*Online Supplemental Material for*

Lundbaek et al. **http://www.jgp.org/cgi/doi/10.1085/jgp.200308996**

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:

$$I'_{Na}(t) = 1 - \exp(-(t-k)/\tau_a)^3,$$

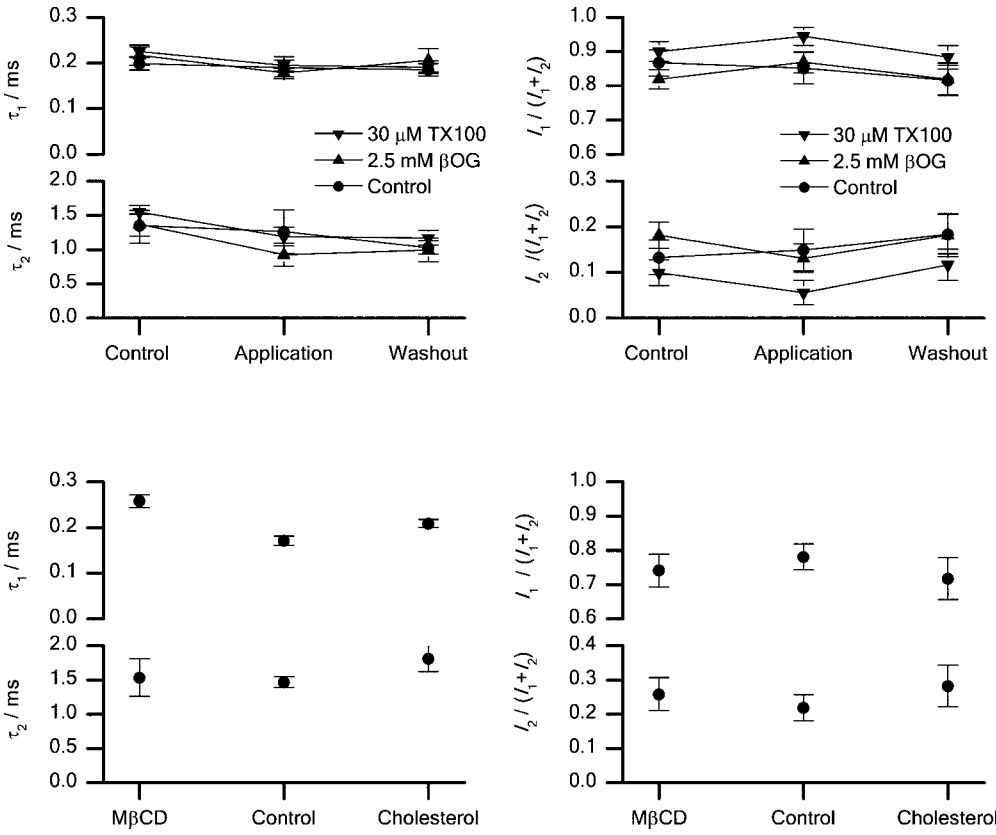


FIGURE S1. The effects of TX100, βOG, and cholesterol concentration on the kinetic parameters of the time course of inactivation at +20 mV. (Top left) Effects of 30 μM TX100 (▼) or 2.5 mM βOG (▲) on $\tau_1$ and $\tau_2$. Control cells (●). (Top right) Effects of 30 μM TX100 (▼) or 2.5 mM βOG (▲) on $I_1/(I_1 + I_2)$ 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, βOG, 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: $\tau_1 = 0.20 \pm 0.01$ ms, $I_1/(I_1 + I_2) = 0.87 \pm 0.04$; $\tau_2 = 1.4 \pm 0.16$ ms, $I_2/(I_1 + I_2) = 0.13 \pm 0.04$ ms. The top panels in Fig. S1 show the effects of 30 μM TX100 or 2.5 mM βOG on $\tau_1$, $\tau_2$, $I_1/(I_1 + I_2)$ and $I_2/(I_1 + I_2)$.

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 $0.17 \pm 0.01$ ms to $0.26 \pm 0.01$ ms with no change in the relative contribution of the fast component.

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

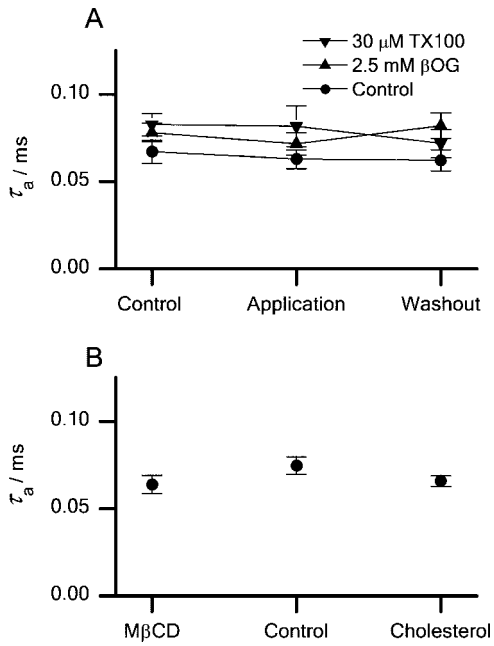


FIGURE S2. (A) Effects of 30 μM TX100 (▼) or 2.5 mM βOG (▲) on the time constant of activation, $\tau_a$. Control cells (●). (B) Effects of cholesterol content on $\tau_a$. Every 5 s the cells were depolarized to +20 mV from a holding potential of −80 mV. Neither the addition of micelle nor manipulation of cholesterol content changed $\tau_a$ relative to timed control experiments ($P > 0.05$). Separate control groups for experiments with micelle-forming compounds and experiments with cholesterol were done at similar times from membrane rupture as the experimental group. Experimental conditions as in Fig. S1.

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 $0.17 \pm 0.02$ ms in the timed control experiments to $0.10 \pm 0.01$ 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 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.)