70 mV produced outward currents that did not inactivate during the duration of the depolarizing pulse (125 ms) (Fig. 1 *A*). The threshold potential for activation of the two channels was different; it ranged from −40 to −30 mV for DRK1 and −20 to −10 mV for DRK/NGK L374V.

Differences in ion conduction and kinetics were apparent in single-channel recordings from cell-attached patches of oocytes expressing currents from the two species of cRNA. The single channel conductance for $K^+$ was ~8 and 4 pS for DRK1 and DRK/NGK L374V, respectively (Fig. 1, *B, D*). Single-channel kinetics also showed large differences: the long openings typical of DRK1 were converted into short openings in DRK/NGK L374V (Fig. 1 *C*). Furthermore, in DRK1 cell-attached patches with Iso-$Rb^+$ in the pipette, the inward $Rb^+$ conductance and the outward $K^+$ conductance were identical ($gRb^+/gK^+$ was ~1; Fig. 1 *D, left*), whereas DRK/NGK L374V showed a strong inward rectification (Fig. 1, *D, right*), and the $gRb^+/gK^+$ ratio rose to 2.4. In addition, the profile for blockade by external and internal tetraethylammonium ions differed markedly between the two channels (7, 11, 13).

### Gating currents from DRK1 and DRK/NGK L374V

The open oocyte vaseline-gap method for recording gating currents has been reported (14) and a detailed study on gating currents in DRK1 has been submitted for publication. Ionic currents were blocked with $K^+$-free, TEA-containing solutions applied to both sides of the oocyte membrane. ON and OFF gating currents were recorded from both expressed channels at the beginning and at the end of the depolarizing pulses. For each channel, the $Q_{ON}$–$Q_{OFF}$ relationships to membrane potential were similar (Fig. 2 *B*). The midpoint of both *Q–V* relationships were shifted −10 to −20 mV with respect to the steady-state activation (*g–V*) curve (Fig. 2 *B*).

The steepness of the *g–V* curves for DRK1 and DRK/NGK L374V were identical and had limiting effective valences of ~3. The midpoint potentials for activation were ~ −12 mV in DRK1 and ~ +8 mV in


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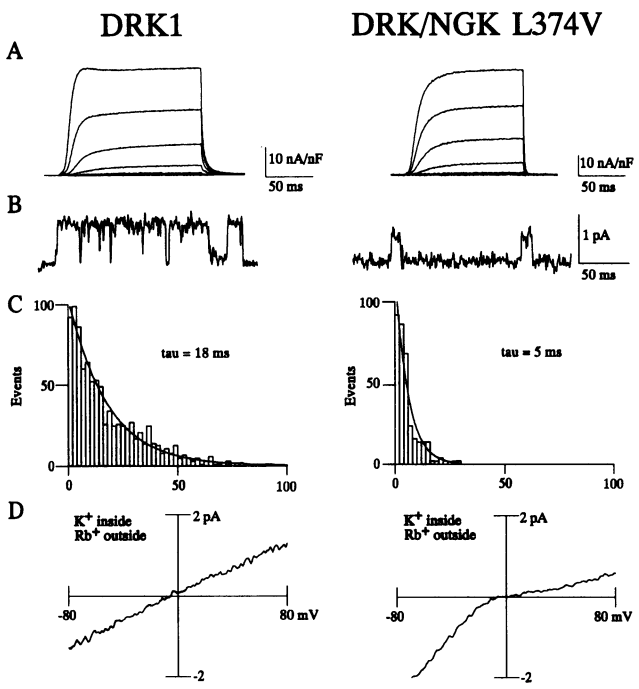


FIGURE 1 Whole oocyte and single channel $K^+$ currents recorded from oocytes injected with DRK1 and DRK/NGK L374V cRNAs. (*A*) Macroscopic currents. Holding potential −70 mV; test pulse potentials −60 to 0 mV (DRK1) and −30 to +30 mV (DRK/NGK L374V) in 10 mV voltage steps. Bath solution: modified Barth's solution; internal solution: $K^+$-MES. (*B*) Single-channel currents in cell-attached oocyte patches. Pipette solution: normal frog Ringer; bath solution: Iso-$K^+$. Test pulse amplitude and duration: +40 mV and 215 ms, respectively. (*C*) Monoexponential distribution of single channel open times. (*D*) Single channel I-V curves under biionic ($K^+$ inside, $Rb^+$ outside) conditions obtained in cell-attached patches using voltage ramps from +80 to −80 mV.

DRK/NGK L374V. This 20-mV shift in the voltage dependence of activation was associated with a comparable shift in the midpoint potentials of the $Q_{ON}$–$V$ and $Q_{OFF}$–$V$ curves. No significant differences were observed in the slopes of the $Q_{ON}$–$V$ and $Q_{OFF}$–$V$ curves and the limiting effective valence for the gating charge movement was ~2.

Other kinetic properties such as absence of charge immobilization in the OFF gating response, the existence of a rising phase in the ON gating current which can be modulated by the prepulse potential (Cole-Moore phenomenon), and the fact that the time constants for the activation and deactivation of the macroscopic currents were identical to those observed in the decays of the ON and OFF gating responses, respectively, are similar in both DRK1 and DRK/NGK L374V (Taglialatela and Stefani, unpublished observations).

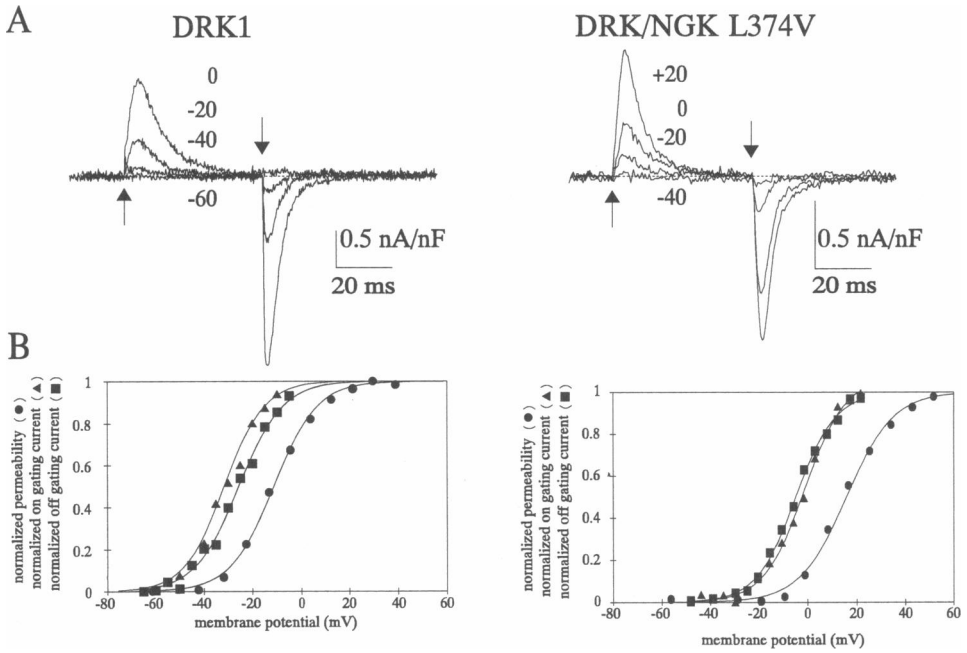


FIGURE 2 Gating currents of DRK1 and DRK/NGK L374. (*A*) Gating currents records. Holding potential was −70 mV. (*B*) Steady-state activation curves of the ionic currents determined as normalized permeabilities using the Goldman-Hodgkin-Katz equation. The Q–V curves were obtained by integrating the first 20–50 ms of the ON (*filled triangles*) and OFF (*filled squares*) gating currents and plotting the normalized integral values vs the respective membrane potentials. The solid lines show the fits of both permeability and gating charge to the Boltzmann equation.

## CONCLUSION

A chimeric DRK1 differing from its parent at eight positions in the putative channel pore had dramatically altered pore properties: single channel $K^+$ conductance was halved, $Rb^+/K^+$ conductance ratio was increased, and profiles for blockade by internal and external TEA were the opposite. If the voltage sensor and the pore are located in different domains of the channel protein, then the changes in ion conduction and blockade might not be associated with changes in voltage sensitivity. This turned out to be the case as judged from the absence of any differences in the steepness of the *g–V* and *Q–V* curves. Nevertheless, the set point for the voltage sensor was clearly shifted by the pore mutations and the voltage-independent open-closed transition (15) in DRK/NGK L374V was enhanced. These differences might arise from structural differences in the coupling between the voltage sensor and pore domains of the channel protein.

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