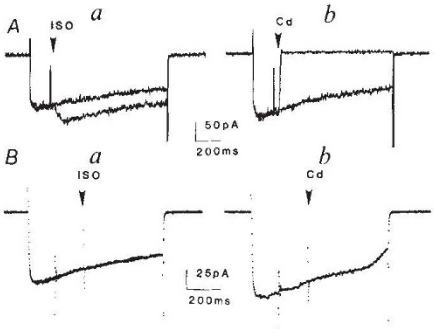

Rapid increase of Ca^{2+} channel currents produced by concentration jumps of isoproterenol (Iso, 100 nM) (*Aa*, arrow) and rapid blockade produced by Cd^{2+} (1.0 mM) (*Ab*, arrow). Recording and solution conditions as described in ref. 1. Switching transients are produced by the solenoid valve of the concentration clamp. When the concentration change was slow, the rapid response was absent (*Ba*) and the Cd^{2+} blockade occurred after a long delay (*Bb*).

(compare their Fig. 1*b* with Fig. 1C of ref. 7). They do not seem to have used the rapidity of Cd^{+} block as a control and their Fig. 2*e* shows that the 'jog' of Iso actually decreases Ca^{2+} channel current. Furthermore, their assumptions regarding Ca^{2+} channel-current rundown in the presence of Rp-cAMP-S are probably invalid; Ca^{2+} currents should fall below, not return to, control levels. The fast response has also been demonstrated in adult guinea pig cardiac myocytes using a different experimental approach[8].

With regard to mechanism, we favoured the direct G protein effect but did not exclude a rapid cytoplasmic pathway[7]. The reasons for our choice were that neither Forskolin, IBMX (1) nor 8Br-cAMP produced the rapid response. Subsequently we have added Wiptide, a peptide inhibitor of PKA, to our patch pipettes and greatly reduced the slow response without reducing the rapid response (n=7).

Hartzell *et al.* claim that the conditions in intact cells under which direct G protein effects on Ca^{2+} channel currents have been demonstrated[2] were non-physiological. However, the same direct effects have also been observed under solution conditions virtually identical to their own[6]. Second, Hartzell *et al.* make the novel proposal that Ca^{2+} channels might be under direct G-protein control in the absence of agonist. They appear unaware of the fact that this proposal, unmodified, overthrows the fundamental concept of G proteins as signal transducers. We recently proposed[9] a modification that might obviate their dilemma.

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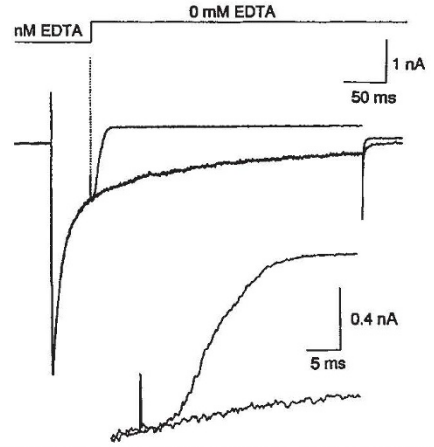
HARTZELL *ET AL.* REPLY — The purpose of our paper[1] was to test the hypothesis[7] that a direct G-protein pathway is necessary to explain the effects of sympathetic nerve stimulation on heart. We do not conclude that direct G-protein mechanisms do not exist. Rather, we document that under physiological conditions a fast pathway, presumed to be due to a direct effect of G proteins, is absent on cardiac Ca channels. This, together with the observation that the inotropic response to sympathetic stimulation can be explained entirely by cAMP-dependent phosphorylation, implies that the direct pathway is neither a proven physiological mechanism nor needed to explain the physiology. A direct pathway may exist, but it is of little import for the cardiac response to sympathetic nerves. This position is supported by the very small size (<50 pA) of the fast response reported by others[7].

We initially examined whether the fast response was a general phenomenon. We developed a new perfusion system[1] to test the question in frog, rat and guinea-pig hearts. We switch between two flowing streams, whereas Yatani and Brown switch from a static bath to a rapidly moving one. The sudden flow past the cell could possibly introduce uncontrolled variables. Quantitative data for the Wiptide experiments are not provided, and in any case would not exclude a flow effect. Our perfusion system appears sufficiently rapid. The cell responds to [K] changes in <30 ms, the time courses of our acetylcholine response[1] and their carbamylcholine response[7] are the same, and the effects of (−)BAY K 8644 occur within 150–300 ms. Moreover, the figure shows that the Na current through Ca channels in the presence of EDTA is blocked within 30 ms of washing out the EDTA.

Yatani and Brown's figure *Bb* is perplexing. It is unclear why Cd blocked so slowly. Furthermore, the data in *Aa* seem different from their previous results[7]. The fast response here develops with a $\tau \approx 40$ ms, compared to 150 ± 35 ms in their previous report. Moreover, Iso does not affect I_{Ba} inactivation, whereas they previously found that it slowed inactivation.

The suggestion that we missed a fast response because our cells are leaky is fallacious. We show non-leak-subtracted traces. The 10–50-pA outward current at 0 mV is not non-specific leak; it is residual K current not blocked by Cs. Holding current was $<\pm 5$pA. In any event, a role for leak is difficult to rationalize: direct G-protein effects are observed in artificial lipid bilayers[10].

Our suggestion that Ca channels may be affected by G proteins in the absence of β-adrenergic agonists does not question the role of G proteins in signal transduction, it merely points out that G proteins may perform other functions.



Block of I_{Ca} carried by Na ions in frog cardiac myocyte by rapid perfusion switch from a Ringer solution containing 5 mM EDTA, 0 Ca to the same solution lacking EDTA with 3.6 mM Mg. Inset, region of the switch enlarged.

The unpublished data of Pelzer *et al.* that basal I_{Ca} is partly inhibited by R_p-cAMP-S suggest that the Ca channel is partially phosphorylated in the absence of β-agonists. If the cAMP system is activated under basal conditions, it is likely that the direct pathway would also be activated, because the direct pathway was said to be more sensitive to β-agonists[7]. If this is true, sympathetic stimulation would be unlikely to stimulate further the direct pathway and it would not participate in the physiological response to sympathetic stimulation. Furthermore, the physiological significance of the fast pathway would be minimal in the presence of basal sympathetic tone.

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