this case channel gates controlled by E_p would look as if controlled by E_j.

When pCa_i or pH_i is altered, the effect is to shift the $g_j(E)$ curve along the E axis without other changes (see e.g., Fig. 3). From this finding we conclude that E, Ca^{2+}, and H^+ all affect the same gates—although we do not know whether any of these acts directly on the gates—and that Ca^{2+} and H^+ do so without influencing the voltage sensitivity (A) of the gates.

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SINGLE Ca^{++} DEPENDENT K^+ CURRENTS IN HELA CANCER CELLS

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Using the extracellular patch-clamp method (1), we have investigated the single-channel events underlying the electrophysiological properties of HeLa cells, a human cell line obtained from an epidermoid carcinoma of the cervix. In a first paper, we presented recordings of discrete current jumps observed with patch electrodes containing solely KCl (75 mM up to 300 mM)(2). This particular channel was found to be mainly permeable to K^+ and showed multiple levels of conductance (40 pS and 28 pS). In addition, the channel I-V curves obtained at various KCl concentrations in the patch electrode were all characterized by a strong inward-rectification effect. We present in this paper recordings of another type of single channel event, made during cell-attached and outside-out patch-clamp experiments. In the cell-attached configuration using electrodes filled with normal saline (140 NaCl + 5 KCl), we detected clear outward-current jumps occurring mainly in bursts. We also found, through various outside-out patch clamp experiments, that this particular channel was mainly permeable to potassium ion and showed Ca^{++}-dependent open-closed kinetics.

MATERIALS AND METHODS

HeLa cells were obtained from the Institut Armand-Frappier in Montreal and subcultured in Falcon bottles (75 cm^2, #3024). The culture medium was MEM, Earle base (Gibco #F-11, Gibco Diagnostics, Chagrin Falls, OH) with 25 mM HEPES buffer and 6 mM bicarbonate. This medium was supplemented with 10% fetal calf serum (Gibco #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.

Unless specified otherwise all cell-attached patch-clamp experiments reported in this work were carried out with patch electrodes containing 140 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 0.81 Mg SO_4 and 10 HEPES buffered at pH 7.2. We will refer to this solution as "Earle-modified." For outside-out patch-clamp experiments, we used pipettes filled with a solution containing 150 mM KCl, 3 mM HEPES (pH 7.2), to which various concentrations of $CaCl_2$ were added (0.1–2 mM). In both types of experiments, the external medium was an Earle-HEPES solution with 116 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.81 $MgSO_4$, 6 $NaHCO_3$, 1 NaH_2PO_4, 5.5 glucose and 25 HEPES (pH 7.3). All experiments were done at room temperature (23°C). The essentials of our setup have been described in detail elsewhere (2). Each experiment was usually recorded on FM tape at a bandwidth of 1 KHz (H.P. 3964A) before being low-pass filtered (4 pole-Bessel) and digitized for computer analysis (MINC 11/23, Digital Equipment Corp., Marlboro, MA). The current jump amplitudes were derived from current amplitude histograms. The time intervals between opening or closing events were detected by setting, for a selected portion of the time record, an intermediate reference level corresponding to the current value with the lowest probability of occurrence. Open and closed time intervals were thus taken as the time the signal remained above or below this reference current level, following the detection of two transitions of opposite polarity.

RESULTS

Fig. 1 *A–E* shows single-channel recordings obtained on two different HeLa cells in cell-attached patch-clamp experiments carried out with patch electrodes filled with normal saline. Outward current-jumps due mainly to potassium ions can readily be observed. The single-channel conductance at low membrane potentials was estimated at 10 pS. Two basic fluctuations patterns are presented. With certain cells (Fig. 1 *A* and *B*), the recorded single-channel events appeared mainly as short bursts of channel openings separated by longer silent periods. With other cells we observed a different channel behavior, characterized by longer channel openings frequently interrupted by brief transitions towards a closed state (Fig. 1 *C–E*). In many cases, we found (Fig. 1 *D*) a random mixture of these two basic fluctuation patterns. A time-interval histogram analysis of these particular records, an example of which is given in Fig. 2 *A* and *B* for the time record shown in Fig. 1 *D*, indicates that at least three closed states and probably two open states are present. In fact, two of the estimated

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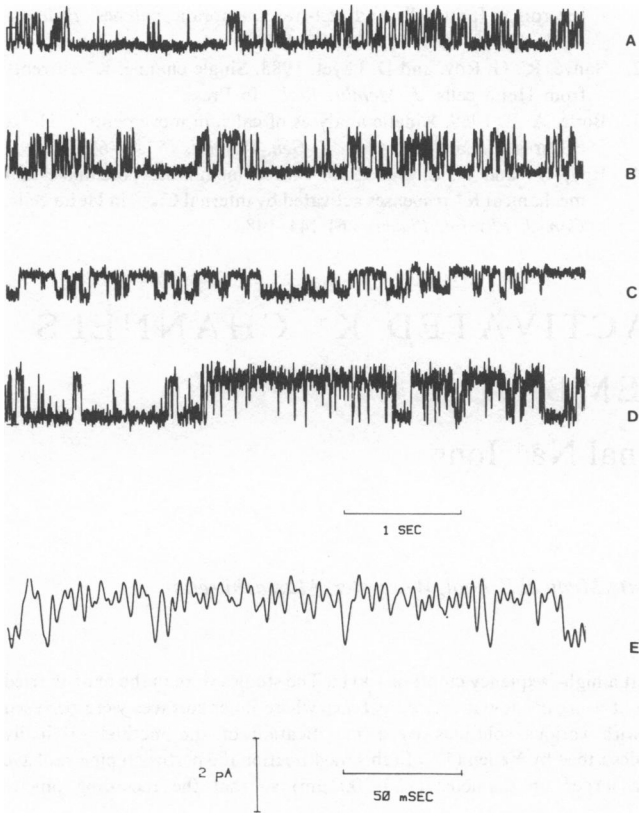

FIGURE 1 Single-channel recordings obtained in cell-attached patch clamp experiments on HeLa cells with patch pipettes containing: *A*, *B*, modified Earle solution (see Materials and Methods); *C*, *D*, *E*, 145 NaCl + 5 KCl. The cell bathing medium was an Earle-HEPES solution. The applied transmembrane potential was (assuming a cell resting potential of −35 mV) −5 mV in *A* and *C*, 15 mV in *B*, and 25 mV in *D* and *E*. The current trace was low-pass filtered at 180 Hz in *A*, *B*, *C*, and *D*; 500 Hz in *E*. Outward current is indicated as upwards. Temperature 23°C.

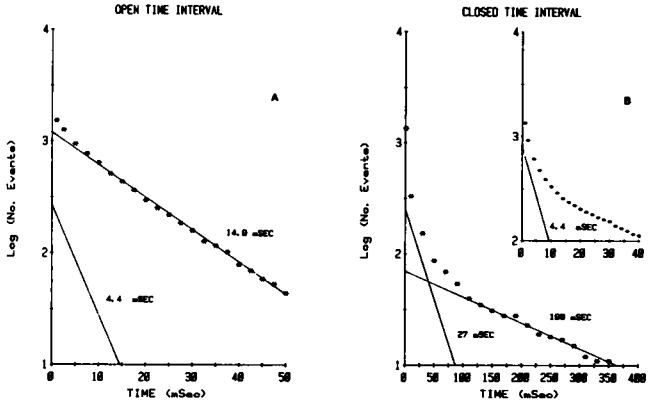

FIGURE 2 Open- and closed-time interval histograms obtained from the single-channel recording shown in Fig. 1 *D*. The original current trace was in this case filtered at 500 Hz, (1.3 ms filter rise time) and sampled at 2,500 points/s. Events <1 ms were not considered. The intervals were measured according to a reference level computed from the current-trace–amplitude histogram (see Materials and Methods). An analysis of the closed-time intervals led to three different time constants of 4.5 ms, 27 ms, and 198 ms. *Inset*, The contribution of the fast closing process. Two time constants of 4.5 ms and 14.9 ms were obtained for the open-time intervals.

time constants can be directly related to the process illustrated in Fig. 1 *C* and *D*, in which the channel is mostly in an open state (14.9 ms in Fig. 2) with brief transitions to a closed state (4.5 ms in Fig. 2), while two others take into account the brief channel openings (4.5 ms) occurring during longer closed time periods (27 ms), as described in Fig. 1 *A* and *B*. A fifth time constant (200 ms) describes the long silent periods between the bursts.

To investigate the potential role of Ca^{++} as a modulating agent of the observed channel behavior, outside-out patch clamp experiments were carried out using pipettes filled with 150 mM KCl, 3 mM HEPES (pH 7.2) at various $CaCl_2$ concentrations (0.1–2 mM). Examples of recordings are shown in Fig. 3. At low Ca^{++} concentrations (0.1 mM), the observed fluctuation patterns corresponded to that described in Fig. 1 *A* and *B*. Increasing the Ca^{++} content led to a greater number of long-lasting channel openings interrupted by brief closures, as in Fig. 1 *C* and *D*, and thus to a greater open-time probability. This effect became apparent at Ca^{++} concentrations >0.5 mM, which is close to the internal Ca^{++} content of 0.25 mM measured on these cells using radioactive tracers (3). The presence of a K^+–Ca^{++}-activated current was also confirmed through measurements of membrane potentials with microelectrodes (4).

We have presented results compatible with a second class of single-channel events in HeLa cells with patch electrodes filled with normal saline. Outward-current jumps could readily be seen, ruling out any direct contribution of a potassium channel with inward rectification properties as described earlier (2). Furthermore, two basic fluctuation patterns seem to be present with Ca^{++}-dependent occurrence relative probability. Future work will show whether or not these two classes of single channel events can be linked to a single molecular structure.

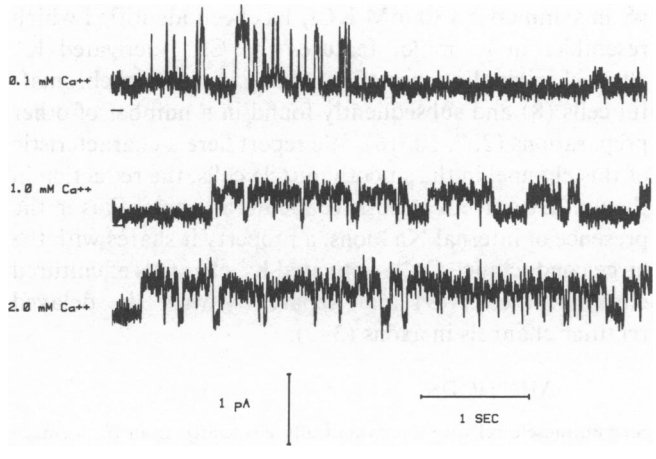

FIGURE 3 Current fluctuations recorded from outside-out membrane patches. Pipette solution: 150 mM KCl, 3 mM HEPES buffered at pH 7.2 and Ca^{++} (0.1–2 mM). The outside bathing medium was a Earle-HEPES solution. All records were obtained at zero applied voltage. The current traces were filtered as in Fig. 1. Temperature 23°C.

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LARGE CONDUCTANCE Ca^{++}-ACTIVATED K^+ CHANNELS IN SMOOTH MUSCLE CELL MEMBRANE

Reduction in Unitary Currents Due to Internal Na^+ Ions

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To circumvent the many problems associated with recording and analyzing transmembrane ionic currents in syncytial tissue preparations, we have used a preparation of freshly dissociated, single-vertebrate smooth muscle cells for electrophysiological studies. Early in the course of experiments on these cells the presence of a Ca^{++}-activated K^+ conductance became apparent (11, 13). In subsequent studies of macroscopic currents with conventional two-microelectrode voltage-clamp techniques, we found that this conductance gave rise to a large-peak outward current whose magnitude depended on the amplitude of the preceding inward calcium current (14). To characterize this conductance further, we have recently examined single-channel currents in these smooth muscle cells using the patch-clamp technique (12, 15). In these studies a channel with a large unitary conductance (~250 pS in symmetric 130 mM KCl) has been identified which resembles in its major features the Ca^{++}-activated K^+ channel originally reported by Marty in cultured chromaffin cells (8) and subsequently found in a number of other preparations (2, 7, 10, 16). We report here a characteristic of this channel in the smooth muscle cells, the reduction in the unitary current at positive membrane potentials in the presence of internal Na^+ ions, a property it shares with the large conductance Ca^{++}-activated K^+ channels of cultured chromaffin cells (9) and apparently with the delayed rectifier channels in axons (3–5).

METHODS

Smooth muscle cells were enzymatically dissociated from the stomach muscularis of the toad *Bufo marinus* by minor modifications of the technique originally developed by Bagby and his collaborators (1). The cells were used on the same day as isolated to avoid long-term changes that might occur in culture. We used a List EPC-5 patch clamp and standard single-channel recording techniques (6), except that patch pipettes were usually not coated with insulator. Recordings were filtered at a high-frequency cutoff of 1 kHz. The studies were in the main carried out using inside-out excised patches whose inner surfaces were perfused with various solutions by a modification of the method originally described by Yellen (17). In this modification the perfusion pipettes have a larger tip diameter (150–200 μm) so that the recording pipette

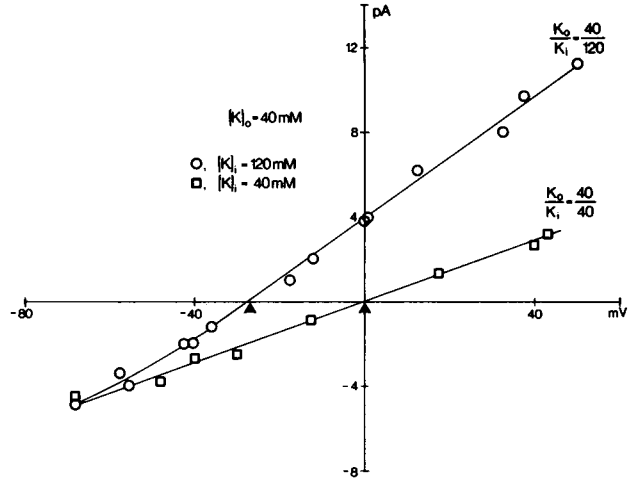

FIGURE 1 Current-voltage relationship of the open Ca^{++}-activated K^+ channel to demonstrate its K^+ selectivity. Recordings were made from an excised inside-out patch in the presence of two different perfusing solutions, one containing 40 mM K^+ and the other 120 mM K^+. The arrowheads below the abscissa give the expected values for E_K, given the patch pipette concentration of 40 mM K^+. The shift in the zero-current level is in good agreement with the expected shift for a highly K^+-selective channel. KCl was exchanged for NaCl to obtain the different K^+ concentrations in the perfusing solutions. (For a given positive potential the ratio of the amplitudes of the unitary currents at the higher and lower $[K^+]_i$ are predicted within 10% by calculations using the Goldman constant field equation.) Patch-pipette solution contained: 40 mM KCl, 82 mM NaCl, 1.8 mM EGTA, 0.9 mM $MgCl_2$, 1.8 mM HEPES. 40-mM K^+ perfusate contained: 40 mM KCl, 92 mM NaCl, 0.023 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM HEPES. 120-mM K^+ perfusate contained: 120 mM KCl, 14 mM NaCl, 0.023 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM HEPES.

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