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Oscillatory Activation of Calcium-Dependent Potassium Channels in HeLa Cells Induced by Histamine H_1 Receptor Stimulation: A Single-Channel Study

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Summary. We have used the patch-clamp method (O.P. Hamill et al., *Pfluegers Arch.*, **391**:85–100, 1981) in order to investigate the activation pattern of a calcium-dependent potassium channel following H_1 receptor stimulation in HeLa cells. Our results essentially indicate that the stimulation of H_1 receptors by exogenous histamine at concentrations greater than 1 μM induces an oscillatory activation pattern of calcium-dependent potassium channels characterized by the occurrence of channel current bursts separated by long silent periods. It was also found that the occurrence of these bursts could be directly correlated with transmembrane potential oscillations, the latter being the resulting effect of the calcium-dependent potassium channel synchronous openings. In addition, the cyclic activation of the calcium-dependent potassium channels could be initiated by the addition of histamine to a calcium-free external medium, indicating that the stimulation of the H_1 receptors in HeLa cells is mainly related to the release of calcium from internal stores. Finally, the membrane-permeable cyclic AMP analog dibutyryl cyclic AMP was found to be ineffective in initiating single-channel events such as those triggered by exogenous histamine. It is proposed that the oscillatory activation of the calcium-dependent potassium channels in HeLa cells results from a repetitive transient increase in cytosolic free calcium concentration consequent to the H_1 receptor stimulation.

Key Words oscillation · histamine · H_1 receptor · Ca^{2+}-activated potassium channel · patch clamp · HeLa cells

Introduction

The role of intracellular calcium as second messenger in numerous cellular events is now well established (*see*, for instance, Campbell, 1983; Rasmussen & Barrett, 1984). In particular, considerable evidence has accumulated that the electrical response in many systems to various external stimuli such as hormones and neurotransmitters is partly mediated by calcium-activated potassium channels (*see* Atwater et al., 1983; Schwarz & Passow, 1983; Petersen & Maruyama, 1984). There should be therefore in many cases a direct correlation between the activation pattern of calcium-dependent potassium channels and the transient mobilization of intracellular calcium ions following the stimulation of plasma membrane receptors. With the advent of the extracellular patch-clamp method (Hamill et al., 1981) it is now possible to measure the activation process of calcium-dependent potassium channels at the single-channel level. Single-channel measurements can thus potentially serve to investigate the cytoplasmic free calcium fluctuations occurring in a single cell.

It was shown in a previous study that HeLa cells, an epithelial cell line derived from an epidermoid carcinoma of the cervix, possess a particular type of calcium-activated potassium channel (Sauvé et al., 1986). This channel was found to behave as an inward rectifier with a conductance in symmetrical 200 mM KCl of 50 and 10 pS at large negative and large positive membrane potentials, respectively. The channel activity could be triggered at cytoplasmic calcium concentrations ranging from 0.1 to 1 μM and the open-channel probability was found to be voltage insensitive within the voltage range -100 to $+100$ mV. Under normal conditions, the transmembrane potential is not therefore expected to play an important role in the control of the channel activity.

The HeLa cell external membrane also contains H_1 receptors whose activation was shown to mediate an increase of potassium conductance following an exogenous addition of histamine (Hazama et al., 1985). Since the stimulation of H_1 receptors has often been associated to an increased mobilization of intracellular calcium ions (Schwartz, 1979; Douglas, 1980; Hough & Green, 1984; McNeill, 1984), it is likely that the potassium channel described previously is involved in the histamine-evoked hyperpolarization reported by Hazama et al. (1985).

We have thus undertaken a single-channel study aimed first to determine if the calcium-acti-

vated potassium channel found in HeLa cells was responsible for the hyperpolarizing response induced by histamine, and second to investigate how the activation pattern of these potassium channels could be related to the fluctuation pattern of the intracellular calcium concentration consequent to the H_1 receptor stimulation. Our results indicate that the activation of these potassium channels follows an oscillatory fluctuation pattern, suggesting that the stimulation of H_1 receptors in HeLa cells induces a repetitive transient increase in cytosolic free calcium concentration.

Materials and Methods

Cell Culture

The details of the cell culture procedure have been described elsewhere (Sauvé et al., 1986). Essentially, the cells were grown in monolayers in plastic petri dishes (Falcon #3002) and used for patch experiments five to seven days after being subcultured. The culture medium was 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 mg of gentamycin per liter.

Solutions

The standard extracellular solution was an Earle medium which contained (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. Calcium-free external solutions were prepared by adding 1 mM EGTA to Earle solutions containing no $CaCl_2$. Unless specified otherwise, the patch electrodes were filled with a solution containing 200 mM KCl, 0.5 mM $MgSO_4$, 1 μM $CaCl_2$, 25 mM HEPES buffered at pH 7.3 with 10 mM KOH. This solution was chosen in order to optimize the signal-to-noise ratio for potassium current recording and will be referred to as the 200-KCl pipette solution. Histamine dihydrochloride, the calcium ionophore A23187 and N^6,2'-O-dibutyryladenosine 3'-5'-cyclic monophosphate were purchased from Sigma (Sigma Chemical Company, St. Louis, Mo.). All the solutions were passed through a filter of pore size 0.2 μm.

Patch Clamp

Single-channel currents were recorded from untreated cell membrane surfaces using the patch-clamp method in the cell-attached configuration as described by Hamill et al. (1981). The patch electrodes were pulled from Pyrex® capillaries (Corning 7040) and had an electrical resistance ranging from 5 to 10 mΩ when filled with a 200-mM KCl solution. The essential of our electronic setup has been described in an earlier work (Sauvé et al., 1983). The voltage inside the pipette is always maintained to virtual ground in our case, so that the expression "externally applied potential" corresponds here to the potential of the bath compared to ground. The current traces we obtained were all recorded directly on FM tape (H.P. 3964A) at 3-3/4 ips (bandwidth DC to 1.25 kHz). Records were subsequently filtered using a combination of two low-pass four-pole Bessel filters (VVS #300-B) before being digitized and stored in a continuous mode on hard disks (Digital Minc 11/23). Unless specified otherwise, we used a sampling rate of 2250 pts/sec which corresponded to five times the filtering frequency chosen for the signal. Current traces were then selected and plotted using a digital X-Y plotter (H.P. 7225-B) interfaced with our main computer. The experimental procedure we used in order to change the external medium while being in the cell-attached configuration consisted of continuously perfusing the 2 ml petri dish containing the cells for 40 sec at approximately 0.25 ml/sec. The perfusion was then stopped and the resulting single-channel activity recorded. In some cases, the signal was recorded throughout the perfusion period, but additional noise sources were usually present under these conditions resulting in a lower signal-to-noise ratio. However, the time at which single-channel events started to occur could be in all cases clearly determined. All the experiments reported in this work were carried out at room temperature (23°C).

Results

Activation of Calcium-Dependent Potassium Channels by Histamine

In order to investigate if the exogenous addition of histamine could activate calcium-dependent potassium channels in HeLa cells, cell-attached experiments were first undertaken in which the Earle bathing medium was replaced by an Earle solution containing 1 mM histamine. The patch electrode was filled with a 200-KCl solution. Figure 1 illustrates a typical example of the resulting single-channel activation process. As shown in (*A*), no single-channel event could be detected in most of our cell-attached experiments performed in the absence of histamine. This result indicates that the calcium-activated potassium channels in HeLa cells are not activated under normal conditions. However, the current trace presented in Fig. 1(*B*) clearly shows that an oscillatory single-channel activation pattern can be obtained after the exogenous addition of histamine (1 mM), with an important and sustained channel activity directly after the introduction of the histamine medium. This period of sustained activity lasted on the average 20 to 40 sec and was followed by silent periods interrupted by bursts in which several channels were activated simultaneously (*see* Fig. 1*B*). These bursts occurred at regular intervals at the beginning but were separated by increasingly longer silent periods after 1 or 2 min. The number of single-channel events within a burst was also found to be time dependent with the number of events per burst decreasing gradually until no

single-channel event could be clearly detected. This oscillatory activation pattern was observed in most of our experiments (>80%) with 10^{-3} M histamine and lasted on the average from 3 to 5 min with a frequency ranging from 1.8 to 3.0 bursts/min. After that period no apparent single-channel event could be detected. Subsequent perfusions with histamine-free and histamine-containing external solutions failed to trigger single-channel openings. In one particular case where we had returned to the histamine-free control medium for more than 15 min, the subsequent addition of histamine succeeded to initiate a transient single-channel activation. No oscillatory activation could, however, be observed.

Experiments were next undertaken in order to determine if the ionic channel, whose activity could be initiated by histamine, corresponded to the calcium-dependent potassium channel described previously (Sauvé et al., 1986). These experiments consisted of measuring the channel I/V curve during the initial period of sustained single-channel activity. The amplitude of the current jumps was estimated by least-squares fitting a summation of Gaussians to the current amplitude histogram obtained from selected segments of the current record. The results are presented in Fig. 2. As seen, the channel behaved as an inward rectifier with a conductance at large negative potentials of 47 pS with pipettes containing 200 mM KCl. This is in agreement with the results obtained previously in inside-out experiments where the conductance of the calcium-activated potassium channel in HeLa cells was estimated at 50 pS for inward currents in symmetrical 200 mM KCl. Furthermore, we could observe that the single events initiated by adding 4 μM of the calcium ionophore A23187 in the bathing medium corresponded to those recorded following the addition of histamine. In both cases, the current jump amplitude was nearly equal to 3.5 pA at zero applied voltage and the channel activity appeared as brief channel openings with a mean lifetime ranging from 3 to 5 msec. This is also in agreement with our previous work (Sauvé et al., 1986) where it was shown that the effect of internal calcium on this channel is more to increase the number of channel openings per second than to increase the channel mean open time. This particular behavior corresponds to what was observed in cell-attached experiments where histamine was present in the cell bathing medium. In fact, it can be seen in Fig. 1(*C*) that the number of channel openings within a burst increases gradually, reaches a maximum value with multiple channel openings and decreases slowly as a function of time. The channel mean open time remained, however, approximately unchanged

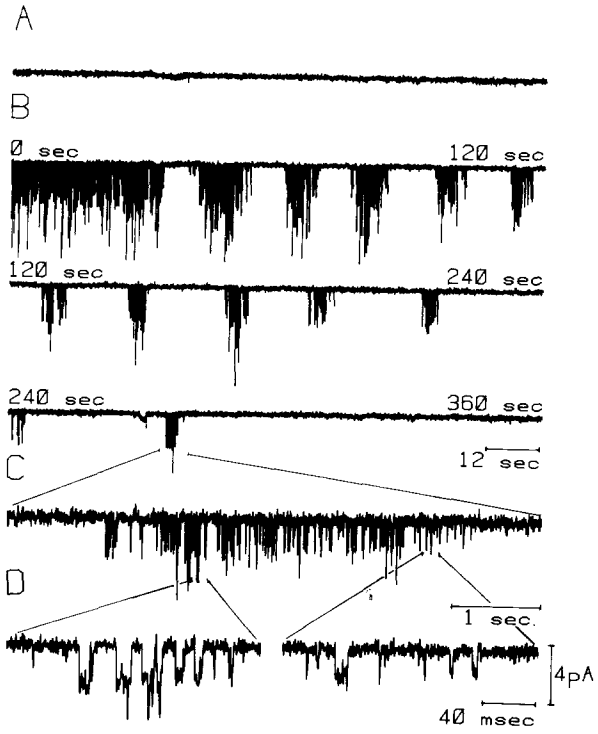

Fig. 1. Single-channel recording obtained from a cell-attached experiment with a patch electrode containing a 200-mM KCl solution as described in Materials and Methods. In (*A*) the external bathing medium was an Earle solution and no single-channel event could be detected. In (*B*) the external bathing medium was replaced by an Earle solution containing 1 mM histamine. As seen, the presence of histamine in the external medium has initiated the cyclic appearance of bursts in which several channels are activated simultaneously. The silent period between successive current bursts increases as a function of time and the number of single-channel events per burst decreases gradually until no channel opening can be clearly detected. A more detailed description of the single-channel events occurring in a single burst is presented in (*C*). The electrical activity within a burst is shown to increase gradually, to reach a maximum and then to decrease to its original value as a function of time. In (*D*), segments of low and high activity are presented in which the channel mean open time is shown to remain approximately constant within a burst. The current traces (*A*), (*B*) and (*C*) were filtered at 400 Hz. The record shown in (*D*) was obtained at a sampling rate of 10,000 pts/sec filtered at 800 Hz

throughout this process (*see* Fig. 1*D*). Based on these observations, it thus appears that the ionic channel triggered by histamine corresponds to the calcium-activated potassium channel described previously.

In order to determine if the observed silent periods between successive current bursts were due to channel desensitization, experiments were also carried out in which the patch configuration was changed from cell-attached to inside-out during a

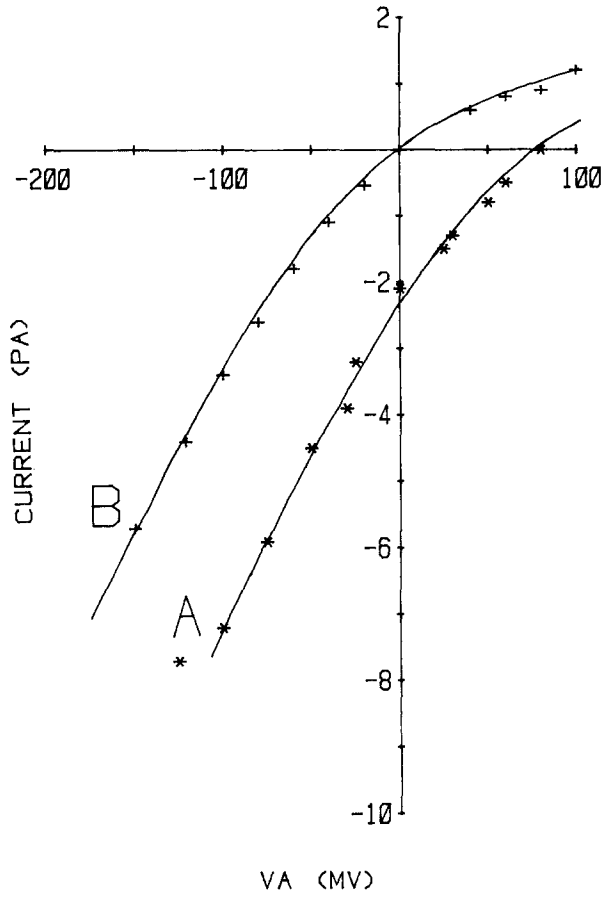

Fig. 2. Single-channel *I/V* curves measured with a patch electrode containing a 200-mM KCl solution. The *I/V* curve (*A*) was obtained in the cell-attached configuration. The external bathing medium was an Earle solution containing 1 mM histamine. The current jump amplitude was estimated from current amplitude histograms measured from segments taken at the beginning of records where a sustained single-channel activity could be obtained as shown in Fig. 1. In this case, the effective potential applied across the patched membrane area includes, in addition to the known applied potential V_a, the contribution of the cell resting potential. The *I/V* curve of the calcium-activated potassium channel described previously (Sauvé et al., 1986) on HeLa cells is presented in (*B*). This *I/V* curve was obtained in the inside-out configuration with a cytoplasmic solution containing 200 mM KCl, 0.4 μM $CaCl_2$, 0.5 mM $MgSO_4$ buffered at pH 7.3 with 10 mM HEPES. In this case, the applied potential V_a corresponds to the effective potential across the patched membrane area. Both channels have the same inward rectifying properties and the same single-channel conductance for inward currents. No clear outward currents could, however, be measured in (*A*)

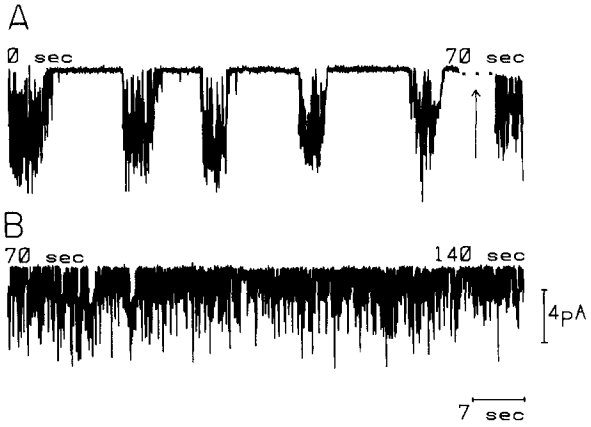

Fig. 3. Patch-clamp experiment in which the configuration was changed from cell-attached to inside-out during a silent period. The external solution was an Earle medium containing 0.4 μM calcium. The pipette was filled with a 200-mM KCl solution and the applied voltage was equal to 0 mV when in the cell-attached configuration. The arrow indicates the time at which the patch was excised. As seen in (*B*), this procedure led to a sustained activation of the channel, indicating that the channel (*A*) was always sensitive to calcium within the micromolar range. A potential of −50 mV was applied in the inside-out configuration to increase the current jump amplitude

silent period in the presence of 0.4 μM Ca^{2+} in the Earle external bathing medium (*see* Figs. 3*A* and 3*B*). As can be observed in Fig. 3(*B*), this procedure led systematically to a sustained activation of the channel. Clearly the channel was still sensitive to internal calcium since its exposure to an Earle solution containing 0.4 μM calcium succeeded promoting single-channel openings. The observed silent periods between successive current bursts are thus likely to be mainly caused by a decrease in intracellular free-calcium concentration. It may then be tentatively suggested that the oscillatory activation pattern shown in Fig. 1(*B*) arises from a repetitive transient increase in intracellular free-calcium ions consequent to the H_1 receptor activation in HeLa cells.

To infer the role of the calcium-dependent potassium channel in the histamine-evoked hyperpolarization reported by Hazama et al. (1985), we used current records which contained in addition to calcium-activated potassium channels, the slow inward rectifying potassium channel (SK) described in a previous work on HeLa cells (Sauvé et al., 1983). Our approach consisted essentially of using the calcium-independent SK channel to monitor intracellular potential variations while measuring the activation of calcium-dependent potassium channels. The identification of these two types of potassium-selective channels was based on their respective kinetic behavior (Sauvé et al., 1983, 1986). Figure 4 presents examples of cell-attached single-channel records typical of each of these potassium channels. The SK channel, which remained in inside-out experiments insensitive to internal calcium, showed for inward currents a kinetic behavior characterized by long channel openings interrupted by briefer shut intervals. As also seen in

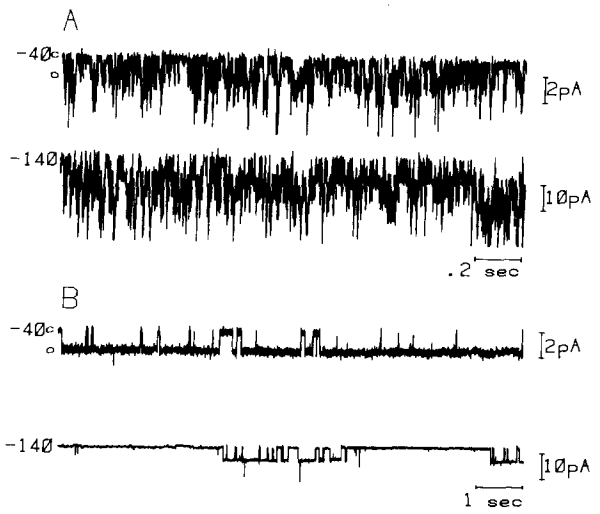

Fig. 4. Examples of single-channel recordings typical of the calcium-activated potassium channel in (*A*) and of the slow inward rectifying potassium channel (SK) in *B*. Both recordings were obtained in the cell-attached configuration with a patch electrode containing a 200-mM KCl solution. The calcium-activated potassium channel is mainly characterized by brief channel openings whose frequency was shown to be controlled by internal calcium, whereas the activity of the calcium-independent SK channel appears as long channel openings interrupted by briefer shut intervals. The sampling rate was 2,000 pts/sec in (*A*) filtered at 600 Hz and 400 pts/sec in (*B*) filtered at 200 Hz

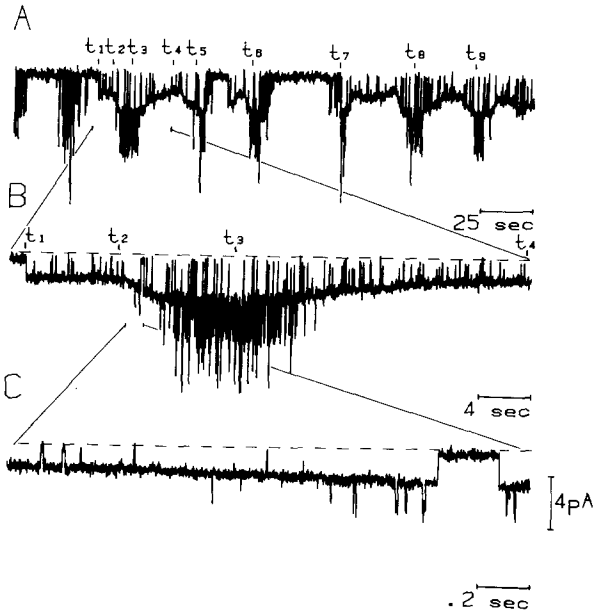

Fig. 5. Single-channel recordings in which SK and calcium-activated channels are present. Current records were obtained in the cell-attached configuration at zero applied potential with a patch pipette containing a 200-mM KCl solution. The cell bathing medium consisted of an Earle solution with 1 mM histamine. As seen in (*A*), the cyclic bursting activation of the calcium-dependent potassium channels occurs concomitantly with an increase in the amplitude of the current jumps associated with the calcium-independent SK channel. It is shown on a faster time scale in record (*B*), that a variation of the cell potential, as measured by SK channel current jump amplitude, is invariably correlated with a change in the number of calcium-dependent potassium channel openings per second. In (*C*), individual channel openings can be observed. The current records were filtered at 500 Hz

Fig. 4(*B*), the open-channel probability P_o changes as a function of voltage in this particular case, with P_o increasing at more positive potential values. This channel is similar to the one discussed by Sakmann and Trübe (1984*a*,*b*) and Payet et al. (1985) on cardiac cells. In contrast, the calcium-activated potassium channel activity appeared as brief channel openings whose occurrence was shown to depend upon the cytoplasmic calcium level. Both types of channel can be therefore easily distinguished based on their respective mean open time.

In addition, it was already established that both types of channel behave approximately as ohmic resistors at large negative voltages. Consequently, there should be a direct correlation between the amplitude of the potassium current jumps resulting in particular from the SK channel fluctuations, and the effective potential difference V_m applied across the membrane area under the patch electrode. Such a correlation becomes especially useful when both the patch electrode and the cell bathing medium are maintained to zero potential in the cell-attached configuration, since the potential V_m is then explicitly equal to the cell transmembrane potential. Under these conditions and with a patch pipette containing 200 mM KCl, a hyperpolarization of the cell will thus result for potassium-selective channels in current jumps of greater amplitude. It appeared therefore convenient to use the current jump amplitude of the slow calcium-independent SK channel to monitor variations of the cell resting potential. Furthermore, this experimental procedure guaranteed that there would be no oscillatory hyperpolarization due to leakage pathways introduced by microelectrode impalements (Ince et al., 1984). Figure 5 illustrates an example of single-channel record in which both types of potassium channel were present. This record was obtained in the cell-attached configuration at zero applied potential following the perfusion of the external medium with an Earle solution containing 1 mM histamine. The patch electrode was filled with a 200-mM KCl solution. As seen, this record contains two distinct types of fluctuation patterns. There are bursts of single-channel activity occurring at more or less regular intervals superimposed on long channel openings lasting for more than 40 sec. The long channel openings are characteristic of an SK chan-

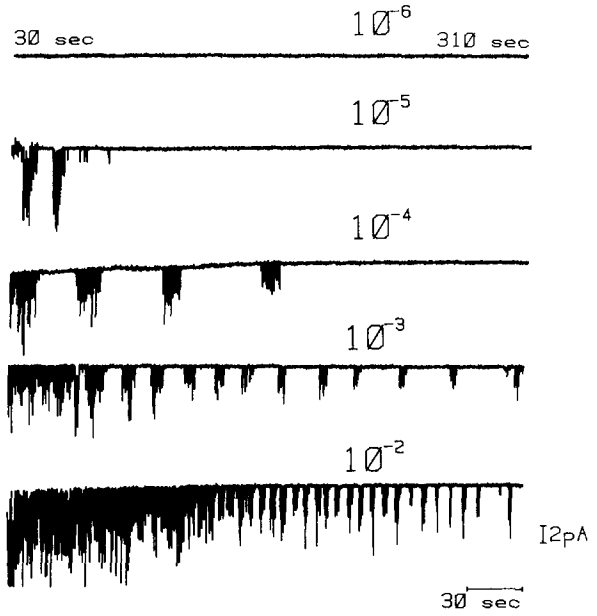

Fig. 6. Single-channel cell-attached recordings obtained as described in Fig. 1 for different histamine concentrations (10^{-6} to 10^{-2} M) in the external medium. Each record was measured on a different cell due to histamine response desensitization. As observed, bursts of single-channel events could be in all cases induced at histamine concentrations greater than 1 μM. As the histamine concentration was increased, the oscillatory phenomenon lasted over a longer time period. The oscillations with 10^{-2} M histamine lasted one minute more than the actual record presented

nel whereas the bursting brief channel openings are typical of calcium-activated potassium channels (*see* Fig. 5*C*). This record provides direct evidence that the membrane potential of these cells oscillates in the presence of histamine and that these oscillations are directly correlated with the activation of calcium-dependent potassium channels. This conclusion can easily be derived from an analysis of the single-channel events which took place within the time interval $t_4 - t_1$ as defined in Fig. 5(*A*). The time t_1 marks the opening of an SK channel. As seen, the amplitude of the current jumps within the time interval $t_2 - t_1$ remained equal to -1.5 pA, which corresponds to the current flowing through a 47-pS SK channel for a membrane potential of -33 mV. This estimate of the cell resting potential is in good agreement with our previous microelectrode measurements on HeLa cells where a value of -38 ± 11 mV was obtained (Roy & Sauvé, 1983). At t_2, the amplitude of the current jumps of the SK channel started to increase and reached a maximum value of -3.2 pA at t_3, indicating an intracellular potential of -68 mV. The cell has thus become hyperpolarized within the time interval $t_3 - t_2$. On a faster time scale as shown in Fig. 5*B*, it can be observed that the number of rapid channel openings added to the current contribution of the SK channel became more important as the amplitude of the SK channel current jumps increased (*see also* Fig. 5*C*). As shown previously, these fast events corresponded to the openings of calcium-activated potassium channels. Within the time interval $t_4 - t_3$ the current jump amplitude of the SK channel returned to its original value and the number of fast openings decreased as shown in Figs. 5(*A*) and 5(*B*). This typical behavior occurred at more or less regular intervals (*see* t_3, t_5, t_6, t_7, t_8, t_9 in Fig. 5*A*), indicating that both the cell membrane potential, as monitored via the SK channel, and the activation of the calcium-dependent potassium channels oscillate in a synchronous manner. There is therefore an obvious correlation between the cell potential oscillations induced by histamine and the activation of calcium-dependent potassium channels. It was shown previously that the calcium-activated potassium channel in HeLa cells is nearly voltage insensitive for voltages within -100 to $+100$ mV. Consequently, the oscillatory fluctuations in membrane potential illustrated in Fig. 5(*A*) cannot be responsible for the bursting activation of the calcium-dependent potassium channels, but rather represent the resulting effect of the calcium-activated potassium channel synchronous openings. These results directly confirm the key role so many times assigned to the calcium-related potassium conductance in membrane potential oscillation phenomena (Hülser & Lauterwasser, 1982; Okada et al., 1982; Ueda et al., 1983; Ince et al., 1984).

Influence of Histamine and External Calcium Concentration on the Calcium-Dependent Potassium Channel Activation

The dose-response effect of histamine on the oscillatory activation of the calcium-dependent potassium channels in HeLa cells was next investigated in a series of cell-attached experiments carried out at various exogenous histamine concentrations. The patch electrode was filled with a 200-mM KCl solution. Figure 6 presents single-channel recordings obtained on different cells after changing the regular histamine-free Earle external medium by an Earle solution containing histamine in concentrations ranging from 1 μM to 10 mM. This series of experiments had to be carried out on different cells because the response to histamine desensitized as mentioned earlier. As seen, no single-channel event could be initiated at histamine concentrations lower than 1 μM. The appearance of bursts separated by repetitive silent periods became truly visible at his-

tamine concentrations greater than 10 μM, which is in agreement with the results reported by Hazama et al. (1985) on the hyperpolarizing response induced by histamine in HeLa cells. As also illustrated in Fig. 6, the main effect of an increase in the exogenous histamine concentration is to prolong the overall period over which oscillations can be detected. For instance, the time needed to get a first silent period between two consecutive current bursts longer than 60 sec was on the average equal to 120 and 240 sec with 10 μM and 10 mM histamine, respectively. Interestingly, histamine did not seem to affect to a great extent the frequency of the oscillations since the average number of bursts per minute remained equal to 2.4 for histamine levels ranging from 10 μM to 1 mM.

As mentioned previously, the most likely mechanism responsible for the repetitive bursting activation of the calcium-dependent potassium channels would consist of a periodic transient mobilization of the intracellular calcium ions. The entry of external calcium represents one of the most likely hypotheses for the source of this calcium. Cell-attached experiments were thus performed in which the cell bathing medium was sequentially replaced first by a calcium-free ($<10^{-8}$ M) Earle solution and then by a calcium-free Earle solution plus 1 mM histamine. Figure 7 illustrates the result of such an experiment. It can be observed in Fig. 7(*B*) for instance that a 2-min preincubation of the cells in a calcium-free medium did not result in a release of calcium from internal stores substantial enough to initiate channel openings. However, as seen in Fig. 7(*C*), histamine could induce an oscillatory channel activation process, despite the absence of calcium in the external medium, indicating that the release of calcium from internal stores is likely to contribute to a large extent to the initial repetitive transient rises in intracellular calcium level. The effect of a calcium-free external medium appears mainly as a reduction of the time over which current oscillations can be observed. An influx of calcium may thus be involved to some degree in the oscillatory channel activation process without being essential to the initial bursting activity. In fact, it is possible that the hyperpolarization caused by histamine induces an influx of Ca^{2+}, but its major role would consist more of sustaining the oscillations than of initiating the oscillatory process.

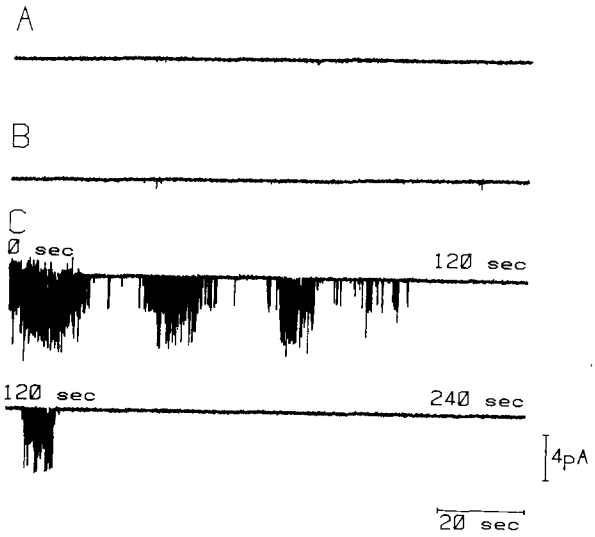

Fig. 7. Single-channel recordings from a cell-attached experiment with a patch electrode containing a 200-mM KCl solution. In (*A*), the extracellular bathing medium was an Earle solution and no single-channel event could be detected. In (*B*), the external medium was replaced by a calcium-free Earle solution ($<10^{-8}$ M) buffered with 1 mM EGTA. This procedure did not induce *per se* internal calcium release from internal stores as shown by the absence of single-channel activity. Finally, in (*C*), the calcium-free Earle solution was replaced by a calcium-free Earle solution containing 1 mM histamine. Under these conditions an oscillatory activation of the calcium-dependent potassium channels could be initiated. The silent period between successive current bursts were, however, longer. It is concluded that the oscillatory fluctuation pattern induced by histamine is not directly related to an influx of calcium ions into the cell. There should be therefore an important contribution coming from the release of calcium from internal stores

Is Cyclic AMP Involved in the Activation of the Calcium-Dependent Potassium Channels in HeLa Cells?

In many cases the flow of information from cell surface to cell interior occurs via the adenyl cyclase-cyclic AMP pathway. Numerous reports in the literature suggest that some of the effect of cyclic AMP may be related to its ability to modulate the intracellular calcium level (*see*, for instance, Berridge, 1975; Rasmussen & Goodman, 1977; Rasmussen & Barrett, 1984). The role of cyclic AMP was thus investigated in cell-attached experiments in which the external medium was replaced by an Earle solution containing 0.1 mM of the lipid-soluble cyclic AMP analog dibutyryl cyclic AMP. Cyclic AMP was ineffective in initiating the activation of the calcium-dependent channels in HeLa cells within a time period comparable to that observed in the presence of histamine. In fact, some effects of dibutyryl cyclic AMP could be measured in some cases but the channel activation occurred usually 6 to 12 min after perfusing the external medium. Under cell-attached conditions, it cannot be ruled out that this effect was due to a cyclic AMP-independent mechanism. In general, no effect due to cyclic AMP could be observed within 15 min.

Discussion

We have shown that the stimulation of H_1 receptors by exogenous histamine induced in HeLa cells the repetitive transient activation of calcium-dependent potassium channels. Transmembrane potential oscillations were also measured and their occurrence could be directly correlated with the bursting activity of the calcium-dependent potassium channels. In addition, single-channel records have clearly established that external calcium is not required for the initial repetitive bursting channel activation process evoked by histamine. Finally, it was found that the membrane-permeable cyclic AMP analog dibutyryl cyclic AMP cannot initiate single-channel events such as those triggered by exogenous histamine.

It should first be mentioned that the single-channel response obtained following the stimulation of H_1 receptors in HeLa cells has to be linked to a second messenger system. This conclusion is based on the observation that the single-channel events reported in this work were always measured using histamine-free patch electrodes. A direct interaction between histamine and calcium-activated potassium channels can be therefore ruled out. The activation of calcium-dependent channels following agonist receptor stimulation has been reported on other preparations (*see*, for instance, Petersen & Maruyama, 1983) without any mention of an oscillatory single-channel activation process. Such a difference may be partly due to the molecular mechanisms responsible for the global cellular response to receptor stimulation which may be different for various cell types. There may also be differences in the channel response to various internal chemical signals. In this regard, the calcium-dependent potassium channel in HeLa cells has several interesting properties. The channel is highly selective to potassium ions ($P_K/P_{Na} > 30$) and its kinetic is nearly voltage insensitive within the voltage range -100 to $+100$ mV (Sauvé et al., 1986). In addition, the channel activity is extremely sensitive to internal calcium within the concentration range 0.1 to 1 μM. Higher internal calcium levels were found to be ineffective in promoting channel openings. The channel can thus change from a low to a high open probability kinetic within an extremely narrow internal calcium concentration range without being affected by potential variations.

Single-channel records were also obtained which showed that the cyclic activation of the calcium-dependent channel in HeLa cells cause transmembrane potential oscillations. Cyclic fluctuations in transmembrane potential have been observed on many cell types and, in most cases, these changes in membrane potential could be related to a cyclic variation of the cell membrane permeability to potassium (Hülser & Lauterwasser, 1982, Ueda et al., 1983, 1986; Yada et al., 1986). Our work provides direct evidence that calcium-dependent potassium channels can indeed be activated in a more or less cyclic manner resulting in transmembrane potential oscillations. As the source of these oscillations, recent studies have clearly established that cyclic variations in membrane potential occur in many cases concomitantly with oscillatory increases in the intracellular calcium level (Ueda et al., 1986; Yada et al., 1986). If one assumes that internal calcium is the main factor controlling the potassium channel activated by histamine in HeLa cells, our present results not only confirm that there is intracellular calcium mobilization following H_1 receptor stimulation, but also that histamine induces cytosolic free calcium level oscillations. Our results would thus be in agreement with the observations reported on fused L-cells by Ueda et al. (1986), although it cannot at present be entirely ruled out that there could be factors other than calcium involved in the channel control mechanisms. The experiments in which the patch-clamp configuration was changed from cell-attached to inside-out during a silent period have nevertheless clearly indicated that the channel activated by histamine remains always sensitive to internal calcium within the micromolar range. Within this framework, the observed single-channel fluctuation pattern would be due to cytosolic free calcium level oscillations with values ranging from less than 0.1 to more than 0.4 μM. Interestingly, this effect could be initiated within the 40 sec needed to fully exchange the cell bathing solution from a histamine-free to a histamine-containing medium. The initial release of calcium appears therefore as a rapid phenomenon whose time constant is comparable to that obtained from Quin 2 fluorescence measurement on the effect of histamine in several preparations (Ohsako & Deguchi, 1984; Matsumoto et al., 1986). Finally, it should be apparent that a summation of the oscillatory responses coming from a large number of individual cells would have yielded an apparent continuous transient increase in cytosolic-free calcium level followed by a slow calcium decrease with a time constant of 3 to 5 min. This response is expected since no two individual cells are likely to oscillate in a synchronous manner. However, each cell would have been characterized by a substantial increase in cytosolic free calcium concentration after H_1 receptor stimulation.

As for the mechanism relating calcium release and H_1 receptor stimulation, let us first mention that our results do not support a model in which an influx of calcium is essential to the initial phase of the

oscillatory process. Secondly, an increase in cyclic AMP did not give rise to an increased intracellular calcium concentration high enough to activate the observed calcium-dependent potassium channels. Taken together these results indicate that the stimulation of the H_1 receptors in HeLa cells was capable of mobilizing intracellular calcium ions via a mechanism not directly related to cyclic AMP. This conclusion is further supported by the work of Hazama et al. (1985) in which vasoactive polypeptides linked to the adenylate cyclase pathway (Prieto et al., 1981), failed to stimulate potassium conductance in HeLa cells. In fact, the activation of adenylate cyclase is generally associated with H_2 not H_1 receptor stimulation (Schwartz, 1979; Douglas, 1980; Hough & Green, 1984; McNeill, 1984). In addition, several recent studies have clearly established that H_1-histaminergic activation stimulates inositol-1-phosphate accumulation in several preparations (Brown et al., 1984; Daum et al., 1984; Donaldson & Hill, 1985; Noble et al., 1986). These results are particularly important since there is considerable evidence that many cell types undergo, following hormone stimulation, a pronounced increase in the hydrolysis of the plasma membrane lipid phosphatidylinositol-4,5-biphosphate (PIP_2), releasing into the cytosol the water-soluble product, inositol-1,4,5-triphosphate (Insl,4,5,P_3) which in turn was shown to trigger calcium release from intracellular stores (*see*, for instance, Streb et al., 1983; Berridge, 1984; Berridge & Irvine, 1984; Burgess et al., 1984; Busa et al., 1985). Such a mechanism would be quite compatible with our results on the histamine-evoked activation of the calcium-dependent potassium channels in HeLa cells. According to this proposal, there would be no need for extracellular calcium and cyclic AMP would not be directly involved in the calcium release process. Furthermore, the recent work of Woods et al. (1986) on isolated rat hepatocytes has clearly shown that transient rises in cytoplasmic free calcium can occur through an Insl,4,5,P_3-mediated mechanism. A similar phenomenon may thus take place in HeLa cells. The oscillatory behavior of the observed channel activation process must include, in addition, a cellular mechanism capable of dissipating a hypothetical internal calcium rise. One likely mechanism involved in the cytosolic calcium extrusion consists of the activation of calcium pumps. In this regard, Okada et al. (1982) have convincingly established that calcium pumps play an important role in the membrane potential oscillation process observed in L-strain mouse fibroblasts. Since our previous study has shown that the open probability of the calcium-dependent potassium channel in HeLa cells can increase 10^3-fold within an intracellular free calcium concentration range of 0.1 to 1 μM, small fluctuations in the internal calcium level caused by the release of calcium from internal stores coupled to a calcium concentration decrease via calcium pumps may then be sufficient to generate the observed oscillatory single-channel activation. More direct measurements are needed, however, in order to confirm the role played by Insl,4,5,P_3 in this process.

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