Effect of Bay K8644 on Ca^{2+} channel gating charge

Dear Sir:

It is clearly important to understand the molecular mechanisms by which agonist and antagonists modify the opening of voltage-dependent calcium channels. Following the identification and characterization of the gating charge movement associated with L-type calcium channels in cardiac muscle (Field et al., 1988; Bean and Rios, 1989), it has been possible to examine how or whether particular agents, such as 1,4-dihydropyridines (DHP), affect the voltage sensors controlling the calcium channels. Field et al. (1988) found that the DHP, nifedipine, did affect the voltage sensors of the calcium channels in cardiac muscle, as it appeared to slow the return of the charge movement (OFF charge) upon repolarization. Recently, Josephson and Speralakis (1990) have claimed that 1 μM Bay K8644 accelerated the charge movement in cardiac cells and that this accounted for both the faster kinetics *and* the negative shift in the potential dependence of the calcium current. However, it seems appropriate to point to some possible problems with the data and interpretation of Josephson and Sperelakis:

(*a*) The current records in Fig. 1 *A* and *B* show that the first 0.2–0.3 ms of current has been deleted at the start (and end) of each pulse. The sections removed at the start of each pulse almost certainly showed an inward current, a remnant of the linear capacitive current which resulted from a mismatch in the magnitude or timecourse of the currents produced by the control and test steps. If the linear capacitive currents did differ slightly in their timecourse, there *must* also be a subsequent artefactual *outward* current carrying the same amount of charge as the deleted inward current, and this outward current would contaminate the true gating current, particularly early in the step. Moreover, the facts that the deleted section is larger and that the current following the deleted section rises more steeply in Fig. 1 *B* than in Fig. 1 *A*, suggests that the deleted inward capacitive current was larger in the presence of Bay K8644, and this would result in any corresponding outward current artefact making the gating charge appear to rise faster in the presence of Bay K8644. Given that the gating charge (maximum 1 nC/μF) can only be identified after very accurately subtracting the *100-fold larger* linear capacitive charge, it is clear that even very small changes in the size or timecourse of the linear capacitive currents could have profound effects on the apparent gating current. The "faster" decline of the apparent gating charge in the presence of Bay K8644 in Figs. 1 and 2, does not necessarily support the proposed acceleration of the gating charge, as it obviously includes a remnant of the inward calcium current, which is known to be activated faster in the presence of Bay K8644, and which thus will cause the net current to decline more rapidly. To make it clear that the artefact referred to above is not large enough to substantially influence their results, the authors should show the entire records of Fig. 1, *A* and *B*, without deleting any part.

(*b*) If Bay K8644 does accelerate the gating charge, this would clearly explain the parallel acceleration in the activation of the calcium current. However, it would not explain the shift in the *voltage dependence* of the calcium current, as the authors claim in the Abstract and Discussion. This confusion seems to come about from the authors' use of "isochronal" charge (e.g., Fig. 3 *B*) to further quantify the *acceleration* of the gating charge. The isochronal charge is simply the integral of the charge at each potential up to some arbitrary time. By choosing a time in which most of the charge has moved in one circumstance and not in the other, such analysis can highlight the potential range over which the gating charge is much slower in one case than the other (i.e., Fig. 3 *B*). However, such analysis gives no information about the *total* charge moved at each potential. The authors state elsewhere that the total charge moved at each potential was in fact *no different* in the presence or absence of Bay K8644, as found previously in skeletal muscle (Lamb and Walsh 1987; and unpublished data). (The authors also report that at each potential, both in the absence and presence of Bay K8644, all the gating charge had moved before the peak of the calcium current, as required if the charge is involved in opening the channels.) Thus, this means that the number of channels opened at each potential should be unaffected by the drug, irrespective of whether the channels opened more quickly. In other words, the reported acceleration of the charge movement does not explain the shift in the voltage dependence of the calcium current.

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