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Single-Channel Analysis of the Potassium Permeability in HeLa Cancer Cells: Evidence for a Calcium-Activated Potassium Channel of Small Unitary Conductance

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Summary. Cell-attached and inside-out patch-clamp experiments (O.P. Hamill et al., *Pfluegers Arch.* **391**:85–100, 1981) were undertaken in order to characterize the molecular mechanisms responsible for the calcium-dependent potassium permeability observed in HeLa cancer cells. Our result essentially indicate that the HeLa cell external membrane contains potassium channels whose activity can be triggered within an internal calcium concentration range of 0.1 to 1 μM. This particular channel was found to behave as an inward rectifier in symmetrical 200 mM KCl with a conductance of 50 and 10 pS at large negative and large positive membrane potentials, respectively. I/V curves were also measured in 10, 20, 75, 200 and 300 mM KCl and the data interpreted in terms of a one-site-two-barrier model. The channel activity appeared to be nearly voltage independent within the voltage range -100 to $+100$ mV, an increase of P_o, the open channel probability, being observed at large negative potentials only. In addition, the results obtained from inside-out experiments on the relationship between P_o and the cytoplasmic free-calcium concentration have led to conclude that four calcium ions are probably required in order to open the channel. In this regard it was found that an increase of the internal free-calcium level affects more the number of channel openings per second than the actual channel mean lifetime. Finally, it is concluded following a time interval distribution analysis, that this particular channel has at least three closed states and two open states.

Key Words potassium channel · inward rectification · patch clamp · Ca^{2+} activated · HeLa cells

Introduction

It becomes now well established that the potassium permeability of many cell types is controlled to a large extent by internal calcium (*see*, for instance, Meech, 1976; Eckert & Tillotson, 1978; Schwarz & Passow, 1983; Rasmussen & Barrett, 1984). In most cases, this effect has been related to K^+-selective ionic channels, whose activity was shown to be a function of the intracellular calcium concentration (*see*, for a review, Latorre & Miller, 1983; Petersen & Maruyama, 1984). Two distinct classes of calcium-activated potassium channels have been so far reported in the literature: large potassium channels with conductances nearly equal or superior to 200 pS in 140 KCl solutions (Marty, 1981; Pallotta et al., 1981; Barrett et al., 1982; Latorre et al., 1982; Methfessel & Boheim, 1982; Wong et al., 1982; Maruyama et al., 1983) and small potassium channels with conductances ranging from 20 to 50 pS (Lux et al., 1981; Grygorczyk & Schwarz, 1983; Hamill, 1983; Grygorczyk et al., 1984). Although a substantial amount of information has been gathered on the calcium-activated potassium channels of large unitary conductance, very few single-channel studies have been reported on calcium-dependent K^+ channels of smaller conductance.

We have established in a previous work that HeLa cells, a well-known human cell line obtained from an epidermoid carcinoma of the cervix, become hyperpolarized following the addition of the calcium ionophore A23187 to the bathing medium (Roy & Sauvé, 1983). In addition, a recent study by Hazama et al. (1985) has shown that HeLa cells contain H_1 receptors capable of mediating an increase of potassium conductance following an exogenous addition of histamine. Since the stimulation of H_1 receptors has often been linked to a mobilization of internal calcium (Schwartz, 1979; Douglas, 1980), the histamine-evoked hyperpolarization reported by Hazama et al. (1985) is likely to be caused by the presence in the HeLa cell external membrane of calcium-activated potassium channels. K^+ channels of small unitary conductance have already been identified on this preparation (Sauvé et al., 1983; 1984), but the effect at the single-channel level of internal calcium remains still to be fully determined. An electrophysiological study aimed to characterize the molecular mechanisms responsible for the calcium-dependent potassium permeability in HeLa cancer cells was thus undertaken using the extracellular patch-clamp method introduced by Neher et al. (1978) and described later by Hamill et

al. (1981). The results presented in this work essentially indicate that the HeLa cell external membrane contains inward-rectifying calcium-activated potassium channels of small unitary conductance, whose activity can be initiated by four calcium ions at internal free calcium concentrations less than 1 μM.

Materials and Methods

Cell Culture

HeLa cells were obtained from the Institut Armand Frappier in Montreal and subcultured in Falcon bottles (75 cm^2 #3023). The cells were removed from the bottles with 3 ml of a solution containing 0.5 g trypsin (1:250) and 0.2 g EDTA per liter of modified Puck's saline. A stock culture was always maintained in a growing state. The culture medium as MEM Earle base (Gibco #410-1100) with 25 mM Hepes buffer and 6 mM bicarbonate at pH 7.3. This medium was supplemented with 10% fetal bovine serum (Gibco #230-6140) and 10 μg/ml of gentamycin. The cells were grown in monolayers in plastic petri dishes (Falcon #3002) and used for patch experiments two or three days after being subcultured. The culture medium was changed daily, starting from the first day.

Unless specified otherwise, the cell bathing medium used for cell-attached experiments was an Earle-Hepes solution containing (in mM): 121 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$, 6 $NaHCO_3$, 1 NaH_2PO_4, 5.5 glucose and 25 Hepes, buffered at pH 7.3 with 10 NaOH. In inside-out experiments, all our pipette and bath solutions with 150 or 200 mM KCl were buffered at pH 7.3 with 10 mM Hepes plus 4 mM KOH. In experiments in which the K^+ concentration was varied, Na^+ was used as the substituting ion. The total concentration $[K^+] + [Na^+]$ was always equal to 150 mM. The solutions were in that case buffered with 10 mM Hepes plus 4 mM NaOH at pH 7.3. Solutions with a desired free-calcium concentration equal or less than 1 μM were buffered with 1 mM EGTA. $CaCl_2$ was added to the solution to obtain the desired level of free calcium. The stability constants used to calculate the free-calcium concentration were equal to 5.2×10^{10} for the binding of calcium, 2.88×10^9, 7.07×10^8, 478 and 100 for the four hydrogen ions and 1.58×10^5 for magnesium (Fabiato & Fabiato, 1979). All the solutions were passed through a filter of pore size 0.2 μM.

Patch Clamp

The single-channel experiments reported in this work were carried out using the cell-attached or inside-out patch-clamp configuration as described by Hamill et al. (1981). No special enzymatic treatment of the cell external surface was necessary to achieve stable gigaohm seals. The patch electrodes were made of Pyrex® (Corning 7040) and had an electrical resistance ranging from 5 to 10 MΩ in 200 mM KCl. Compared to the patch electrodes obtained from hematocrit capillaries, we found that these pipettes provided lower electrical noise at high frequencies, better seals (average value of 10 gigaohms) and more stable patches when used on HeLa cells. The essential of our electronic setup has been described elsewhere (Sauvé et al., 1983). Let us simply remind that in our case the voltage inside the pipette is always maintained to virtual ground, so that the term "externally applied potential" used in this work refers to the potential of the bath compared to ground. The signal was usually recorded directly on FM tape (H.P. 3964A) at a bandwidth of 1 to 2.5 kHz. If further filtering was necessary, we used a combination of two low-pass four-pole Butterworth filters connected in series (Frequency Devices models 745PB-3, 745PB-5) together with a low-pass four-pole Bessel filter (VVS #300-B). The power spectra were measured using a real time power spectrum analyzer (H.P. 3582) interfaced with our main computer (Digital Minc 11/23). All the records to be analyzed were digitized and stored in a continuous mode on hard disks (Digital RL 01). The sampling rates we used ranged from 2400 to 10,000 pt/sec and corresponded usually to four times the filtering frequency chosen for the signal. All the experiments reported in this work were carried out at room temperature (23°C).

Analysis of the Signal

The analysis of the single-channel recordings was carried out mainly through computerized data processing. Our procedure consisted first in computing for each record stored on disk a current amplitude histogram. Records lasting on the average 60 sec or more were usually used for the analysis. We corrected for baseline drift or undesirable current fluctuations by dividing the entire record into several segments. The final current amplitude histogram was computed by summing all the histograms obtained from selected segments. This histogram was then least-square fitted to a summation of Gaussians. In order to determine P_o, the open channel probability, when more than one channel were present under the patched area, it was assumed that $P(r)$, the probability of having r channels open among N followed a binomial distribution, namely

$$P(r) = \frac{N!}{(N-r)!\, r!} P_o^r \{1 - P_o\}^{N-r} \tag{1}$$

so that an estimate of P_o could be obtained from

$$P_o = 1 - \frac{\sigma_T^2 - \sum_{r=0}^{N} P(r)\, \sigma_r^2}{\{\langle I \rangle - I_0\}\, \Delta I} \tag{2}$$

where σ_T^2 is the total variance of the recorded signal, σ_r^2 the variance due to the noise on the current trace when r channels are open, $\langle I \rangle$ the mean current value of the record, ΔI the amplitude of the current jump and I_0 the current value of the baseline level. For a noiseless patch-clamp signal, the summation term $\Sigma_{r=0}^{N} P(r)\sigma_r^2$ equals zero and Eq. (2) reduces to the usual expression relating current variance to open-channel probability as commonly used in noise analysis (*see*, for instance, Hille, 1984; Chap. 9). This expression is particularly interesting since in cases where $\Sigma_{r=0}^{N} P(r)\sigma_r^2$ is small compared to σ_T^2, one can estimate P_o independently of N. The values of P_o obtained from Eq. (2) were nevertheless usually compared to that predicted from (*see* Patlack & Horn, 1982)

$$P_o = \frac{1}{N} \sum_{r=0}^{N} rP(r). \tag{3}$$

In most cases where the signal-to-noise ratio was high (>3), both

methods gave essentially the same results indicating that the values of N used in Eq. (3) were basically correct.

Open and closed time interval durations were measured using two distinct reference current levels coupled to a minimum time interval criterion. The selected reference levels were computed according to a confidence level parameter α_{co} defined as

$$\alpha_{co} = \frac{P[\text{open}/I\varepsilon\ \{I_{co}, I_{co} + dI\}]}{P[\text{closed}/I\varepsilon\ \{I_{co}, I_{co} + dI\}]} \tag{4}$$

where $P[\text{open}/I\varepsilon\ \{I_{co}, I_{co} + dI\}]$ and $P[\text{closed}/I\varepsilon\ \{I_{co}, I_{co} + dI\}]$ are, respectively, the probability that the channel be open knowing that I is within I_{co}, $I_{co} + dI$ and the probability that the channel be closed knowing that I is within I_{co}, $I_{co} + dI$. A confidence level of 90% was used throughout this work meaning that $P[\text{open}/I\varepsilon\ \{I_{co}, I_{co} + dI\}]$ corresponded to 0.9. The value of I_{co} which satisfied the equality Eq. (4) was taken as reference level for the closed-to-open state transitions. The open-to-closed state transitions were detected using Eq. (4) with α_{co} being replaced by α_{oc} where $\alpha_{oc} = 1/\alpha_{co}$ (*see* Colquhoun & Sigworth, 1983, for a detailed discussion). Unless otherwise specified, time interval histograms were constructed according to a minimum time interval criterion of 0.5 msec and least-square fitted to a summation of exponentials. An analysis of how the open and closed time interval probability density are modified by the omission of time interval smaller than a certain critical value has been presented in detail in a previous work (Roux & Sauvé, 1985).

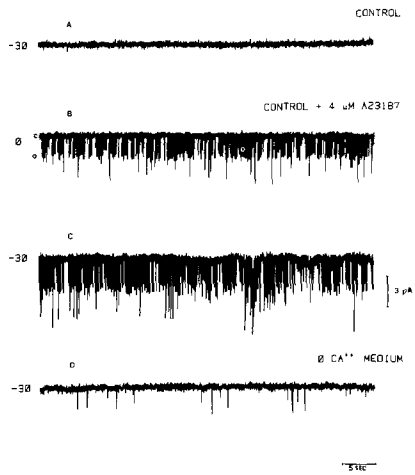

Fig. 1. Single-channel recordings obtained from a cell-attached experiment with a patch electrode containing (in mM) 145 KCl, 1.8 $CaCl_2$, 10 Hepes, 4.2 KOH at pH 7.3. In *A* the external bathing medium was an Earle solution and no single-channel events were apparent. In *B* the external bathing medium was replaced by an Earle solution to which 4 μM of A23187 were added. Following an increase of the Ca^{2+} level inside the cell, single-channel events could then be observed. In *C* we show the effect of the applied voltage on the channel activity. Finally, record *D* illustrates the return to the control case after perfusing the external medium with a calcium-free Earle solution. This procedure led to a drastic decrease in single-channel activity. The current traces were filtered (4 poles Bessel) at 400 Hz. The values on the left correspond to the magnitude in mV of the applied potentials

Results

A) Evidence for Calcium-Activated Potassium Channels

In order to determine if the effect of calcium on the potassium permeability of HeLa cells observed previously (Roy & Sauvé, 1983) was due to potassium channels, cell-attached experiments were performed in which the cell bathing medium was sequentially replaced first by an Earle solution containing 4 μM of the calcium ionophore A23187 and then by a calcium-free Earle medium. The patch electrode was filled with a 150 mM KCl + 2.0 mM $CaCl_2$ solution. Figure 1 illustrates an example of such an experiment. As can be observed, the presence of the calcium ionophore caused, usually within less than 30 sec, a substantial increase in single-channel activity which disappeared following the perfusion of the external medium with a Ca^{2+}-free Earle solution. An increase in intracellular calcium level has thus initiated single-channel-like events whose occurrence was greatly reduced by decreasing the cell internal calcium content using a calcium-free external medium in presence of the calcium ionophore A23187. Let us also mention that during those experiments the applied potential was changed from −30 to 0 mV in order to compensate for the hyperpolarization of the cell observed in the presence of the calcium ionophore A23187 (Roy & Sauvé, 1983). Any major effect of the membrane voltage on the channel activation process was thus eliminated.

We present in Fig. 2 on a different time scale a portion of the single-channel records shown in Fig. 1*C* (*A*) and Fig. 1*D* (*B*). Discrete current jumps of equal amplitude can now be readily seen with sometimes the occurrence of multiple current levels. However, it is particularly interesting to note that despite a large difference in their respective open channel probability (P_o in *A* is equal to 0.07 compared to 0.002 in *B*) the open time intervals are in both cases of comparable values. In fact, an analysis of selected portions of these records where no multiple channel openings could be detected has revealed that the arithmetic mean value of the channel open time was equal to 2.6 msec in *A* compared to 1.7 msec in *B*, while the channel mean closed time changed from 35 msec in *A* to 869 msec in *B*. Therefore, it appears that the main effect of calcium at low cytoplasmic concentration (low value of P_o) is more related to an increase in the number of channel openings per second than to an augmentation of

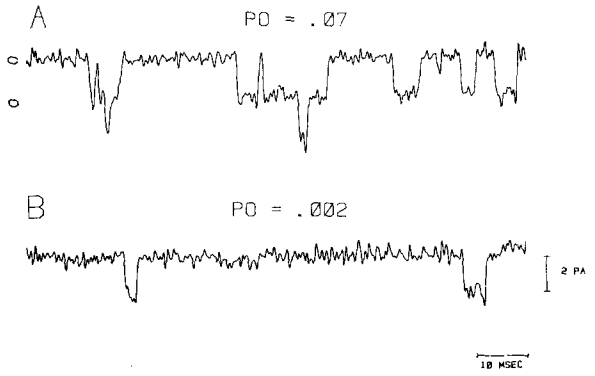

Fig. 2. More detailed description of the single-channel records presented in Fig. 1*C* (*A*) and Fig. 1*D* (*B*). Experimental conditions as described previously for these two current traces in Fig. 1. Despite a large difference in their respective open-channel probability (P_o in *A* is 35 times larger than P_o in *B*), both records show discrete current jumps with open time intervals of comparable value. The increase in P_o induced by internal calcium is thus more directly related to an increase in the number of channel openings per second than to an augmentation of the channel mean open time. The current traces were filtered at 1 kHz (4 poles Bessel)

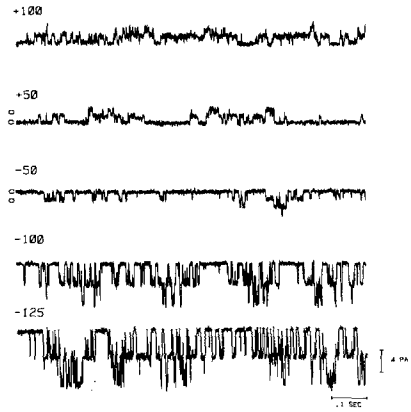

Fig. 3. Voltage effect on the current jump amplitude. Results obtained from inside-out patch experiments with as cytoplasmic medium a solution containing 200 mM KCl, 0.4 μM $CaCl_2$. The patch electrode was filled with a solution of 200 mM KCl, 1 μM $CaCl_2$ and 0.5 mM $MgSO_4$. The numbers shown on the left represent the values in mV of the applied membrane potential (inside the pipette maintained to ground). Note the difference between inward and outward currents for voltages of equal absolute value. The current traces were filtered (4 poles Bessel) at 600 Hz

the channel mean open time. We can also mention that the channel activity in Fig. 1*D* occurred clearly in burst separated by long silent periods (mean closed time > 1 sec). Within a burst, however, shorter closed intervals (<100 msec) could be observed as illustrated in Fig. 2*B*. The channel fluctuation pattern is thus likely to result from a complex kinetic behavior with time constants ranging from msec to sec. Finally, records with three to five equally spaced current levels could routinely be seen, indicating a rather high channel density.

B) Current-Voltage Relationship and Ionic Selectivity

Two different types of experiments were carried out in order to investigate the current-voltage relationship of this calcium-dependent channel in various ionic conditions. First, we used the inside-out configuration with patch electrodes containing NaCl and KCl in various ratios as described in Materials and Methods. In all cases, the cytoplasmic-surface medium consisted of 200 mM KCl with 0.4 or 1 μM $CaCl_2$ plus 0.5 mM $MgSO_4$. Cell-attached experiments were also performed in which the patch pipette contained various KCl solutions as described previously with a cell bathing medium of 200 mM KCl + 2.0 mM $CaCl_2$ to which 4 μM of A23187 were added. We found that both methods gave essentially the same results although the channel conductance was found on the average to be slightly smaller when measured in the excised patch configuration. Figure 3 illustrates the effect of voltage on the single-channel current amplitude. This particular record was obtained in the inside-out configuration with 200 mM KCl + 1 μm $CaCl_2$ and 0.5 mM $MgSO_4$ in the patch electrode. As can be observed, the current jumps in symmetrical K^+ conditions do not have the same amplitude for equivalent positive and negative membrane voltages. In this record for instance, the amplitude for the inward current jumps at −100 mV corresponds to 3.6 pA compared to 1.2 pA for the outward currents obtained at +100 mV. Since the number of detectable channels remains constant for inward and outward currents, it can be reasonably well assumed that current traces such as those shown in Fig. 3 are representative of the same channel population. We present in Fig. 4 the channel *I/V* characteristics with 10, 20, 75, 200 and 300 mM KCl in the patch electrode. The 10 mM KCl experiment was performed in the cell-attached configuration with a potassium bathing solution containing the ionophore A23187 as described above. Clearly the channel *I/V* curves are not linear even in symmetrical 200 mM KCl solutions. The outward current jump amplitude never exceeds 1.5 pA and appears to reach a constant value at large positive potentials. There is therefore a substantial decrease in single-channel conductance within this voltage range. In contrast, the channel *I/V* curves appear linear at large negative potential values but this behavior is likely to be due to the limited voltage range we used. We present in Fig. 5*A* a plot of

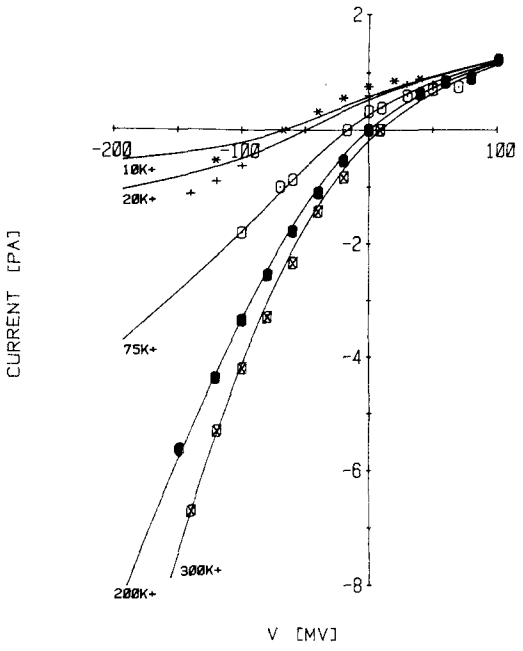

Fig. 4. Single-channel *I/V* curves measured at various external potassium concentrations (10 (*), 20 (+), 75 (□), 200 (■), 300 (⊠) mM). Except for the 10 mM KCl external concentration experiment, all the curves presented in this figure were obtained in the inside-out configuration with a cytoplasmic solution containing 200 mM KCl, 0.4 or 1 μM $CaCl_2$ with 0.5 mM $MgSO_4$, 10 Hepes at pH 7.3. The patch electrodes were filled with solutions of K^+ and Na^+ in various ratios. With 10 mM KCl + 140 mM NaCl in the patch electrode we used the cell-attached configuration with a cell bathing medium containing 200 mM KCl, 1.8 mM $CaCl_2$ at pH 7.3 to which 4 μM of A23187 were added. Under these conditions the cell resting potential was essentially equal to zero. The continuous line represents the theoretical prediction of the two-barrier-one-site model presented in Fig. 12

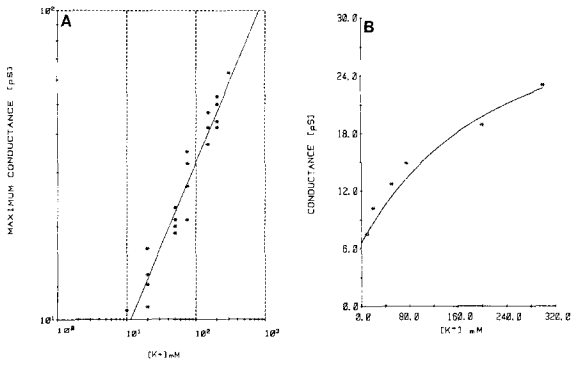

Fig. 5. Variation of the single-channel conductance as a function of external potassium concentration and transmembrane voltage. As seen in *A*, values of the single-channel conductance for inward currents measured within the voltage range −100, −150 mV could be fitted using $\Lambda = \Lambda_o[K]^S$ with $\Lambda_o = 2.3$ and $S = 0.57$. We present in *B* the conductance measured at zero applied potential for various external potassium concentrations. As seen, the half-channel occupancy can be estimated at 38 mM external potassium. The continuous line shows the prediction of the two-barrier-one-site model presented in Fig. 12

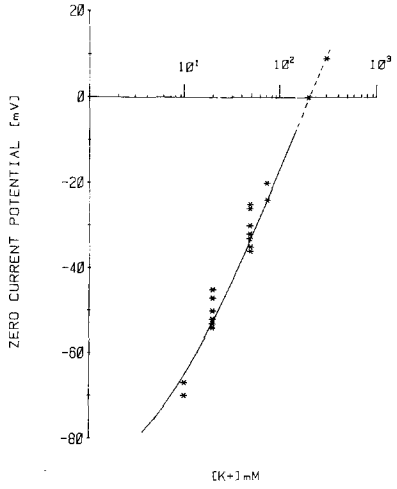

Fig. 6. Variation of the zero-current potential as a function of the external potassium concentration. The values of the reverse potential were computed from *I/V* curves obtained in experimental conditions as described in Fig. 4. The continuous line represents the theoretical prediction of the Goldman-Hodgkin-Katz equation with a ratio $P_{Na}/P_K = 0.03$

Λ, the single-channel conductance for inward currents, measured within the voltage range −100 to −150 mV as a function of $[K^+]$, the external potassium concentration. The experimental points could be fitted using $\Lambda = \Lambda_o [K^+]^S$ with $S = 0.57$ and $\Lambda_o = 2.3$. Thus, the results presented in Fig. 5*A* predict a maximum value of Λ equal to 5.7 pS for a physiological potassium concentration of 5 mM. In order to estimate more precisely the channel ion occupancy, the single-channel conductance at zero applied potential Λ_o^o, was also measured as a function of the external potassium concentration. The values of Λ_o^o were obtained from a linear regression of the *I/V* curves around zero applied voltage. As seen in Fig. 5*B*, the maximum conductance estimated under these conditions reaches a value slightly higher than 23 pS with a half-channel occupancy at 38 mM external potassium. The extrapolated value of Λ_o^o at zero external potassium equals 6 pS and corresponds to the conductance associated with a unidirectional flux with 200 mM KCl as internal medium.

In order to determine the channel ionic selectivity, extrapolated values of zero-current potential obtained at various K^+/Na^+ ratios were plotted as a function of the potassium concentration. The resulting experimental points are shown in Fig. 6. The continuous line describes the theoretical prediction

Table. Binomial prediction for four channels[a]

j	$P_o = 0.24$		$P_o = 0.14$	
	Probability		Probability	
	Observed	Predicted	Observed	Predicted
0	.306	.333	.546	.547
1	.446	.421	.353	.356
2	.206	.199	.093	.086
3	.033	.042	.006	.009
4	.006	.003	0	.0004

[a] Comparison between the observed probability of having j channels open among N ($N = 4$) and the theoretical prediction of Eq. (1) with $P_o = 0.14$ and $P_o = 0.24$ as computed from Eqs. (2) and (3). The observed probability was computed from 150 independent observations of current amplitude taken from single-channel records measured in the inside-out configuration with 200 mM KCl, 1 μM $CaCl_2$ in the patch electrode and 200 mM KCl, 0.4 μM $CaCl_2$ in the cytoplasmic medium. A χ^2 analysis indicates that Eq. (1) can be used with a degree of confidence greater than 97%.

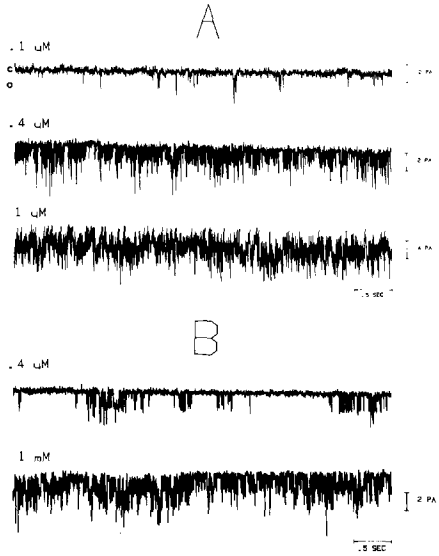

Fig. 7. Response of the calcium-activated potassium channel to an increase in intracellular calcium concentration. Records obtained in the inside-out configuration with a patch electrode containing 200 mM KCl, 1 μM $CaCl_2$ plus 0.5 mM $MgSO_4$. The cytoplasmic medium was a solution of 200 mM KCl, 0.5 mM $MgSO_4$ with various calcium-free concentrations buffered with 1 mM EGTA. The applied potentials were −50 mV. The current traces were filtered at 500 Hz (4 poles Bessel). As seen in *B*, a cytoplasmic concentration of calcium as large as 1 mM does not induce long channel openings. The number of channels was greater than 5 in *A* and equal to 3 in *B*

of the Goldman-Hodgkin-Katz equation with a ratio $P_{Na}/P_K = 0.03$. We should mention, however, that values of P_{Na}/P_K ranging from 0.02 to 0.04 would also be compatible with our experimental results. Clearly the calcium-activated channel present in HeLa cells is mainly potassium selective.

C) Are Calcium-Activated Channels Independent?

Since more than 95% of our experimental current records showed multi-channel events, it became important to establish quantitatively if the channels in a membrane patch have the same open-channel probability and act independently of one another. In order to test this hypothesis a χ^2 analysis was undertaken assuming that the probability of having r channels open among N was given by Eq. (1) with P_o estimated from Eqs. (2) and (3). The number of degrees of freedom used to compute the χ^2 function was taken as the number of detected current levels (number of channels). The test was based on 150 independent measurements of the current amplitude chosen so that there would be no time correlation among the different selected points. This latter point is particularly important since only independent measurements of current amplitude contribute to the precision at which P_o can be estimated. A good fit procedure of current amplitude histograms by a summation of Gaussians does not therefore imply that the channels are independent, unless one can show that the difference between the theoretical prediction of the binomial and the probability measured experimentally is not statistically significant. It is thus essential to include this number in an analysis aimed to test the independence of the ionic channels. The Table contains the essential results of this analysis performed on two inside-out current records obtained at −50 and −100 mV for which the open-channel probability was 0.14 and 0.24, respectively. Based on the value obtained for the χ^2 in both cases, it can be concluded that within a confidence level of more than 97%, the four channels in this particular membrane patch behave independently with the same statistical properties. It appears therefore that an evaluation of P_o using Eqs. (1), (2) and (3) is correct, since the calcium-activated potassium channels in HeLa cells behave as a homogeneous population of independent elements.

D) Effect of Internal Ca^{2+} and Voltage on the Potassium Channel Open Probability

We have established in section A that single-channel events related to a potassium-selective channel could be triggered by increasing the intracellular level of free calcium. We report here results of in-

side-out experiments in which the concentration of calcium facing the cytoplasmic membrane surface was changed systematically. In order to take into account the possible rundown of channel activity as a function of time (Maruyama & Petersen, 1984), the following experimental protocol was used. Inside-out experiments were carried out with patch pipettes containing 200 mM KCl, 1 μM $CaCl_2$ plus 0.5 mM $MgSO_4$. The cytoplasmic medium consisted of a 200 mM KCl + 0.5 mM $MgSO_4$ to which various amounts of $CaCl_2$ and EGTA were added as described in Materials and Methods. Single-channel activity was first measured with 1 μM of free calcium into the bathing medium. The bath was then perfused with solutions containing different test concentrations of free calcium and the channel activity recorded. At the end of the experiment the test solution was replaced by the original 1 μM free calcium medium. In order for the experiment to be regarded as significant, the original single-channel activity must have been restored. We present in Figs. 7*A* and 7*B* experimental records obtained at various free-calcium concentrations. More than five channels were present in the membrane patch in *A* compared to three in *B*. As in Fig. 2, we found that the main effect of $[Ca^{2+}]$ was to increase more the number of channel openings per second than the channel mean open time. In Fig. 7*A*, for instance, the arithmetic mean of the channel open time was estimated at 1.6 and 1.9 msec for 0.1 and 0.4 μM calcium, respectively. The number of channel openings per second changed, however, from 6.8 to 142 for the same calcium increase. A fourfold increase of the cytoplasmic calcium concentration has thus resulted in a 20-fold increase in the number of openings per second without any significant change in the channel mean open time. Figure 8 summarizes most of our results on the effect of calcium on P_o, the open channel probability. We found essentially that this particular potassium channel could be activated at submicromolar free-calcium concentration, the major increase of P_o occurring within the calcium concentration range 0.1 to 1 μM. Calcium concentrations of higher values did not seem to promote channel openings to a much greater extent. In fact, the open-channel probability measured at 1 mM cytoplasmic calcium concentration was not in most cases substantially different from that measured at 1 μM. At these calcium levels, the open-channel probability P_o usually reaches values close to 0.3 for a transmembrane potential of −50 mV (*see* Fig. 7*B*). It thus appears that a high calcium concentration is not enough to bring this particular channel into a permanently open state. The results presented in Fig. 8 also indicated that P_o was on the average higher at −100 mV (filled circles) than at

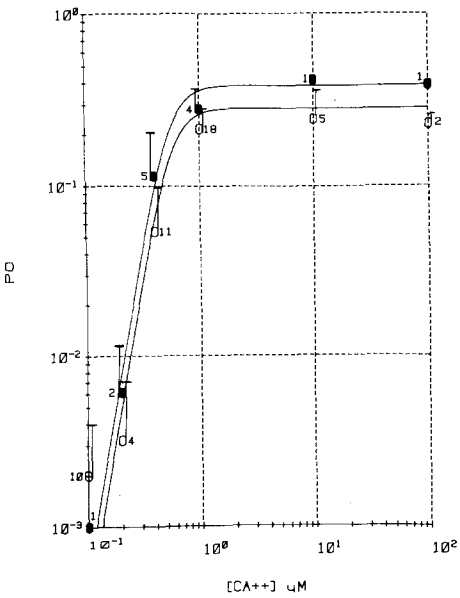

Fig. 8. Variation of the open channel probability P_o as a function of the intracellular free calcium concentration for two applied potentials −50 and −100 mV (filled circles) as described in Fig. 7. The value of P_o was computed according to Eqs. (2) and (3) in Materials and Methods. The number of experiments from which average values were computed is shown for each point. The continuous line shows the prediction of Eq. (10) with $P_1 = 0.123$ $(\mu M)^4$, $P_2 = 6.45 \times 10^{-3}$, $P_3 = 0.038$ mV^{-1} and $P_4 = 0.36$ assuming the binding of four calcium ions

−50 mV (open circles) at all internal calcium levels. Finally, it can be observed in Fig.8 that an increase in free-calcium concentration from 0.2 to 0.4 μM yielded a 17-fold increase of the mean value of P_o. Several mechanisms can take into account such a power relationship between P_o and $[Ca^{2+}]_i$. One possibility is that the binding of four calcium ions is required to open the channel. Due to the large scattering of the results and to the less precise estimate of P_o obtained especially at very low $[Ca^{2+}]_i$, we cannot entirely rule out that only three calcium ions are required for channel opening. On the average, however, our results are more compatible with a fourth than with a third power relationship. A more detailed model will be presented later.

We present in Fig. 9 the effect of the transmembrane potential on P_o. The experimental records used to compute the values of P_o were obtained from inside-out experiments with 200 mM KCl, 1 or 0.4 μM $CaCl_2$ plus 0.5 mM $MgSO_4$ in the cytoplasmic solution. The patch electrode contained 200 mM KCl, 0.5 mM $MgSO_4$ with 1 μM or 2 mM $CaCl_2$. No additional effect of external $[Ca^{2+}]$ could statistically be inferred. The results shown on this Figure represent the mean value of 1 to 17 different measurements. The obvious conclusion that can be

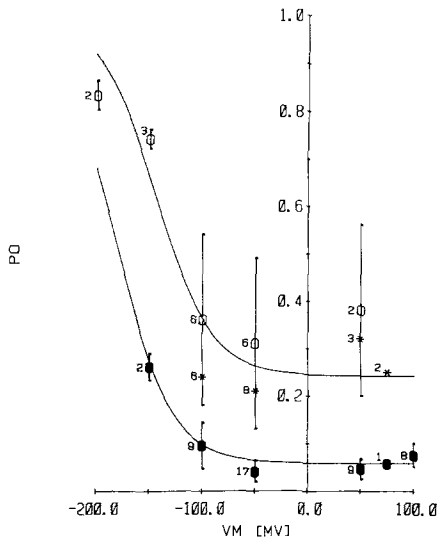

Fig. 9. Effect of the transmembrane potential on the open-channel probability based on results obtained from inside-out experiments with pipettes and the bath containing (in mM) 200 KCl, 0.5 $MgSO_4$; □ 1 mM pipette calcium, 1 μM cytoplasmic calcium; * 1 μM pipette calcium, 1 μM cytoplasmic calcium; ■ 1 μM pipette calcium, 0.4 μM cytoplasmic calcium. As seen, the value of P_o is voltage-insensitive within −100 to +100 mV. The results presented correspond to the average values of 1 to 17 different experiments and as observed there is a large variability from cell to cell. The continuous line was obtained from Eq. (10) using the same set of parameters as used in Fig. 8

drawn from this analysis is that P_o increases for potentials more negative than −100 mV but remains constant at more positive values. In some experiments, we could also observe a small increase of P_o at positive potential values, but on the average P_o remained constant within that voltage range. The voltage sensitivity of P_o within the voltage range −100 to +100 mV is not therefore as important as that reported for calcium-activated potassium channels of large unitary conductance (*see*, for instance, Barrett et al., 1982; Maruyama et al., 1983; Moczydlowski & Latorre, 1983; Vergara & Latorre, 1983).

E) Effect of Voltage on Time Interval Distributions

In order to determine more precisely the effect of the membrane potential on the channel kinetics parameters, an analysis of the effect of voltage on the channel open and closed time interval distribution was undertaken. We must mention, however, that this type of analysis could be carried out on a few single-channel records only, since the large majority of the records we obtained contained a number of channels ranging from 2 to 6. In addition, we considered only current records obtained at large negative potential values, since the effect of voltage in P_o was dominant within that voltage range only (*see* Fig. 9). The analysis discussed here is based on records obtained from an inside-out experiment with 200 mM KCl, 1 μM $CaCl_2$ plus 0.5 mM $MgSO_4$ on the cytoplasmic side and 200 mM KCl + 2 mM $CaCl_2$ inside the patch electrode. The current traces were filtered at 1 kHz and sampled at 10 kHz. We show in Fig. 10 the cumulative open and closed time interval distributions measured at three different voltages together with an example of the single-channel events observed at −100 mV. In this particular case, the open-channel probability was estimated at 0.3, 0.51 and 0.75 for applied voltages of −50, −100 and −150 mV, respectively. The value of the cumulative probability was computed from two thousand intervals and the resulting cumulative probability fitted by a sum of exponential using a nonlinear least-square fit procedure. A simple inspection of current records, such as the one shown in Fig. 10, first reveals that there are two types of opening events; one type consisting of short-lived spikes of mean lifetime less than 1 msec (O-F) and a second type of well resolved open-state events (O). The contribution of the short-lived openings never exceeded 5% in all the records used for this analysis. We will thus consider, as shown in Fig. 10, that the channel openings can be taken into account by a single open state under the conditions we worked. The closed time interval distribution showed a more complex behavior. As seen in Fig. 10, the results we obtained at −150 and −100 mV indicate that there were at least three closed states with time constants ranging from 1 msec to 100 msec. The long shut intervals (mean lifetime of 100 msec) had a contribution of less than 3% in all the records studied here, so that a precise estimate of the effect of voltage on this component could not be carried out properly. The two other components at −50, −100 and −150 mV had time constants of 1, 1.7 and 2 msec for the fast closing events (C-F) and of 11, 8.2 and 5.1 msec for the component of intermediate values (C-I). The latter represented approximately 80% of the interval we detected. As shown in Fig. 11*A* we found that both the channel arithmetic mean of all the open and closed time intervals changed as a function of voltage. Essentially, we measured an increase in the channel mean open time and a decrease in the channel mean closed time at more negative potential values. With an applied potential equal to −50 mV the channel was on the average open during 5.1 msec and closed during 9.5 msec. These values changed to 12.7 and 4.1 msec at −150 mV. We also found that in all cases the ratio, mean

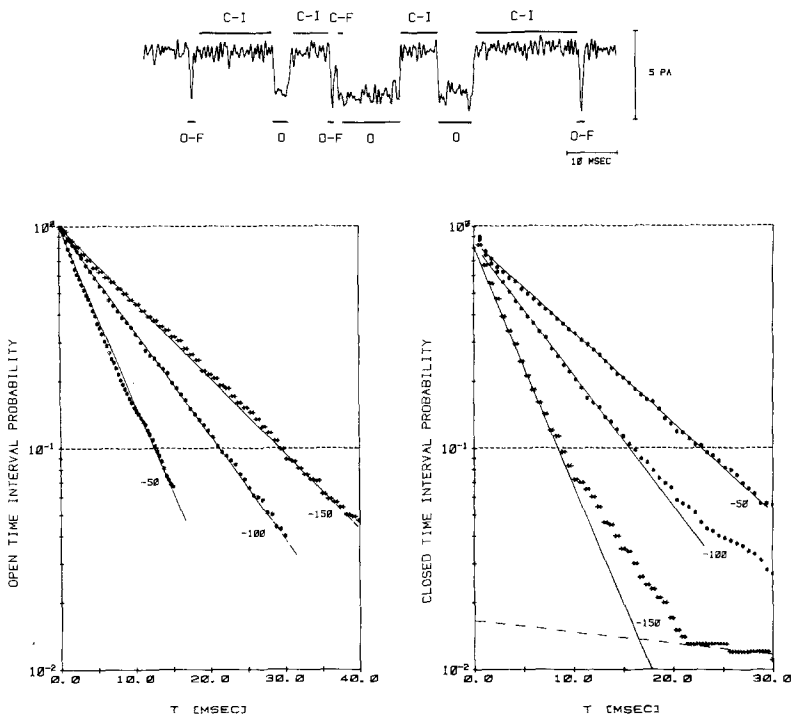

Fig. 10. Effect of transmembrane potential on the channel cumulative open and closed time interval probability (probability that an interval is greater than a given time t). Results obtained from an inside-out experiment with 200 mM KCl, 0.5 mM $MgSO_4$ at pH 7.3 in both the patch electrode and the cytoplasmic medium. The cytoplasmic calcium concentration was 1 μM. The example of single-channel recording presented was obtained at −100 mV. The open time interval distribution could be least-square fitted to a single exponential function with a time constant of 5.2, 7.9 and 13 msec for an applied potential of −50, −100, and −150 mV, respectively. The fast opening events (O-F) contributed to less than 5% of the total number of intervals. Three exponential functions were needed in order to take into account the closed time interval distribution at −100 and −150 mV. As shown, the long shut intervals (mean life of 100 msec or more) contributed usually to less than 3% of the total number of detected transitions so that only the intermediate (C-I) and fast (C-F) closed time intervals were considered. The dotted line represents the contribution of the long shut intervals. The continuous lines represent the contribution of the intermediate closed time intervals with time constants equal to 11.8, 7.9 and 5.1 msec at −50, −100 and −150 mV, respectively. The time constants of the fast closing events had values of 1, 1.7 and 2 msec

open time/(mean open time + mean closed time), corresponded precisely to the value P_o obtained by means of Eqs. (2) and (3). This agreement indicates a self-consistency between the two types of analysis. Since we found that the channel had essentially a single open state under the condition we used, our last results can be regarded as evidences that at least one rate constant from the dominant open state is voltage dependent. The modulation by calcium of this effect will be discussed in a future work. Figure 11*B* shows how the different time constants obtained by curve fitting the probability distributions presented in Fig. 10 vary as a function of voltage. As seen, the effect of voltage on the channel mean closed time is due mainly to a decrease in the mean lifetime of the intermediate component with values of 11 and 5.1 msec at −50 and −150 mV, respectively. Since 80% of all the detected transitions belonged to this category, this effect led to an overall decrease of the channel mean closed time at more negative voltages as shown in Fig. 11*A*.

Discussion

The main purpose of the present work was to investigate the calcium dependency of the potassium permeability in HeLa cancer cells. Based on the results we obtained at the single-channel level, it can be concluded that the HeLa cell membrane contains calcium-activated potassium channels of small unitary conductance, whose activity can be triggered at submicromolar free calcium concentrations. This particular channel was shown to behave as an inward rectifier with a conductance in symmetrical 200 mM KCl solution of 50 and 10 pS at large negative and large positive potentials, respectively. The

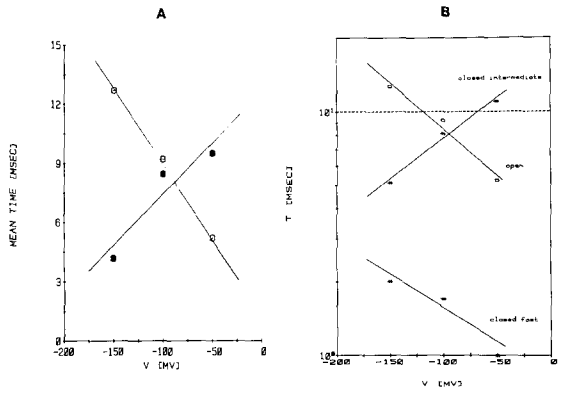

Fig. 11. Variation of the channel mean open time (open circles) and mean closed time (filled circles) as a function of the applied voltage. Experimental conditions as described in Fig. 10. In *B*, we present the variation of the time constants as a function of voltage for the channel single open state and for the fast (C-F) and intermediate (C-F) channel closed states. The time constants were obtained by a least squares fit of the probability distribution shown in Fig. 10

channel activity was also found to be nearly voltage independent within the voltage range −100 to +100 mV, an increase in open-channel probability being observed at large negative potentials only. In addition, it was shown that an increase in cytoplasmic free-calcium concentration affects more the number of channel openings per second than the actual channel mean open time.

A) Comparisons with Other Calcium-Activated Channels

We have reported previously the presence in HeLa cells of calcium-activated potassium channels with similar properties (Sauvé et al., 1984). These results were based on the analysis of outward current records obtained from outside-out experiments with 5 mM potassium in the bathing medium. No clear inward currents could, under these conditions, be detected. It is likely that the channel presented here corresponds to the channel described in our earlier work, but we cannot entirely rule out that there is more than one type of calcium-activated channel in these cells (*see*, for example, Hamill, 1983). Interestingly, the results presented in our earlier work with 5 mM external K^+ showed that values of P_o greater than 0.2 and 0.6 could be obtained at positive potentials with 0.1 and 1 mM internal calcium, respectively (Sauvé et al., 1984). This is in accordance with the results shown in Figs. 8 and 9 where values of P_o at positive potentials were on the average equal to 0.4. We have also presented in a previous work single-channel evidences for a K^+-selective channel with inward rectification properties (Sauvé et al., 1983). Although of similar single-channel conductance, this particular channel cannot be associated with the calcium-activated channel described here. This channel was not activated by internal calcium, remained open at small negative potentials (Sauvé, *unpublished results*) and closed at hyperpolarizing potential values (*see*, for instance, Payet et al., 1985). Records in which both types of channels were present could also be obtained indicating a clear difference in gating behavior. In addition, the channel reported previously (Sauvé et al., 1983) behaved like a true inward rectifier with no detectable outward currents.

It should also be apparent that the potassium channel described in this work differs on several major points from the calcium-dependent potassium channels of large unitary conductance reported so far in the literature (Marty, 1981; Barrett et al., 1982; Latorre et al., 1982; Methfessel & Boheim, 1982; Wong et al., 1982; Maruyama et al., 1983). First, there is a fivefold difference in conductance between the two types of channels and the channel *I/V* curves we obtained experimentally showed an inward rectification not found for the large conductance calcium-activated potassium channels. Second, the calcium-activated potassium channel described in this work is nearly voltage insensitive within the voltage range −100 to +100 mV. This is in contrast with some reported large conductance potassium-calcium-activated channels (Barrett et al., 1982; Maruyama et al., 1983; Vergara & Latorre, 1983). However, there are in the literature a few examples of voltage-insensitive calcium-activated channels (Colquhoun et al., 1981; Maruyama & Petersen, 1982; Yellen, 1982) but these channels were found to be equally permeable to several alkali metal ions and differ therefore from the potassium-selective channel described here. In addition, the voltage dependence of P_o we observed at large negative potentials works in a direction opposite to that reported for calcium-activated potassium channels of large unitary conductance, where channel opening is enhanced as voltage is made increasingly positive. Interestingly, Vergara and Latorre (1983) and later Marty et al. (1984) have shown that P_o could decrease at more positive potential values for high (100 μM) cytoplasmic free-calcium concentrations. This effect was interpreted in terms of a slow channel blocking action of internal calcium. There are several reasons why this explanation is not likely to hold in our case. First, the same qualitative voltage dependency of P_o could be observed even if long silent periods (>100 msec) characteristic of a slow blocking process were not included in our analysis

(*see* Fig. 1*D*). Second, this effect of the voltage on P_o was observed at internal free-calcium concentrations as low as 0.2 μM where the open channel probability did not exceed 0.01. A blocking effect of calcium at these low levels is not probable, since neither the channel activity nor the channel amplitude decreased following an additional increase in internal free-calcium concentration at levels (2 mM) where a total block of the channel would have been expected.

A greater correspondence can be found with the calcium-activated potassium channel reported by Grygorczyk et al. (1984) in human red cells. The channel *I/V* curves they obtained showed an inward rectification comparable to what we described in the present work, with unitary conductances identical to the ones we measured for the calcium-dependent potassium channels in HeLa cells. They also found (Grygorczyk & Schwarz, 1983) an enhancement in channel open probability at hyperpolarizing voltages. Our results indicate, however, a greater sensitivity to internal calcium. In our case, channel activity could be initiated at concentrations ranging from 0.1 to 1 μM, whereas in red blood cells the reported effect of $[Ca^{2+}]_i$ on P_o took place within 1 and 10 μM. It seems nevertheless clear that these two calcium-dependent potassium channels of small unitary conductance possess similar molecular features which differ from those usually reported for the large conductance potassium channels.

B) Mathematical Modeling of the Channel *I/V* Curve

In order to account for the channel inward rectification, a mathematical analysis of the channel *I/V* curves was undertaken. To minimize the number of adjustable parameters involved in such a computational procedure, we used a simple one-site-two-barrier type model (Fig. 12). The *I/V* equation for this model is as follows:

$$J = \frac{B[[\mathrm{K}]_{\mathrm{in}} e^{\phi} - [\mathrm{K}]_{\mathrm{out}}]}{Z}$$

with

$$Z = e^{\left[.5 - \frac{\lambda}{2d}\right]\phi} + Ae^{\left[1 - \frac{\lambda}{2d}\right]\phi} + W[\mathrm{K}]_{\mathrm{in}} e^{\left[.5 + \frac{\lambda}{2d}\right]\phi} + WA[\mathrm{K}]_{\mathrm{out}} e^{\frac{\lambda}{2d}\phi} \quad (5)$$

where $\phi = VQ/KT$ with V the membrane voltage, Q the electronic charge, K the Boltzmann's constant, T the absolute temperature, $A = k_o'/k_o = \exp(E_1 - E_3)$ with E_1 and E_3 in KT units, $W = \gamma k_i/k_o = \gamma \exp(-E_2)$ with γ the partition coefficient of the ion into the channel in M^{-1} and E_2 also in KT units and $B = \gamma Q k_i' = \gamma Q \nu_o \exp(-E_3)$ with k_i' the entry rate of the ion from the external solution and $\nu_o = 6.25 \times 10^{12}$ Hz (Fig. 12). The location of the binding site λ/d, the relative amplitude of the inside barriers A, the binding constant W and the amplitude parameter B are all adjustable parameters. The values of A and λ/d both have an effect on rectification with λ/d having a larger effect particularly on outward currents. If $A = 1$ and $\lambda/d = 0.5$, the *I/V* curves are symmetrical in symmetrical solutions; but if $A < 1$ and $\lambda/d > 0.5$, an inward rectification appears. The *I/V* curves obtained at various KCl concentrations were thus least-square fitted simultaneously using a single set of parameters. We present in Fig. 12 the results of our calculations and in Fig. 4 the theoretical prediction of the model. Essentially, our analysis indicates that the channel inward rectification can be taken into account by considering a potassium binding site located inside the channel at 80% of the membrane internal surface ($\lambda/d = 0.8$) with $A = 0.52$, $B = 25$ pA/M^{-1} and $W = 28$ M^{-1}. The rates at which potassium ions can enter into the channel from the internal and external side were estimated

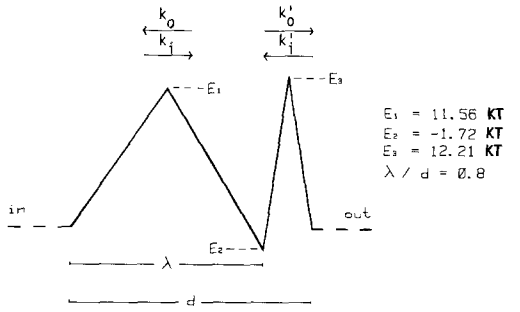

Fig. 12. Features of the permeation model for the calcium-activated potassium channel in HeLa cells. The model is an Eyring rate theory model with one site for ion binding. Using a least-square fit procedure, the fractional electrical distance from the internal surface was estimated at 80%, although values as high as 90% could also well reproduce the experimental results presented in Fig. 4. According to the proposed model, k_i and k_i', the rates of potassium entry into the channel should be equal to 5.8 $\times 10^7$ and 3.0 $\times 10^7$ ions/sec, respectively, in symmetrical 200 mM KCl. The model also predicts values of k_o and k_o' equal to 1.0 $\times 10^7$ and 5.2 $\times 10^6$ ions/sec, respectively. It thus follows that the rate-limiting step to the ion movement across the channel is mainly determined by the rate at which the ion can exit from the channel. Consequently, the inward rectification predicted by the model results more from the localization of the ion building site than from a difference in amplitude between the two energy barriers

at 2.9×10^8 sec^{-1} M^{-1} for γk_i and 1.5×10^8 sec^{-1} M^{-1} for $\gamma k_i'$. We also obtained for the ion exit rates k_o and k_o' values equal to 10^7 and 5.2×10^6 ions/sec, respectively. Consequently, with 200 mM of potassium on both sides of the membrane, one predicts entry rates of 5.8×10^7 ions/sec for k_i and 3.0×10^7 ions/sec for k_i'. It must be pointed out in this regard that the values of E_1, E_2 and E_3 presented in Fig. 12 were based on these entry rates. The differences $E_1 - E_2$ and $E_3 - E_2$ are, however, independent of the internal and external ionic concentrations. It thus follows that the rate-limiting step for ion movement across the channel in a symmetrical 200 mM potassium experiment corresponds essentially to the ion exit rate. However, since the computed values of k_o and k_o' differ only by a factor of two, we must conclude that the inward rectification of the theoretical curve shown in Fig. 12 results more from the localization of the ion binding site than from the difference in amplitude between the two energy barriers. Since the difference in potential between the ion binding site and the cytoplasmic medium accounts for 80% of the total drop in potential across the membrane, it is therefore not surprising to observe a larger single-channel conductance for inward currents. However, it must be borne in mind that the value of 0.8 we obtained for λ/d represents a lower limit. Our experimental results do not allow for the moment a more precise estimation of this parameter. This would have required measurement of the channel *I/V* curve over a broader voltage range (±300 mV) than that reported here. The curve-fitting procedure we used indicates nevertheless that values of λ/d lower than 0.8 are not compatible with our results. Such a model also predicts correctly how the single-channel conductance at zero applied voltage should vary as a function of the external potassium concentration (*see* Fig. 5*B*). It should be clear that more elaborate models with more binding sites could also have reproduced the *I/V* curves found experimentally. However, such models would be at this stage too speculative and would require, in order to be fully justified, more experimental data than that provided in this work. The inward rectification of the *I/V* curves thus appears as a property of the channel *per se* and does not seem to be altered by working in the excised patch configuration. A direct blocking effect of a cellular compound may thus be ruled out, except possibly for calcium which was always present in our inside-out experiments (Armstrong, 1975; Hille & Schwarz, 1978). But a block by calcium at submicromolar concentrations is unlikely. In fact, the resulting *I/V* curves were found to be independent of the cytoplasmic calcium levels even at concentrations greater than 1 mM.

The proposed one-site-two-barrier model can also be used in cases where two ions such as K^+ and Na^+ compete for the same channel binding site. It can be shown in that case that the zero-current potential will be given by an equation of the form

$$V_r = \frac{KT}{Q} \ln \left[\frac{[K]_{out} + (P_{Na}/P_K)\,[Na]_{out}}{[K]_{in} + (P_{Na}/P_K)\,[Na]_{in}} \right] \tag{6}$$

with the ratio P_{Na}/P_K given by

$$\frac{P_{Na}}{P_K} = \frac{k_i'^{Na}}{k_i'^{K}} F(\phi) \tag{7a}$$

where

$$F(\phi) = \frac{1 + A^K e^{\phi/2}}{1 + A^{Na} e^{\phi/2}} \tag{7b}$$

with A as defined previously. Assuming that the energy peak offset condition holds in our case so that $A^K = A^{Na}$ (*see* Hille, 1975), the ratio P_{Na}/P_K then reads

$$\frac{P_{Na}}{P_K} \cong \frac{k_i'^{Na}}{k_i'^{K}} = e^{-[E_3^{Na} - E_3^K]}. \tag{8}$$

With P_{Na}/P_K equal to 0.03 as found previously, it may be concluded that the channel selectivity is mainly governed by the ion entry rate and that $E_3^{Na} = E_3^K + 3.4\,KT$.

C) Channel Kinetic Scheme

Although a detailed analysis of the channel open and closed time interval distributions is not within the scope of the present work, we may nevertheless outline several constraints that must be included in any molecular schemes aimed to describe the kinetic behavior of this particular channel. First, our results obtained in high K^+ conditions indicate that three to four calcium ions are needed in order to open the channel. In addition, an increase of P_o as a function of $[Ca^{2+}]_i$ seemed to affect more the number of channel openings per second than the actual channel mean open time. Second, the open channel probability P_o reaches a maximum value of 1 μM calcium and does not increase at higher cytoplasmic calcium levels. Finally, the value of P_o was found to be voltage dependent at large negative potentials only. At positive potentials P_o remained on the av-

erage constant. These features can be taken into account by means of a linear kinetic model similar to the one often used to describe receptor reactions (*see* Colquhoun & Hawkes, 1981; Magelby & Pallotta, 1983)

$$N[Ca^{2+}] + C \underset{R_2}{\overset{R_1}{\rightleftharpoons}} C^* \underset{R_4(+V)}{\overset{R_3(-V)}{\rightleftharpoons}} O_1 \qquad (9)$$

$$R_5 \Big\downarrow\Big\uparrow R_6$$

$$O_2$$

with $N = 4$. According to the proposed model, the binding of calcium ions (the agonist) increases the system probability to be in the C^* state where channel openings can occur. At low calcium concentrations the main effect of calcium will thus be to increase the number of channel openings per second rather than the channel mean lifetime as observed experimentally. It should be mentioned that several closed states are likely to be involved in the binding of calcium. We will consider that these reactions can be represented by a single reaction namely $4[Ca^{2+}] + C$. The open states O_1 and O_2 are assumed to be of equal conductance since our experimental results never showed multiple conductance levels.

It can be shown that the open channel probability associated to the model proposed in Eq. (9) is given by

$$P_o = \frac{1}{1 + (1 + P_1/[Ca^{2+}]^4)(P_2 e^{-P_3 V} + P_4)^{-1}} \qquad (10)$$

where $P_1 = R_2/R_1$, $P_2 = R_3^o/R_4^o$ and $P_4 = R_5/R_6$. It was assumed here that R_3 and R_4 could be expressed as $R_3 = R_3^o \exp(-\alpha_1 V)$ and $R_4 = R_4^o \exp(\alpha_2 V)$ so that $\alpha_1 + \alpha_2 = P_3$. Under these conditions, the open state O_1 becomes dominant at large negative potentials (<-50 mV) and the mean open time given approximately by $[1/R_4^o] \exp(-\alpha_2 V)$ should increase within that voltage range as observed experimentally (*see* Fig. 11). However, at positive potential values, the state O_2 would then become dominant leading to a value P_o independent of voltage as shown in Fig. 9. In addition, at cytoplasmic calcium levels where $P_1/[Ca^{2+}]^4 \leq 1$, P_o may be approximated by

$$P_o \approx \frac{1}{1 + (P_2 e^{-P_3 V} + P_4)^{-1}} \qquad (11)$$

Values of P_o nearly equal to 1.0 can then be obtained only in cases where the inequality $P_2 e^{-P_3 V} + P_4 \geq 1$ is satisfied. In order to test the proposed kinetic scheme, a curve-fitting procedure of the results shown in Figs. 8 and 9 was undertaken with P_1, P_2, P_3 and P_4 as adjustable parameters. We found that our experimental results could be reproduced using $P_1 = 0.123$ $(\mu M)^4$, $P_2 = 6.45 \times 10^{-3}$, $P_3 = +0.038$ mV^{-1} and $P_4 = 0.36$. It thus follows that the equilibrium constant R_3/R_4 increases an e-fold per 26 mV. Under these conditions, the state O_1 becomes dominant at negative voltages but remains improbable at positive potential values. A similar curve-fitting procedure was carried out assuming that three rather than four calcium ions were involved. These calculations confirmed that our experimental results are more compatible with a four-calcium binding than a three-calcium binding model. It would certainly have been possible to fit our results with a more elaborated model. But the simple scheme presented in Eq. (9) can take into account all the main features of the results we have obtained so far.

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References

Armstrong, C.M. 1975. Potassium pores of nerve and muscle membrane. *In:* Membrane—A Series of Advances. G. Eisenman, editor. Vol. 3, pp. 325–358. Marcel Dekker, New York

Barrett, J.N., Magleby, K.L., Palotta, B.S. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (London)* **331:**211–230

Colquhoun, D., Hawkes, A.G. 1981. On the stochastic properties of single ion channels. *Proc. R. Soc. London B* **211:**205–235

Colquhoun, D., Neher, E., Reuter, H., Stevens, C.F. 1981. Inward current channels activated by intracellular Ca^{2+} in cultured cardiac cells. *Nature (London)* **294:**752–754

Colquhoun, D., Sigworth, F.J. 1983. Fitting and statistical analysis of single channel records. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 191–263. Plenum, New York

Douglas, W.W. 1980. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. *In:* The Pharmacological Basis of Therapeutics. A.G. Gilman, L.S. Goodman, and A. Gilman, editors. pp. 609–646. MacMillan, New York

Eckert, R., Tillotson, D. 1978. Potassium activation associated with intraneuronal free calcium. *Science* **200:**437–439

Fabiato, A., Fabiato, F. 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J. Physiol. (Paris)* **75:**463–505

Grygorczyk, R., Schwarz, W. 1983. Properties of the Ca^{2+}-activated K^+ conductance of human red cells as revealed by the patch clamp technique. *Cell Calcium* **4:**499–510

Grygorczyk, R., Schwarz, W., Passow, H. 1984. Ca^{2+}-activated

K^+ channels in human red cells: Comparison of single-channel currents with ion fluxes. *Biophys. J.* **45:**693–698

Hamill, O.P. 1983. Potassium and chloride channels in red blood cells. *In:* Single Channel Recording. B. Sackmann and E. Neher, editors. pp. 451–471. Plenum, New York

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording form cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hazama, A., Yada, T., Okada, Y. 1985. HeLa cells have histamine H_1 receptors which mediate activation of the K^+ conductance. *Biochim. Biophys. Acta* **845:**249–253

Hille, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In:* Membrane—A Series of Advances. G. Eisenman, editor. Vol. 3, pp. 255–325. Marcel Dekker, New York

Hille, B. 1984. Ionic channels of excitable membranes. Sinauer Associates, Sunderland, Mass.

Hille, B., Schwarz, W. 1978. Potassium channels as multi-ion single file pores. *J. Gen. Physiol.* **72:**409–442

Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71:**11–30

Latorre, R., Vergara, C., Hidalgo, C. 1982. Reconstitution in planar lipid bilayers of a Ca^{2+}-dependent K^+ channel from transverse tubule membranes isolated from rabbit skeletal muscle. *Proc. Natl. Acad. Sci. USA* **79:**805–809

Lux, H.D., Neher, E., Marty, A. 1981. Single channel activity associated with calcium dependent outward current in Helix pomatia. *Pfluegers Arch.* **389:**293–295

Magleby, K.L., Pallotta, B.S. 1983. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (London)* **344:**585–604

Marty, A. 1981. Ca^{2+}-dependent K^+ channels with large unitary conductance in chromaffin cell membranes. *Nature (London)* **291:**497–500

Marty, A., Tan, Y.P., Trautmann, A. 1984. Three types of calcium-dependent channel in rat lacrimal glands. *J. Physiol. (London)* **357:**293–325

Maruyama, Y., Gallacher, D.V., Petersen, O.H. 1983. Voltage and Ca^{2+}-activated K^+ channel in basolateral acinar cell membranes of mammalian salivary glands. *Nature (London)* **302:**827–829

Maruyama, Y., Petersen, O.H. 1982. Single channel currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature (London)* **299:**159–161

Maruyama, Y., Petersen, O.H. 1984. Single calcium-dependent cation channels in mouse pancreatic acinar cells. *J. Membrane Biol.* **81:**83–87

Meech, R.W. 1976. Intracellular calcium and the control of membrane permeability. *In:* Calcium in Biological Systems. pp. 161–191. Cambridge University Press

Methfessel, C., Boheim, G. 1982. The gating of single calcium-dependent potassium channels is described by an activation/blockade mechanism. *Biophys. Struct. Mech.* **9:**35–60

Moczydlowski, E., Latorre, R. 1983. Gating kinetics of Ca^{2+}-activated K^+ channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca^{2+} binding reactions. *J. Gen. Physiol.* **82:**511–542

Neher, E., Sakmann, B., Steinbach, J.H. 1978. The extracellular patch clamp: A method for resolving currents through individual open channels in biological membranes. *Pfluegers Arch.* **375:**219–228

Pallotta, B.S., Magleby, K.L., Barrett, J.N. 1981. Single channel recordings of Ca^{2+} activated K^+ currents in rat muscle cell culture. *Nature (London)* **293:**471–474

Patlack, J., Horn, R. 1982. Effect of N-bromoacetamine on single channel currents in excised membrane patches. *J. Gen. Physiol.* **79:**333–351

Payet, D., Rousseau, E., Sauvé, R. 1985. Single-channel analysis of a potassium inward rectifier in myocytes of newborn rat heart. *J. Membrane Biol.* **86:**79–88

Petersen, O.H., Maruyama, Y. 1984. Calcium activated potassium channels and their role in secretion. *Nature (London)* **307:**693–696

Rasmussen, H., Barrett, P.Q. 1984. Calcium messenger system: An integrated view. *Physiol. Rev.* **64:**938–984

Roux, B., Sauvé, R. 1985. A general solution to the time interval omission problem applied to single channel analysis. *Biophys. J.* **48:**149–158

Roy, G., Sauvé, R. 1983. Stable membrane potential and mechanical K^+ responses activated by internal Ca^{2+} in HeLa cells. *Can. J. Physiol. Pharmacol.* **61:**144–148

Sauvé, R., Bedfer, Roy, G. 1984. Single Ca^{2+} dependent K^+ currents in HeLa cancer cells. *Biophys. J.* **45:**66–68

Sauvé, R., Roy, G., Payet, D. 1983. Single channel K^+ currents from HeLa cells. *J. Membrane Biol.* **74:**41–49

Schwartz, J.C. 1979. Histamine receptors in brain. *Life Sci.* **25:**895–912

Schwarz, W., Passow, H. 1983. Ca^{2+}-activated K^+ channels in erythrocytes and excitable cells. *Annu. Rev. Physiol.* **45:**359–374

Vergara, C., Latorre, R. 1983. Kinetics of Ca^{2+}-activated K^+ channels from rabbit muscle incorporated into planar bilayers. Evidence for a Ca^{2+} and Ba^{2+} blockade. *J. Gen. Physiol.* **82:**543–568

Wong, B.S., Lecar, H., Adler, M. 1982. Single calcium-dependent potassium channels in clonal anterior pituitary cells. *Biophys. J.* **39:**313–317

Yellen, G. 1982. Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. *Nature (London)* **296:**357–359

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