CONDITIONING HYPERPOLARIZATION-INDUCED DELAYS IN THE POTASSIUM CHANNELS OF MYELINATED NERVE

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ABSTRACT Hyperpolarizing conditioning pulses delay the onset of potassium channel current in voltage-clamped myelinated nerve fibers. Both the development of and recovery from this conditioning are approximately exponential functions of time: the time constants are functions of the conditioning voltage. The delay is larger and develops faster for more hyperpolarized conditioning pulses. The magnitude of the delay (but not the rate of development or recovery) depends upon the test potential—small test depolarizations produce larger delays than large depolarizations. The currents with and without the conditioning pulse cannot be made to superimpose by a simple time translation.

INTRODUCTION

Potassium channel currents are sigmoid functions of time in many nerve and muscle preparations including squid axons (Hodgkin and Huxley, 1952*a*), *Myxicola* axons (Goldman and Binstock, 1969), myelinated nerve of toad (Frankenhaeuser, 1962*a*, *b*) and frog (Dodge, 1961, 1963). The kinetics of these currents have been mathematically formulated by considering that the potassium permeability (or conductance) is proportional to n^x where n is the solution of a first order differential equation. Values of x equal to two (Goldman and Schauf, 1973; Frankenhaeuser, 1963) and four (Hodgkin and Husley, 1952*b*; Dodge, 1961, 1963) have been reported. Frankenhaeuser and Hodgkin (1957) and Cole and Moore (1960) have shown that for squid axons the sigmoid nature of the permeability change can be exaggerated by preceding the usual voltage clamp step with a hyperpolarizing conditioning pulse. The time delay produced by the hyperpolarization required a value of x equal to 25 (Cole and Moore, 1960). Similar effects have been observed in many other nerve tissues such as *Myxicola* giant axons (Goldman and Schauf, 1973), crayfish giant axons (Shrager, 1974), and frog myelinated nerve (Moore, 1971; Palti et al., 1976).

When the delayed currents in squid axons (Cole and Moore, 1960) were shifted along the time axis, they approximately superimposed with the normal currents. Hill and Chen (1971*a*, *b*) used this property to argue against inter- and intrachannel cooperativity. This conclusion should now be reconsidered, at least for myelinated nerve, since the experiments of Palti et al. (1976) and my results reported here show that the currents with and without a conditioning hyperpolarization do not superimpose.

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I have made a quantitative study of this effect in single nodes of Ranvier of frog myelinated nerve. Included in this analysis are the voltage dependence, time-course of development and time-course of recovery of the hyperpolarization-induced time delay. Such data are necessary for a complete kinetic description of potassium channels in nerve.

METHODS

Single, myelinated, motor, and sensory sciatic nerve fibers isolated from *Rana pipiens* were voltage clamped using the procedures described by Hille (1971). Most of the experiments were done with northern rather than southern frogs. The K currents in northern frogs produce little accumulation of K ions just outside the nodal membrane (Armstrong and Hille, 1972). Most of the experiments were done with the node bathed in a solution that contained (mM) 120 NaCl, 2.5 KCl, 2.0 CaCl, 5.0 Tris (hydromethyl) amino methane buffer (pH 7.4), plus 100 nM tetrodotoxin (TTX) to block the current through sodium channels. A few experiments used an isotonic KCl solution that consisted of (mM) 120 KCl, 2 CaCl, 5 Tris plus TTX. The ends of the fibers were cut in 120 mM KCl. The temperature was maintained at 15°C.

The holding potential was adjusted so that resting sodium inactivation (h_∞) was 0.6. The absolute value of this potential is uncertain due to the many liquid junction and electrode potentials coupled with a shunted high impedance system (Hille, 1967). This potential, then, was taken (arbitrarily) as -80 mV. With this value for the holding potential membrane potentials reported here will be on the "E" scale of absolute millivolts. These values have been corrected for an average, measured "attenuation artifact" (Hille, 1971) of 19%. This is between previously reported values of 11 (Hille, 1971) and 24% (Woodhull, 1973).

Membrane currents were corrected for leakage current using an analog subtraction method (Armstrong and Hille, 1972). In most cases little or no capacity current subtraction was done. This is because K currents are slow enough not to be obscured by the faster capacitative current and the capacity transient serves as a convenient marker for the beginning of the test pulse.

Examples of the type of experiments described in this report are shown for two different nodes in Fig. 1. For each node test pulses to 16 and 74 mV are shown, each with and without a conditioning prepulse (CP) to -138 mV for 50 ms. The prepulse produces a delay in the development of potassium current. The amount of this delay can be measured by comparing the time to half maximum ($t_{1/2}$) with and without the CP. The time delay is the difference between half-time values and will be called $\Delta t_{1/2}$. If, after the initial lag, the delayed curve had exactly the same shape as the control, $\Delta t_{1/2}$ would be a complete description of the effect of the conditioning hyperpolarization. Unfortunately, as shown in Fig. 2, this is not the case. In this figure current records for four conditioning potentials have been shifted along the time axis until the later part of the records superimpose with the control traces. Records *a* and

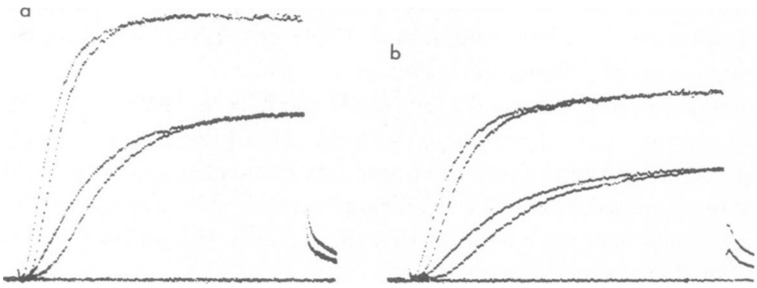

FIGURE 1 Oscilloscope photographs of potassium channel current of two different nodes (48, *a*; 47, *b*) with and without a hyperpolarizing conditioning pulse to -138 mV for 50 ms for two different test potentials (16 and 74 mV). In each pair of records the delayed current is that produced by the conditioning potential. Each record lasts 20 ms. Maximum current is 7.5 (*a*) and 11 nA (*b*), assuming $R_{ED} = 50$ MΩ.

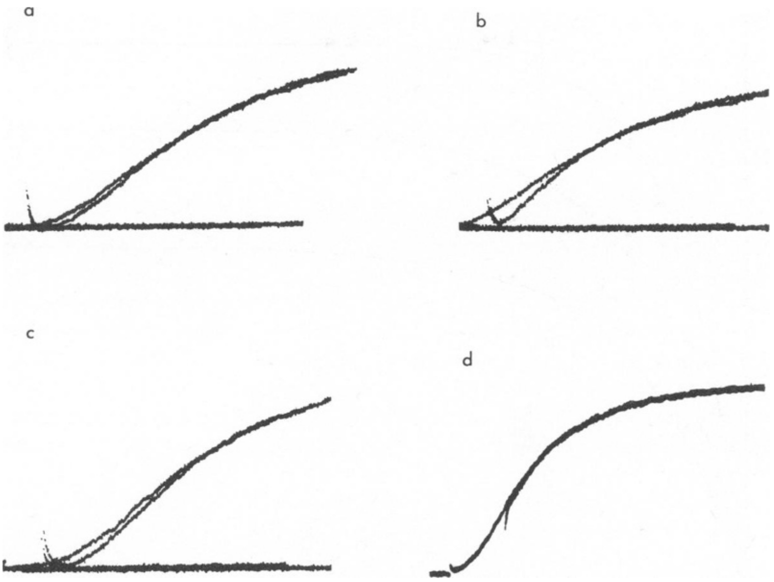

FIGURE 2 Oscilloscope photographs made by superimposing records with and without prepulse after first shifting the record with the prepulse along the time axis. Records *a* and *c* are from node 39 with a test pulse to 16 mV and a 50-ms CP to −128 and −157 mV, respectively. Record *b* is from node 40 with a test pulse to 16 mV and a 20-ms CP to −176 mV. *d* is from node 28 with a test pulse to 55 mV and a 10-ms depolarizing CP to −3 mV. Records *a*, *b*, and *c* are 10-ms long, record *d* is 20-ms long.

c are from the same node. The test pulse was 16 mV with a 50-ms CP to −128 (*a*) and −157 mV (*b*). Records *b* and *d* are from two different nodes. In *b* the test pulse was to a value of 16 mV and the CP was to −176 mV and lasted 20 ms. In *d* the test pulse was 55 mV and the CP was a depolarization to −3 mV for 10 ms. It is clear that the records cannot be made to super-impose over their entire time-course. The greater the hyperpolarization, the greater the deviation from a simple time translation. $\Delta t_{1/2}$, then, is a convenient, but only approximate, measure of the effect of the conditioning potentials.

As seen in Figs. 1 and 2 the hyperpolarizing prepulses did not alter the steady-state level of currents. This was true in all cases, even for conditioning pulses lasting hundreds of milliseconds, consistent with the findings of Schwartz and Vogel (1971). To improve the measurement of small shifts, records containing the test pulse with and without conditioning pulses were photographed on one frame. Also, in some experiments with experimental series was done twice and the results averaged.

RESULTS

Time-Course of Development of the Delay

The delaying effect of the conditioning hyperpolarization develops with an approximately exponential time-course that depends on the value of the conditioning potential. This is shown in Fig. 3 *a*. In this experiment a single test pulse to −3 mV was used with three values of conditioning potential: −119 mV, −138 mV, and −176 mV. The values of $\Delta t_{1/2}$ for these CP values are plotted against the duration of the conditioning pulse. The solid curves are exponential functions of time with time constants of 3.5, 3.1, and 2.2 ms, respectively. The more hyperpolarized the CP, the faster the delay develops.

Occasionally, the development of the delay appeared to occur in two phases: a relatively fast

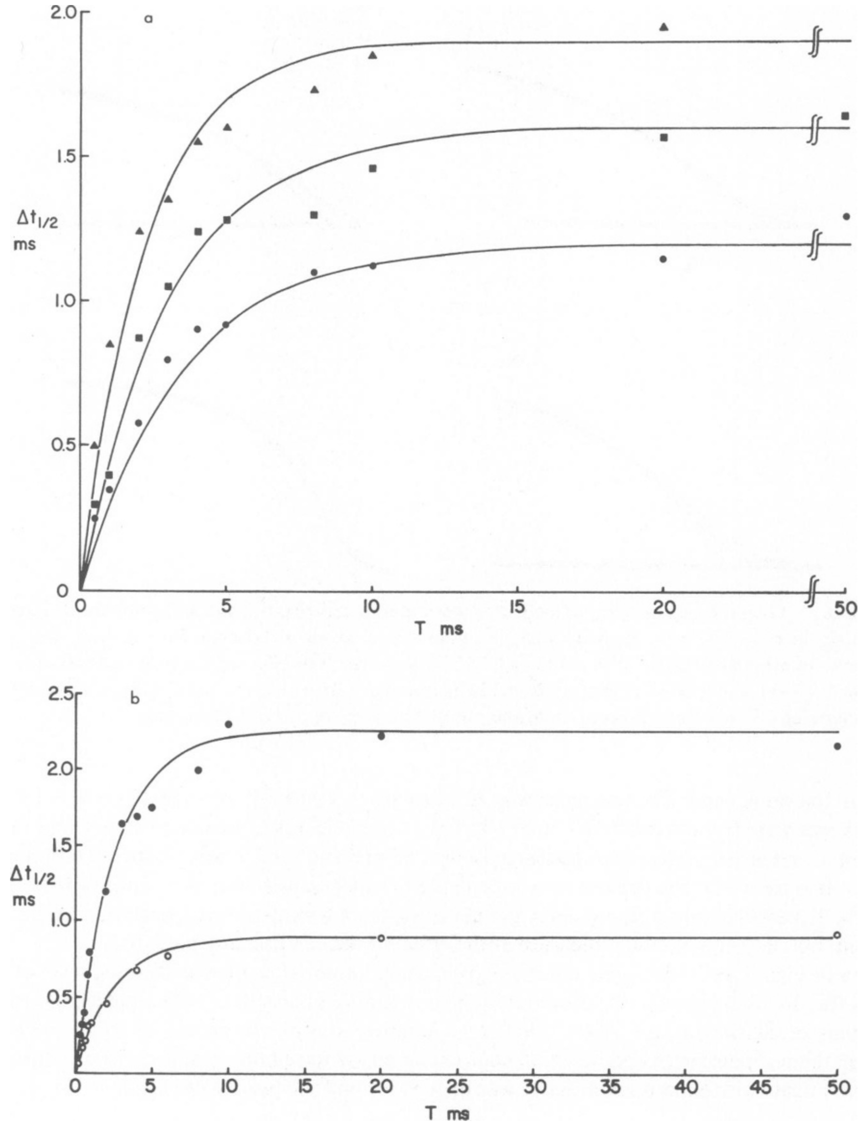

FIGURE 3 (*a*) Development of hyperpolarization-induced delay ($\Delta t_{1/2}$). $\Delta t_{1/2}$ is plotted against CP duration for three CP values: −119 mV (●), −138 mV (■), and −176 mV (▲). Solid lines are exponential functions of time with time constants given in text. (*b*) Development of $\Delta t_{1/2}$ for two test-pulse values: −3 mV (●) and 55 mV (○). Solid line through filled circles is an exponential function of time with a 2.5-ms time constant. The lower solid line is the same curve scaled to match open circle $\Delta t_{1/2}$ value at 20 ms. Node 32, CP = −157.

beginning followed by a slower approach to the steady-state. An example of this is the CP = −119 mV curve in Fig. 3 *a*.

The time-course of development of $\Delta t_{1/2}$ does not depend on the value of the test pulse. Fig. 3 *b* shows data taken with test potentials of −3 and 55 mV with a conditioning pulse to −157 mV. The solid curve passing through the filled circles is an exponential function of time with time constant of 2.5 ms. The lower solid curve is the same curve scaled to match the $\Delta t_{1/2}$ value at 20 ms. In this and most experiments the values of $\Delta t_{1/2}$ saturated for prepulse durations >20 ms. Most experiments were done with CP durations up to 100 ms, but 2 were done with maximum durations of 200 and 900 ms. In these experiments the delay was constant from 25 ms up to the maximum of 200 or 900 ms.

Although the time-course of development of $\Delta t_{1/2}$ does not depend on the value of the test pulse, the magnitude of the delay does. This can be seen in Fig. 3 *b*, and Figs. 1 *a* and *b* where the time delay is greater for smaller test depolarizations than larger ones. Fig. 4 *a* is a plot of $\Delta t_{1/2}$ as a function of conditioning potential for three test potentials. The delay is larger at all conditioning voltages for the less depolarized test potentials.

A striking result can be obtained if the $\Delta t_{1/2}$ data of Fig. 4 *a* are replotted for each test pulse as $\Delta t_{1/2}$ divided by the value of the time to half maximum current without the CP. This manipulation causes the data for all test-pulse potentials to fall along the same curve. The factor of five or more of spread in $\Delta t_{1/2}$ values of Fig. 4 *a* is reduced to a few percent scatter in Fig. 4 *b*. This was a very consistent finding and may be an important clue to deciding upon a kinetic model for K channels in nerve.

Experiments of the type shown in Fig. 4 *a* were carried out with conditioning potentials to -250 mV. Even at these large potentials $\Delta t_{1/2}$ had not saturated, though the rate of change with voltage was much reduced. Often $\Delta t_{1/2}$ started to approach a steady value at $\simeq -200$ mV but then increased again for more negative values. This may be due to deleterious effects of these large potentials on membrane structure.

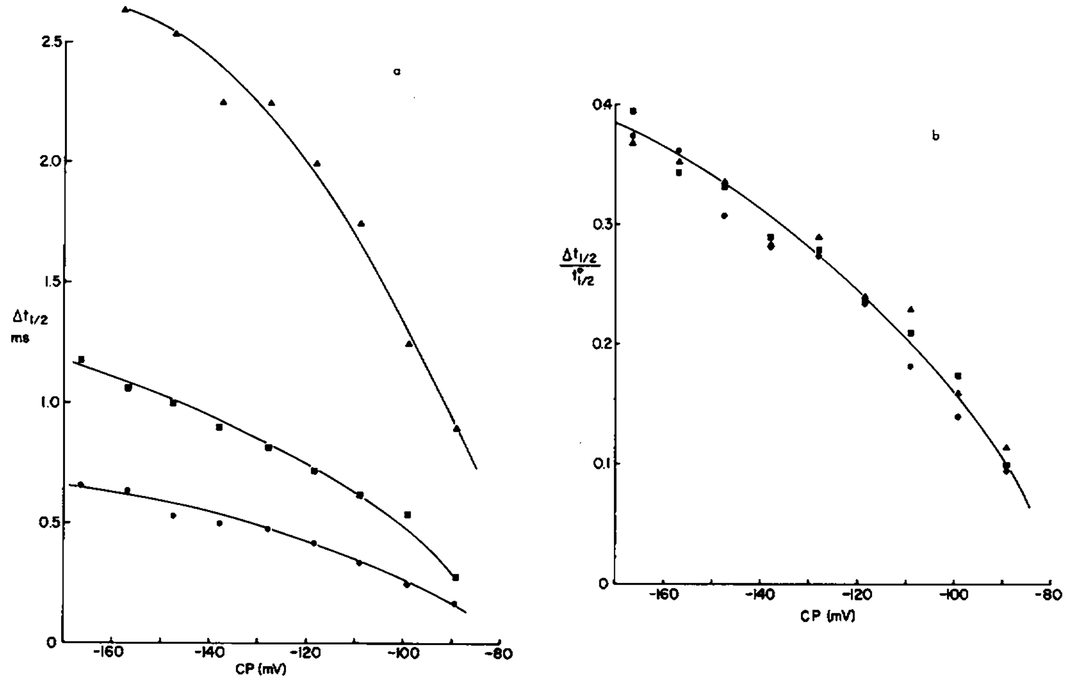

FIGURE 4 (*a*) $\Delta t_{1/2}$ values plotted against CP magnitude for three test-pulse values: -3 (▲), 55 (■), 113 mV (•). Width of CP was 50 ms. Node 27. (*b*) Data of 4*a*, scaled to the value of $\Delta t_{1/2}$ of potassium current without CP.

Time-Course of Recovery of the Delay

If the test pulse does not immediately follow the hyperpolarizing conditioning pulse, $\Delta t_{1/2}$ is reduced. By varying the time between the end of the CP and the beginning of the test pulse, the recovery from the conditioning effect can be observed. The recovery process was found to follow an exponential time-course. This is shown in the semi-log plot, Fig. 5, of $\Delta t_{1/2}$ as a function of the time between the conditioning and test pulses. Like the development of $\Delta t_{1/2}$ the recovery process does not depend on the test-pulse value. This figure includes data for test-pulse values to 16 and 55 mV. The straight lines represent exponential functions of time with time constants of 11.5 and 10.8 ms, respectively. Given the scatter in the data, these two values are probably not significantly different.

Voltage Dependence of Development and Recovery of the Delay

A summary of the voltage dependence of the time constants of development, τ_D, and recovery, τ_R, is shown in Fig. 6. In this figure the abscissa is the conditioning potential for τ_D and the recovery potential for τ_R. The recovery potential is the voltage level between the CP and the test pulse. Both time constants appear to decrease as the potential is made more negative. At the one potential where both could be readily measured (–119 mV), they appear to overlap.

Time Delay and K Concentration

As discussed in Methods the choice of frogs used for many of these experiments should minimize K accumulation near the external membrane surface. The outward tail currents of Fig. 1 support this position. A more rigorous test of the effects of external K was made in a few experiments like those in Figs. 3 and 4 *a* and *b* using isotonic KCl Ringer's solution. The same qualitative results were found in normal and KCl Ringer's solution, regardless of whether the net current was inward or outward. There were, however, some quantitative differences. The

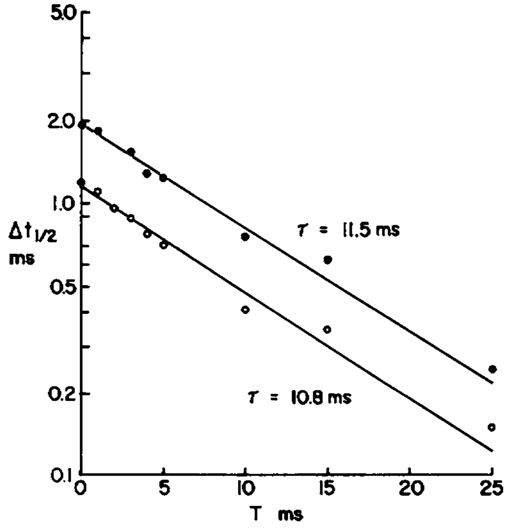

FIGURE 5 $\Delta t_{1/2}$ plotted against time between CP and test pulse for two values of test pulse: 16 (•) and 55 mV (o). Straight lines represent expoential functions of time with time constants given. CP was to –176 mV for 10 ms. Recovery potential was –80 mV. Node 30.

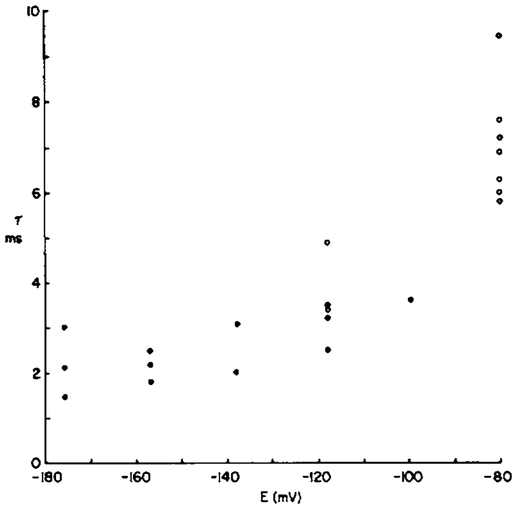

FIGURE 6 Development time constant, τ_D (•), and recovery time constant, τ_R (○), plotted against membrane potential.

time constants of normal K currents as well as the time constants for development of $\Delta t_{1/2}$ were slowed by 15–25%.

DISCUSSION

The presence of a conditioning hyperpolarization-induced time delay in potassium channel kinetics was first noticed in squid axons by Frankenhaeuser and Hodgkin (1956). Cole and Moore (1960) studied this effect in some detail, but, because at that time there was no convenient way to eliminate Na currents, their observations were restricted to test pulses near the Na current reversal potential. Palti et al. (1976) found similar delays in the K channel kinetics of myelinated fibers, but concentrated more on depolarizing conditioning pulses. The present work extends these observations to include a wide range of test and conditioning pulse values. A range of conditioning pulse durations were used to study the kinetics of the conditioning process. I have also included new experiments designed to study the recovery from the conditioned state.

My results show that hyperpolarizing conditioning pulses delay the onset of potassium conductance in frog myelinated nerve fibers. The delay can be as long as a few milliseconds and depends upon the kinetics of the conductance without a prepulse. That is, for large depolarizations where K channel kinetics are fast, the delay is small. For small depolarizations (slow kinetics) the delay is large. This time delay (measured as $\Delta t_{1/2}$) develops and recovers with an approximately exponential time-course, the time constants being functions of potential. The delay is greater for larger hyperpolarizing prepulses and may eventually saturate at very negative potentials. The kinetics of the development and recovery of the delay do not depend upon the values of the test pulses used. The current with and without a conditioning hyperpolarization cannot be made to superimpose by a simple time translation.

Similar time delays have been seen in the potassium channels of *Myxicola* (Goldman and Schauf, 1973) and crayfish (Shrager, 1975) giant axons. Armstrong and Bezanilla (1974), Keynes and Rojas (1976), and Goldman and Hahin (1977) have reported similar (but smaller) hyperpolarization-induced delays in the Na channels of squid and *Myxicola* axons. This effect does not seem to be present in the K channels of *semitendinosus*[1] or *lumbricalis digiti IV*[2] muscles of *Rana pipiens*.

The study by Cole and Moore (1960) of the time delay in the potassium conductance raised the first serious questions about the general validity of the Hodgkin and Huxley (1952*b*) n^4 formalism. The usual interpretation of the scheme is that the K channel is composed of four identical and independent two-state subunits, each obeying first-order kinetics. Raising n to the 25th power as found necessary by Cole and Moore (1960) suggests 25 identical and independent subunits. Furthermore, in agreement with Palti et al. (1976), I find n^4 to be a poor description of even normal K channel kinetics, and raising n to powers as large as 30 cannot duplicate the long time delays introduced by conditioning hyperpolarizations.

Hill and Chen (1971*b*) considered the results of relaxing the restriction that the channel subunits be independent. For their calculations they assumed various degrees of coupling between subunits. These calculations showed that such coupled models could duplicate the conditioning hyperpolarization-induced delays, but, unlike noncoupled models, the delayed currents could not be made to superimpose on the nonconditioned currents with a simple time translation. Because this appeared to contradict the experiments of Cole and Moore (1960), Hill and Chen (1971*b*) concluded that cooperativity within the K channel was unlikely. Because the results reported here and those of Palti et al. (1976) demonstrate that the delayed currents do not superimpose on the nondelayed currents after a time translation, the possibility of coupled subunits within the K channels of myelinated nerve can no longer be discounted.

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