Reply to Dr. Graham D. Lamb's comments on "Effect of Bay K 8644 on Ca^{2+} Channel Gating Charge"

Dear Sir:

We would like to respond to the comments made by Dr. Lamb in his Letter to the Editor concerning our Brief Communication, "Fast Activation of Cardiac Ca^{++} Channel Gating Charge by the Dihydropyridine Agonist, Bay K 8644" (1990. *Biophys. J.* 58: 1307–1311). In our experiments, we have used embryonic chick ventricular myocytes because the surface membrane of these small diameter cells (15 μm diameter, ~700 μm^2, not 200 μm^2 as printed) can be rapidly voltage clamped (100–200 μs decay of the linear capacity current). Furthermore, the results and their interpretation are not complicated by the presence of an internal membrane system (as in adult cardiac myocytes and skeletal muscle fibers) (Josephson and Sperelakis, 1991*b*). In his skeletal muscle experiments (Lamb and Walsh, 1987), the question of kinetic shifts of the charge movement in the presence of Bay K 8644 could not be clearly examined because of technical limitations inherent in the preparation and the methods employed. Therefore, it is difficult to make a useful comparison between the two studies.

The following comments address the specific points raised by Dr. Lamb.

(*a*) It was suggested that the kinetic shift of the charge movement recorded with Bay K 8644 might be a result of a mismatch in the time course of the linear capacitance currents produced by the control and test steps (see Almers, 1978). In some experiments of this study, including the one displayed in Fig. 1, only 30–150 μs (1–5 digitized points) were imperfectly subtracted at the onset and termination of the test voltage step. After the digital filtering used for the traces shown in the figures, these few digitized points result in brief gaps in the current record. This brief artifact, when present, does not contribute to the time course of the gating current at times greater than 200 μs, and it cannot account for the kinetic shift recorded in the presence of Bay K 8644.

There was no change in the time course or magnitude of the linear capacity current after the addition of Bay K 8644 in our experiments. Moreover, if as Dr. Lamb speculates, a mismatch produced a larger inward capacity current after exposure to Bay K 8644 (during the hyperpolarizing control steps), then an apparent slowing of early charge movement would be predicted. This prediction is at variance with the observed acceleration of the charge movement. Furthermore, the argument proposed cannot account for the more rapid decay phase of the gating currents after exposure to Bay K 8644, because the gating current decays 1–5 ms after the onset of the voltage step and, therefore, after >98% decay of the linear capacity current.

As stated in the Results section of our paper, the Ca^{++} currents in the experiment shown in Fig. 1 were intentionally only partially blocked in order to compare the effects of Bay K 8644 on the gating and ionic currents. As stated in Methods, the gating currents (e.g., Fig. 2) were recorded after complete suppression of all ionic currents. Therefore, the faster decay phase of the ON gating currents in the presence of Bay K 8644 is not caused by an overlapping residual inward ionic current, but by an acceleration of the activation of the ON charge.

(*b*) As we stated in Results and Discussion of our paper, Bay K 8644 did not significantly alter the total (or steady-state) charge moved at each potential. This agent did accelerate the kinetics of the ON charge movement (as measured by the isochronal Q_{ON} curves). The kinetic shift in the gating current correlates with, and may account for (at least in part), the shift in the I-V curve for the peak I_{Ca}. We did not report, as stated by Dr. Lamb, "that at each potential, both in the absence and presence of Bay K 8644, all the gating charges had moved before the peak of the Ca current". The gating charge movement is still clearly decaying after the peak of I_{Ca}. After Bay K 8644, a greater fraction of the total Ca channel population gate earlier in time (i.e., with shorter first latencies after a depolarization) and with a greater open-state probability (P_0). The negative shifts in the isochronal charge vs. V_m curve and in the P_0 vs. V_m curve would produce a negative shift in the I-V relationship for I_{Ca}. This effect will be more pronounced at more negative potentials (i.e., in the negative slope region of the curve), because the effects of Bay K 8644 to accelerate the charge movement and to prolong the open time of the Ca channel are proportionately greater at more negative potentials than at positive potentials.

The total number of Ca channels that contribute to the nonlinear charge movement did not appear to be increased by Bay K 8644. This does not necessarily imply, as Dr. Lamb states, "that the number of channels opened at each potential should be unaffected by the drug . . .," because additional conformational steps may be necessary to couple the voltage-dependent charge movement to Ca^{++} channel opening and Ca^{++} ion permeation. That is, charge movement of the Ca^{++} channel may not always lead to ion conduction.

Finally, isoproterenol produced a kinetic shift in the gating currents similar to that produced by Bay K 8644 (Josephson and Sperelakis, 1991*a*). This effect of ISO is probably mediated indirectly by elevation of cAMP and phosphorylation of the Ca channel. Therefore, these results support the general hypothesis that Ca channel modulators produce their effects, at least in part, through alterations in the Ca channel charge movement.

We thank Dr. Lamb for giving us the opportunity to clarify these points.

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