LETTERS TO THE EDITOR

Kinetics of Calcium Inward Current Activation

Dear Sir:

Recently Lee et al. (1978) and Akaike et al. (1978) have published in this Journal two very interesting papers, reporting elegant experiments with the internally perfused nerve cell bodies. We are especially happy with the success of these authors: the idea of how simple it is to substitute the cytoplasm of the cell body by artificial saline has once more appeared to be fruitful since our first reports on the approach (Kostyuk et al., 1975; Krishtal and Pidoplichko, 1975) and on the separation of Na and Ca currents (Kostyuk et al., 1977).

Akaike et al. (1978) have presented a description of the activation of Ca inward current using first order kinetics (the Hodgkin-Huxley variable m raised to the first power). The authors note that the time resolution of their modification of the cell body perfusion method could be insufficient. As the question of how Ca channels function seems now to be essential (because of the powerful new methods of their investigation), we want to present some evidence that the kinetic scheme of Ca current activation is m raised to the second power, as suggested in our earlier paper (Kostyuk et al., 1977).

In this study we used a further modification of the method of intracellular dialysis (Krishtal, 1978) which achieves quite short resolution times near 70–150 μs. In such experiments the isolated cell is located between the two pores. One of them is used for the measurement of potential, and the feedback current is injected through the other. This technique eliminates about 95% of the series resistance and simultaneously makes the substitution of the internal medium very rapid and effective.

We have performed precise measurements of Ca inward currents and corresponding gating currents in neurones enzymatically isolated from the ganglia of the snail *Helix pomatia*. Temperatures from 2 to 22°C were used. Gating and ionic currents were digitally separated as shown in Fig. 1 A to obtain reliable information on the initial period of the Ca current development. A definite delay in the development of the Ca current is seen after the subtraction of the gating current. The residual net ionic current is perfectly approximated by m^2 model as shown in Fig. 1 B. It is very important that the time constants τ_m for ionic current and τ_{on} for corresponding gating current are practically the same.

A good fit of the Ca current to m^2 kinetics is well supported by the voltage dependence of the gating-charge distribution curve which coincides with the m_∞ voltage dependence (Fig. 2). Such correspondence seems to be peculiar since

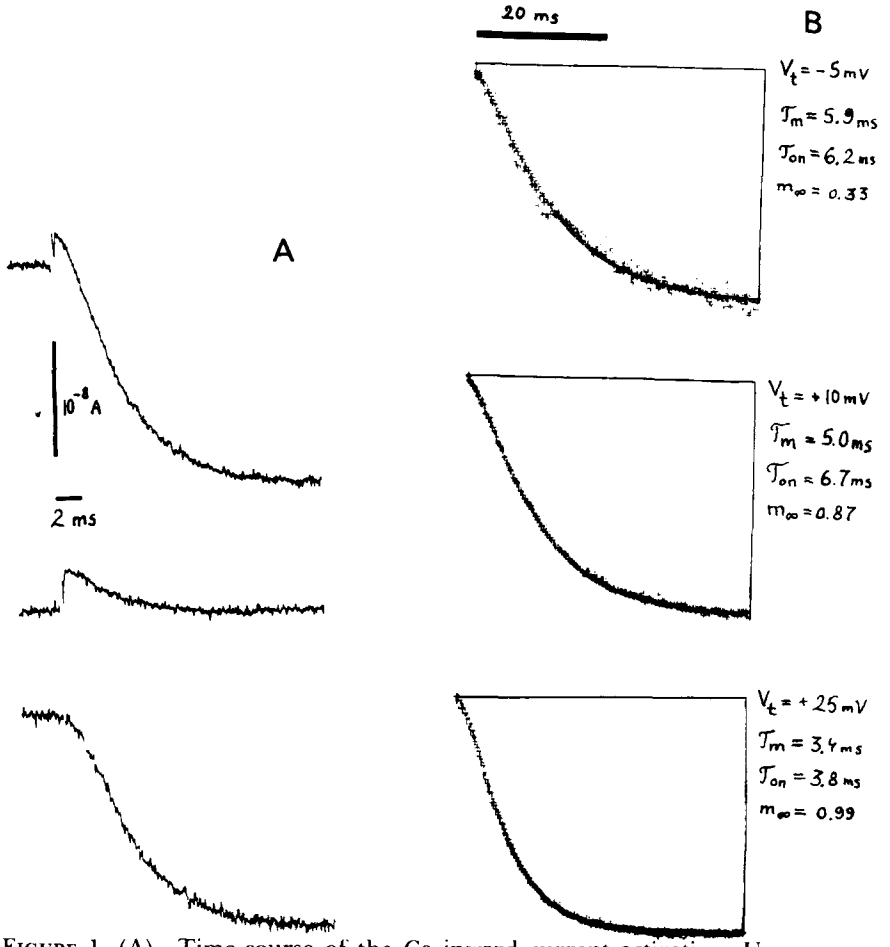

FIGURE 1. (A) Time-course of the Ca inward current activation. Upper curve: scheme). Temperature: 6.5°C. Holding potential: −40 mV. Test potential, τ_m and gating current (Ca inward current was blocked by 2 mM external Cd^{++}). Both curves are obtained by the algebraic summation of the responses to 50 positive and 50 negative test pulses of the same amplitude. Lower curve: Net Ca inward current (the result of digital subtraction of the middle curve from the upper curve). Holding potential: −40 mV. Test potential: +25 mV. 27 mM Ca in the external Na-free solution. Temperature: 6.5°C. Calibrations are shown at the left of the upper curve. (B) Ca inward current superposition of observations and model (m^2 scheme). Temperature: 6.5°C. Holding potential: −40 mV. Test potential, τ_m, and gating current τ_{on} values are shown near the corresponding curves. All the curves are normalized to demonstrate a good quality of approximation at small values of m_∞. Each digitalized current trace coincides with its approximation.

the m^3 scheme which is formally good for Na channels activation kinetics is not supported by the voltage dependence of gating-charge distribution (Meves, 1974). One cannot exclude that it may be partially due to the especially slow inactivation of Ca current which does not interfere in practice with the activation process. Further detailed investigation of Ca channels may open new complexities of their behavior as with Na channels (Bezanilla and Armstrong, 1977;

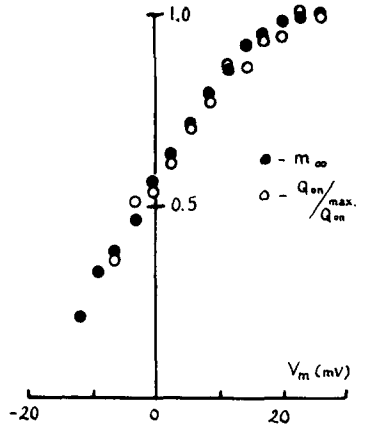

FIGURE 2. Voltage dependence of m_∞ and gating charges distribution (m^2 scheme). Curves are normalized.

Armstrong and Bezanilla, 1977), but for now the m^2 scheme seems to be an adequate working hypothesis.

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Reply to the Letter on the Kinetics of Calcium Inward Current Activation

Dear Sir:

The innovative method reported by Kostyuk et al. (1975) was the starting point for our own technical efforts. Their technique proved too difficult for us, however, and we were forced to adopt a method quite different and less demanding, to us at least, based on a suction pipette (Lee et al., 1977, 1978). Recently, Kostyuk et al. (1978) used a type of suction pipette on neuroblastoma cells, and we have applied the suction pipette method to dispersed individual heart muscle cells (Lee et al., 1979) so that the suction pipette technique may have broad application. The letter of Kostyuk et al. (1979) refers to our paper (Akaike et al., 1978 *b*) in which we noted that G_{Ca} was proportional to a variable which obeys a first-order equation. We also pointed out that using the second power fitted the changes in G_{Ca} just as well and the letter of Kostyuk et al. (1979) seems to confirm this further. The situation might be even more complicated because it is known that the kinetics of the K (Cole and Moore, 1960) and Na (Keynes and Rojas, 1974; Neumcke et al., 1976) systems in axon depend upon the holding potential.

Resolution of G_{Ca} kinetics by Kostyuk et al. requires the subtraction of an asymmetry current they call a gating current. In order to separate the asymmetry current, I_{Ca} was suppressed with Cd^{2+}. Two relatively large currents are then subtracted and produce the very small initial I_{Ca} shown in their Fig. 1 A. If Cd^{2+} has an effect on the asymmetry current, even a small effect, the apparent initial course of I_{Ca} would be altered. The time-course of the asymmetry currents shown in the middle trace of Fig. 1 A (Kostyuk et al., 1979) requires some comment. Relaxation of the asymmetry current appears to be more than a single exponential process, as is the case in axon (Armstrong and Bezanilla, 1977). Unlike axon, however, the beginning of the decline is less steep than later stages (their Fig. 1 A). This could lead to difficulties in estimating τ_{on}, and the relationships among τ_{on}, τ_m and voltage may be more complicated than the one shown in their Fig. 1 B. This has turned out to be the case in axon (Neumcke et al., 1976; Keynes, 1978). The asymmetry currents of Kostyuk et al. are also very large for gating currents. From Fig. 1 A and information about the unit Ca conductance ($\sim 10^{-13}$S) (Krishtal and Pidoplichko, 1977; Akaike et al., 1978 *a*; Kostyuk, 1978), it can be calculated that the gating particles that activate each Ca channel have a net charge equivalent to that of 60–100 electrons. This should be compared with the value of six electronic charges per Na channel arrived at by consideration of either the dependence of sodium conductance on voltage (Hodgkin and Huxley 1952) or a calculation based upon charge movement during Na gating and Na channel density (Armstrong and Bezanilla, 1973). In fact, from the dependence of Ca conductance on voltage (Fig. 2, letter of

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Kostyuk et al.; Akaike et al., 1978 *b*), one can calculate a value of only two to six electronic charges per Ca channel. The normalized $m_\infty - V$ or $Q - V$ curves in Fig. 2 of the letter of Kostyuk et al. do not show these large discrepancies in magnitude. Another possibility for the large size of the so-called Ca gating currents might be that Ca transport is carrier-mediated. However, the power density spectra for stochastic fluctuations in G_{Ca} have a Lorentzian form which is inconsistent with transport "noise" and is characteristic of channel noise (Akaike et al., 1978 *a*; Szabo, 1977; Kolb and Laüger, 1978). The good fit between the curves in Fig. 2 and the poor fit for equivalent curves for the Na system in axon may be due to rather slow inactivation of I_{Ca} as Kostyuk et al., suggest, but correction for Na inactivation still does not eliminate the discrepancies for the Na system in axon (Neumcke et al., 1976). We do agree with Kostyuk et al. that the asymmetry currents they have described are likely to prove quite complicated.

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