ANOMALOUS POTASSIUM CHANNEL-GATING RATES AS FUNCTIONS OF CALCIUM AND POTASSIUM ION CONCENTRATIONS

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ABSTRACT With near normal monovalent ionic concentrations, the rate of increase of the potassium conductance after a depolarizing voltage-clamp step is slowed when the external calcium concentration is increased. This trend in the rise-time with changes in calcium is reversed when the axointernal potassium concentration is reduced (150 mM) and the periaxonal concentration is increased (50 mM); that is, the rise-time decreases with increasing calcium concentration. Furthermore, the degree of sigmoidality of the K-conductance time-course always increases when the rise-times increase for a given test potential. In the case of calcium surface-charge screening, these effects may be caused by a shifted distribution of K-ions within the channels following the altered ion gradient, and by a consequent shift in the reciprocal electrostatic interactions between the ionic charges and channel-gate charges.

INTRODUCTION

It has been established for some time that changing the calcium concentration in the bathing solution of neural preparations shifts the apparent voltage dependence of ionic conductances under voltage-clamp conditions (Frankenhaeuser and Hodgkin, 1957). In particular, the rise-time of potassium current behaves as though the membrane potential were increased ~10–15 mV for each five-fold reduction of external Ca^{++}-concentration over the range of 4–112 mM for the squid axon membrane and for the membrane at a node-of-Ranvier (Frankenhaeuser, 1957). The simplest interpretation of this effective shifting along the voltage axis is the screening of membrane surface charge, which is in a position to influence the electric field at the voltage-sensing charges for the channel gate. The reduction of surface charge may involve further the binding of divalent cations to negative polar groups. The degree of charge screening, both with and without binding, is a direct function of the calcium concentration (McLaughlin et al., 1971). As a consequence of incomplete screening, the transmembrane potential difference at the voltage sensor is altered in relation to that imposed by the voltage-clamp electrodes.

Irrespective of surface charge effects, the sigmoidal time-course of the K-ion conductance is primarily due to channel gating, which depends directly on the electric field at or within the membrane (Hodgkin and Huxley, 1952*a*). The kinetics of this gating is well described by a first-order differential equation (for the Hodgkin-Huxley [1952*b*] state variable n) with rate constants that are strictly voltage dependent. In this report we present further results, which indicate that the macroscopic description of this gating depends also on the ionic strength and concentration of the permeant ions (in this case K^+-ions). This result is obtained when both the calcium and potassium concentrations are changed from their normal values.

METHODS

Cleaned single giant axons from the hindmost stellar nerve of the squid *Loligo pealei* were used in this study. Internally perfused axons were voltage clamped by single-pulse and "ric-rac"-pulse (Adelman, 1979) techniques. The clamp was compensated for a series resistance for 2 Ω-cm^2 (Binstock et al., 1975). Temperature was 4.6 ± 0.3°C.

Internal solutions were Na^+-free and contained KF, K-glutamate, and K_2HPO_4 in concentrations of 50, 200, and 25 mM, respectively, giving a total K^+-concentration of 300 mM. 505 mM sucrose was added to maintain osmotic strength. To obtain total K^+-concentrations of 150 and 450 mM, the same proportions of potassium compounds were used, plus sucrose concentrations of 792.5 and 217.5 mM, respectively. Electrochemical effects due to changes in total internal ionic strength are considered in the Discussion. pH was adjusted to 7.2 with free glutamic acid.

Twelve different combinations of external Ca^{++}- and K^+-concentrations were used. The sodium-ion concentration was varied to maintain a constant and full external ionic strength. TTX (0.2 μM) blocked the Na-channels completely. The external solutions are listed in Table I.

The voltage pulses used in the single-pulse technique were depolarizations from a holding potential of −60 mV to −20, +5, +30, and +55 mV axointernal relative to external ground with pulse durations of 18 ms, and a resting period of ≥8 s between pulses. Measured junction potentials were compensated. Ric-rac voltages consisted of a train of low amplitude square-wave voltage pulses centered on the main voltage pulses listed

TABLE I
EXTERNAL SOLUTIONS

K	Ca	Na	Cl
	(*mM*)		
50	2	467	521
	10	455	525
	40	410	540
	100	320	570
10	2	507	521
	10	495	525
	40	450	540
	100	360	570
5	2	512	521
	10	500	525
	40	455	540
	100	365	570

Tris = 20 mM, TTX = 0.2 μM, ionic strength = 1080, pH = 7.4.

above with 5-mV peak-to-peak amplitudes and a square-wave frequency of 2 kHz. Fig. 1 shows sample records. A ric-rac frequency (f) of 2 kHz was chosen so as to be greater than expected values of $2/\tau_n$, and to be such that the membrane capacitative current transient responses decayed toward zero (settling time was <15 μs) in times much less than $1/(2f)$. The 5-mV ric-rac amplitude was chosen so that the plus-or-minus current traces were approximately equidistant from a central current trace generated by the main pulses in the absence of ric-rac.

As the membrane currents recorded with the single-pulse technique could be superposed on the mean membrane currents recorded with the ric-rac technique, both of these techniques were used to calculate the development in time of the chord-potassium conductance (g_K^{chord}) for each main pulse voltage. The time-course of $g_K^{chord}(t)$, defined by

$$g_K^{chord}(t) = I_K(t)/[E - E_K(t)], \quad (1)$$

was computed for the single-pulse current records and for the mean

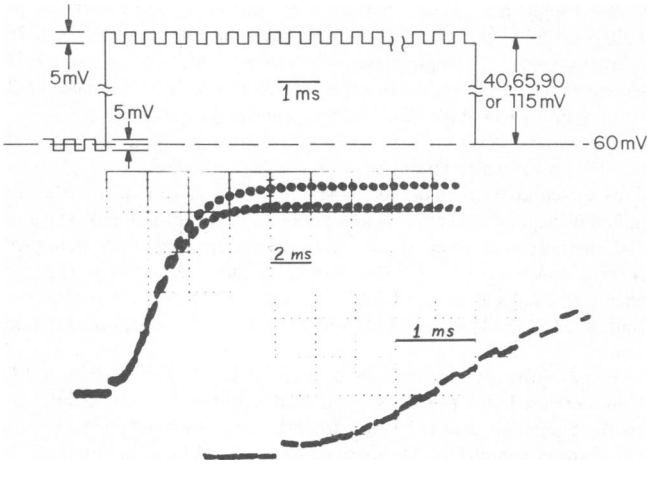

FIGURE 1 (*Top*) Ric-rac voltage-clamp pulse. (*Middle*) Oscilloscope trace showing current requirement for an axon bathed in Mg^{++}-free, TTX "sea water" solutions with $[K^+]_i/[K^+]_e$ = 300 mM/10 mM and $[Ca^{++}]_e$ = 40 mM for a voltage-clamp depolarization to +55 mV at 4.5°C. (*Bottom*) Same as *Middle* except trace is for the initial 4 ms after the onset of depolarization.

current of the ric-rac current traces with the aid of a calculated reversal potential, $E_K(t)$, varying with the accumulation of potassium ions in the periaxonal space that results from sustained outward current flow (Frankenhaeuser and Hodgkin, 1956). $E_K(t)$ was calculated from the relation

$$E_K(t) = \frac{RT}{F} \ln \{[K]_i/[K]_s(t)\}, \quad (2)$$

where $[K]_s(t)$, the variable periaxonal potassium concentration, was computed from the relation

$$[K]_s(t) = [K]_e + \frac{1}{\theta F}\int_{-\infty}^{t} I_K(t') \exp[-(t - t')/\tau]dt', \quad (3)$$

where I_K are measured values of the potassium current, τ (= 11 ms) = θ/P_K^s = time constant of washout from the space, θ (=379 Å) is the space width, and F is Faraday's constant. P_K^s (= 3.41×10^{-4} cm/s) is the permeability to potassium of the diffusion barrier between the periaxonal space and the external bulk solution. Values for these parameters were taken from previous measurements (Adelman et al., 1973; Adelman and FitzHugh, 1975).

As a check on the calculated values of $E_K(t)$, instantaneous I/V curves were measured for each set of ionic concentrations and for E_T = 55 and 5 mV at t = 5 and 15 ms for three of the experimental axons. Measured reversal potentials, listed in Table II, agree with those computed from Eqs. 2 and 3 to within ±3 mV for $[K]_i/[K]_e$ = 450/5, ±2 mV for 300/10, and to within ±1 mV for 300/50 and 150/50.

The ric-rac technique gives small jumps in membrane current corresponding to each of the jumps in the small amplitude voltage train superimposed on the main pulse. This method gives the slope of the instantaneous current/voltage relationship at the mean main pulse potential. This is analogous to a tail current experiment (Hodgkin and Huxley, 1952*a*, Fig. 12; Adelman et al. 1973) in which the instantaneous current difference is measured insofar as the current traces from the single-pulse experiments were equal to the mean ric-rac currents throughout the voltage-clamp pulse. With ric-rac, a continuous record of the instantaneous conductance of the population of open channels is obtained throughout the onset of the potassium current. The instantaneous potassium conductance, g_K, was calculated from the measured $\Delta I(t)$ as

$$g_K(t) \equiv \Delta I(t)/(5 \text{ mV}), \quad (4)$$

with ΔI corrected for leakage current. The results presented in Fig. 2 are derived from these data, which were also crosschecked with conductance data obtained from the instantaneous I/V curves that were measured to

TABLE II
REVERSAL POTENTIALS

		E_T = 55 mV		E_T = 5 mV	
$[K]_i/[K]_e$	[Ca]	5 ms	15 ms	5 ms	15 ms
(*mM/mM*)	(*mM*)		(*mV*)		
300/10	2	−50	−28	−58	−35
	10	−52	−28	−60	−37
	40	−54	−26	−61	−38
	100	−56	−26	−62	−39
150/50	2	−22	−19	−25	−22
	10	−22	−19	−25	−22
	40	−20	−18	−24	−21
	100	−19	−17	−23	−20

Membrane potentials for which the measured instantaneous tail currents were zero after test pulses (E_T) of 5 and 15-ms duration.

construct Table II. Differences of instantaneous conductance, g_K, derived from ric-rac and from the instantaneous I/V curves were <0.5 mS/cm^2 in all cases.

As we were interested in the effects of [Ca^{++}] on the time-course of potassium conductance gating, both $g_K^{chord}(t)$ and the instantaneous conductance, $g_K(t)$ were determined as functions of voltage. The results of these determinations showed a direct correspondence between the time courses of $g_K^{chord}(E)$ and those of $g_K(E)$ for each value of membrane potential (E) and [Ca]. However, the magnitudes of $g_K^{chord}(E)$ and $g_K(E)$ were not equal, which is a consequence of the nonlinear instantaneous I/V curves of the K-channel (Binstock and Goldman, 1971; Begenisich, 1975; Fohlmeister and Adelman, 1981). Nevertheless, the gating times were independent of the definition of conductance for the experimental conditions chosen.

RESULTS

Rise-times of g_K

The times of half-maximum ($t_{1/2}$) of the instantaneous K-conductances (g_K) under voltage clamp are shown in Fig. 2 as functions of external Ca^{++}-concentration. "Half-maximum" of g_K was defined as the midpoint between the value of g_K immediately after depolarization and the value at 18 ms. The four panels plot the $t_{1/2}$ values for four conditions of bulk K-ionic strengths or transmembrane K-ionic gradients. For a given ionic environment and test potential, the values of g_K at t = 18 ms varied somewhat among axons, but $t_{1/2}$ values remained remarkably constant.

Each of the four panels of Fig. 2 shows the $t_{1/2}$ data for instantaneous conductance for four equally spaced test potentials, −20, +5, +30, and +55 mV, intraaxonal relative to external ground. Note the increase (positive slope, Frankenhaeuser and Hodgkin, 1957) in $t_{1/2}$ with increasing [Ca^{++}]$_e$ for both [K$^+$]$_i$/[K$^+$]$_e$ = 300 mM/10 mM and 450 mM/5 mM in the upper panels. This positive slope of $t_{1/2}$ values vs. [Ca^{++}]$_e$ is abolished and becomes negative for [K$^+$]$_i$/[K$^+$]$_e$ = 300 mM/50 mM and [K$^+$]$_i$/[K$^+$]$_e$ = 150 mM/50 mM, as shown in the lower panels of the figure. The adjacent curves correspond to test voltages spaced by 25 mV. Note, therefore, that the shifts in $t_{1/2}$ values are within the range of 10–15 mV for a fivefold change in [Ca^{++}]$_e$ for both [K$^+$]$_i$/[K$^+$]$_e$ = 300/10 and [K$^+$]$_i$/[K$^+$]$_e$ = 150/50, with the shifts going in opposite directions for these two ionic gradients. Furthermore, with the exception of small increases in the values of $t_{1/2}$ for [K$^+$]$_i$/[K$^+$]$_e$ = 300/50, all $t_{1/2}$ values for a given test potential are equal for a calcium concentration of ~25 mM.

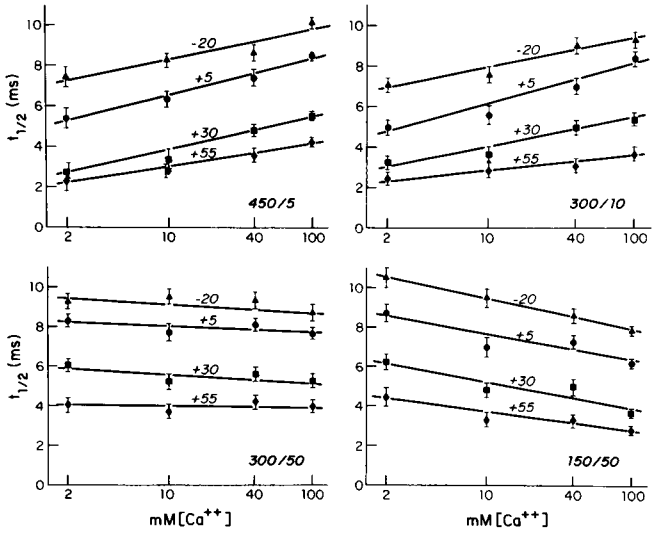

FIGURE 2 Rise-times, $t_{1/2}$, of the K-conductance as functions of log [Ca^{++}] for mean depolarizations to −20, +5, +30, and +55 mV at 4.6 ± 0.3°C. K$^+$-concentrations in mM are [K$^+$]$_i$/[K$^+$]$_e$ = 450/5, 300/10, 300/50, and 150/50, from left to right and top to bottom. Note the systematic change in the slope of the set of curves from positive to negative. Each point represents the averaged data from four axons. The total data base is derived from experiments performed on 12 axons.

Single-pulse current data were obtained for six axons, and chord conductances, $g_K^{chord}(t)$, were computed for a variable E_K as described in Methods. Values of $t_{1/2}$ were determined from $g_K^{chord}(t)$ values for each of the test potentials for the experimental conditions given in Fig. 2. The pattern of $t_{1/2}$ vs. [Ca] as determined from the chord conductances in almost identical to that in Fig. 2.

Sigmoidality of g_K

The degree of sigmoidality of the K-conductance as a function of time appeared to be a continuous function of both [Ca^{++}]$_e$ and [K$^+$]$_i$/[K$^+$]$_e$. Fig. 3 shows two sets of curves, each set for a [Ca^{++}]$_e$-series with fixed K$^+$-bulk concentration. For each set, the initial delay (or initial slope) before the upturn to maximum rate-of-rise is a systematic (monotonic) function of the Ca^{++}-concentration. The trend of this function (monotonically increasing or decreasing) is in opposite directions for [K$^+$]$_i$/[K$^+$]$_e$ = 450 mM/5 mM and for 150 mM/50 mM; the initial slope is greater for the smaller $t_{1/2}$ in each case (Frankenhaeuser and Hodgkin, 1957). The data therefore show a direct correlation between the amount of sigmoidal inflection and the slope of the $t_{1/2}$ curves as functions of [Ca^{++}]$_e$ (compare Figs. 2 and 3). This feature is seen for both the chord conductance g_K^{chord}, and the instantaneous conductance, g_K.

DISCUSSION

Divalent Cation Concentrations

A magnesium-free environment was chosen to eliminate the somewhat uncertain competition effects between the magnesium and calcium divalent ions. In this context, it is interesting to observe that the value of [Ca^{++}]$_e$ = 25 mM, for which the $g_K(t)$ rise-times are roughly independent of potassium concentrations, may be effectively equivalent to the normal divalent ionic environment of the nerve. Following Frankenhaeuser and Hodgkin (1957), who in their summary on the squid axon K-channel say that "magnesium had similar stabilizing effects to calcium but

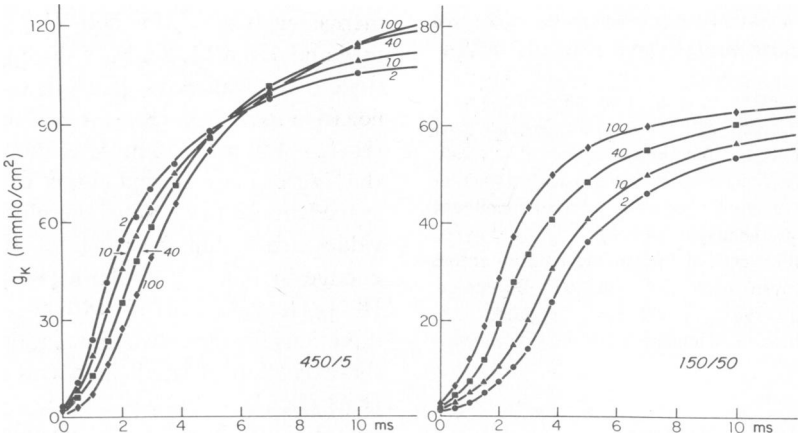

FIGURE 3 Conductances, g_K, as functions of time under ric-rac voltage-clamp to a depolarization of +55 mV for $[K^+]_i/[K^+]_e$ = 450/5 (4.4°C) and 150/50 (4.8°C). The number accompanying each curve is $[Ca^{++}]$ in the periaxonal bathing solution. Data are from two axons. Curves are drawn by eye. Conductance and current levels varied somewhat among axons, but the conductance rise is typical.

was less effective," let us assume that 25 mM calcium is effectively equivalent to the normal 10 mM Ca^{++} plus 50 mM Mg^{++} of sea water. If this is reasonably accurate, the rise-time $t_{1/2}$ is independent of K-ion concentrations near the divalent cation concentrations found in normal sea water. In other words, both the K^+- and Ca^{++}-concentrations must be varied from the normal simultaneously in order to see significant anomalous changes in $t_{1/2}$.

Surface Charge, Surface Potential, and Changes in Ionic Strength

The ionic strength of the external solutions was held constant for all solution changes. Nevertheless, changes in external surface potential that influence the voltage sensing charges for the channel gate may occur by changes in the divalent calcium ion concentration. These changes may be a result simply of surface-charge screening, which has been described by the Grahame equation (Grahame, 1947),

$$\sigma = \left[\frac{kT\epsilon}{2\pi}\sum_i C_i\left(\exp\frac{-z_i e\psi_s}{kT} - 1\right)\right]^{1/2}, \qquad (5)$$

or they may include further specific calcium binding to external surface charges:

$$\sigma = \sigma_o/[1 + K \cdot C_{Ca} \cdot \exp(-2e\psi_s/kT)] \qquad (6)$$

(cf., e.g., McLaughlin et al., 1971) where $kT\epsilon/2\pi = 1.20$ (10^{-5}) for a surface-charge density σ in electronic charges per square Ångstrom, σ_o is surface-charge density in the absence of calcium binding, K is the binding constant in molar^{-1}, C_i is ionic concentration of the ith species in the bulk solution in moles per liter, z_i is ionic valence, kT/e = 23.8 mV at 4.6°C, and ψ_s is the surface potential. The one, or both mechanisms may alter the external surface potential (with changes in calcium) in ways that should be independent of external potassium ion concentration insofar as ionic strength remains constant. At the internal surface, however, ionic strength was varied to permit changes in K-concentration. The addition of sodium ions to maintain ionic strength was considered and rejected because, since sodium has a blocking action on K-channels (French and Wells, 1977), the results would be considerably more difficult to evaluate than changes in the internal surface potential due to changes in ionic strength. Internal surface potential shifts were evaluated with the Grahame equation for the two values of surface charge of $\sigma_i = -e/714$ Å^2 (measured for the sodium channel by Chandler et al., 1965) and $\sigma_i = -e/1600$ Å^2 (Rojas and Atwater, 1968). For the larger charge density, the internal surface potential varies from $\psi_{is} = -13.6$ mV for $[K]_i$ = 450 mM to $\psi_{is} = -16.7$ for $[K]_i$ = 300 and $\psi_{is} = -23.5$ mV for $[K]_i$ = 150 mM. For the smaller σ_i the potentials vary from −6.0 to −7.3 and −10.3 mV, respectively. All potentials are relative to the bulk internal potential. With a surface charge of $\sigma_i = -e/714$ Å^2, the internal surface potential is calculated to be $\psi_{is} = -12$ mV for a full ionic strength of ~1,050 mM. In the worst case, therefore, the voltage-clamp potentials that label the individual curves in Fig. 2 may be reduced by 2 mV for each curve in the panel called 450/5, by 5 mV for 300/10 and 300/50, and by 12 mV for 150/50, all relative to the potentials for full internal ionic strength. However, more likely is the absence of any voltage shift with changes in internal ionic strength. Measurements by Chandler et al., (1965) indicate the virtual absence of internal surface charge near the potassium channel molecule.

Irrespective of the actual magnitude of such voltage shifts, the anomalous behavior of the gating times $t_{1/2}$ vs. [Ca] remains unchanged. A possible explanation of the anomaly may lie in changes of preferential positions of K^+ within closed channels when the K-ion concentration gradient is changed. The electric field component due to

intrachannel ions in the vicinity of the voltage-sensing charges for the channel gates may then be altered significantly. In an aqueous medium (dielectric constant ϵ/ϵ_0 = 80), such ionic fields are 1.1×10^6 V/cm at a distance of 4 Å from the ion's center and will be higher if the dielectric constant of the channel medium is smaller. The reciprocal electrostatic interaction between channel-ion and channel-gate charges may then influence the state of gate conditioning that may precede channel opening. However, detailed descriptions of such phenomena are beyond the scope of the data.

Received for publication 10 November 1980 and in revised form 17 April 1981.

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