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Cole–Moore effect in the frog node

(voltage clamp/potassium current kinetics)

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ABSTRACT **Potassium currents were recorded from the voltage-clamped frog node (*Rana esculenta*) during various test pulses that followed hyperpolarizing prepulses of different amplitudes and durations. Both the delay in potassium current onset and the shape of the current trace as a function of time were found to be a function of prepulse parameters. This finding is different from the current trace superposition described by Cole and Moore for a specific test pulse, sodium equilibrium potential in the squid giant axon. The Cole–Moore effect, which was found here only under a specific set of conditions, thus may be a special case rather than the general property of the membrane. The implication of these findings to the various excitable membrane potassium channel models, which are based on the Cole–Moore effect, is discussed.**

The turn-on kinetics of axon membrane potassium current and conductance are characterized by an initial delay. In the Hodgkin–Huxley (1) axon model this delay is accounted for by raising the potassium conductance parameter, n, to the 4th power. Within this framework the delay has been interpreted as being due to the need for four potassium channel subunits to be in the so-called open state for the channel, as a whole, to be open. Because these subunits are assumed to have only two states, "open" or "closed," and to be independent of each other, the fraction of channels in which all four subunits are in an open state is given by n^4.

In 1960 Cole and Moore (2) found that the delay of potassium conductance turn-on was increased by conditioning hyperpolarization. However, the trace of potassium current against time obtained after different conditioning potentials could be made to superimpose by shifting the curves along the time axis. These findings could be accounted for within the Hodgkin–Huxley axon model by raising n to the 25th power. These high powers pose a severe difficulty in giving the Hodgkin–Huxley model the same physical interpretation as above.

The above phenomenon, usually referred to as the Cole–Moore effect, has been referred to by many workers since it restricts the number of possible models for the potassium gating mechanism in the axon membrane (3–8). However, in spite of the numerous models based on this phenomenon, the experimental data supporting it are limited. Because specific poisons such as tetrodotoxin were unavailable at the time, Cole and Moore (2) used only test pulses that equalled the sodium equilibrium potential (E_{Na}). Several investigators who have since repeated their procedure (i.e., pulsing to the vicinity of E_{Na}) on *Myxicola* axons (9), crayfish (10), and the frog node (11) have claimed similar results. In contrast, it has recently been demonstrated (12) that the frog nodal membrane behaves differently: After hyperpolarizing conditioning potentials of different amplitudes and durations there was, in addition to the increase in current turn-on delay, a significant difference in the form of the relationship of potassium current against time, and the curves could not be made to superimpose by shifting them on the time axis.

This work attempts to clarify whether the reported differences, between squid and *Myxicola* on one side and nodal membrane on the other, are due to a basic difference between their properties or to the different conditions under which the experiments were carried out.

METHODS

Single, myelinated nerve fibers, isolated from the frog *Rana esculenta* (13), were mounted and voltage clamped as described by Nonner *et al.* (14). The node was externally perfused with Ringer's solution containing 300 nM tetrodotoxin. The pH was adjusted to 7.4 by Tris buffer. The temperature of the solution bathing the node was held constant at 15°.

In between voltage clamp pulses membrane potential was held at its resting value (V_H). All potentials are given relative to resting potential; depolarization is positive, while hyperpolarization is in the negative direction.

From V_H the membrane potential was stepped to a conditioning prepulse (V_{pp}). At the end of each prepulse the membrane potential was clamped to a test pulse (V_p), and the currents associated with this step were monitored. Pulse interval was 4 sec. At the end of each experiment the node was destroyed by strong hyperpolarization and the absolute membrane potential (E_M) was determined. Both the holding potential and command pulses were generated by a D/A converter under computer program control (Honeywell DDP-516, Honeywell Information Systems, Waltham, MA).

Membrane currents were filtered by a 40-kHz low-pass filter and then sampled at 20-μsec intervals for 40 msec by means of a 10 bit A/D converter operating also under program control. Sampling started upon stepping membrane potential from V_{pp} to V_p. After initial online processing the sampled data were stored on digital tape for further computer analysis. Leakage conductance was determined from a hyperpolarizing pulse. Leakage current was subtracted from all current analyzed.

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Abbreviations: V_p, test pulse potential; V_{pp}, conditioning pulse potential; t_{pp}, conditioning pulse duration.

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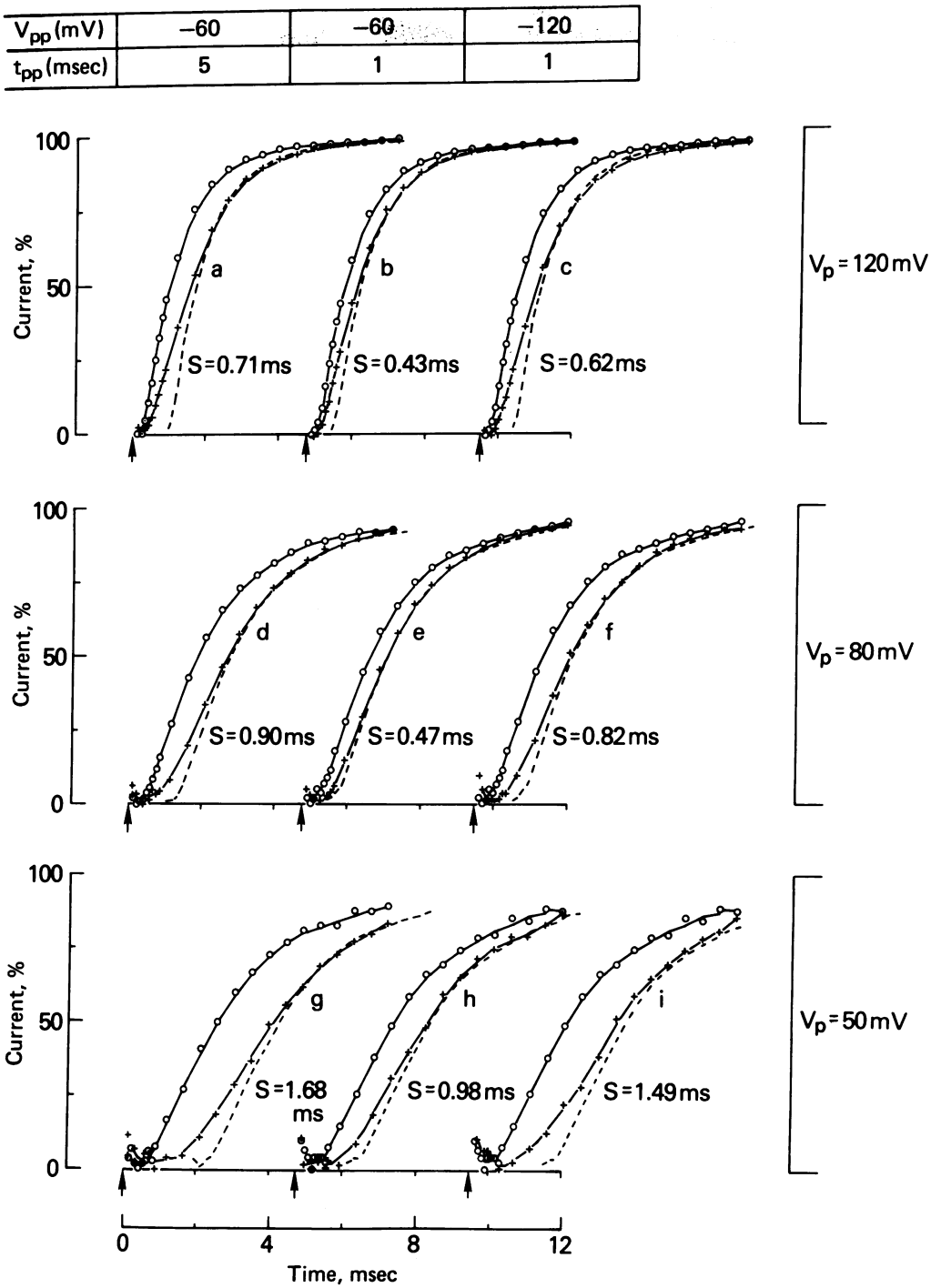

FIG. 1. Computer-reconstructed potassium current tracing obtained during test potentials (V_p), after conditioning potentials of different amplitudes (V_{pp}) and durations (t_{pp}). The tracings begin at the moment membrane potentials are stepped from V_{pp} to V_p. The current values obtained upon stepping membrane potential directly from holding potential (V_{pp} = 0) to V_p (○) are given together with those obtained after various conditioning potentials (×). The I_K against time functions are compared by shifting the current values obtained without a conditioning pulse along the time axis to give best superimposition (interrupted trace) with the conditioned current trace. The amount of the shift in msec, S, is given adjacent to the trace. Arrows, the time membrane potential was stepped to V_p. Fiber 22/75, 15°.

RESULTS

Fig. 1 illustrates the time course of potassium current during test potentials (V_p), after conditioning potentials of different amplitudes (V_{pp}) and durations (t_{pp}). The records begin at the moment membrane potential is stepped to V_p. Fig. 1(curve a) gives the current obtained under conditions similar to those used by Cole and Moore. Their conditioning pulse duration, t_{pp}, was 3 msec, and amplitude, V_p, was 100 mV. As described by Cole and Moore, the onset of potassium current is delayed after the conditioning hyperpolarization (V_{pp} = −120 mV, t_{pp} = 5 msec). However, the current time course without a conditioning hyperpolarization cannot be made to superimpose completely on those obtained when V_{pp} was −60 mV (a conditioning hyperpolarization) by shifting along the time axis. In other words, even when the delay is compensated for, the I_K turn-on kinetics

Table 1. Slopes of linear parts of potassium current initial segment (Fig. 1) (relative units) and shift of 50% current points (Δt_{50}, in msec) along the time axis

		V_{pp} −60 mV				V_{pp} −120 mV	
	V_{pp} 0 mV	t_{pp} 5 msec		t_{pp} 1 msec		t_{pp} 1 msec	
V_p, mV	Slope	Slope	Δt_{50}	Slope	Δt_{50}	Slope	Δt_{50}
120	5.4	4.4	0.50	3.9	0.29	3.6	0.43
80	2.8	2.1	0.79	2.8	0.43	2.4	0.71
50	1.9	1.5	1.5	1.6	0.86	1.7	1.29

are a function of conditioning potential and, therefore, the complete Cole–Moore effect cannot be demonstrated here. Note that the delays in potassium current onset in squid axons (2) are on the order of 0.1 msec while in the node they are about 1 msec.

Fig. 1 (curves b–i) illustrates that superposition cannot be significantly improved by varying t_{pp}, V_{pp}, or V_p, except under one specific set of conditions (Fig. 1, curve e): $V_{pp} = -60$ mV, $t_{pp} = 1$ msec, and $V_p = 80$ mV. Thus, the superposition may be a special case rather than a general property of the membrane. Note, however, that for any V_p, the smaller the V_{pp} and t_{pp}, the shorter the delay and the better the superposition.

It can be seen in Fig. 1 that, depending on the conditions, both the initial and later segments of the current traces may not be superimposable. Table 1 illustrates the differences between the conditioned and unconditioned current tracings: For any specific V_p the initial slope may differ by as much as 50%; in general, the longer or larger the conditioning hyperpolarization, the greater the Δt_{50} shift.

Fig. 2 superimposes computer-reconstructed potassium currents during a test pulse of $V_p = 50$ mV initiated directly from holding potential and after 30 msec of a $V_{pp} = -50$ mV conditioning pulse. The predicted tracing was computed by the Hodgkin and Huxley model equations for the frog node (15) from our experimentally determined $\bar{g}_k$ and τ_n values, taking 4 as the power of n. The observed shift of the conditioned current tracing relative to the unconditioned one is about twice as large as that predicted by the Hodgkin–Huxley model.

DISCUSSION

The results of this work demonstrate that in the frog node the so-called Cole–Moore effect is obtained, at best, only under very specific conditions. Under all other conditions tested, the conditioning hyperpolarization affects both the delay of the current onset and the current kinetics. These results agree with those of Palti *et al.* (12), who demonstrated that potassium current kinetics are a function of both conditioning pulse amplitude and duration (figures 5 and 6 in ref. 12). Careful examination of the conditioning induced shifts in the crayfish axon (10) reveals that there, too, the shifts are accompanied by a change in the current kinetics. Unfortunately, Goldman and Schauf (9), who found reasonable superposition between the potassium current tracings, present only currents produced by potentials in the vicinity of E_{Na}.

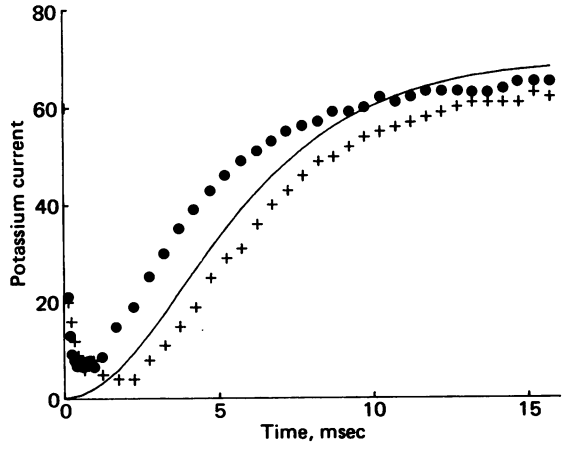

FIG. 2. Computer-reconstructed potassium currents (relative units) during a test pulse of $V_p = 50$ mV initiated directly from holding potential (●) and after 30 msec of $V_{pp} = -50$ mV conditioning pulse (×). The continuous line represents the theoretical Hodgkin–Huxley current trace after the hyperpolarizing conditioning pulse. Fiber 77/77, 15°.

Different kinetic models suggested for the potassium channel rely, among other data, on the two main features described by Cole and Moore (2): (*i*) The delay of the potassium current onset is a direct function of conditioning hyperpolarization. (*ii*) Beyond the delay, the potassium current kinetics are independent of the conditioning hyperpolarization. Based on the above two features, Hill and Chen (6) abandoned their original potassium channel models (4, 5) and proposed two potassium channel models (7) that incorporate an additional special process which is assumed to be introduced by the conditioning hyperpolarization. Within the framework of their models, our data suggest cooperativity between the channel subunits when the channel is opened. Furthermore, in view of our results, the conclusions of Albano (8), regarding the potassium channel energy profile, and of Hoyt (3), regarding the coupling between sodium and potassium channels, must be reconsidered.

The results of our work can be used for the elimination of certain potassium channel models. However, in general, they widen rather than narrow the number of possible models. Although the results are inconsistent with the classic two-state model, they are compatible with the following classes of channel models (see also ref. 3):

(*i*) The potassium conductance is determined by a multistate process. Such multistate processes were recently proposed by Goldman (16) and Neumcke *et al.* (17) on the basis of the variable delay in sodium current turn-on for the sodium activation.

(*ii*) The potassium conductance is determined by two or more types of potassium channels which may have different kinetics and voltage dependency.

(*iii*) The measured currents are affected by some extra-axonal components, such as the Schwann sheath.

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