To investigate the extent to which the changes in inactivation are coupled to changes in channel activation, we examined the kinetics of channel activation and inactivation at +20 mV.

Methods

The time course of inactivation was fitted by a simple fitting procedure in pClamp 6.0 (Axon Instruments, Inc.). From the time point where the rate of current decay by visual inspection was judged to be maximum, the current \( I_{Na}(t) \) was fitted by the expression:

\[
I_{Na}(t) = I_1(\exp(-t/\tau_1)) + I_2(\exp(-t/\tau_2)) + I_3,
\]

where \( \tau_1 \) and \( \tau_2 \) are the inactivation time constants. \( I_1 \) and \( I_2 \) are the corresponding amplitudes, and \( I_3 \) signifies noninactivating current and noise. (The time course of inactivation was best fit by a sum of two exponential distributions; but the fit of the steady state inactivation was not improved when fitted as a three-state process [not depicted]). Following Sarkar, S.N., A. Adhikari, and S.K. Sikdar. 1995. J. Physiol. 488:633–645, the time course of current activation \( I_{Na}(t) \) was determined by transforming the measured currents as:

\[
I_{Na}(t) = I_{Na}(t)/I_1(\exp(-t/\tau_1)) + I_2(\exp(-t/\tau_2)) + I_3,
\]

using the values for \( I_1, \tau_1, I_2, \tau_2, \) and \( I_3 \) obtained from the fit to the inactivation time course. Subsequently, \( I_{Na}(t) \) was fitted with the expression:

\[
I_{Na}(t) = 1 - \exp(-(t - k)/\tau_3)^3,
\]

Figure S1. The effects of TX100, βOГ, and cholesterol concentration on the kinetic parameters of the time course of inactivation at +20 mV. (Top left) Effects of 30 μM TX100 (▼) or 2.5 mM βOГ (▲) on \( \tau_1 \) and \( \tau_2 \). Control cells (●). (Top right) Effects of 30 μM TX100 (▼) or 2.5 mM βOГ (▲) on \( I_1/(I_1 + I_2) \) and \( I_2/(I_1 + I_2) \). Control cells (●). Effects of cholesterol content on \( \tau_1 \) and \( \tau_2 \) (bottom left) and \( I_1/(I_1 + I_2) \) and \( I_2/(I_1 + I_2) \) (bottom right). Cholesterol depletion significantly altered \( \tau_2 \) (P < 0.05). Mean ± SEM, \( n = 5, 6, 6 \) (TX100, βOГ, timed controls); 9, 8, 6 (cholesterol-enriched, cholesterol-depleted, timed controls for cholesterol experiments).
where $k$ is a phenomenological delay and $\tau_a$ is the activation time constant. (Initial analysis showed that an exponent of three gave a better fit than two or four [not depicted].) Neither $k$ nor $\tau_a$ were significantly altered by changing the filter and sample frequency from 10 and 40 kHz ($n = 3$) to 50 and 200 kHz, respectively ($n = 3$) ($P > 0.4$ and $P > 0.4$ [not depicted]).

**RESULTS**

**Effects of βOG, TX100, and Cholesterol on the Kinetics of Inactivation**

In control cells, the time course of inactivation was best described by a double-exponential decay with a major fast component and a minor slow component:

\[ \tau_1 = 0.20 \pm 0.01 \text{ ms}, \frac{I_f}{I_f + I_s} = 0.87 \pm 0.04; \tau_2 = 1.4 \pm 0.16 \text{ ms}, \frac{I_s}{I_f + I_s} = 0.13 \pm 0.04 \text{ ms}. \]

The top panels in Fig. S1 show the effects of 30 μM TX100 or 2.5 mM βOG on $\tau_1$, $\tau_2$, $I_f/(I_f + I_s)$ and $I_s/(I_f + I_s)$.

Neither TX100 nor βOG altered these parameters. The bottom panels in Fig. S1 show the effects of cholesterol on the inactivation kinetics; increasing the cell cholesterol content altered neither $\tau_1$ nor $\tau_2$ nor their relative contributions to the current. Decreasing the cholesterol content increased $\tau_1$ from $0.17 \pm 0.01 \text{ ms}$ to $0.26 \pm 0.01 \text{ ms}$ with no change in the relative contribution of the fast component.

**Effects of βOG, TX100, and Cholesterol on the Kinetics of Activation**

None of the experimental maneuvers altered the activation time constant ($\tau_a$) significantly, but the time course of activation is so fast that small changes in $\tau_a$ may have been masked by the time constant of the voltage clamp. Fig. S2 A shows the (lack of) effects of 30 μM TX100 and 2.5 mM βOG on $\tau_a$; Fig. S2 B shows the corresponding (lack of) effects of changes in cholesterol content.

Whereas neither the application of the micelle-compounds or cholesterol enrichment altered the phenomenological delay ($k$) relative to its value in timed control experiments, cholesterol-depletion decreased $k$ from $0.17 \pm 0.02 \text{ ms}$ in the timed control experiments to $0.10 \pm 0.01 \text{ ms}$ ($P < 0.05$). We do not understand why.

In conclusion, none of the experimental maneuvers, except for the effect of cholesterol-depletion on $\tau_1$, have major effects on the kinetics of channel activation and inactivation at $+20 \text{ mV}$. That said, we are not able to discern modest changes (decreases) in $\tau_a$, which would tend to be obscured by the time course of the membrane charging. (The cholesterol-enrichment-induced changes in $V_{act}$ certainly indicates that channel activation is effected by the maneuver.)