EFFECT OF CONDITIONING POTENTIAL ON POTASSIUM CURRENT KINETICS IN THE FROG NODE

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ABSTRACT The kinetics of potassium conductance changes were determined in the voltage clamped frog node (*Rana esculenta*), as a function of conditioning prepotential. The conditioning potential duration varied from 1 to 50 ms and the amplitude between −60 and +130 mV (relative to rest). The conductance kinetics were determined at a single test potential of +20 mV (depolarization) by means of the slope of log $[n_\infty - n_t]$ vs. time relationship which defines the time constant of the process (τ). The values of τ, after conditioning hyperpolarizations, were around 5 ms, up to 10 times greater than values obtained following a strong depolarization. The τ vs. prepotential curve was sigmoid in shape. These differences were only slightly dependent on $[K^+]_o$ or conditioning pulse duration. The steady-state current values were also found to be a function of conditioning potential. After conditioning hyperpolarizations, the log $[n_\infty - n_t]$ vs. time curve could not be fitted by a single exponent regardless of the power of n chosen. The prepotential dependency of potassium current kinetics is inconsistent with the Hodgkin-Huxley axon model where the conductance parameters are assumed to be in either one of two possible states, and where the rate of transfer from one state to the other follows first order kinetics. In contrast the described kinetics may be consistent with complex multistate potassium "channel" models or membranes consisting of a number of types of channels.

INTRODUCTION

The membrane ionic conductances as described by the Hodgkin-Huxley axon model for the squid giant axon (Hodgkin and Huxley, 1952), the frog (Doge and Frankenhaeuser 1958), and toad (Dodge and Frankenhaeuser, 1958; Koppenhöfer, 1967; Koppenhöfer and Schmidt, 1968) are based on two-state kinetic models.

Within this framework, sodium and potassium ions can cross the membrane at the specific "channels" only when in a given channel a number (x) of identical channel functional subunits (m, n, or h) are in one of their two possible states. The probability for each subunit to be in a given state is assumed to be independent of the state of other subunits. Thus the fraction of open channels is given by n^x. The channel as a whole, therefore, also has two functional states: open or closed. For the squid potassium channel the value of x is usually taken as 4, while values between 2 and 4 were de-

scribed for frog (Dodge and Frankenhaeuser, 1958) and toad (Koppenhöfer, 1967; Koppenhöfer and Schmidt, 1968).

In the above model, the dimensionless variable n represents the fraction of potassium channel subunits in the activated state. The transfer of a subunit from state to state;

$$n_c \underset{\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} n_o, \tag{1}$$

is formally described by first order reaction kinetics. The rate of transfer is given by:

$$dn/dt = \alpha_n(1 - n) - \beta_n \cdot n, \tag{2}$$

where $1 - n$ is the fraction of inactive subunits and α_n and β_n are voltage-dependent rate constants.

Since these rate constants are a function of momentary voltage only, the previous membrane potential has no effect on their values. Therefore, under step clamp conditions, the time constant which the α's and β's define ($\tau_n = 1/[\alpha_n + \beta_n]$) is determined by the momentary potential only.

However, there are indications in the literature that this fact may not be true. Working with *Xenopus*, Frankenhaeuser (1963) reported: "In the case of β_n its value was higher when it was measured from the current at a repolarization after a brief cathodal step than that obtained after a long lasting cathodal step."

Recently Palti et al. (1975) described evidence indicating multistate processes in the potassium conductance system while Goldman and Schauf (1972, 1973) and Hille (1975) presented evidence indicating the same for the sodium system.

It is the object of this work to investigate in detail, the kinetics of the potassium conductance in the node of Ranvier, in order to determine whether the accepted two-state models or some other kinetic model describes the data best.

METHODS

Single myelinated, motor and sensory, nerve fibers isolated from the frog *Rana esculenta* (Stämpfli, 1952) were mounted and voltage clamped by the procedure described by Nonner (1969). In some of the experiments a silicone grease seal was added to the Vaseline seal placed between pool A and E and the air gap was replaced by a silicone seal (Nonner et al. 1974). The node was externally perfused with either Ringer's solution or Ringer's in which 80 mM of sodium was replaced by an equal amount of potassium. 300 nM tetrodotoxin (TTX) was added to all test solutions and their pH was adjusted to 7.4 by Tris buffer. The temperature of the solution bathing the node was held constant at 15°C.

In between voltage clamp pulses membrane potential was held at its initial resting value, V_H,[1] i.e. the same holding potential was used in all solutions.

[1]All potentials are given relative to the testing potential, depolarization is positive while hyperpolarization is in the negative direction.

From V_H the membrane potential was first stepped to a conditioning prepulse, V_{pp}. The prepulse amplitude varied from −60 to +130 mV in seven steps while its duration, t_{pp}, varied from 1 to 50 ms in eight steps.

At the end of each prepulse the membrane potential was clamped to the same test pulse value, V_p = 20 mV. Pulse interval was 4 s. At the end of each experiment the node was destroyed by a 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, Mass.).

Membrane currents were sampled at 20-μs intervals by means of a 10 bit A/D converter operating also under program control. After initial on-line processing the sampled data was stored on digital magnetic tape for further analysis by means of a computer.

Before each series of pulses the membrane leakage conductance g_L, was determined by means of a hyperpolarizing pulse. Assuming a linear leakage current-voltage relationship that crosses the current axis at $V = 0$, the leakage currents were calculated and subtracted from all measured currents. Potassium inactivation was assumed to be negligible for the pulse durations used.

The current analysis focussed on the currents generated upon stepping membrane potential from V_{pp} to V_p. When generated by depolarizing V_{pp}, these are commonly referred to as "tail currents." Since an adequate amount of TTX was used, the measured currents were assumed to represent potassium currents, I_K.

The potassium current time constants, τ_n, were determined for each t_{pp} and V_{pp} from the exponential segments of the curves (see below) by determining the reciprocal of the slope of the $\log_e[n_\infty - n_{(t)}]$ vs. time curves. The slopes and standard deviations (SD) were determined by the linear regression method. Time was measured from the onset of V_p.

The values of $[n_\infty - n_{(t)}]$ were determined from the potassium current values by means of the following equation:

$$n_\infty - n_{(t)} = A[I_\infty^{1/x} - I_{(t)}^{1/x}] \tag{3}$$

where $I_{(t)}$ and $n_{(t)}$ are the values of potassium current and parameter n at any given time t, I_∞ is the steady-state potassium current (at any V_p) and

$$A = 1/[g_K(V - V_K)]^{1/x}, \tag{4}$$

where g_K and V_K have their usual meaning.

However, since A does not affect the slope of the $\log_e [n_\infty - n_{(t)}]$ vs. time curve, it is not necessary to determine its value. The τ_n computation was repeated for different powers of n, i.e. different values of x (Eq. 3).

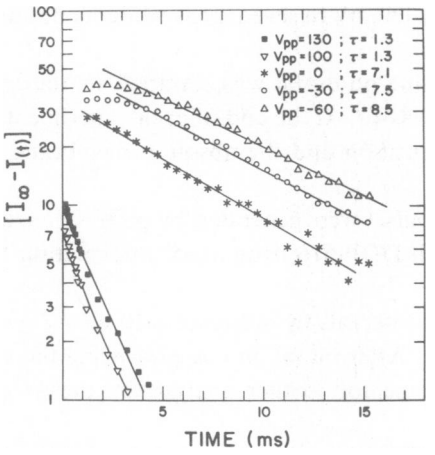

FIGURE 1

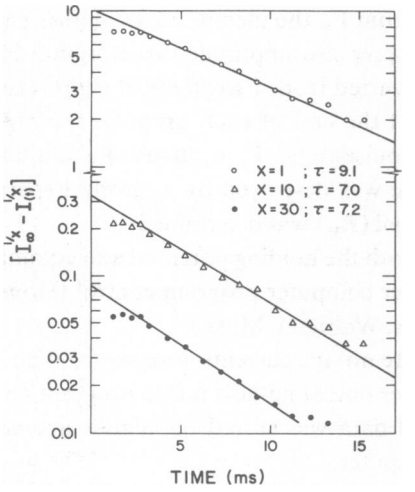

FIGURE 2

FIGURE 1 Ordinate: the log of the difference between steady-state potassium current (I_∞) and the potassium current amplitude at any time t. Abscissa: time. At the time $t = 0$ membrane potential was stepped from various conditioning values (V_{pp}) to a test potential (V_p) of 20 mV. Conditioning pulse duration, t_{pp}, is 50 ms. The given τ values were computed by the least squares method. For V_{pp} values of −60, −30, and 0 mV, log values corresponding to $t > 3$ ms were used (to avoid the nonlinear initial segment of the curve). For V_{pp} values of 130 and 100 mV points beginning with $t > 0.1$ ms were used. Fiber 74/92 in normal Ringer's solution, temperature 15°C.

FIGURE 2 The effect of changing the power (x) to which the potassium conductance parameter n is raised (see Eqs. 3 and 4) on the linearity of the log $[n_\infty - n_{(t)}]$ (given by means of log $[I_\infty^{1/x} - I_{(t)}^{1/x}]$) vs. time relationship. The τ values were calculated by the least squares method as explained in legend of Fig. 1. Note that the initial deviation from linearity does not significantly change when the power varies from 1 to 30. Conditioning potential amplitude = −60 mV, its duration t_{pp} = 50 ms, and V_p = 20 mV. Fiber 74/85, 80 K Ringer's.

RESULTS

Typical log $[I_\infty - I_{(t)}]$ vs. time relationships obtained from a motor fiber externally perfused with Ringer's solution is illustrated in Fig. 1. If we assume that the power of the potassium conductance parameter, n, in Eq. 3 is one ($x = 1$), the ordinate becomes: log $[n_\infty - n_{(t)}]$. The experimental points are seen to fit a linear relationship following depolarizing prepulses. The same is true for hyperpolarizing prepulses except for the initial segment of the curve.

The reciprocal of the slope of this relationship gives the time constant, τ, of the process. Fig. 2 illustrates that the deviation of the initial segment from linearity following hyperpolarizing prepulses can not be corrected by increasing the power of n (increasing x).

The time constant, τ, is seen to vary with the conditioning potential, V_{pp}, in spite of the fact that all measurements were taken at a single test potential, V_p, of 20 mV

TABLE I

AVERAGE τ_n VALUES ± THE AVERAGE OF THE INDIVIDUAL SD OBTAINED IN TTX RINGER'S SOLUTION AND IN 80 K TTX RINGER'S SOLUTION

The number of fibers is given in brackets. Test pulse potential, V_p, was 20 mV and t_{pp} = 50 ms.

$[K^+]$	x	τ_n						
		V_{pp} (mV):						
		−60	−30	0	50	70	100	130
mM								
2.5	1	6.6 ± 0.8 (5)	6.2 ± 1.0 (5)	4.6 ± 1.4 (5)		0.60 ± 0.09 (4)	0.75 ± 0.03 (4)	0.87 ± 0.04 (4)
	2	5.5 ± 0.9 (5)	4.6 ± 1.0 (5)	4.1 ± 1.0 (5)		0.60 ± 0.10 (4)	0.42 ± 0.11 (2)	
	4	4.9 ± 1.0 (5)	4.0 ± 1.1 (5)	3.8 ± 1.2 (5)		0.59 ± 0.10 (4)	0.36 ± 0.12 (2)	
80	1	6.3 ± 0.8 (5)	6.7 ± 1.0 (5)	6.8 ± 1.7 (5)	1.5 ± 0.09 (6)	1.8 ± 0.07 (6)	1.6 ± 0.06 (6)	1.7 ± 0.05 (6)
	2	5.4 ± 0.9 (5)	5.7 ± 1.6 (5)	5.7 ± 1.5 (5)	1.7 ± 0.09 (6)	1.0 ± 0.07 (6)	1.9 ± 0.07 (6)	2.0 ± 0.05 (6)
	4	5.0 ± 1.0 (5)	5.3 ± 1.1 (5)	5.5 ± 1.5 (5)	1.8 ± 0.09 (5)	2.3 ± 0.07 (6)	2.2 ± 0.06 (6)	2.2 ± 0.06 (6)

(E_p = −50). Note that the slopes tend to form two groups, one for hyper- and the other for depolarizing prepotentials.

The average τ values and their corresponding standard deviations obtained from a number of fibers under different conditions (in Ringer's) are given in Table I. It is seen that irrespective of the choice of the power of n, the values of τ fall into two groups; following hyperpolarizing conditioning prepulses the τ values are almost 10 times as large as compared with depolarizing prepulses. In view of the given SD, these differences are highly significant. No significant difference was found between sensory and motor fibers.

Note that the small τ values were obtained for prepulse values of 70, 100, and 130 mV, i.e. depolarizations which are associated with outward potassium currents. These currents may result in potassium ion accumulation at the outer surface of the node (Sternheim et al., 1973; Dubois and Bergman, 1975; see also Hille, 1976). However, the larger τ values were obtained using prepulses (V_{pp} = −30 and V_{pp} = −60 mV) which are associated with inward as well as outward potassium current (V_{pp} = 0 mV). Thus, it seems that the changes in τ are not related to current direction or to potassium ion concentration changes.

The experiments were repeated in 80 mM potassium Ringer's (Table I). It is seen that under these conditions the average τ_n values are also significantly higher after conditioning hyperpolarizations (see also Fig. 3). Moreover, for all conditioning prepulses the τ values are seen to be higher in high potassium as compared with low potassium Ringer's. This finding contradicts the possibility that the low τ values obtained after depolarizing conditioning potentials result from potassium ion accumula-

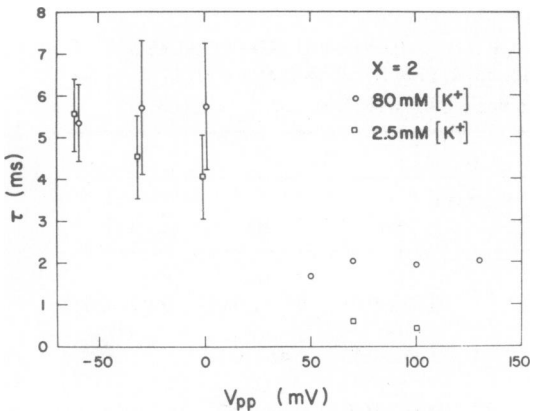

FIGURE 3

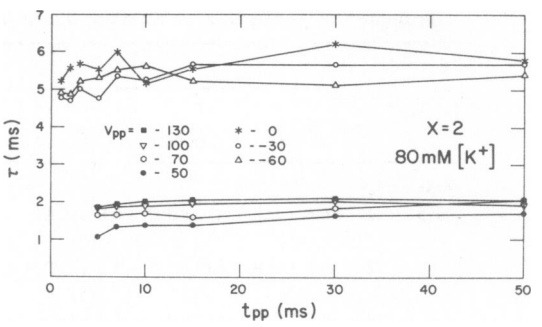

FIGURE 4

FIGURE 3 Average values of the potassium conductance time constant, τ, as a function of conditioning potential, V_{pp} (±SD). Values are based on Tables II and III. Conditioning pulse duration, t_{pp} = 50 ms, V_p = 20 mV. The standard deviations for depolarizing V_{pp} are too small to plot (under 0.15 ms). Note higher τ values in 80 mM K.

FIGURE 4 Average values of potassium conductance time constant, τ, as a function of conditioning pulse duration (t_{pp}) for various conditioning potential values (V_{pp}). Fibers in 80 K Ringer's. Power of n, x = 2. Values are based on Tables II and III. Each point represents the average from five to six fibers.

tion external to the nodal membrane. Note also that the group of small τ includes values obtained after outward (V_{pp} = 70, 100, and 130 mV) as well as inward (V_{pp} = 50 mV) potassium currents.

Fig. 3 illustrates the dependency of τ on conditioning prepulse values for a constant t_{pp} of 50 ms. It is seen that for both low and high potassium Ringer's solutions the τ values fall into two groups, one for hyperpolarizing and the other for depolarizing prepulses. Within each group the voltage dependency is relatively small. In the intermediate prepulse range, i.e., 0–50 mV, the current changes were too small to allow for proper determination of the τ values. Note that for depolarizing V_{pp} the SD values are too small to show.

Fig. 4 illustrates a typical τ vs. t_{pp} (prepulse duration) plot in 80 K Ringer's. The average τ values (± SD) are given as a function of prepulse duration in Tables II and III for different powers of n in normal and 80 mM K Ringer's. When V_{pp} is in the depolarizing direction the τ values seem to increase with t_{pp}, in both solutions. Note however, that when t_{pp} is shorter than about 5 ms the currents become too small for proper τ determination. When V_{pp} is in the hyperpolarizing direction the changes in the τ values are not significant. This is illustrated by the fact that in this case the variations in τ are similar to those obtained with V_{pp} = 0, i.e. with no prepulse at all.

The initial deviation of the log ($I_\infty - I_t$) vs. time relationship from linearity in the case of hyperpolarizing prepulses, cannot be simply related to increased delay in onset of potassium currents (the "Cole-Moore effect"). Here, in contrast to the findings in

TABLE II

THE AVERAGE VALUES OF THE POTASSIUM CONDUCTANCE TIME CONSTANT, τ, AS A FUNCTION OF CONDITIONING PULSE DURATION (t_{pp}) AND AMPLITUDE (V_{pp})

The standard deviations are given with each value and the number of fibers from which the average was calculated is given in brackets. The τ values were determined from the slopes of the log $[I_\infty^{1/x} - I_{(t)}^{1/x}]$ vs. time relationships as given in Figs. 1 and 2. The values were calculated for three powers (x) of the potassium conductance parameters n (x = 1, 2, 4). Values with SD of the same order as τ were rejected. Values obtained in 2.5 mM K Ringer's solution.

V_{pp}	x	t_{pp} (ms)								
		1	2	3	5	7	10	15	30	50
mV										
130	1				0.68 ± 0.03 (4)	0.73 ± 0.02 (4)	0.77 ± 0.03 (4)	0.77 ± 0.02 (4)	0.86 ± 0.02 (4)	0.87 ± 0.02 (4)
100					0.61 ± 0.04 (4)	0.62 ± 0.03 (4)	0.62 ± 0.06 (4)	0.76 ± 0.04 (4)	0.72 ± 0.03 (4)	0.75 ± 0.03 (4)
70					0.92 ± 0.15 (1)	0.52 ± 0.13 (3)	0.48 ± 0.12 (4)	0.54 ± 0.10 (3)	0.55 ± 0.07 (4)	0.60 ± 0.09 (4)
0		5.4 ± 1.4 (5)	5.4 ± 1.3 (5)	5.7 ± 1.4 (5)	4.9 ± 1.4 (5)	4.7 ± 1.4 (5)	5.3 ± 1.4 (5)	5.0 ± 1.3 (5)	4.8 ± 1.3 (5)	4.6 ± 1.4 (5)
−30		5.1 ± 1.3 (5)	5.1 ± 1.1 (5)	5.4 ± 1.2 (5)	6.3 ± 1.1 (5)	5.4 ± 1.0 (5)	5.7 ± 1.0 (5)	5.8 ± 1.0 (5)	6.7 ± 0.9 (5)	6.2 ± 1.0 (5)
−60		4.9 ± 1.2	6.7 ± 1.1	6.1 ± 1.0	6.3 ± 1.1	5.8 ± 0.9	6.5 ± 0.9	5.9 ± 1.0	5.6 ± 0.9	6.6 ± 0.8
100	2				0.42 ± 0.04 (1)	0.44 ± 0.05 (1)		0.52 ± 0.06 (1)	0.50 ± 0.04 (1)	0.42 ± 0.11 (2)
70						0.30 ± 0.18 (2)	0.26 ± 0.10 (2)	0.26 ± 0.13 (2)	0.31 ± 0.30 (3)	0.60 ± 0.10 (4)
0		4.8 ± 1.4 (5)	4.7 ± 1.3 (5)	5.0 ± 1.4 (5)	4.5 ± 1.4 (5)	4.3 ± 1.4 (5)	4.7 ± 1.2 (5)	4.2 ± 1.3 (4)	4.0 ± 1.1 (4)	4.1 ± 1.0 (4)
−30		4.5 ± 1.3 (5)	4.3 ± 1.2 (5)	4.7 ± 1.3 (5)	5.1 ± 1.2 (5)	4.5 ± 1.1 (5)	4.6 ± 1.1 (5)	4.6 ± 1.2 (5)	5.1 ± 0.9 (4)	4.6 ± 1.0 (4)
−60		4.3 ± 1.2 (5)	5.3 ± 1.3 (5)	4.9 ± 1.2 (5)	5.0 ± 1.2 (5)	4.6 ± 1.0 (4)	4.5 ± 0.8 (4)	4.5 ± 1.2 (2)	4.9 ± 0.8 (2)	5.5 ± 0.9 (2)
100	4				0.41 ± 0.05 (1)	0.42 ± 0.06 (1)	0.27 ± 0.12 (1)	0.47 ± 0.06 (1)	0.45 ± 0.05 (1)	0.36 ± 0.12 (2)
70						0.30 ± 0.18 (2)	0.26 ± 0.10 (2)	0.26 ± 0.13 (2)	0.29 ± 0.12 (4)	0.59 ± 0.10 (4)
0		4.5 ± 1.5 (5)	4.5 ± 1.3 (5)	4.7 ± 1.4 (5)	4.2 ± 1.2 (5)	4.0 ± 1.4 (5)	4.4 ± 1.5 (5)	3.7 ± 1.5 (5)	3.8 ± 1.2 (4)	3.8 ± 1.2 (4)
−30		4.1 ± 1.4 (5)	3.9 ± 1.2 (5)	4.3 ± 1.3 (5)	4.6 ± 1.3 (5)	4.1 ± 1.2 (5)	4.1 ± 1.1 (5)	4.1 ± 1.2 (5)	4.5 ± 1.0 (4)	4.0 ± 1.1 (4)
−60		3.8 ± 1.3 (5)	4.8 ± 1.3 (5)	4.4 ± 1.2 (5)	4.4 ± 1.2 (5)	4.1 ± 1.0 (4)	3.9 ± 0.9 (4)	4.0 ± 1.2 (2)	4.3 ± 0.9 (2)	4.9 ± 1.0 (2)

squid giant axons where the curves can be made to superimpose by translating them on the time axis (Cole and Moore, 1960), the shape of the curves changes and thus the current tracings cannot be superimposed by any translation. This finding is true for both low (Fig. 5A) and high (Fig. 5B) potassium concentrations. The change of shape was similar for test potential values, V_p, in the range of 20–90 mV.

The potassium current vs. time tracing obtained at the termination of hyperpolarization, varied with prepulse duration, t_{pp}. The longer the conditioning hyperpolarization, the slower the onset of potassium current (which is outward in 2.5 K Ringer's (Fig. 6A) and inward (Fig. 6B) in 80 K Ringer's). Again the shape of the curves changes such that translocation along the time axis could not result in superimposition. It is obvious that the curves are not simple exponent functions and therefore one cannot describe them by a single time constant. Moreover, the linearity at the initial segment of the log $[I_\infty^{1/x} - I_{(t)}^{1/x}]$ vs. the time curve does not significantly improve when the power, i.e., x, is increased (Fig. 2).

TABLE III

THE AVERAGE VALUES OF THE POTASSIUM CONDUCTANCE TIME CONSTANT, τ, GIVEN AS A FUNCTION OF CONDITIONING POTENTIAL DURATION (t_{pp}) AND AMPLITUDE (V_{pp}).

The standard deviation is given with each value and the number of fibers from which the average was calculated is given in brackets. Values obtained in 80 mM K Ringer's solution. Other detail as in Table II.

		t_{pp} (ms)								
V_{pp}	x	1	2	3	5	7	10	15	30	50
mV										
130	1				1.50 ± 0.05 (6)	1.43 ± 0.04 (5)	1.62 ± 0.05 (6)	1.69 ± 0.06 (6)	1.72 ± 0.08 (6)	1.67 ± 0.05 (6)
100					1.46 ± 0.05 (6)	1.48 ± 0.04 (6)	1.50 ± 0.04 (6)	1.55 ± 0.05 (6)	1.65 ± 0.06 (6)	1.58 ± 0.06 (6)
70					1.46 ± 0.06 (6)	1.43 ± 0.06 (6)	1.49 ± 0.07 (6)	1.34 ± 0.06 (6)	1.51 ± 0.08 (6)	1.75 ± 0.07 (6)
50					0.95 ± 0.10 (6)	1.08 ± 0.13 (6)	1.04 ± 0.11 (6)	1.22 ± 0.11 (6)	1.45 ± 0.08 (6)	1.48 ± 0.09 (6)
0		5.6 ± 1.2 (5)	6.0 ± 1.3 (5)	6.2 ± 1.2 (5)	6.0 ± 1.4 (5)	6.5 ± 1.3 (5)	5.6 ± 1.4 (5)	5.9 ± 1.3 (5)	6.2 ± 1.4 (5)	6.9 ± 1.7 (5)
−30		5.3 ± 1.1 (5)	5.3 ± 1.2 (5)	5.6 ± 1.0 (5)	5.4 ± 0.9 (5)	6.1 ± 1.0 (5)	6.0 ± 1.0 (5)	6.5 ± 1.0 (5)	6.6 ± 1.0 (5)	6.7 ± 1.0 (5)
−60		5.2 ± 1.0 (5)	5.5 ± 0.8 (5)	5.9 ± 0.9 (5)	6.1 ± 0.9 (5)	6.3 ± 1.0 (5)	6.5 ± 0.9 (5)	6.0 ± 0.8 (5)	6.0 ± 0.8 (5)	6.3 ± 0.8 (5)
130	2				1.84 ± 0.05 (6)	1.77 ± 0.04 (5)	1.98 ± 0.05 (6)	2.06 ± 0.07 (6)	2.10 ± 0.08 (6)	2.04 ± 0.05 (6)
100					1.87 ± 0.04 (6)	1.87 ± 0.05 (6)	1.88 ± 0.05 (6)	1.94 ± 0.05 (6)	2.04 ± 0.07 (6)	1.93 ± 0.07 (6)
70					1.63 ± 0.07 (6)	1.65 ± 0.06 (6)	1.69 ± 0.07 (6)	1.58 ± 0.06 (6)	1.83 ± 0.09 (6)	2.04 ± 0.07 (6)
50					1.05 ± 0.10 (6)	1.31 ± 0.13 (6)	1.37 ± 0.11 (6)	1.39 ± 0.11 (6)	1.64 ± 0.10 (6)	1.68 ± 0.09 (6)
0		5.2 ± 1.2 (5)	5.6 ± 1.3 (5)	5.7 ± 1.3 (5)	5.5 ± 1.4 (5)	6.0 ± 1.3 (5)	5.2 ± 1.4 (5)	5.5 ± 1.3 (5)	6.2 ± 1.5 (5)	5.7 ± 1.6 (5)
−30		4.8 ± 1.1 (5)	4.7 ± 1.1 (5)	5.0 ± 1.1 (5)	4.8 ± 0.9 (5)	5.4 ± 1.1 (5)	5.2 ± 1.0 (5)	5.7 ± 1.0 (5)	5.7 ± 1.0 (5)	5.7 ± 1.6 (5)
−60		4.9 ± 1.1 (5)	4.8 ± 0.9 (5)	5.2 ± 0.9 (5)	5.3 ± 1.0 (5)	5.5 ± 1.1 (5)	5.6 ± 1.0 (5)	5.2 ± 0.8 (5)	5.1 ± 0.9 (5)	5.4 ± 0.9 (5)
130	4				1.94 ± 0.05 (6)	1.89 ± 0.04 (5)	2.16 ± 0.05 (6)	2.25 ± 0.05 (6)	2.43 ± 0.09 (6)	2.2 ± 0.06 (6)
100					2.09 ± 0.06 (6)	2.12 ± 0.05 (6)	2.09 ± 0.05 (6)	2.19 ± 0.05 (6)	2.28 ± 0.07 (6)	2.16 ± 0.07 (6)
70					2.00 ± 0.06 (6)	1.95 ± 0.06 (6)	2.03 ± 0.07 (6)	1.83 ± 0.06 (6)	2.02 ± 0.07 (6)	2.30 ± 0.07 (6)
50					1.10 ± 0.10 (6)	1.28 ± 0.13 (6)	1.46 ± 0.11 (6)	1.48 ± 0.11 (6)	1.75 ± 0.10 (6)	1.79 ± 0.09 (6)
0		5.0 ± 1.2 (5)	5.4 ± 1.3 (5)	5.5 ± 1.3 (5)	5.3 ± 1.4 (5)	5.8 ± 1.3 (5)	5.0 ± 1.4 (5)	5.2 ± 1.3 (5)	6.0 ± 1.5 (5)	5.5 ± 1.1 (5)
−30		4.5 ± 1.2 (5)	4.5 ± 1.1 (5)	4.7 ± 1.1 (5)	4.5 ± 1.0 (5)	5.0 ± 1.1 (5)	4.9 ± 1.1 (5)	5.3 ± 1.1 (5)	5.3 ± 1.1 (5)	5.3 ± 1.1 (5)
−60		4.6 ± 1.1 (5)	4.6 ± 0.9 (5)	4.9 ± 1.0 (5)	5.0 ± 1.1 (5)	5.1 ± 1.1 (5)	5.3 ± 1.0 (5)	4.9 ± 0.7 (5)	4.8 ± 0.9 (5)	5.0 ± 1.0 (5)

Note that here the increase in delay obtained upon membrane hyperpolarization was found to vary between 1 and 5 ms, depending on V_{pp} and V_p (15°C). The delays found in the squid giant axon by Cole and Moore (1960) at temperatures of 20°C, were in the order of 0.1 ms.

DISCUSSION

The potassium conductance kinetics were shown to be a function of conditioning potential amplitude. As this fact implies that there is a memory in the conductance

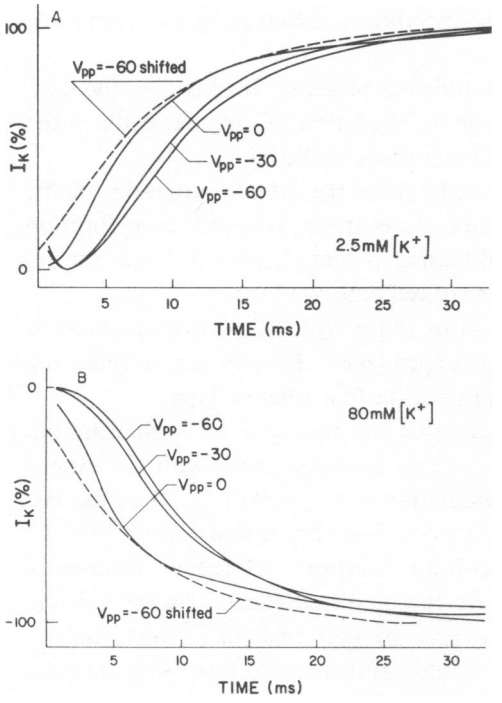

FIGURE 5

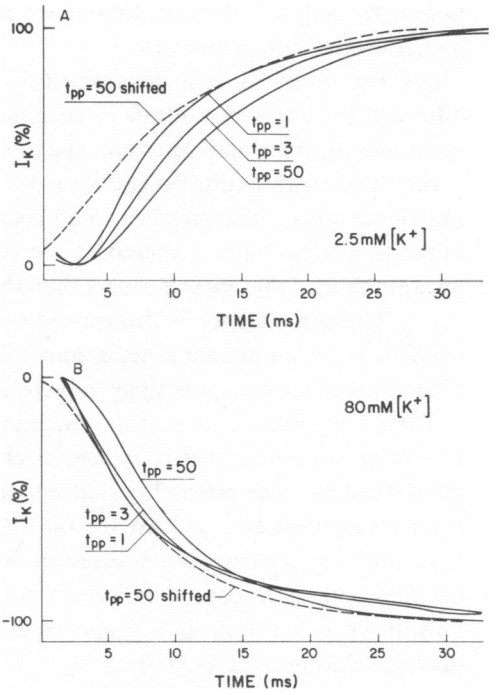

FIGURE 6

FIGURE 5 Tracing of potassium currents (I_K), obtained upon stepping membrane potential from various conditioning potentials V_{pp} to a test value, V_p = 20 mV, as a function of time. The current tracings are given as percentages to allow superimposition. Note that the current kinetics following different V_{pp} are different, such that shifting the current tracings along the time axis does not result in superimposition. In normal Ringer's solution (Fig. 5A) the currents are outward while in 80 K Ringer's (Fig. 5B) the currents are inward; conditioning pulse duration, t_{pp}, was 50 ms. Fiber 74/92.

FIGURE 6 Tracings of potassium currents (I_K), obtained from conditioning potential V_{pp} of −60 mV of duration t_{pp} = 1, 3, and 50 ms to V_p = 20 mV, as a function of time. Fiber 74/88. Other details as in Fig. 5.

mechanism it is inconsistent with Hodgkin-Huxley type two-state membrane functional units (see Introduction). In such models the rate of conductance change is determined by the kinetics of transfer of the channel functional elements from one state to the other. As this rate is determined only by the prevailing conditions (potential, temperature etc.) the kinetics of conduction change are not affected by the conditioning pulse. Thus a conditioning pulse may only result in a change of the initial conditions, i.e. the number of available units.

There are a number of possible mechanisms which may render the conductance kinetics dependent on previous events:

(*i*) The conditioning potential affects some factor, other than the ion conductance mechanism, but which may affect the channel conductance. For example, accumula-

tion of K^+ or Ca^{++} in or at the surface of the membrane, a change in the membrane matrix around the channel, etc.

(*ii*) The conductance is determined by a multistate process. In this case the conditioning potential may result in accumulation or depletion of an intermediary the quantities of which may determine the rate of the process studied.

(*iii*) The conditioning potential or ΔV directly alters the kinetic properties of the functional units. Such an effect could take place, for example, when the configuration of the functional units is altered by the conditioning potential, provided that the relaxation time of this effect is longer than the measured time constant.

(*iv*) The conductance is determined by two or more types of potassium channels which may have different kinetics and voltage dependency. In such a case following different conditioning potentials the active units may be of a different type.

There are a number of factors which indicate that the first type of mechanism (an ion concentration or matrix structural change) may be inconsistent with the experimental results. The possibility that ion accumulation is responsible for the observed results was ruled out, at least for the case of potassium, by demonstrating that the phenomena in question are independent of current direction. Moreover, changes in external potassium concentration do not have the predicted effect. As for calcium ions, if we accept that $g_{Ca} \ll g_K$, we may expect external calcium concentration to decrease during hyperpolarizing V_{pp}. This would result in a decrease in potassium current turn-on delay (Moore, 1971) in contrast to the above findings.

A significant effect of a conditioning potential on the membrane matrix does not seem likely as membrane capacity was shown to be little effected by membrane potential (Cole, 1968; Palti and Adelman, 1969).

In the case of simple multistate processes (mechanism no. 2) such as three-state process, one can usually expect that upon setting up the conditioning potential the quantity of the intermediary, which determine the rate of the process, would initially increase and then eventually decrease. Thus τ should change its value first in one and then in the opposite direction as conditioning pulse duration, t_{pp}, is increased.

One can illustrate this point by means of the following three-state process:

$$A \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} B \underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}} C.$$

The fraction of sites in the active state and their rate of appearance would depend not only on the momentary conditions, but also on previous events, i.e. how many sites were driven into the B or some other state by the conditioning potential.

The description of such a tri-state system can be given by a second order differential equation:

$$d^2C/dt^2 = -(\alpha_1 + \beta_1 + \alpha_2 + \beta_2)(dC/dt) + \alpha_1\alpha_2(A + B + C) - (\alpha_1\alpha_2 + \alpha_1\beta_2 + \beta_1\beta_2)C. \quad (5)$$

In the above case the change of concentration or, in our case, the number of rate-

limiting units, is a complex function of time. It will therefore follow a single exponential function only under conditions where the rate of one of the reactions ($A \rightleftharpoons B$ or $B \rightleftharpoons C$) is much slower when compared with the other.

The fact that the duration of conditioning potential, t_{pp}, had no effect on the rate of the process (Fig. 4) may be taken as a strong argument against this type of mechanism. However, it does not rule out the possibility that a multistate process may explain the experimental data since the resolution of the experimental data did not allow analysis for short t_{pp}.

It is obvious that more complex multistate systems can account for a wide variety of experimental results. However, since the presently available data gives the overall rate of the reaction, without any indication as to the concentration changes of the intermediaries, one can not hope to learn much from fitting the data with various multistate reactions.

Note, however, that a dependency of the rate of a process on previous conditions is usually obtained only for multistate processes involving a closed loop and an irreversible stage (Glansdorff and Prigogine, 1971).

In the case of a direct effect of V_{pp} or ΔV (see Jakobsson, 1973) on the kinetics (mechanism no. 3), not only the time course but also the "steady-state" values of the currents may change. However, assuming that the effect is reversible, the currents will eventually return to their original value with a slow time constant. In view of the experimentally determined I_K, the time constant of such a relaxation must be over 100 ms.

The fourth type of possible mechanism can obviously be made to fit a wide variety of experimental evidence depending on the chosen properties of the different membrane functional units.

Fig. 7 illustrates that during the test pulse the currents tend to reach different steady

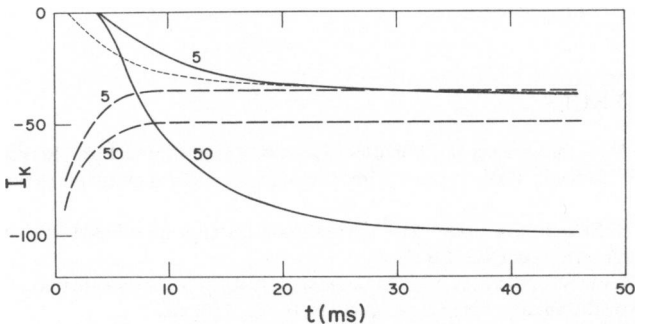

FIGURE 7 Tracings of potassium currents (I_K) obtained upon stepping membrane potential from various conditioning potentials: V_{pp} = −60 mV (interrupted lines), V_{pp} = 130 mV (continuous lines), and V_{pp} = 0, (finely interrupted line), to a test potential, V_p = 20 mV. Numbers denote conditioning pulse durations in milliseconds. Note that the same steady-state values are reached only for short or no conditioning potentials. However, for long t_{pp} the steady-state values differ in spite of the fact that they were obtained at the same membrane potential. Fiber 74/88 in 80 K Ringer's.

states depending on the conditioning potential amplitude and duration. For both hyperpolarizing and depolarizing conditioning potentials of short durations, t_{pp} = 1–5 ms, the steady-state current values are the same as those obtained without conditioning potential. However, following conditioning potentials of longer durations (t_{pp} above 10 ms) the currents reach within about 10 ms different pseudo-steady-state values; which decay towards the base-line current values with a time constant of about a second. Smaller changes in steady-state potassium currents obtained upon strong depolarizations after different hyperpolarizing holding potentials were reported by Moore (1967).

The above finding can serve to differentiate between some of the proposed mechanisms. Multistate reversible processes generally tend to reach the same steady state as determined by the prevailing conditions (Glansdorff and Prigogine, 1971). However, the changes in the steady-state values could be the result of a multistate process which includes an irreversible stage and a branching point or loop.

A membrane system consisting of two or more types of channels of the Hodgkin-Huxley type (two-state first order reversible kinetics) but with different time and voltage dependency will also tend towards a steady state determined by the prevailing conditions. Therefore, it seems that the mechanism which may describe best the given experimental evidence is either a potential effect on the gating mechanism, which is independent of the normal gate behavior or a complex multistate mechanism. Such a multistate mechanism may have a few end products, i.e. types of "open" channels, etc.

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