Gating Currents Associated with Sodium and Calcium Currents in an Aplysia Neuron

Abstract. *In a voltage-clamped aplysia neuron (R_{15}), depolarization beyond −30 millivolts produces an inward sodium current. Depolarization beyond −10 millivolts produces an additional inward calcium current with slower kinetics than the sodium current. When these ionic currents have been suppressed and capacitive currents subtracted out, a small outward displacement current can be seen with depolarizations beyond −30 millivolts. An additional, slower displacement current is seen with depolarizations beyond −10 millivolts. The currents have an exponential decay with an increase in rate per 10°C increase in temperature of about 3 and are thought to be sodium and calcium gating currents.*

It has recently been demonstrated that depolarization of the surface membrane of squid axon (*1–4*) and amphibian myelinated nerve (*5*) generates a nonionic, outward displacement current which precedes the ionic currents. This displacement current is thought to be generated by the movement of charged "gating" particles associated with sodium channels and hence has been called the sodium gating current (*1, 3*). In some aplysia neurons, membrane depolarization initiates inward sodium and calcium ionic currents (*6, 7*). We have recorded currents generated by depolarization of the neuron R_{15} in aplysia (*Aplysia juliana*) abdominal ganglia (*8*) and believe that two outward displacement currents can be distinguished, one being generated at the same potential as sodium current, the other appearing at a more depolarized potential where calcium current is first seen clearly.

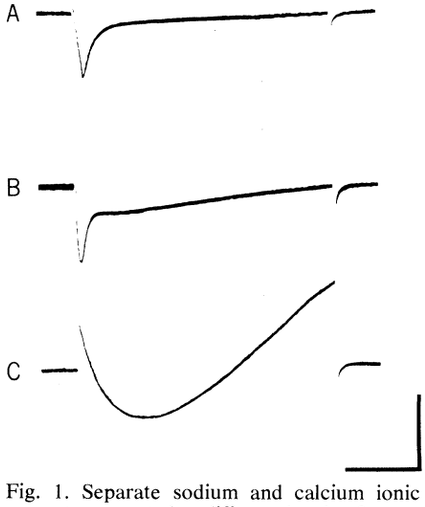

Fig. 1. Separate sodium and calcium ionic currents generated at different levels of membrane potential in a voltage-clamped aplysia neuron (R_{15}). The holding potential was −45 mv. In (A) and (B), but not (C), the bath solution contained TEAC (50 m*M*). (A) Sodium current generated by a clamp pulse to −10 mv. (B) Sodium current and calcium current generated by a clamp pulse to +5 mv. (C) Calcium current generated by a clamp pulse to +5 mv in a sodium-free solution (sodium chloride was replaced by 494 m*M* choline chloride) containing 10^{-6}*M* TTX. Calibrations: horizontal, 40 msec; vertical, 1 μa for (A) and (B) and 0.2 μa for (C). The temperature was 11°C.

The neuron R_{15}, which ranged from 180 to 300 μm in diameter, was voltage-clamped with two intracellular microelectrodes. Control of membrane potential following clamp pulses of 50 to 100 mv was achieved in less than 100 μsec. Electrodes used for recording membrane potential were filled with 3*M* KCl and had resistances of 7 to 10 megohms. Electrodes used for passing current to control membrane potential were filled with a mixture of K citrate (2*M*) and KCl (0.8*M*) (*9*) and had resistances of 2 to 5 megohms. Membrane potential was recorded differentially across the cell membrane between an intracellular microelectrode and an extracellular electrode. Current was monitored with a 1-kilohm resistor in the feedback loop of an operational amplifier (Philbrick 1011) in ammeter configuration between the bath solution and ground. The bath temperature was held constant (±0.5°C) within the range 6° to 12°C.

When the membrane potential of R_{15} was clamped at the resting potential (−40 to −45 mv), a voltage step to a level between −30 and −10 mv produced an early inward current, as illustrated in Fig. 1A. In this experiment (temperature, 11°C) the membrane potential was stepped from −45 to −10 mv in a solution containing 50 m*M* tetraethylammonium chloride (TEAC) to inhibit potassium current (*10*). The early inward current, which disappeared in sodium-free solutions [choline or tris(hydroxymethyl)aminomethane (tris) replacing sodium] and in the presence of tetrodotoxin (TTX), was taken to be sodium current. When the membrane potential was stepped to levels more positive than −10 mv (from the same holding potential) another component of inward current appeared. This can be seen in Fig. 1B. The same neuron as in Fig. 1A was clamped to +5 mv and the early inward (sodium) current was followed by a slower inward current. This slower current was not affected by the absence of sodium or the presence of TTX. This is illustrated in Fig. 1C (note change in vertical scale), again recorded from the same neuron. Here the external solution contained TTX (10^{-6}*M*) and no sodium (choline substitution). The current shown was generated by a clamp step from −45 to +5 mv. This later, slower current, which appeared only at clamp potentials more positive than −10 mv, was taken to be calcium current because its peak amplitude was dependent on extracellular calcium concentration and it was inhibited by either manganese ions (15 m*M*) or cobalt ions (15 m*M*) (*11*). The peak amplitudes of these sodium and calcium currents (temperature, 8°C) are plotted as a function of clamp potential in Fig. 2. The sodium current has an appreciably lower threshold than the calcium current. Clearly distinguishable calcium current was seen only at clamp potentials more positive than −10 mv. Another difference between the sodium and calcium currents was that when the sodium current had been completely inactivated by setting the holding potential at −20 to −10 mv, inward calcium currents could still be elicited by depolarizing to more positive potentials.

In an attempt to discern displacement currents which are small compared with linear capacitive currents, positive and negative clamp steps of exactly equal amplitude were applied alternately (0.5 sec^{-1}) and the currents were summed algebraically (PDP 8I minicomputer, 30 μsec per point). The rationale of this technique is that the early linear capacitive current which charges the membrane capacity to the clamp level should be symmetrical and cancel out with the averaging procedure, leaving nonlinear

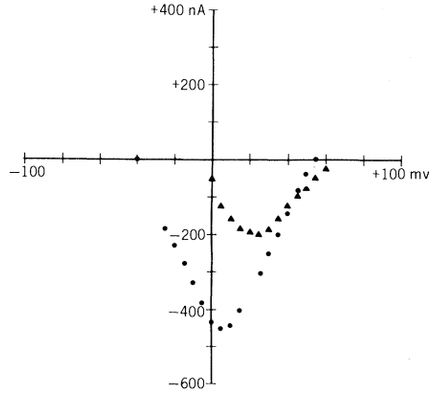

Fig. 2. Peak amplitudes of sodium (circles) and calcium (triangles) currents plotted as a function of membrane potential (holding potential, −40 mv). The diagram illustrates the different voltage dependence of the two currents, particularly the difference in threshold. The temperature was 8°C.

(displacement) currents. When voltage steps and holding potentials were used so that the membrane potential was never more depolarized than −40 mv, the currents were symmetrical and canceled out. This can be seen in Fig. 3C. In this experiment, the membrane potential was stepped plus and minus 50 mv from a holding potential of −101 mv and the capacitive currents canceled out (average of 15 positive and 15 negative pulses). But when the same neuron was clamped plus and minus 50 mv from a holding potential of −62 mv, the nonlinear components of the capacitive currents did not cancel and "on" and "off" currents, shown in Fig. 3A, were recorded. Normally such displacement currents are superimposed on much larger ionic currents so that it is difficult to distinguish them clearly. In this experiment (Fig. 3) the extracellular solution was sodium-free (sodium was replaced with choline) and contained TTX ($10^{-6}M$), manganese chloride (15 mM), and TEAC (50 mM). It therefore seems unlikely that the displacement currents are ionic currents. Because they are outward they could not be sodium or calcium ionic currents. When the membrane potential was clamped plus and minus 75 mv (to +13 and −137 mv), the average displacement current now contained a later, slower component (Fig. 3B; note the increased vertical gain). The slower component was more prolonged but smaller in amplitude in the off-current (Fig. 3B). The slower component of the displacement current was generated only by clamp pulses to a level more positive than −10 mv.

The directions of both displacement currents were outward at the beginning of a pulse and inward at the termination of the pulse. The decays of both early and late displacement currents were exponential, and the outward currents decayed more rapidly than the inward currents. Displacement currents had a high temperature coefficient. It was possible to measure the time constant of decay of calcium displacement currents with some accuracy and in three experiments values of Q_{10} (the increase in rate per 10°C increase in temperature) of 3.3, 3.0, and 2.6 were obtained.

We believe that the early, relatively more rapid displacement currents shown in Fig. 3A are associated with the opening and closing of sodium channels because they are seen only with clamp pulses which activate sodium currents and they precede the sodium currents. The later, slower displacement currents which appear at precisely the potentials where calcium ionic current is normally generated are thought, for similar reasons, to be associated with the opening and closing of calcium channels. In support of this hypothesis is the observation that when calcium currents are reduced in amplitude with holding potentials more negative than −70 mv (*7*) the calcium displacement currents are also reduced in amplitude. That these displacement currents are generated by movements of ions across the surface membrane is unlikely for reasons that have been presented above and by others (*3, 4, 12*)—particularly because the currents are most prominent in solutions in which sodium, calcium, and potassium currents have been inhibited.

In several experiments a divided pulse technique was used (*3*), and gave much the same results as above. In these experiments the displacement of charge was approximately equal at the beginning and end of a pulse. When a holding potential of −20 to −10 mv was used for depolarizing steps and a holding potential of −100 mv was used for hyperpolarizing pulses, the recorded displacement current still had two components, as in Fig. 3B, although the sodium current had been inactivated. The amplitude of both displacement currents was reduced, however. It is interesting that sodium gating currents have been recorded in squid axons in which sodium current has been inactivated (*12*). An attempt was made to relate the charge movement to membrane area. By measuring the total capacitive current following a voltage step and assuming a membrane capacitance of 1 microfarad per square centimeter, the effective membrane area was calculated. From this, the peak amplitude of sodium gating current was calculated to be 20 to 60 μa per square centimeter (6°C; holding potential, −62 mv) which is similar to values obtained for sodium gating current in squid axons (*1–4, 12*).

It is not surprising that separate sodium and calcium channels with different kinetics should be controlled by separate gating mechanisms with different kinetics. The observation of two distinct displacement currents in a neuron which has separate sodium and calcium ionic currents supports the hypothesis that these displacement currents are generated by movements of charged particles within the membrane and that it is movements of these gating particles which cause sodium and calcium channels to open and close.

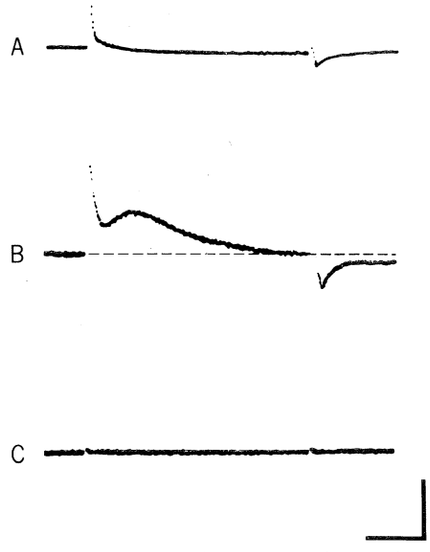

Fig. 3. Separate sodium and calcium gating currents generated at different levels of membrane potential in a voltage-clamped aplysia neuron (R_{15}) in a sodium-free solution (494 mM choline chloride) containing TTX ($10^{-6}M$), Mn (15 mM), and TEAC (50 mM). Solutions contained the normal 11 mM $CaCl_2$. The holding potential was −62 mv in (A) and (B) and −101 mv in (C). The records were obtained by summing currents generated by 15 positive and 15 negative clamp pulses (delivered at 2-second intervals) and obtaining the average asymmetrical (nonlinear capacitive) current. (A) Average sodium gating current produced by voltage steps to −112 and −12 mv (±50 mv). (B) Average sodium and calcium gating currents produced by voltage steps to −137 and +13 mv (±75 mv). (C) Average response to voltage steps to −151 and −51 mv (±50 mv from a holding potential of −101 mv). Calibrations: horizontal, 2 msec; vertical, 100 na for (A) and 50 na for (B) and (C). The temperature was 6°C.

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10. The normal aplysia solution contained 494 mM NaCl, 11 mM KCl, 11 mM $CaCl_2$, 19 mM $MgCl_2$, 30 mM $MgSO_4$, and 10 mM tris buffer, pH 7.7.
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