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Single Channel K^+ Currents from HeLa Cells

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Summary. The extracellular patch-clamp technique was used in order to investigate the presence of ionic channels in HeLa cells, a well-known cultured cell type obtained from an epidermoid carcinoma of the cervix. Under Gigohm-seal conditions, discrete current jumps could be observed with patch electrodes containing KCl. These channels were found to be mainly permeable to K^+ and showed multiple levels of conductance. From single-channel *I–V* curve measurements, a strong rectification effect, characterized by a large inward and no detectable outward current, was observed. For negative membrane potentials (0 to −90 mV), the measured current-voltage relationship was found to be mostly linear, corresponding to a single-channel conductance of 40 pS. An analysis of some selected time records has revealed in addition that the probability of the channel to be in the open state was a function of the KCl concentration in the patch pipette.

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

Introduction

Since the introduction by Neher, Sakmann & Steinbach (1978) of the extracellular patch clamp technique, several studies have been concerned with the electrical characterization of single channels in excitable membranes. (*See* Hamill et al. (1981) for a detailed discussion of the technique.) By means of this approach, the presence in these membranes of Na^+ (Horn & Patlack, 1980; Horn, Patlack & Stevens, 1981; Sigworth & Neher, 1980) and of many types of K^+ channels (Conti & Neher, 1980; Fukushima, 1981; Pallotta, Magleby & Barrett, 1981) has been directly confirmed and for the first time the single-channel events underlying cellular excitability observed.

It is, however, likely that the passage of ions through pores represents a general cellular feature shared by nonexcitable cells, as well. In fact, most cells are characterized by a membrane resting potential, which results in part from the more or less selective permeability of their external membrane to certain ionic species such as K^+. In turn, this membrane potential is known to be involved in numerous transmembrane transport mechanisms, and its importance to many cellular activities is now well established. In order to gain further information on the molecular mechanisms underlying the ionic permeability of nonexcitable cell membranes, an electrophysiological study based on the patch-clamp technique was undertaken using HeLa cells, a human cell line obtained from an epidermoid carcinoma of the cervix and which has already been used in previous studies on ion transport (Wickson-Ginsburg & Solomon, 1963; Hülser, 1971; Okada, Ogawa, Aoki & Izutzu, 1973; Hülser et al., 1974; Aiton & Pitman, 1975; Cook, Vaughn, Proctor & Brake, 1975; Nelson-Rees & Flandermayer, 1976; Walliser & Redmann, 1978; Aiton & Lamb, 1980). This first paper intends to provide some partial answers to the following questions: Are there ionic channel-like structures present in the external membrane of HeLa cells? If channels are indeed present, what ionic species can be considered as the main charge carrier and what would be the related current-voltage relationship? And finally, are there chemical agents capable of modulating the channel statistical properties?

Materials and Methods

Cell Culture

HeLa cells were obtained from the Institut Armand-Frappier in Montreal and subcultured in Falcon bottles (75 cm^2 # 3024). The cells were removed from the bottles with 3 ml of a solution of trypsin phosphate buffer solution (PBS) containing 0.2% trypsin. A stock culture was always maintained in a growing state. The culture medium was MEM, Earle base (GIBCO # F-11) with 25 mM Hepes buffer and 6 mM bicarbonate. This medium was supplemented with 10% fetal calf serum (GIBCO

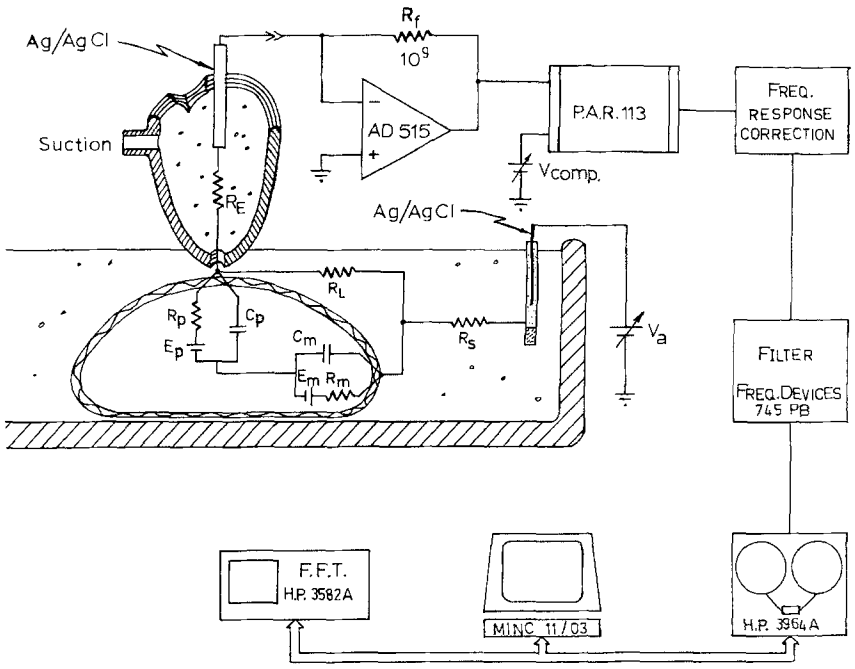

Fig. 1. Schematic diagram of the set-up used for patch experiment. The seal resistance, the resistance of the electrode, and that of the extracellular solution are represented by R_L, R_E and R_S, respectively. R_P and C_P are the resistance and capacitance of the cell area located under the patch pipette, and R_m, C_m the resistance and capacitance of the remaining cell surface. The cell resting potential is symbolized by E_m and E_p^i stands for the equilibrium potential of current I

G14H1) and 1 μg/ml of gentamycine. The cells were grown in monolayers in plastic petri dishes and used for patch experiments two or three days after being subcultured. The culture medium was changed daily, starting from the first day.

Patch-Clamp Experiments

The single-channel experiments reported in this work were carried out using the cell-attached patch-clamp configuration as described by Hamill et al. (1981). Stable Gigohm seals could be achieved without any special enzymatic treatment of the cell external surface. The cell bathing medium was an Earle-Hepes solution containing (in mM): 116, NaCl; 5.4, KCl; 1.8, $CaCl_2$; 0.81, $MgSo_4$; 6, $NaHCO_3$; 1, NaH_2PO_4; 5.5, glucose; and 25, Hepes, buffered at pH 7.3. The patch pipettes were filled with four different types of KCl solution, namely (in mM), 75 KCl + 150 glucose, 150 KCl, 300 KCl and 75 KCl + 75 NaCl, at pH ranging from 5.4 to 5.7. All experiments were done at room temperature (23 °C), and the cells were kept no more than 2 hr for one series of experiments.

The essential of our electronic set up is shown in Fig. 1. Contrary to the basic circuit proposed originally by Neher et al. (1978) and discussed by Hamill et al. (1981), the interior of the patch pipette in our case is maintained to virtual ground so that the term "externally applied voltage" used in this work refers to the potential of the bath compared to ground. The amplitude ΔI of a current pulse due to a change of conductance ΔG_p in the patch area will thus be given, assuming $R_L \geqq R_E$, by

$$\Delta I = [V_a + E_m + E_p^i] \left[\frac{R_p}{R_p + R_m}\right]^2 \Delta G_p \qquad (1a)$$

where E_m is the resting potential of the cell, E_p^i the equilibrium potential associated with the current ΔI, and V_a the applied potential via the bath. As seen, for $V_a = E_p^i = 0$, ΔI does not necessarily vanish, the potential E_m being always present. Furthermore, in normal conditions $R_p \geqq R_m$ so that

$$\Delta I = [V_a + E_m + E_p^i]\, \Delta G_p. \qquad (1b)$$

Finally, let us mention that, unless specified otherwise, the single-channel recordings were filtered at 600 Hz by means of two low-pass, four-pole Butterworth filters connected in series (Frequency Devices models 745PB-3, 745PB-5), before being recorded on FM tape (H.P. 3964A) or digitized for computer analysis (MINC 11/03).

Furthermore, the spectral intensity of the recorded signal was measured throughout each experiment (H.P. 3582A) in order to insure that no undesirable noise contribution was present.

The analysis of the single-channel recording was carried out mainly through computerized data processing. Current amplitude histograms were used to evaluate single-channel amplitude, and time interval distributions were obtained by means of a semi-automated program that allows the operator to select which current jumps may be regarded as true open-close transitions. Unless specified otherwise, the sampling rate selected for A/D conversion was usually 700 μsec per point. We also used strip-chart recordings obtained by playing back at lower speed signals prerecorded on FM tape. This latter method allowed one to examine slow events (> 100 msec) in detail.

Results

Single Channels

Figure 2 shows single-channel recordings obtained from HeLa cells with patch electrodes containing 0.15 M KCl. The first current trace illustrates fluctuations recorded from a highly active patched membrane area. A four-level superposition of inward current jumps of 1 pA amplitude can clearly be seen. There was no externally applied voltage ($V_a = 0$) in this particular case, so that the net potential across the patch area corresponded to E_m, the cell resting potential (≈ -27). Figure 2*B* is an example of the fluctuation pattern

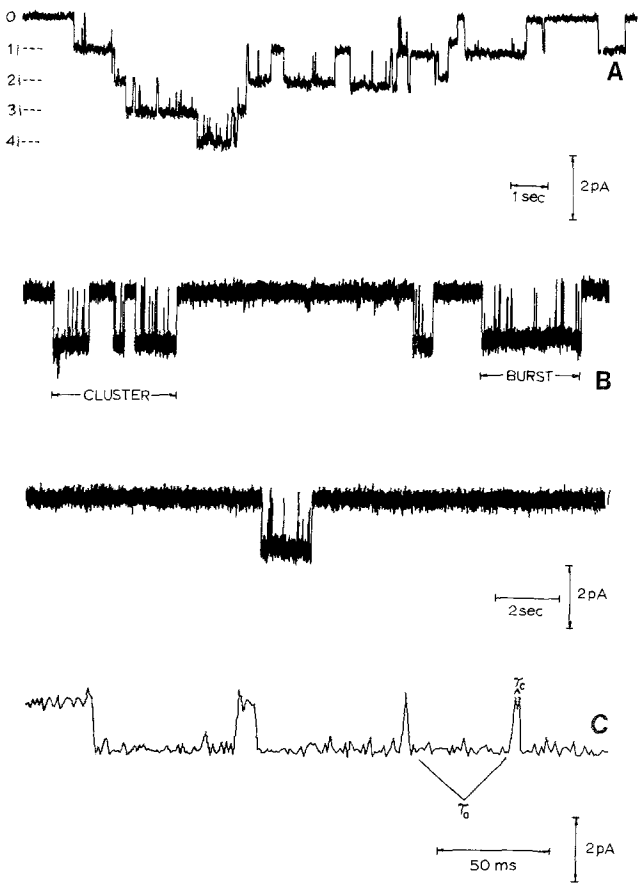

Fig. 2. (*A*): Single channel events recorded during a cell-attached patch experiment with a patch pipette containing 0.15 M KCl at zero applied voltage ($V_a = 0$ mV). Multiple channel openings are shown corresponding to current jumps of 1 pA. The signal was filtered at 150 Hz. (*B*): Longer time record of channel activity at an applied voltage of −30 mV with isotonic KCl patch pipette. The channel activity is shown to occur in bursts, which sometimes appear in clusters. (*C*): Open τ_o and closed state τ_c interval within a burst. The signal was low-pass filtered at 600 Hz in *B* and *C*

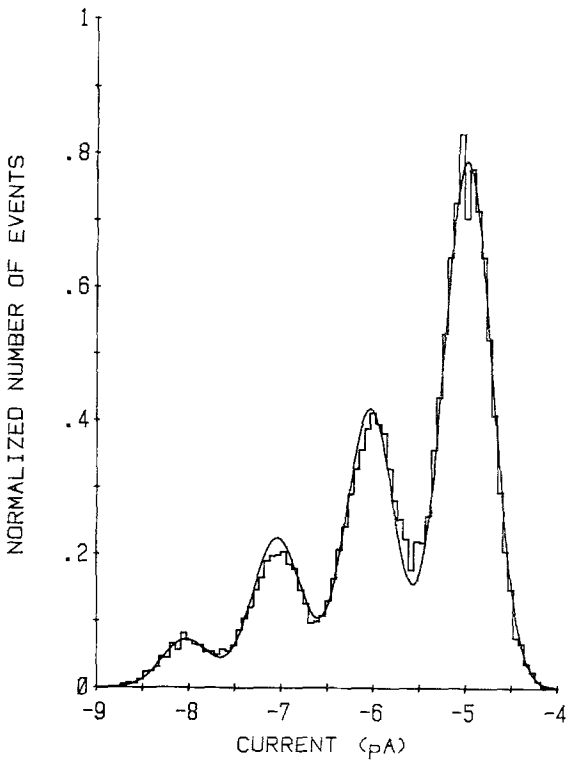

Fig. 3. Current amplitude histogram of a very active single-channel recording obtained with a patch pipette containing 0.15 M KCl and at $V_a = 0$. The difference between any two consecutive peaks is equal to 1.0 pA. The input signal was low-pass filtered at 600 Hz. Background level = −5.0 pA: first level = −6.0 pA, etc.

one observes from longer channel records. It should be apparent that the channel openings occur mainly in bursts separated by relatively long closing periods (typically 10–20 sec). Occasionally these bursts appeared in clusters with interburst gaps in the range of 1 sec. Figure 2*C* shows the rapid opening and closing events taking place on a much faster time scale within a single burst. Clearly, the channel returns to the original closed-state level for a short period of time. These results suggest, in our view, that there are at least two kinds of closures involved in this particular channel kinetics, the first kind describing the fast interruptions taking place during channel openings and the second kind taking into account the nonactive period between the bursts. Similar complex fluctuation patterns have already been reported in studies on Ach-activated channels (Sakmann, Patlack & Neher, 1980), K^+–Ca^{++} dependent channels (Pallotta et al., 1981) and Ca^{++}-activated nonselective channels (Yellen, 1982). On the average, one out of ten successful Gigohm seals with 0.15 M KCl patch electrodes showed this type of active channels. Smaller current jumps with much faster on–off transitions were occasionally present, but their contribution was not taken into account in this work.

In order to determine to what extent the occurrence of this particular channel was not more related to the low external concentration of ions such as Ca^{2+} ($< 10^{-6}$ M) than to the presence of K^+ in the patch pipette, experiments were carried out in which the patch electrode was filled with a solution containing 148 mM of KCl plus 2 mM of $CaCl_2$. Under these conditions we found current jumps similar to those described previously, so that the presence of K^+ in the pipette and not the lack of external Ca^{2+} seems to be the main factor involved in this case.

A current amplitude histogram of a single-channel recording obtained from a patch pipette

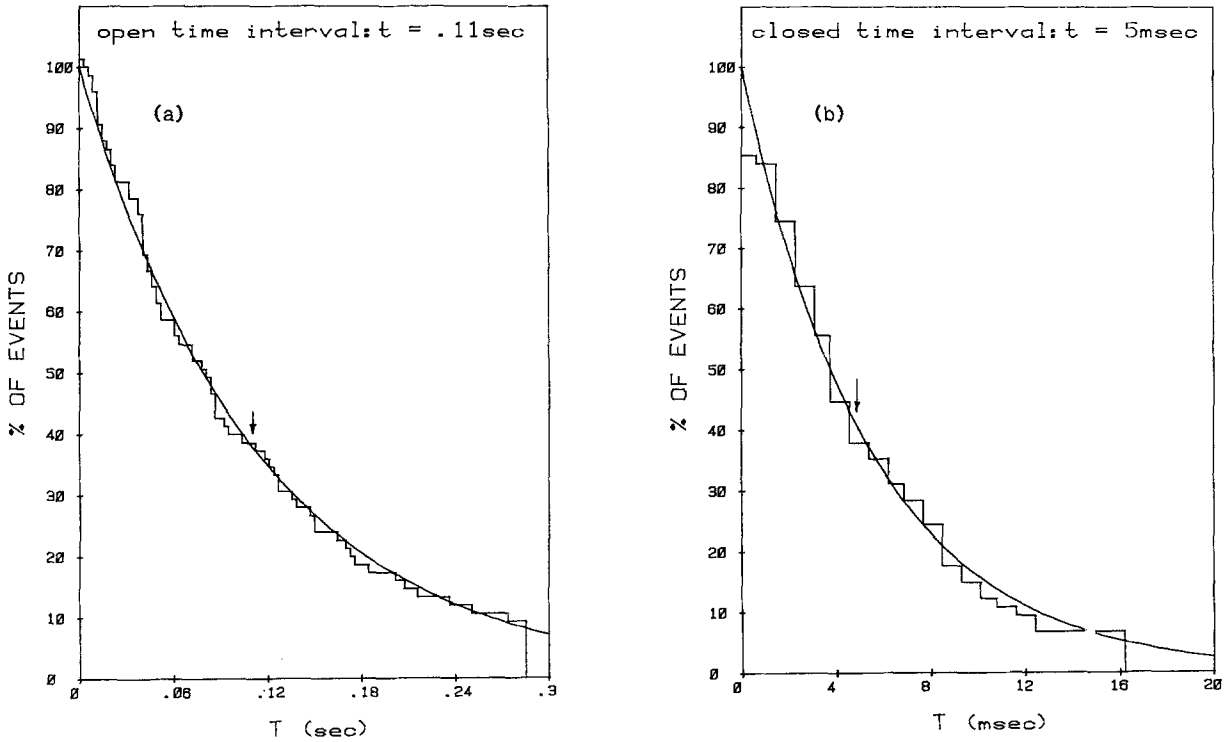

Fig. 4. Time-interval histogram of open τ_o and closed τ_c time intervals within a burst. Histograms computed from time records obtained with a patch pipette containing 0.15 mM KCl and at $V_a = -30$ mV. The computed mean life time reads 110 and 5 msec, respectively. The curves could be fitted by a single exponential function. τ_c also contains interburst intervals, but their contribution is not significant at his time scale

containing 0.15 M KCl is shown in Fig. 3. Equally spaced gaussian-like current levels, typical of current fluctuations caused by channel-like structures, can readily be seen. Figure 4*a* and *b* are examples of the time interval histograms one obtains for channel openings and closings within a burst. The current fluctuations in this particular case were recorded from a patch pipette containing 0.15 M KCl at an applied voltage of -30 mV (V total ≈ -55 mV). The analysis of open time intervals could easily be confined to intra-bursts events, the appearance of channel activity being clearly evident as illustrated in Fig. 2. However, within a cluster some intra-burst, closed time intervals could not be unambiguously distinguished from short inter-burst periods. Both time interval histograms could nevertheless be satisfactory least squares fitted by a simple exponential function as shown in Fig. 4*a* and *b*. The contribution of interburst periods to be closed time interval histogram does not seem therefore to be significant at least for time intervals smaller than 20 msec. It has to be mentioned, in addition, that current jumps less than 1 msec duration could not be adequately resolved since a sampling rate of 0.7 msec per point was used throughout. As a result, the computed open time period histogram is partially biased since very fast closures interrupting a channel opening could not be clearly detected. There should be, however, no more than 20% of these interrupting events, considering that the mean value of τ_c is approximately 5 msec. Consequently the error on the computed mean value of τ_o should also be less than 20%, assuming that these fast interrupting events are not caused by a rapid kinetic process superimposed upon the slower kinetic characterized by τ_o and τ_c.

Current-Voltage Relationships

The single-channel current amplitude was measured as a function of the applied voltage V_a for various KCl concentrations in the patch electrode (*see* Fig. 5*a*). One must bear in mind that the net voltage acting on the patched membrane area always included the cell resting potential E_m, so that the experimentally obtained zero current V_a value corresponds to the sum $-(E_p^i + E_m)$, E_p^i being the equilibrium potential associated with the single-channel current, Figure 5*b* shows four *I–V*

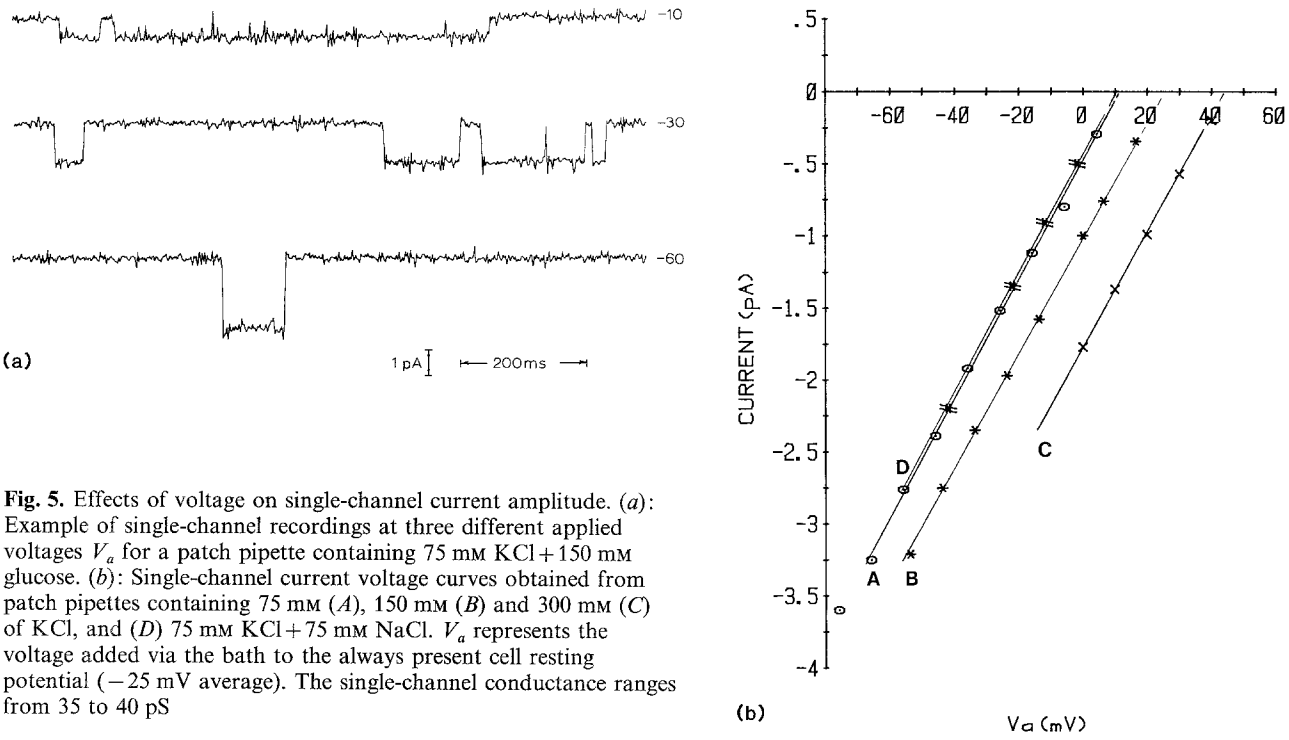

Fig. 5. Effects of voltage on single-channel current amplitude. (*a*): Example of single-channel recordings at three different applied voltages V_a for a patch pipette containing 75 mM KCl + 150 mM glucose. (*b*): Single-channel current voltage curves obtained from patch pipettes containing 75 mM (*A*), 150 mM (*B*) and 300 mM (*C*) of KCl, and (*D*) 75 mM KCl + 75 mM NaCl. V_a represents the voltage added via the bath to the always present cell resting potential (−25 mV average). The single-channel conductance ranges from 35 to 40 pS

curves obtained from patch electrodes containing, respectively, 75 mM KCl, 150 mM KCl, 300 mM KCl, and 75 mM KCl + 75 mM NaCl. It should first be apparent that the *I–V* curves are shifted in the positive direction by approximately 15 mV for every twofold increase of the KCl concentration in the pipette. Based on this result alone one may already conclude that the observed channel is mainly permeable to cations, the direction of the shift not being compatible with a Cl^--selective channel. A major contribution of the Na^+ inside the cell has also to be rejected, since a Na^+ current down its electrochemical gradient, especially with patch electrodes containing solely KCl, would have resulted in current jumps with a polarity opposite to that observed experimentally. Finally, since the *I–V* curves obtained with patch electrodes containing 75 mM KCl and 75 mM KCl + 75 mM NaCl led to the same zero current V_a value, it may be reasonably concluded that K^+ is the main charge carrier in this case. This conclusion was also confirmed by experiments that failed to show inward current jumps as described previously when the isotonic KCl solution in the patch electrode was completely replaced by NaCl. In fact, when patch pipettes containing 144 mM NaCl + 6 mM KCl were used, another type of single-channel events, characterized by outward current jumps occurring in bursts, could clearly be detected. A detailed analysis of these channels will be presented in future work.

The main characteristic of these *I–V* curves is, however, the absence of clearly detectable outward current jumps ($\Delta I > 0.3$ pA) at any of the KCl concentrations considered even at applied voltages greater than +100 mV. In fact, in order to account for our experimental results, the single-channel conductance for ($V_a + E_m > 0$) should be substantially less than 10 pS, since no outward current jumps of amplitude greater than 0.5 pA could be observed at applied voltage V_a greater than 100 mV. For negative transmembrane potentials (external medium considered to ground) the measured current-voltage relationship was mostly linear, corresponding to a single-channel conductance of 40 pS. This value is more than five times smaller than that reported by Pallotta et al. (1981) for the Ca^{2+}-activated K^+ channel on rat myotubes, but is at least five times larger than the single-channel conductance found by Fukushima (1981) for the K^+ current of the anomalous rectifier in tunicate egg cells (*see also* Ohmori, Yoshida & Hagiwara, 1981). We also found that the extrapolated value of the zero current applied potential V_a was variable from one series of experiments to another, with values ranging from +20 to +40 mV with isotonic KCl patch pipettes. Since

HeLa cells are continuously dividing, this variability may in part be attributed to a change in the cell electrical properties during the cell cycle. Wickson-Ginsburg and Solomon (1963) have shown in this regard that the K^+ content of HeLa cells varies during cell growth. More directly, measurements of HeLa cell resting potential have shown a relatively large scattering of the E_m (-27 to -47 mV) with an estimated mean of -37 mV (Roy & Sauvé, 1982).

Finally, in some cases, smaller single-channel currents (20–30 pS) associated with more positive zero current value of V_a (typically $+45$ mV) were also observed with patch electrodes containing 150 mM KCl. These smaller current jumps had a kinetic similar to that described previously, and could have been in part caused by 40 pS channels located in a poorly-defined seal area (*see* Neher et al., 1978). This explanation does not hold, however, for single-channel recordings in which double jumps of equal amplitude corresponding to a single-channel conductance of 20–30 pS were measured. For these particular recordings one has to assume that two or more channels can be located exactly in the same intermediate seal area, which is quite unlikely. Another interesting point is that all observed channels (20–30 or 40 pS) in a given recording showed the same reversal potential. These observations seem to indicate that this particular K^+ channel has more than one conducting state whose appearance can probably be related to the cell internal state. This point will be discussed in greater detail later. The possibility of several types of K^+ channels in the external membrane of HeLa cells cannot be ruled out, however.

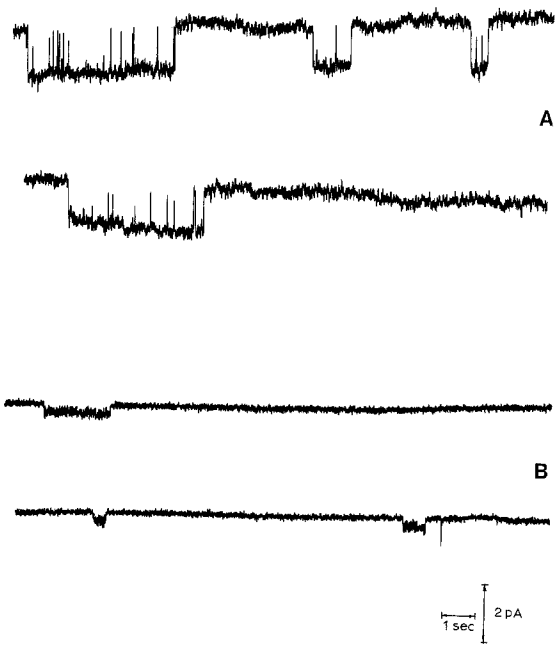

Fig. 6. Time records obtained at $V_a=0$ with patch pipettes containing 300 mM KCl in *A* and 75 mM KCl in *B*. As seen, the average burst length increases with more KCl in the patch electrode. Signal was low-pass filtered at 150 Hz

Table. Relationship between the KCl concentration in the patch pipette, and P_o, the probability of the channel to be in the open state[a]

K (mM)	P_o
75	0.04
150	0.11
300	0.32

[a] P_o was computed from current traces obtained at $V_a=0$ mV. P_o was taken as the ratio of the time spent by the channel in the open state divided by the total time of the record.

Effect of K^+ on the Channel Statistics

In order to determine to what extent the channel statistics were a function of the experimental conditions selected for the patch experiments, the relationship between the probability of a channel to be in the open state and the KCl concentration in the patch pipette was investigated. For each KCl concentration considered, several current records were selected for analysis.

Each chosen current trace was obtained at zero applied voltage ($V_a=0$) from cells having approximately the same E_m value (≈ -25 mV). From the ensemble of time records so selected, the total time spent by the channel in the open state was measured. The ratio: time spent in the open state/total time of the records was taken as a measure of P_o, the probability of a channel to be open. This computational procedure therefore took into account the nonactive periods separating the bursts and did not yield information on the equilibrium constant between the open and closed state within a burst. For those records with double or triple jumps the probability P_o was fitted according to:

$$P(n)=\frac{N!}{n!(N-n)!}P_o^n\,[1-P_o^n]^{N-n} \qquad (2)$$

where $P(n)$ is the probability of simultaneously having n channels open among N.

The estimate of P_o was usually based on more than 50 individual events, although for 0.3 M KCl

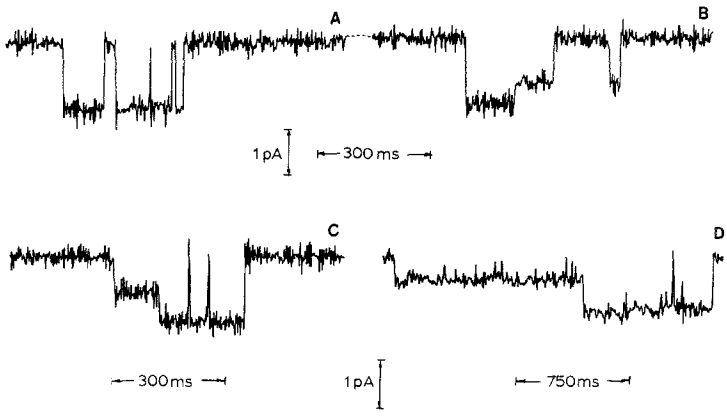

Fig. 7. Single-channel recordings of multistate transitions obtained with patch pipettes containing 75 mM of KCl in *A*, *B* and *C* and 150 mM in *D*. The applied voltage V_a was 0 mV in *A*, *B* and *C* and −20 mV in *D*. The two conducting states may exist independently (*A* and *B*) with sometimes transitions from one to the other (*B*)

only 20 events could be collected, the channel being open for most of the time.

Figure 6 shows two time records obtained with patch pipette containing 75 and 300 mM KCl, respectively. It should be apparent that the burst length and gap change on the average as a function of KCl concentration in the patch pipette. One cannot guarantee, however, from this result alone, that the probability of the channel to be in an open state will increase as a function of the KCl concentration. Longer burst lengths may in fact be correlated with longer interburst periods, leaving the overall ratio τ_o/τ_c unchanged.

The Table summarizes the findings of this analysis. As seen, a fourfold increase in KCl concentration results in an approximately six- to eightfold increase in the probability of the channel to be activated. This observation provides further support to results obtained by Roy and Sauvé (*to be published*) and Okada et al. (1973) which showed that the permeability of HeLa cells to potassium increases as a function of the external potassium concentration.

We also found that P_o increases slightly as a function of V_a, more positive value of V_a leading to higher values of P_o. However, these results were scattered and comparisons from one series of experiments to another difficult to make.

Multistate Events

In addition to the regular fluctuation pattern presented in Fig. 2, more complex waveforms were often recorded as illustrated in Fig. 7. These complex transitions occurred mainly between two states, although in some cases a third state could be assumed. Figure 7 *A* and *B* show that both states may occur independently, the lower conduction state being not necessarily the result of a transition from the main state. Figure 7 (*C* and *D*) illustrates the flickering process of the channel towards the closed state following multiple conducting state transition. We found that these complex transitions occurred both ways, namely from the higher conducting state to the lower and vice versa. Figure 8 presents the current voltage relationships obtained from a current recording with two main levels of conductance. The extrapolated zero current potential is in both cases approximately equal to 10 mV, and the single channel conductances reads 28 and 40 pS, respectively. It has to be pointed out, however, that an observed transition from one conducting state to the other could in fact arise from the unresolved occurrence of two independent events. In our case, the transition to base line and the return to one of the conducting states should be within 700 μsec in order for two independent events to become indistinguishable. For the system considered here, such an occurrance is thus quite unlikely since, with patch electrode containing 75 mM of KCl, the P_o probability of the channel to be open was estimated at 0.04. It thus appears that the observed K^+ channel has at least two distinct conducting states with possible transitions from one state to the other. Similar results have been published by Hamill and Sakmann (1981) on the Ach channel of skeletal muscle.

Discussion

Let us briefly summarize the main findings of this study. In cell-attached patch experiments with

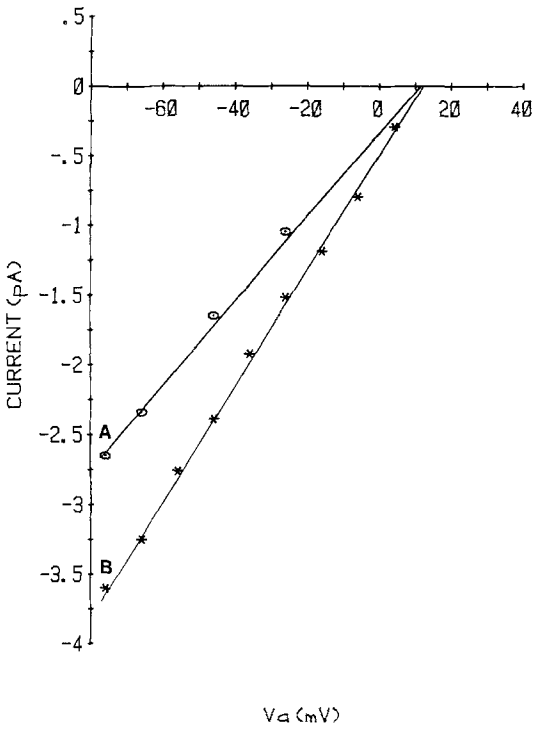

Fig. 8. Single-channel current voltage relationship obtained with patch pipettes containing 75 mM of KCl. The current traces showed two conductance levels with transition from one to the other. The single-channel conductance reads 28 pS in *A* and 40 pS in *B*, respectively

patch pipettes solely containing KCl, all-or-none type current fluctuations were observed, indicating the presence of ionic channel-like structures in the external membrane of HeLa cells. These channels were found to be mainly permeable to potassium ions with a related inward rectification *I–V* curve. An analysis of some selected time records has revealed also that the probability of the channel to be in the open state was dependent on the KCl concentration in the patch pipette. Finally, current traces were presented which showed the occurrence of burst-like events and multiple levels of conductance.

There are several possible explanations to the observed inward rectification effect associated with the present channel *I–V* curves.

As indicated in Eq. (1 a), the current-jump amplitude is related to the net potential acting on the patch area by the product $[R_p/(R_p+R_m)]^2$. In principle, the ratio R_m/R_p should be negligible since it corresponds to the ratio of the membrane area under the patch divided by the total surface of the cell. If, however, one assumes that the cell membrane has highly nonlinear current voltage characteristics, an increase of R_m/R_p may in fact result, the current in R_m being always in the opposite direction to that of R_p. This is, however, unlikely considering the known *I–V* curves measured on nonexcitable cells such as L cells (Roy & Okada, 1978), the amplitude of the current through the patch region (less than 10^{-10} A) and the presence of outward current jumps with patch pipettes containing mainly NaCl (results to be published).

A second alternative would be that the rectification property of this channel is due to the channel itself. Inward rectifying K^+ channels have already been observed in cultured rat myotubes (Ohmori et al., 1981) and in tunicate egg cells (Fukushima, 1981). However, in addition to a larger single-channel conductance (40 pS compared to 10 pS or less), the present K^+ channel seems to differ in several ways from the corresponding channels in muscle and in eggs. For instance, due to the channel inactivation Ohmori et al. (1981) had to use Ba^{2+} as a blocking agent in order to see current fluctuations in steady-state conditions where all the channels were activated. A similar inactivation process for the K^+ rectifier in HeLa is not likely, since in our case single-channel events were observed over steady-state periods exceeding 30 min. We also failed to obtain an increase of single-channel conductance proportional to the square root of the external K^+ concentration. The presence of surface charges on the external membrane of HeLa cells and the nonconstant ionic strength of the ionic solutions in the patch electrode may have contributed to this relatively constant value of the single-channel conductance for different KCl concentrations. It remains, however, that our results can be at least qualitatively interpreted in terms of a multi-ion blocking model with an internal blocking agent as proposed originally by Armstrong (1975) and discussed in detail by Hille and Schwarz (1978). Within this framework, the effect of external K^+ would be to compete for sites and thus to clear the channel of blocking ions. Positive transmembrane potential would favor the blocking action of the channel, while increasing the external K^+ concentration would help to clear the channel. The probability of the channel to be open will thus increase at higher KCl concentrations, as we observed experimentally. The kinetics scheme underlying these events appears, however, to be complex. In fact our results indicate that there are at least two closed states together with open states having mul-

tiple levels of conductance. Moreover, some rate constants appear to be functions of the external K^+ concentration, as illustrated in Fig. 6. A more detailed analysis would obviously require more information than has been presented so far in this work.

The present study was mainly concerned with establishing the presence of K^+-selective ionic channels in the external membrane of HeLa cells. Several important questions have remained unanswered. For example, is the channel Ca^{2+} activated? What is the origin of the flickering while the channel is open? Is the inward rectification a property of the channel *per se?* These questions will be discussed in greater detail in future work.

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