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Pressure Dependence of the Potassium Currents of Squid Giant Axon

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Summary. The effect of pressure upon the delayed, K, voltage-clamp currents of giant axons from the squid *Loligo vulgaris* was studied in axons treated with 300 nM TTX to block the early, Na, currents. The effect of TTX remained unaltered by pressure. The major change produced by pressures up to 62 MPa is a slowing down of the rising phase of the K currents by a time scaling factor which depends on pressure according to an apparent activation volume, $\Delta V^{\neq}$, of 31 cm^3/mole at 15 °C; $\Delta V^{\neq}$ increased to about 42 cm^3/mole at 5 °C.

Pressure slightly increased the magnitude, but did not produce any obvious major change in the voltage dependence, of the steady-state K conductance estimated from the current jump at the end of step depolarizations of small amplitude (to membrane potentials, E, $\leqq 20$ mV) and relatively short duration. At higher depolarizations, pressure produced a more substantial increase of the late membrane conductance, associated with an apparent enhancement of a slow component of the K conductance which could not be described within the framework of the Hodgkin-Huxley (HH) n^4 kinetic scheme.

The apparent $\Delta V^{\neq}$ values that characterize the pressure dependence of the early component of the K conductance are very close to those that describe the effect of pressure on Na activation kinetics, and it is conceivable that they are related to activation volumes involved in the isomerization of the normal K channels. The enhancement of the slow component of membrane conductance by pressure implies either a large increase in the conductance of the ionic channels that are responsible for it or a strong relative hastening of their turn-on kinetics.

Key words axon · hydrostatic pressure · K currents · kinetics · activation volume

Introduction

In the preceding paper (Conti, Fioravanti, Segal & Stühmer, 1982) we analyzed the influence of hydrostatic oil pressure up to 62 MPa upon the early phase of the voltage-clamp currents in squid giant axons, dominated by the properties of the Na channels (Hodgkin & Huxley, 1952*a*), and we discussed one possible interpretation of the pressure data based upon the assumption that pressure directly affects the channel gating reaction. In this paper we present a similar analysis for the late component of the voltage-clamp currents, mainly governed by the properties of K channels (Hodgkin & Huxley, 1952*a*). In most of the experiments the dissection of the K currents was achieved by treating the axons with the Na channel blocker tetrodotoxin (TTX), but some data are derived from voltage-clamp currents measured in untreated axons at membrane potential steps to values very close to the reversal potential of the Na currents, E_{Na}.

Previous studies of the pressure dependence of K currents have been reported by Henderson and Gilbert (1975) and by Shrivastav, Parmentier and Bennett (1979) for squid giant axons, and by Harper, MacDonald and Wann (1981) for snail neurons. A preliminary account of our present study has been reported already (Conti, Fioravanti, Segal & Stühmer, 1980) and contained the analysis of analog data in the form of Polaroid pictures obtained from several experiments. The present work contains a more accurate computer analysis of digital data acquired from five different TTX-treated axons.

Materials and Methods

The experiments were performed on giant axons dissected from the squid *Loligo vulgaris*. The axon chamber, the electronic set-up for voltage-clamp measurements, the system for programmable stimulation and digital acquisition of data, and the pressure apparatus were the same as described in detail in the previous paper (Conti et al., 1982). The measurements of K currents in the absence of Na currents were obtained from axons immersed in artificial seawater (450 mM NaCl, 10 mM $CaCl_2$, 1 mM Tris Cl, pH 7.8) containing 300 nM TTX.

Two types of automatic sequences of voltage-clamp pulses were used routinely to characterize K currents at any pressure. The first sequence contained a series of 13 depolarizing pulses with fixed duration (5 to 30 msec, depending on temperature and

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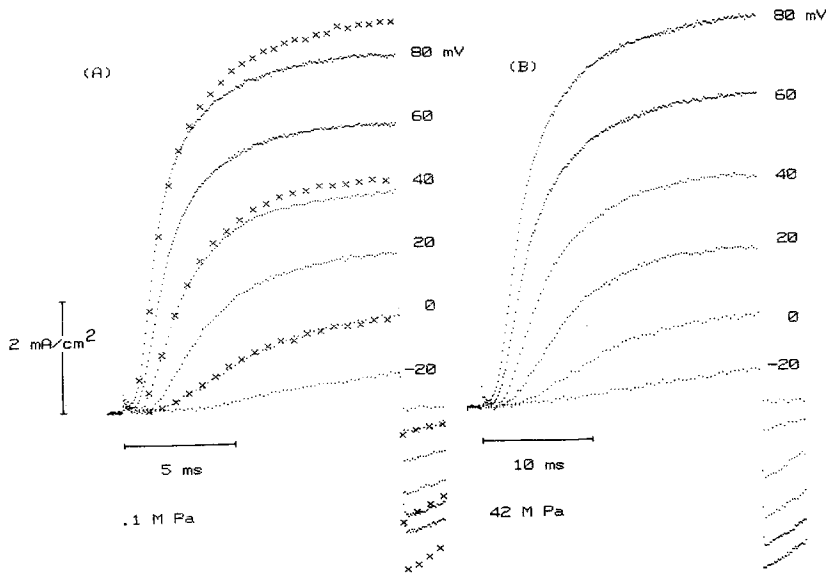

Fig. 1. Change in the K currents of a squid giant axon produced by raising the hydrostatic pressure to 42 MPa. The dotted data are replotted from original digital records obtained at atmospheric pressure (*A*) and at 42 MPa (*B*). The voltages indicated in the figure give the membrane potential during the voltage-clamp depolarizations which produced the respective responses. Note that the time scale in *B* is twice as large as in *A*. The symbols in *A* are sampled data from the corresponding curves in *B* plotted with the same time scale as in *B*. The figure also shows the initial phase of the tail inward currents which follow the membrane repolarization to the holding potential ($E_H = -80$ mV). The amplitude of the tail curents following large depolarizations is much higher at 42 MPa as a consequence of the greater accumulation of K ions in the extracellular space produced by longer clamp pulses. Axon 5; $T = 5$ °C

pressure) from a holding potential, E_H, of -80 mV to a membrane potential, E, between -40 and $+80$ mV. The second type of sequence included six series of pulses, each series consisting of six depolarizations of increasing duration from $E_H = -80$ mV to a fixed membrane potential in the range of -40 to $+60$ mV (a total of 36 pulses in each sequence).

For the analysis of the kinetics of the K currents generated by the first type of pulse routine, the time, t_f, to the inflection point of the rising phase of the K currents was measured from the least squares fit of the data with a cubic parabola. The currents produced by the second type of pulse sequence were measured only during limited time intervals starting shortly before, and ending shortly after, the end of the clamp pulse. After linear extrapolation of the tail currents to the value at the termination of the clamp pulse, estimates were made of the instantaneous membrane conductance, g_K, at the end of each pulse.

Results

Kinetics of K Currents

Figure 1 compares the voltage-clamp currents obtained from a TTX-treated axon at normal pressure (*A*) and at 41 MPa (*B*) for six different depolarizations from a holding potential of -80 mV. The membrane potential, E, during the step depolarization is indicated in the figure next to the corresponding response. The temperature of the preparation in this experiment was 5 °C. It should be first noticed that there is no sign of a decreased efficacy at high pressure of the blocking effect of 300 nM TTX upon Na currents. Note also that the time scale for the data at 41 MPa is twice as large as for the data at normal pressure, showing that the main effect of pressure consisted, in this experiment, in a slowing down of the kinetics of K currents by about twofold. However, a closer inspection of the data shows that a single time scaling factor is not sufficient to account for the effect of pressure upon the entire time course of the voltage-clamp responses to depolarizations more positive than 20 mV. In order to emphasize this aspect, the data of Fig. 1 *B* for $E = 0$, 40, and 80 mV are also replotted in Fig. 1 *A* with different symbols. At 40 and 80 mV the late currents at high pressure are significantly higher than at normal pressure. The discrepancy is even more pronounced if one compares the current jumps produced by the step repolarization at the end of the test pulse, since the tail inward currents of Fig. 1 *B* are clearly much higher than in Fig. 1 *A*. This is a consequence of the fact that the accumulation of K ions in the extracellular Schwann space (Frankenhaueser & Hodgkin, 1956; Adelman, Palti & Senft, 1973) is about twice as large at the end of the voltage-clamp currents of Fig. 1 *B*, the time integrals of which are about twice those of the corresponding curves in Fig. 1 (*A*).

Due to the nonlinear current-voltage characteristic of K channels (*see*, e.g., French & Wells, 1977), an accurate account of the effect of K accumulation would require the full measurement of instantaneous *I-V* curves at several times after the onset of each

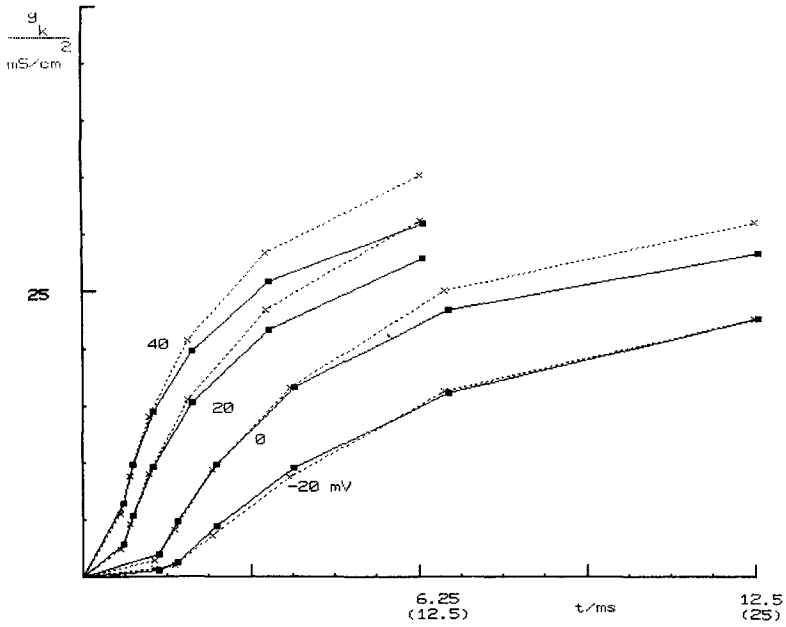

Fig. 2. Effect of pressure upon the kinetics of the rise in K conductance, g_K. The data are estimates of g_K obtained from the current jump at the end of voltage-clamp depolarizations of variable duration, t, to the levels indicated. The time scale out of parentheses refers to the data at normal pressure (■); the time scale in parentheses refers to data obtained at 42 Atm (×). Same axon as in Fig. 1; $T=5$ °C

step depolarization. However, a rough estimate of the time course of K conductance, $g_K(t)$, free of most of the error due to K accumulation, was obtained from measurements of the current jumps at the end of step depolarizations of increasing duration generated according to the second pulse protocol described in Materials and Methods.

Figure 2 shows the results of two such measurements performed on the axon of Fig. 1 at normal pressure and at 41 MPa. The data illustrated are four of the six depolarizations normally contained in our standard sequence of tail current measurement pulses. Each of the two experiments described in Fig. 2 was made shortly after the normal voltage-clamp series shown in Fig. 1. It is evident that the tail measurements yield the same qualitative results as in Fig. 1, but they emphasize the difference between the effects produced by pressure upon the early and late time course of the rise in g_K conductance at large depolarizations.

It is difficult to make an accurate quantitative analysis of the effect of pressure upon the late phase of the rise of membrane conductance at large depolarizations because of the limited duration of our test pulses. When we attempted to fit g_K kinetic data such as those of Fig. 2 according to the classical n^4 description of Hodgkin and Huxley (1952*b*) we found that such a description was fairly adequate only for $E \leqq 20$ mV. At larger depolarizations g_K did not level off at long times as expected from the n^4 kinetics but kept increasing with a much slower time course. This phenomenon was readily apparent at normal pressures also, as has been observed in squid axons by other investigators (e.g., E. Carbone and E. Wanke, *personal communication*), although it has never been described in detail. It is possible that the late rise of g_K is associated with slow K channels similar to those found recently in frog nodes by Ilyin et al., (1980). In the absence of a detailed description of the slow K component in our preparation, the observed enhancement by pressure of this component relative to the faster one is subject to two different interpretations: (i) it might reflect a true increase in the g_K associated with the slow process; (ii) it might result from the fact that the effect of pressure upon the kinetics of the late component is much less pronounced than, or even opposite to, that upon the fast one.

The effect of pressure upon the early kinetics of g_K and/or upon the whole kinetics for relatively small depolarizations can be well-described quantitatively within the framework of the HH scheme by a factoral increase of the K activation time constant, τ_n, which is fairly independent of membrane potential. Measurements of this factoral increase, Θ_n, are reported in Table 1 in three different columns depending on the type of experiment from which it was derived. The data of column 4 were obtained from measurements on unpoisoned axons at the reversal potential, E_{Na}, of Na currents; those in column five were obtained from the normal voltage-clamp series of TTX-treated axons; and column six contains data obtained from tail-current measurements.

The data of columns four and five is the ratio between the time, t_f, to the inflection point of the K currents at pressure P, and that at normal pressure. As we have directly verified in the tail experiments, K accumulation at these early times is quite small.

Table 1. Effect of pressure on the early kinetics of K conductance[a]

Axon	T (°C)	P (MPa)	Θ_n (data at E_{Na} in normal axon)	Θ_n (K currents in TTX)	Θ_n (tail exp. in TTX)	Partial means
Na-6	5	21	1.46			
K-5	5	21		1.37	1.25	1.36
Na-1	10	21	1.37			
Na-2	10	21	1.28			
K-1	10	21			1.20	1.28
Na-5	15	21	1.27			1.27
Mean	8.6	21				1.31 (±0.09)
K-4	2.5	41		2.47		2.47
Na-4	5	41	1.82			
K-5	5	41		1.99	2.02	2.02
K-2	5	41		2.33	1.93	
Na-1	10	41	2.00			
Na-2	10	41	1.93			1.83
K-1	10	41			1.56	
Na-3	15	41	1.60			1.69
Na-5	15	41	1.78			
K-3	18	41		1.62	1.77	1.69
Mean	9.5	41				1.91 (±0.27)
K-5	5	62		2.96	3.64	3.3
Na-1	10	62	2.27			
Na-2	10	62	2.85			2.67
K-1	10	62		2.36	3.17	
Na-5	15	62	2.54			2.54
Mean	9.3	62				2.83 (±0.48)

[a] The data in columns 4, 5 and 6 give the ratio, Θ_n, between the K activation time constant, τ_n, at pressure P and at atmospheric pressure, as estimated from the three different types of experiments described in the text. The error in parentheses is ±SD of all data at any given pressure

Consequently, within the HH scheme t_f is simply proportional to the K activation time constant, τ_n, through the relationship: $t_f = \tau_n \ln 4$. The data in column six of Table 1 was obtained from estimates of τ_n for depolarizations in the range of −20 to +20 mV, obtained from data of the type shown in Fig. 2.

Inspection of Table 1 shows that the measurements of Θ_n obtained with the three different methods are in good quantitative agreement and show no systematic bias dependent upon the method. This is further evidence that the Na blocking efficacy of 300 nM TTX is not appreciably affected by pressure. However, a clear trend of the data obtained at different temperatures indicates that the effect of pressure decreases upon increasing temperature. This is shown by the

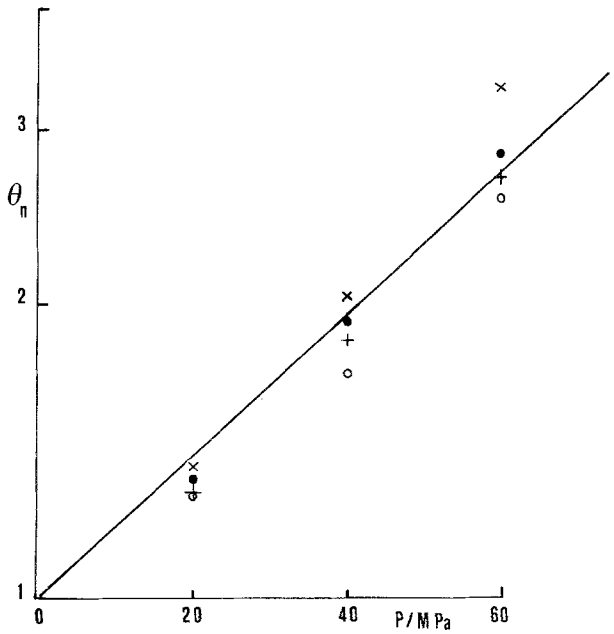

Fig. 3. Semilogarithmic plot of the time scaling factor, Θ_n, as a function of pressure, P, which describes the slowing down of the kinetics of the early rise of g_K. The data points are mean values of Θ_n at 5 °C (×), at 10 °C (+), at 15 °C (○), and overall means of Θ_n from all data (●) (*see* Table 1). The straight line was drawn according to Eq. (1) with $\Delta V^{\neq} = 37$ cm³/mole

partial means reported in the last column of Table 1, evaluated from various measurements at any given temperature, T.

The partial means for $T = 5$ °C, $T = 10$ °C, and $T = 15$ °C, as well as the overall mean values given in the last column of Table 1 are plotted in Fig. 3 as a function of pressure, P. The straight line is the least squares fit of the overall means according to the equation:

$$\ln \Theta_n = P\Delta V^{\neq}/RT \qquad (1)$$

where R is the gas constant and the apparent activation volume, $\Delta V^{\neq}$, is about 37 cm³/mole. The same $\Delta V^{\neq}$ fits the data only at 10 °C, while a better fit for the data at 5 or at 15 °C is obtained with 42 or 31 cm³/mole, respectively.

Steady-State K Conductance

The presence of the slow component of g_K at large depolarizations, which is emphasized at high pressures, prevents an accurate analysis of the effect of pressure on the steady-state properties of K conductance. Such an analysis requires the separation of the slow, late rise of g_K from the faster component using g_K data obtained with much longer depolarizations than those of this study. An empirical description of the effect of pressure on the amplitude of

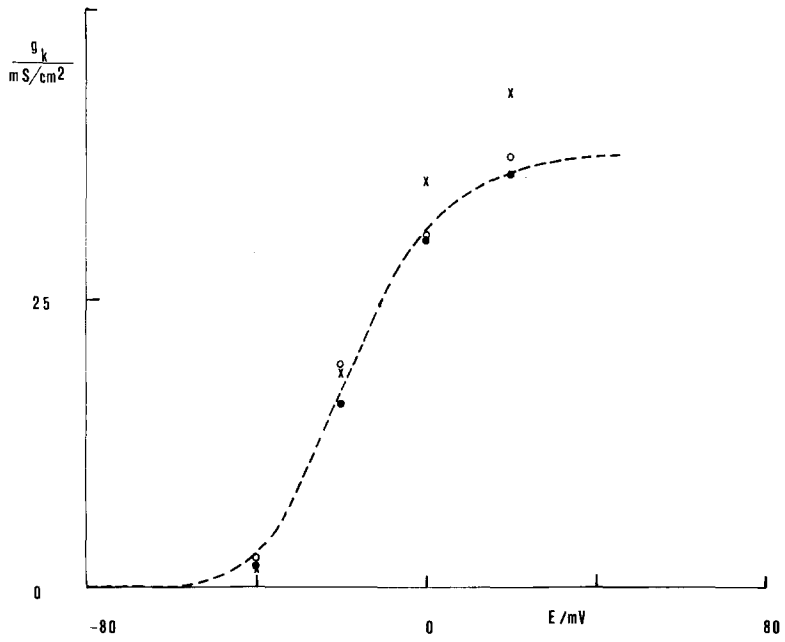

Fig. 4. Effect of pressure upon the estimated steady-state g_K as a function of membrane potential, E. The data were obtained from the fit of the time course of g_K, estimated from tail current measurements, with n^4 HH kinetics. The filled dots and the open circles are data obtained at atmospheric pressure before and after pressurization to 41 MPa, respectively. The theoretical curve is the best fit of the data at normal pressure according to Eq. (2) with $\bar{g}_K = 38$ mS/cm^2, $E_n = -41$ mV, and $U_n = 13.5$ mV (×): data obtained from the same axon at 41 MPa. Axon 3; $T = 18$ °C

the early component of K activation was obtained from estimates of the apparent steady-state K conductance, $g_K(\infty)$, derived from the fit of HH n^4 kinetics to $g_K(t)$ data for $E \leqq 20$ mV and $t \leqq 4\, t_f$.

Figure 4 shows plots of this type of data obtained from a single axon at normal pressure, at 41 MPa, and again at atmospheric pressure about 5 min after decompression. The theoretical line shown in the figure is the best fit of the data at normal pressure according to the equation:

$$g_K(\infty) = \bar{g}_K \{1 + \exp(-(E - E_n)/U_n)\}^{-4}. \quad (2)$$

The parameters used for the fit were: $\bar{g}_K = 38$ mS/cm^2, $E_n = -41$ mV, and $U_n = 13.5$ mV. It is seen that for small depolarizations the data at 41 MPa lie close to that obtained at normal pressure. At larger depolarizations the apparent steady-state g_K is clearly increased by pressure. This phenomenon, which is also clear in the data of Figs. 1 and 2 which were obtained from a different axon and at a different temperature, was invariably observed in all our experiments. A fair fit of the data at 41 MPa in Fig. 4 according to Eq. (2) was obtained with the same values of E_n and U_n used to fit the data at normal pressure and with a $\bar{g}_K$ of 46 mS/cm^2 – 20% larger than at normal pressure. However, due to possible contaminations from the slow component of g_K, the above increase of $\bar{g}_K$ should be considered as an upper limit. Thus, the only reliable conclusion that can be reached from our present analysis is that pressure does not produce any major change in the amplitude and voltage dependence of the fast, HH-like, component of K currents.

Discussion

We have analyzed the reversible changes in the kinetics and amplitude of the K currents in squid giant axons produced by pressures up to 62 MPa, extending by a factor of at least three the range of pressures explored by other authors in the same preparation (Henderson & Gilbert, 1975; Shrivastav et al., 1979) and in snail neurons (Harper et al., 1981). Compared with the previous work on squid axons, our measurements are also free of possible side effects consequent to the use of gas as the pressure transmitting medium (MacDonald, 1975; Mastrangelo, Trudell & Cohen, 1978).

The major effect of pressure is a slowing down of the kinetics of K activation. Qualitatively, our results agree with those reported by Henderson and Gilbert (1975) for squid axons exposed to He pressures up to 21 MPa and by Harper et al. (1981) for the fast component of the delayed outward current in snail neurons exposed to hydrostatic pressures up to 21 MPa. Quantitatively, the data of Table 1 are described by apparent activation volumes which fall at the lower end of the wide range of values reported by Henderson and Gilbert (1975), and ours are almost 50% lower than those found by Harper et al. (1981) in snail neurons.

The pressure dependence of the amplitude of K currents has been the subject of several controversial reports. In squid axons, Henderson and Gilbert (1975) observed a slight decrease of $\bar{g}_K$ at 21 MPa, which they attribute mostly to deterioration of the fibers. In the same preparation, Shrivastav et al. (1979) re-

port an average increase of $\bar{g}_K$ by 11% at 14 MPa, but this result is subject to criticism since the authors observed a hastening of K currents by pressure – contrary to the observations of others. In snail neurons, Harper et al. (1981) found that at 21 MPa the amplitude of the fast component of the delayed outward currents is decreased by about 25%. In all our measurements we consistently observed that for relatively large depolarizations pressure produced an increase of the late g_K. This increase seems mainly associated with the enhancement of a slow component of the outward currents, the importance of which was realized only later during the analysis of the stored data. Due to the limited duration of the test pulses of our experiments, we could find no reliable way to characterize either the kinetics or the amplitude of this component using the data available. For that matter, although the idea that this component is due to K ions seems plausible, we have no proof that this is the case. For example, we have no data concerning the sensitivity of the slow component to pharmacological agents, such as tetraethylammonium, which are known to block the fast K currents.

Whatever its origin, the slow outward current component is likely to be carried through ionic channels which are distinct from the normal K channels, as judged at least by their differential sensitivity to pressure. Indeed, our present data indicate that pressure either increases dramatically the conductance of such channels, or that their kinetic properties are little affected or even hastened by pressure.

In the previous paper (Conti et al., 1982) we argued that the pressures applied in our studies produced no major modification of the lipid environment of the ionic channels. This supports a tentative, but by no means unique, interpretation of our data in terms of a direct pressure dependence of the rection rates which govern conformational changes of K channels. The application of the kinetic theory of chemical reactions (Johnson, Eyring & Polissar, 1954; Johnson, Eyring & Stover, 1974) to a simple HH model of ionic channels has been discussed by Conti et al. (1982). Within that context the observed pressure dependence of the early component of the K conductance would imply that the closed → open transitions of the *n gates* of a K channel involve activation volumes of the order of 37 cm^3/mole at 10 °C. In addition to reservations about its basic assumptions, this interpretation also should be viewed with caution in the light of the limitations in accuracy of the analysis of our experimental data. These include the ambiguity of the dissection of the fast component of K conductance, the known inadequacy of the description of this component according to the HH n^4 kinetics (Cole & Moore, 1960), and the lack of a full quantitative account of K accumulation effects.

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