Recovery from slow inactivation of Shab K⁺ channels

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We have recently examined slow inactivation of Shab channels. Here we extend our characterization of Shab slow inactivation by presenting the properties of recovery from inactivation. The observations support our proposal that Shab reaches the same inactivated state either from open or closed states and suggest that closed and open state inactivation share the same mechanism. Regarding the latter, we also show that external K⁺ and TEA slow down recovery from inactivation in agreement with the hypothesis that the mechanism of Shab inactivation qualitatively differs from C-type inactivation.

Introduction

Recently we reported the first characterization of Shab slow inactivation. Shab inactivates from both open (OI) and closed states (CI) with a mechanism that is facilitated by external K⁺ (Ko⁺) and TEA (TEAo), suggesting that its inactivation qualitatively differs from the C-type inactivation of Shaker, in which these ions exert a characteristic inhibitory effect (for a recent commentary, see ref. 7).

In order to best understand Shab participation in shaping the electrical responses of excitable cells, a knowledge of its kinetics of recovery from inactivation is necessary. Additionally, considering that in Shab both OI and CI are facilitated by K⁺ and TEA⁺, we hypothesized that the same inactivated state is reached from either pathway. Hence, in order to support this hypothesis and to further characterize Shab inactivation, we present additional data on the recovery from inactivation.

Results and Discussion

To investigate whether the recovery pathway includes re-opening from the inactivated state, we compared I_K at pulse end after a short (+20 mV/30 ms) pulse that does not inactivate the channels, and after a long +20 mV/7 sec pulse that inactivates them (Fig. 1, left and right panel respectively), with the cell bathed in 100 mM Ko⁺. Repolarization to −80 mV after the short pulse elicits a deactivation tail so big that falls out of scale. In contrast, upon repolarization after the long depolarization (indicated by the arrow) there is no appreciably tail I_K. This is best seen in the inset that presents I_K at pulse end on an expanded scale. The same phenomenon was observed in all cells tested so far (n > 40). The latter indicates that Shab either does not open from the inactivated state or that it does it so slowly that I_K becomes merged with the noise. Hence, as observed in the Shaker C-type inactivation, and in Kv2.1, but in contrast to the fast C-type inactivation of HERG channels, where the channels open from the inactivated state, recovery from Shab inactivation bypasses the open state.

We further characterized the kinetics of recovery from OI by applying a 7- to 12-second pulse to +20 mV to open and inactivate the channels, followed by a +20 mV/30 ms test pulse applied after spending a variable time at the indicated potentials (illustrated by I_K in Fig. 2D). Figure 2A shows that in the reference Na⁺/K⁺ solutions I_K recovers in a single exponential phase that becomes faster as Vm becomes more negative, as quantified in the inset. At −80 mV recovery proceeds with a time constant (0.08 sec) considerably faster.
than either the weighted OI (1.6 sec) or the CI (0.6 sec) constants.1 Hence, Shab recovers faster than it inactivates.

Of particular note, compared with Na+, (inverted triangles, $\tau_{CI,Na} = 80$ ms) either 15 mM TEA+ (Fig. 2B) or 100 mM K+ (open circles) for comparison the figure also shows recovery from OI, as indicated. It is noteworthy that recovery from CI (0.6 sec) constants.1 Hence, Shab recovers faster than it inactivates.

To achieve this, the first long pulse of the protocol used to study recovery from CI was replaced by 100 short pulses (+20 mV/20 ms) applied at 1.3 × 10−4 Hz. I K inactivated conformation from open and closed states, we studied recovery from CI. To achieve this, the first long pulse of the protocol used to study recovery from CI was replaced by 100 short pulses (+20 mV/20 ms) applied at 1.3 × 10−4 Hz. I K in 100 mM K+ after 500 ms at −80 mV (pointed by the arrow).

The time course of recovery from CI in Na+ is reported in Figure 3A (closed circles) for comparison the figure also shows recovery from OI, as indicated. It is noteworthy that recovery from CI (open circles, $\tau_{CI,Na} = 105$ ms) follows the same time course (p = 0.053) than recovery from OI (inverted triangles, $\tau_{CI,Na} = 90$ ms).

Figure 3B presents recovery in 100 K+ at −80 mV (closed circles, $\tau_{CI,Na} = 147$ ms); for comparison the figure also shows recovery from OI in the same condition (open circles, $\tau_{OI,Na} = 121$ ms). Note that in this ionic condition recovery either from OI or CI also follow the same time course (p = 0.0767). Similarly, Figure 3C shows that with 15 mM TEA+, present recovery from either CI (open circles) or OI (inverted triangles) shows different (p = 0.0208), present a comparable time course. Taken together the above observation show that, similar to the case of Kv2.1 recovery from Shab CI and OI follow the same time course, in all the conditions tested here.

Figure 3D compares recovery from CI in the conditions tested. For clarity only the curves that fit the indicated time courses are plotted. As it is visually apparent, compared with Na+, both K+ and TEA+ significantly slow down (p < 0.05) recovery. In summary, K+ and TEA+ speed the entry to and slow the exit from inactivation.

It is noteworthy that, in contrast to Kv2.1 and Shaker channels in which K+ facilitates recovery from inactivation, in Shab both K+ and TEA+ significantly slow down recovery from both OI and CI in 15 mM TEA+ (Sigma) to the Na+ solution.

**Data analysis.** Points are mean ± SEM of the indicated number of independent experiments. Curves were compared with the extra-sum-of-squares F-test, as reported.1 Significance level was set to 0.05.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Materials and Methods**

**Cell culture and channel expression.** SF9 cells grown at 27°C in Grace’s media (Gibco) were infected with a recombinant baculovirus containing Shab K+-channel cDNA (dShab 11).1,16

**Electrophysiological recordings.** Currents were recorded under whole-cell patch-clamp with an Axopatch 1D amplifier and sampled with a Digidata 1322A interface (Axon Instruments). Electrodes of borosilicate glass (KIMAX 51) were pulled to a 1–1.5 MΩ resistance.1 The internal solution (K+) contained (in mM): 30 KCl, 90 KF, 2 MgCl2, 10 EGTA-K, 10 Hepes-K. The external Na+ solution contained (in mM): 145 NaCl, 10 CaCl2, 10 Hepes-Na, pH 7.2. The 100 K+ solution contained (in mM): 100 KCl, 45 NaCl, 10 CaCl2, 10 Hepes-Na, pH was 7.2. The 15 mM TEA+ solution was prepared by adding 15 mM TEA-Cl (Sigma) to the Na+ solution.

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Figure 3. Recovery from Cl. (A) Recovery in Na/K solutions (closed circles), for comparison the figure also shows recovery from OI (open circles). Recovery was evaluated as: \( y = \frac{I_{\text{test}} - I_{100}}{I_1 - I_{100}} \), where \( I_1 \) is \( I_1 \) in the first pulse, \( I_{100} \) is \( I_1 \) evoked by the 100th inactivating pulse, and \( I_{\text{test}} \) is \( I_1 \) in the test pulse (see Text). The solid line is the fit of the points with the equation: \( y = 1 - \exp(-t/\tau_{rCI}) \), with \( \tau_{rCI,Na} = 119 \) ms. (B) Recovery in 100 K, as indicated. (C) Recovery with 15 mM TEA (see Text). (D) Least squares curves that describe recovery in the indicated conditions. (E) \( I_1 \) illustrating recovery after 500 ms at −80 mV in 100 K.