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Large and small conductance calcium-activated potassium channels in the GH_3 anterior pituitary cell line

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Abstract. Single Ca^{2+}-activated K^+ channels were studied in membrane patches from the GH_3 anterior pituitary cell line. In excised inside-out patches exposed to symmetrical 150 mM KCl, two channel types with conductances in the ranges of 250–300 pS and 9–14 pS were routinely observed. The activity of the large conductance channel is enhanced by internal Ca^{2+} and by depolarization of the patch membrane. This channel contributes to the repolarization of Ca^{2+} action potentials but has a Ca^{2+} sensitivity at −50 mV that is too low for it to contribute to the resting membrane conductance. The small conductance channel is activated by much lower concentrations of Ca^{2+} at −50 mV, and its open probability is not strongly voltage sensitive. In cell-attached patches from voltage-clamped cells, the small conductance channels were found to be active during slowly decaying Ca^{2+}-activated K^+ tails currents and during Ca^{2+}-activated K^+ currents stimulated by thyrotropin-releasing hormone induced elevations of cytosolic calcium. In cell-attached patches on unclamped cells, the small conductance channels were also active at negative membrane potentials when the frequency of spontaneously firing action potentials was high or during the slow afterhyperpolarization following single spontaneous action potentials of slightly prolonged duration. The small conductance channel may thus contribute to the regulation of membrane excitability.

Key words: Pituitary cells – Ca^{2+}-activated K^+ channels – Patch clamp – Thyrotropin-releasing hormone – Ca^{2+}-dependent afterhyperpolarization

Introduction

The GH_3 clonal cell line, originally derived from a rat anterior pituitary tumor (Tashjian et al. 1968), exhibits a wide range of physiological functions, including the ability to secrete prolactin and growth hormone in response to stimuli. One stimulus that has been extensively studied is thyrotropin-releasing hormone (TRH). TRH stimulates prolactin secretion from this cell line in a biphasic manner via enhanced hydrolysis of phosphatidylinositol bisphosphate. An initial burst phase of secretion that lasts for one minute or less results from an inositol-trisphosphate mediated mobilization of intracellular Ca^{2+} (see reviews by Gershengorn 1986 and Drummond 1986). This is correlated at the electrophysiological level with a transient hyperpolarization (Ozawa and Kimura 1979) that is due to an increase in Ca^{2+}-activated K^+ conductance (Ozawa 1985; Dubinsky and Oxford 1985; Ritchie 1987b). A sustained phase of prolactin secretion, which is dependent upon extracellular Ca^{2+}, also occurs (Gershengorn 1986; Drummond 1986). This sustained phase has been correlated with an increase in Ca^{2+} action potential firing frequency (Kidokoro 1975) and an increase in cell input resistance (Dufy et al. 1979; Ozawa and Kimura 1979) that are probably mediated by diacylglycerol stimulation of protein kinase C activity (Ostberg et al. 1986)

Further investigation of the Ca^{2+}-activated K^+ conductances had demonstrated that two pharmacologically distinct components contribute to the TRH induced hyperpolarization (Ritchie 1987b). Under whole cell voltage clamp, Ca^{2+}-activated K^+ conductances may also be activated by a voltage step which induces Ca^{2+} entry into the cell via voltage dependent Ca^{2+} channels. Upon returning to −50 mV, a portion of the tail current that is associated with the Ca^{2+}-activated K^+ conductances decays very slowly, often requiring several seconds for complete decay. Complete pharmacological inhibition of the TRH-induced Ca^{2+}-activated K^+ current and the slowly decaying tail current requires both tetraethylammonium chloride (TEA) and apamin (Ritchie 1987a). Taken together, these data suggest the presence of two pharmacologically distinct types of Ca^{2+}-activated K^+ channels that are sensitive to changes in cytosolic Ca^{2+} concentrations at potentials near rest.

A Ca^{2+}-activated K^+ channel with a unit conductance of 110–150 pS (in 150 mM symmetrical K^+) has been previously reported in GH_3 cells (Dubinsky and Oxford 1985; Rogawski et al. 1985). However, based on a preliminary report (Rogawski et al. 1985) on the calcium and voltage sensitivity of the channel, it seems highly unlikely that the channel would exhibit activity in response to changes in intracellular Ca^{2+} at potentials near rest. The present study was undertaken to investigate whether multiple types of Ca^{2+}-activated K^+ conductances could be detected at the single channel level and to characterize the Ca^{2+} sensitivity and voltage sensitivity of the channels. This characterization is important for elucidating the role of these channels in the regulation of electrical excitability and in the modulation of excitability by stimuli such as thyrotropin releasing hormone. A preliminary account of these studies has appeared elsewhere (Lang and Ritchie 1986).

Methods

Cell cultures. The GH_3 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA).

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Cell cultures were incubated at 37°C in a 5% CO_2 atmosphere and bathed in Ham's F-10 media supplemented with heat inactivated horse serum (15%), and fetal calf serum (2.5%). Cells used for electrophysiological experiments were plated on 35 mm tissue culture dishes and were used within 2–10 days after plating. The electrophysiological experiments were performed at room temperature.

Electrophysiological recording. The cell-attached, inside-out, and whole-cell modes of the patch technique were employed (Hamill et al. 1981). The electrodes were pulled from Corning 7052 glass (Friedrick and Dimmock; Millville, NJ, USA) in two stages on a David Kopf model 700C puller. Patch electrodes were coated with Sylgard and then fire polished. Electrode offsets were balanced before forming a "giga-seal". Seal resistances were typically in the range of 50–100 GΩ.

Single channel currents were recorded with a List model EPC 7 patch clamp amplifier. In some experiments, both the whole-cell and cell-attached patch currents were simultaneously recorded. In these experiments, the whole-cell was voltage clamped by using a method which has been previously described (Ritchie 1987a). The cell voltage signal was fed into a WPI model KS-700 high impedance amplifier (unity gain) and then into the inverting input of a Tektronics AM 502 differential amplifier. The voltage command signal was led to the non-inverting input of the differential amplifier. The output from the differential amplifier was then fed back to the recording electrode via the stimulus input of the WPI KS-700. The gain of the differential amplifier was typically set at 500 with a frequency response of d.c. to 3 kHz. The current was recorded from the current monitor output of the KS-700. The data were stored on magnetic tape with a Racal Store 4DS FM type recorder (d.c. to 2 kHz).

In all experiments, the Ag-AgCl wire of the patch electrode and the Ag-AgCl plug for the reference electrode were exposed to the same solution. The pool for the reference electrode was electrically connected to the bath solution by a 3 M KCl agar salt bridge. This procedure minimized junction potential errors at the reference electrode.

In all figures the indicated voltages refer to the potential across the membrane patch, cytoplasmic relative to external side. Outward current is positive and represents movement of positive charge from the cytoplasmic to external side of the membrane. The closed state of the channel, except when obvious, is indicated by the letter C and the open state by the letter O.

Solutions. The standard patch pipette solution contained (in mM) 150 KCl, 10 N-2-hydroxyethyl piperazine-N′-2 ethane sulfonic acid (HEPES), 4-aminopyridine (4-AP), 2 $MgCl_2$, 0.003 tetrodotoxin (TTX), and pH 7.3. For the ionic selectivity experiments, KCl was lowered to 75 mM and to 5.6 mM by replacement with equimolar amounts of NaCl. When using these solutions, the electrode resistances were typically 10 MΩ. The bath solutions that were used when recording from inside-out patches contained varying amounts of $CaCl_2$ and (in mM) 150 KCl, 10 HEPES, 2 $MgCl_2$, and pH 7.2. Free Ca^{2+} concentrations in the range of 10 nM to 3 μM were buffered with 2 mM ethylene glycol bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA) and were calculated using a K_{app} of 150 nM (Thomas 1982). Calcium concentrations greater than 3 μM were achieved by simply adding the desired amount of $CaCl_2$. In some experiments, the $MgCl_2$ concentration was reduced from 2 mM to 0.5 mM, and the HEPES concentration was increased to 14.5 mM (Figs. 1B, 2, 3B). At these concentrations, Mg^{2+} has a negligible effect on the buffered free Ca^{2+} concentration. This was determined (Fabiato and Fabiato 1979) by using a K_{app} of EGTA for Mg^{2+} of 84 mM (Thomas 1982). The whole-cell intracellular pipette solution contained (in mM) 150 KCl, 15 HEPES, 2 $MgCl_2$, pH 7.2. The cells were bathed in a physiological saline that contained (in mM) 140 NaCl, 10 glucose, 10 HEPES, 5.6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.3. In some cases, 3 μM TTX and 5 mM 4-AP were added to the saline to block the voltage dependent sodium and potassium channels.

The internal face of inside-out membranes was continually perfused. The patch was placed directly in front of a large bore pipette (diameter approximately 250 μm) through which the test solutions flowed at a rate of 0.6 ml/min. Solution changes required approximately 30 s.

Analysis. Single channel amplitudes were measured either manually from chart paper records replayed at a slow speed or by computer. Data to be analyzed by computer were first pre-filtered (Wavetek Dual HiLo model 852 filter) at 1 kHz (−3 dB, 8 pole Bessel) and were then digitized at 100 μs intervals. The "Analysis" program developed by Dr. Hubert Affolter (courtesy of Dr. Roberto Coronado) was implemented on an IBM PC AT 'clone'. Single channel openings were detected as events which fell within a window set by the user. Single channel amplitudes were calculated by subtracting the mean closed current from the mean open current. The open probability was calculated as the open time integral divided by the number of channels in the patch and the duration of the data segment analyzed. The number of channels was estimated by examining the record for multiple openings under conditions of high open probability ($p > 0.8$). Generally 10–15 s data segments were analyzed for open probability estimates.

Results

Two distinct types of K^+ channels that are activated by internal Ca^{2+} are routinely observed in GH_3 membrane patches. A large conductance channel has properties, as will be shown, which are very similar to the properties of BK channels of cultured rat myotubes (Barrett et al. 1982). A small conductance channel has characteristics which are similar to the apamin sensitive channel of rat myotubes (Blatz and Magleby 1986). This channel has been termed SK by these authors. In this paper, large conductance and small conductance Ca^{2+}-activated K^+ channels will be referred to as BK and SK channels, respectively. Marty and Neher (1985) have previously used the term "SK" in reference to a voltage dependent K^+ channel (12 pS in physiological K^+ gradients) found in adrenal chromaffin cells. This channel is not Ca^{2+} dependent and should not be confused with the Ca^{2+}-activated SK channels described by Blatz and Magleby (1986) and those discussed in this paper.

Studies on inside-out patches

The current-voltage relationships for the two channel types under three different K^+ gradients are shown in Fig. 1. The slope conductance of BK channels in symmetrical 150 mM

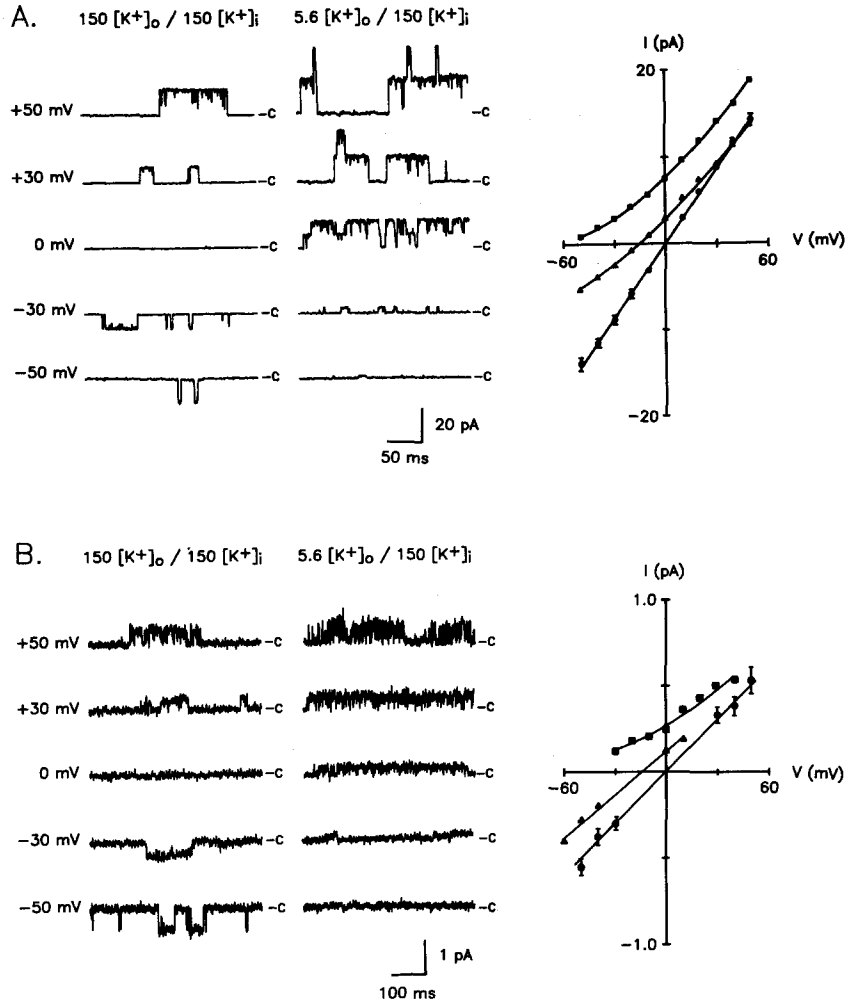

Fig. 1A, B
Ionic selectivity of the BK and SK channels. The data were obtained from inside-out patches, and the channels were confirmed to be Ca^{2+} sensitive. The internal KCl concentration in all cases was 150 mM, and the external KCl concentration was 5.6 mM (■), 75 mM (▲) and 150 mM (●). Data for each K^+ gradient were obtained from a different patch. BK channels are illustrated in **A** and SK channels are illustrated in (**B**). The free internal Ca^{2+} concentration in (**A**) was 10 μM for −50 to 0 mV and 1 μM for +10 to +50 mV, and in **B** was 1 μM. Outward currents are in the upward direction. The points for the I-V curves are from single experiments except in symmetrical 150 mM KCl. In the latter case, the points represent the mean from 5 patches in **A** and 6 patches in **B**. The *error bars* represent the SEM and are omitted for errors smaller than the size of the point. The mean slope conductance of the channels in symmetrical 150 mM KCl is 280 pS for BK channels and 11 pS for SK channels. The representative traces were lowpass filtered at 1 kHz (−3 dB, 8 pole Bessel). The *lines* were drawn by eye

KCl at room temperature was in the range of 250−300 pS with a mean ± SEM of 279 ± 5 pS ($n = 11$). The slope conductance of SK channels under similar conditions was in the range of 9−14 pS with a mean ± SEM of 11 ± 1 pS ($n = 6$). These conductance measurements are in agreement with those previously reported for BK channels (Blatz and Magleby 1984) and SK channels (Blatz and Magleby 1986). A Ca^{2+}-activated K^+ channel found in GH_3 cells with a slope conductance in the range of 100−150 pS has previously been reported (Dubinsky and Oxford 1985; Rogawski et al. 1985). We have not observed channels with a conductance in this range. As the external K^+ concentration is reduced from 150 mM to 75 mM to 5.6 mM, the current-voltage curves for both channel types are shifted to the left, as would be expected for a channel that is mainly permeable to K^+. Clear reversal potentials could be measured for both channels under the 75 mM/150 mM K^+ gradient. The expected reversal potential for a purely K^+ selective channel is −18 mV under these conditions. The BK channel reversed at −17 mV, and the SK channel reversed at −14 mV.

The open probability for both BK and SK channels increases as internal free Ca^{2+} is raised. At −50 mV, SK channels are sensitive to Ca^{2+} concentrations in the range of 300 nM to 10 μM with half-maximal activation occurring at approximately 1 μM (Fig. 2). In contrast, BK channels require Ca^{2+} concentrations greater than 10 μM for appreciable activation at this potential. Ca^{2+}-activated SK channels were observed in approximately 40% of the patches examined.

We have investigated the voltage dependency of the steady-state open probability at fixed Ca^{2+} concentrations for both channel types. The open probability of BK channels increases steeply as the membrane potential becomes more positive (Fig. 3A). The curves in Fig. 3A represent Boltzman distributions[1] which were fit by eye to the data for 1 μM and 10 μM free Ca^{2+} concentrations. The open probability changes e-fold per 8 mV change in the membrane potential at low open probabilities. Increasing the free Ca^{2+} from 1 to 10 μM shifts the curve by −75 mV. The Ca^{2+} and voltage sensitivities of the BK channels are similar to those of rat myotubes (Barrett et al. 1982), although the voltage dependency reported for rat myotubes was slightly less steep ($z = 2$; Methfessel and Boheim 1982). BK channels in a wide variety of preparations all show a similar voltage dependence. However, in some cells, e.g., acinar cells of the pancreas (Maruyama et al. 1983) and lacrimal gland (Findlay 1984), BK channels are activated by much lower Ca^{2+} con-

[1] The open probability (P_O) was calculated as follows: $P_O = \{1 + \exp[(zF/RT)(V_0 - V)]\}^{-1}$, where $z = 3$ and $V_0 = +50$ at 1 μM free Ca^{2+} and −25 mV at 10 μM free Ca^{2+}

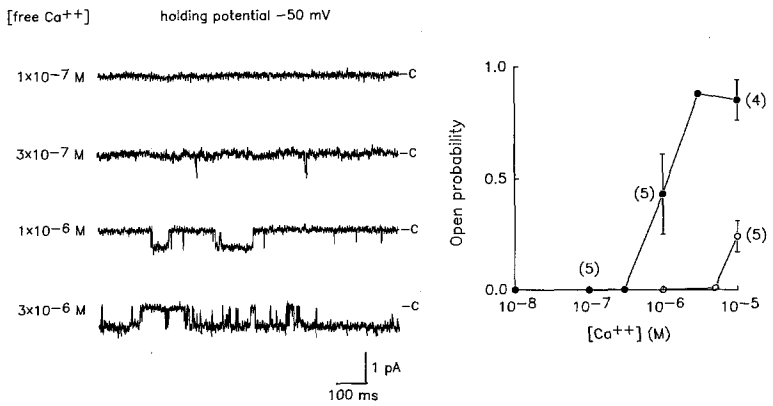

Fig. 2
Calcium sensitivity of the SK channels. These experiments were performed in inside-out patches, and the patch potential was held at −50 mV. The patch membrane was exposed to a symmetrical 150 mM K^+ gradient, so unitary currents are in the inward direction. As internal free Ca^{2+} is increased from 100 nM to 3 μM, the SK channel opens more frequently and for longer periods of time. The open probability (calculated from patches containing either 1 or 2 channels) at −50 mV is plotted in the graph as a function of the log of the Ca^{2+} concentration for SK (●) and BK (○) channels. The representative currents were lowpass filtered at 1 kHz (−3 dB, 8 pole Bessel). The *error bars* represent the standard error of the mean and the number of patches examined is indiciated in parentheses. The *error bar* is omitted for errors smaller than the size of the symbol

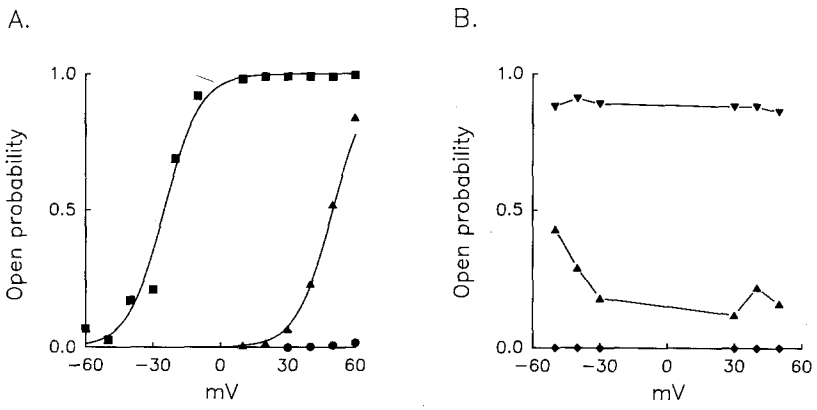

Fig. 3A, B
Comparison of the voltage dependency of the open probability for the BK and SK channels. These experiments were performed on inside-out patches. The open probabilities for the BK (**A**) and SK (**B**) channels were calculated from 10 s data segments from single channel patches. The Ca^{2+} concentrations in **A** are 100 nM (●), 1 μM (▲) and 10 μM (■), and in **B** are 300 nm (◆), 1 μM (▲) and 3 μM (▼)

centrations than those described in GH_3 cells and in most preparations.

The SK channels, in contrast, are not strongly voltage dependent (Fig. 3B). At 3 μM free Ca^{2+}, the open probability is not affected by voltage, and at 1 μM free Ca^{2+}, the open probability slightly decreases as the membrane potential becomes more positive. The SK channel in rat myotubes shows a similar Ca^{2+} sensitivity and little voltage sensitivity (Blatz and Magleby 1986).

Cell-attached patch studies on voltage clamped cells

In order to correlate our previous whole-cell voltage clamp studies with our single channel observations, we have adopted a method of voltage clamping the whole-cell and simultaneously recording single channel activity in a cell-attached patch. We wished to see which channel types were active during the slowly decaying Ca^{2+}-activated K^+ tail currents (Ritchie 1987a) and during Ca^{2+}-activated K^+ currents mediated by TRH-induced mobilization of internal Ca^{2+} (Dubinsky and Oxford 1985; Ritchie 1987b).

Upon depolarization of the whole-cell from −50 mV to 0 mV, a Ca^{2+}-activated K^+ current develops which is shown in the whole-cell record of Fig. 4A. Since the patch membrane potential is 0 mV, which is the reversal potential for K^+ channels under these conditions, single channels are not observed in the patch. Upon repolarization back to −50 mV, a slowly decaying Ca^{2+}-activated K^+ tail current is seen in the whole-cell, and increased channel activity is seen in the patch. The occurrence of increased channel activity is correlated in time with the slowly decaying tail current. The slope conductance of this channel, measured by changing the patch voltage (data not shown), was approximately 14 pS. The mean slope conductance from 5 different patches in which the SK channel was observed during the slow tail current was 12 ± 1 pS ($\bar{x} \pm$ SEM). This value is in agreement with the conductance of the SK channel seen in inside-out patches.

Similar protocols were used to examine single channel activity during the TRH-induced mobilization of intracellular Ca^{2+}. A representative experiment is shown in Fig. 4B. The net potential across both the whole-cell and patch membranes is −50 mV. TRH (50 nM) induces a Ca^{2+}-activated K^+ current as is shown in the whole-cell record and increases channel activity in the cell-attached patch. Typically, two channel types were activated: a very small channel with a chord conductance of about 4 pS and several SK channels with chord conductances of about 11 pS. We have been unable to characterize the ionic selectivity and Ca^{2+} sensitivity of the 4 pS channel. However, a 4 pS Ca^{2+}-dependent K^+ channel has been reported in rat myotubes (Blatz and Magleby 1986). We have investigated TRH effects by using this protocol in 96 cells. Out of this number, 49 cells responded with a positive whole-cell current. A similar percentage of TRH responsive cells in the GH_3 cell line is widely observed (e.g., see Ozawa and Kimura 1979; Dubinsky and Oxford 1985). Increased channel activity was observed in 22% of the responding cells. This reflects, in part, the fact that not every patch has a Ca^{2+}-sensitive channel. Of the cells which showed channel activity, 9 patches contained the SK channels (mean chord conductance of 11.1 ± 0.5 pS) and 4 patches contained the 4 pS channels. In no instance was

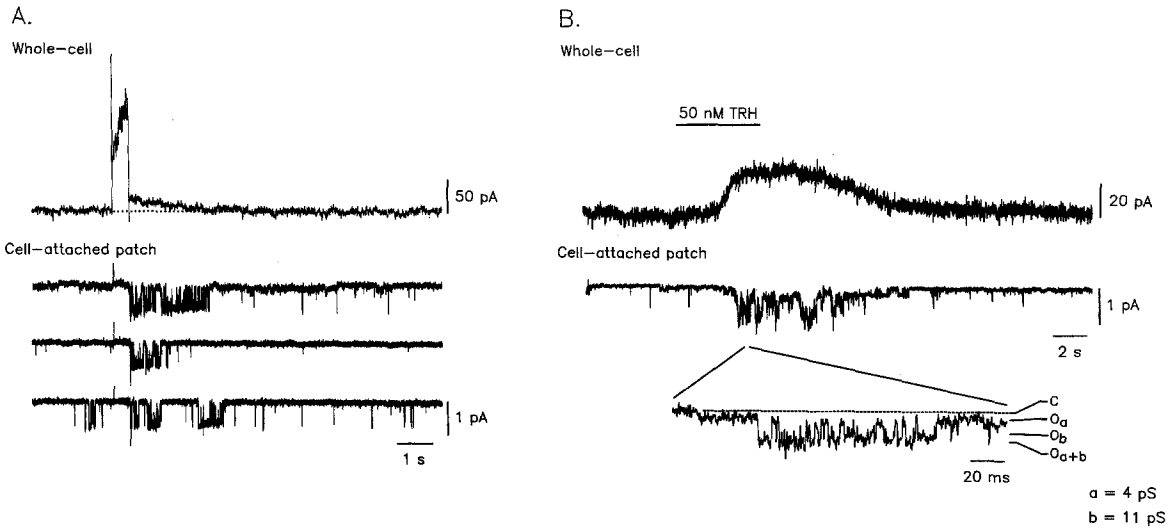

Fig. 4A, B. Correlation of SK channel activity with whole-cell currents. The whole-cell current under voltage clamp and single channel activity in a cell-attached patch were recorded simultaneously from the same cell. Both the whole-cell pipette and the cell-attached patch pipette contained 150 mM KCl. The whole cell was bathed with physiological saline which contained 3 μM TTX and 5 mM 4-AP. Thus, unlike the patch, the whole-cell extrapatch membrane was exposed to a physiological K^+ gradient. The patch pipette was held at 0 mV so that the net potential across the patch membrane was the whole cell voltage. **A** The whole-cell was clamped at −50 mV then stepped to 0 mV for 400 ms. An example of the whole cell current record and 3 selected sweeps of single channel activity (inwardly directed currents) are shown. The current traces were lowpass filtered at 360 Hz (−3 dB, 8 pole Bessel). **B** The cell was clamped at −50 mV, and the net potential across the patch membrane was also −50 mV. TRH (50 nM) was applied to the cell by pressure ejection form a large bore pipette during the time indicated by the horizontol bar. The whole cell outward current and the inwardly directed single channel currents are shown on the same time scale. Two channel types are activated: a very small channel with a conductance of roughly 4 pS and at least one SK channel with a conductance of about 11 pS. The SK channel can be seen more clearly in the expanded trace. The slow current traces were lowpass filtered at 200 Hz and the expanded traces were lowpass filtered at 1 kHz (−3 dB, 8 pole Bessel)

BK channel activity enhanced by application of TRH when the patch membrane was held at −50 mV. The above reported ability of TRH to cause an increase in the activity of the SK channel is consistent with the Ca^{2+} sensitivity of this channel and the effects of TRH on cytosolic Ca^{2+} levels. The basal levels of Ca^{2+} have been reported to range from 70 to 350 nM while maximal concentrations of TRH cause a peak elevation in cytosolic Ca^{2+} that is as high as 1–3 μM (Gershengorn and Thaw 1985; Albert and Tashjian 1984; Ritchie 1987b). These cytosolic concentrations are within the range of Ca^{2+} levels that have a marked effect on the probability of opening of SK channels at −50 mV.

Cell-attached patch studies on unclamped cells

In order to gain some insight into the functional relevance of SK channels, we have recorded channel activity from cell-attached patches in unclamped cells. Although both the whole-cell and patch membrane potentials are no longer controlled, the problem of possible dialysis of intracellular constituents is avoided. Action potentials may be detected in cell-attached patches as outward current transients often referred to as patch action currents (Fenwick et al. 1982; Fischmeister et al. 1984). Thus, it is possible to correlate channel activity with the cell action potential by using only the cell-attached mode.

Cells that are bathed in solutions containing 5 mM 4-AP exhibit an action potential that may be somewhat broader in duration than the action potential of cells bathed in normal solutions (Sand et al. 1980; but see Rogawski et al. 1985). This action potential broadening allows a greater Ca^{2+} influx. An intracellular recording from a cell bathed in 3 μM TTX and 5 mM 4-AP is illustrated in Fig. 5A. Spontaneous action potentials are typically followed by an afterhyperpolarization which slowly decays until the threshold (approximately −38 mV) for the subsequent action potential is reached. Figure 5B illustrates single channel activity measured in a different cell that was also bathed in 3 μM TTX and 5 mM 4-AP but without an intracellular electrode. SK channel activity was found to increase during the period of time following a patch action current. Since no double openings were observed during this experiment, this patch was assumed to contain only a single SK channel. Under this assumption, the channel open probability was calculated with respect to the patch action current. Since the membrane potential across the patch membrane is variable, averaging the raw traces would reflect not only the open probability but also the changes in single channel current as a consequence of the unclamped, pacing cell. We have adopted a method which considers only whether the channel is open or closed. The patch records were first aligned with the patch action current, and channel openings were tallied at time intervals of 125 ms for 45 consecutive patch action currents. The open probability was calculated by dividing the number of channel openings at a given time by the total number of sweeps (45). The result of this analysis is shown in the plot of Fig. 5B. The open probability is lowest immediately before the action potential and is highest during the early part of the slow afterhyperpolarization. Thus, under these conditions, SK channels contribute to the rate of decay of the slow afterhyperpolarization. This could, in turn, influence the rate of cell firing.

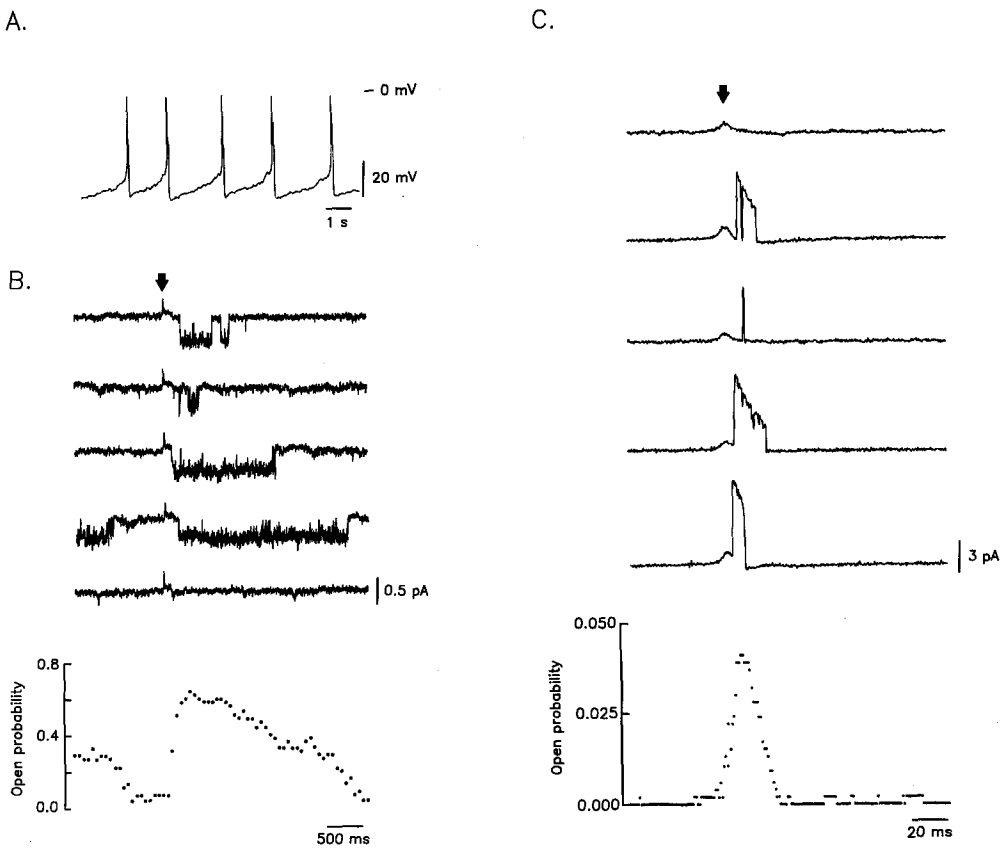

Fig. 5A–C. Ca^{2+}-activated/K^+ channels activated by single action potentials. Current-clamp and single channel records were obtained from GH_3 cells bathed in normal saline containing 3 μM TTX and 5 mM 4-AP. **A** This current clamp record illustrates Ca^{2+}-action potentials and the slowly decaying afterhyperpolarization in a cell that was firing spontaneously at a rate of 0.4–0.5 Hz. The cell-attached patch records in **B** and **C** were obtained with the patch electrode held at 0 mV so that the net potential across the patch membrane was the cell membrane potential. The patch action currents (represented by upward transients) have been aligned at the *arrows*. **B** The patch pipette contained the same solution (150 mM K^+) as used in inside-out patch experiments and the single channel currents are inwardly directed. This is an SK channel with a slope conductance of 9 pS. The open probability of this channel as a function of time with respect to the peak of the patch action current was calculated manually (see text) for 45 consecutive action potentials and is plotted with the same time scale as the cell-attached patch records. SK channel activity is increased for a prolonged period following a patch action current. The spontaneous firing rate in this cell was 0.3 Hz. **C** The patch pipette contained normal saline with 3 μM TTX and 5 mM 4-AP, and single channel currents are outwardly directed. The open probability of this channel was calculated as in **B** from 462 action potentials and is plotted with the same time scale as the single channel records. This channel, which is a BK channel (see text) opens during and for a short period of time after the peak of the patch action current. The records in **A** and **B** were lowpass filtered at 250 Hz, and the records in **C** were lowpass filtered at 1 kHz (−3 dB, 8 pole Bessel)

In these experiments, BK channel activity was generally not seen, even during the first few milliseconds of the afterhyperpolarization. Furthermore, this lack of activity was observed in patches known to contain BK channels (by depolarization of the membrane patch to large positive potentials where BK channels can be activated at resting levels of cytosolic Ca^{2+}). Since Ca^{2+} was not present in the patch electrode, one possible explanation is that during an action potential the local Ca^{2+} concentration at the cytoplasmic face becomes high enough to activate a BK channel only when the BK channel is situated very close to a Ca^{2+} channel that is carrying inward Ca^{2+} current. To test this possibility, experiments were performed with 2 mM Ca^{2+} included in the patch pipette solution. Figure 5C illustrates channel activity from a cell in which both the bath and patch pipette contain physiological saline with 3 μM TTX and 5 mM 4-AP. Outwardly directed single channel openings occur, for the most part, during the first 20 ms after the peak of the action current. The amplitude of the longer duration openings relax with time which is a reflection of the change in cell membrane potential as the action potential repolarizes. This patch contained a single channel, and the open probability with respect to the action currents was calculated as described above. The open probability peaks within a few milliseconds after the peak of the action current than rapidly declines. Channel openings could also be elicited by depolarization of the patch membrane which permits Ca^{2+} entry into the cell via voltage dependent Ca^{2+} channels in the membrane patch (data not shown). The I-V relationship of the current amplitudes were well fit by a Goldman constant field relationship of a K^+ selective channel and assuming a resting membrane potential of −50 mV and an intracellular K^+ concentration of 150 mM. The corresponding single conductance of this channel in 150 mM symmetrical K^+ would be 230 pS. It is thus concluded that this channel is a BK channel which contributes to repolarization of the action potential.

In cells which are bathed in normal saline rather than saline containing 5 mM 4-AP, a somewhat different pattern of SK activity is observed. SK channel openings were not

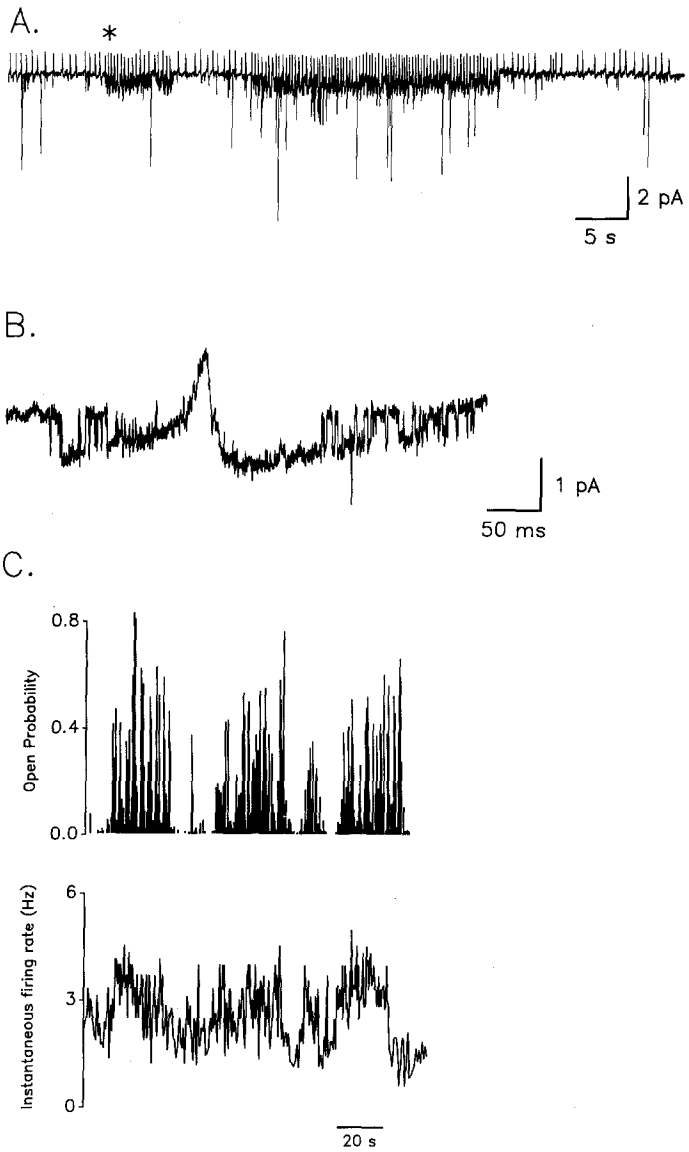

Fig. 6A–C. SK channels activated by high action potential firing rate. **A** A cell-attached patch record from a cell bathed in saline with no channel blockers is shown on a slow time scale. The patch pipette contained the 150 mM KCl solution with 3 μM TTX and 5 mM 4-AP. Patch action currents are visible as upward transients and the SK channel openings are downward. Occasional brief, frequency clipped openings from a larger conductance channel may also be seen. **B** The portion under the *asterisk* in **A** is shown on expanded scales. The chord conductance (12 pS) of the SK channel in this patch was estimated by measuring the unitary amplitude 200 ms after the patch action current and assuming a cell membrane potential of −40 mV and an intracellular K^+ concentration of 150 mM. **C** The open probability for SK channels was calculated for 500 ms windows and is shown in the upper plot. The instantaneous firing rate (inverse of the interspike interval) is shown in the lower plot and is at the same time scale as the open probability window plot. During periods of high action potential firing rates, the SK open probability increases. The records were lowpass filtered at 180 Hz for **A** and 1 kHz for **B** (−3 dB, 8 pole Bessel)

correlated with individual action potentials, but were seen during periods of high firing rates. This pattern of activity is shown in the record of Fig. 6A and the expanded record of Fig. 6B. This correlation is expressed quantitatively in plots which compare the channel open probability and the instantaneous action potential firing rate (Fig. 6C). The channel open probability during 500 ms windows was calculated by the "Analysis" program. A pitfall of this analysis method is that during the action potential depolarization (approximately 80 ms), channel openings cannot be detected. The instantaneous firing rate (inverse of the interspike interval) was calculated manually from chart paper records. The increases in open probability are well correlated with increases in action potential firing rate. Therefore, under normal circumstances, SK channels can contribute to the background membrane conductance during periods of increased firing rates.

Unlike the BK channel which is only activated by action potentials when Ca^{2+} is present in the patch pipette, the correlation of SK channel activity with the slow afterhyperpolarization (when the cells are bathed in saline containing 4-AP and TTX) or with periods of rapid firing is similar whether Ca^{2+} is present or absent in the pipette. Thus, unlike BK channels, SK channels appear to be sensitive to a more diffuse concentration of Ca^{2+} at the cytoplasmic face of the membrane.

On a few occasions (4 patches) a 16 pS voltage dependent channel was also observed in cell-attached patches with nominally Ca^{2+}-free, 150 mM KCl in the patch pipette. The open probability of this channel was decreased by hyperpolarization of the patch and increased by depolarization of the patch relative to the resting membrane potential of the cell (data not shown). This behavior is in contrast to that of the Ca^{2+}-activated SK channel which is not strongly voltage sensitive. Therefore, when Ca^{2+} was not present in the patch pipette, SK channel in cell attached patches was routinely distinguished from the voltage dependent channel by establishing a lack of enhancement of opening probability upon patch depolarization.

We have also tested TRH effects on cell-attached patch activity of unclamped cells. Under these conditions both the 4 pS channels and SK channels are still activated by TRH. In addition, TRH activates another channel type which has a slope conductance of about 38 pS with 150 mM KCl in the patch pipette. A 38 pS channel with high activity during the early part of the slow afterhyperpolarization has also been observed in some cells (data not shown). This channel is not seen in inside-out patches, and we have not yet characterized its ionic selectivity or Ca^{2+} sensitivity. A likely explanation for our failure to observe the 38 pS channels in excised patches or when the cell contains an intracellular patch electrode is that some cytoplasmic factor is probably needed to stabilize its activity.

Discussion

These single channel studies demonstrate the existence of at least two types of Ca^{2+}-activated K^+ channels in the GH_3 anterior pituitary cell line. The two channel types are distinguished by differences in unit conductance, Ca^{2+} sensitivity and voltage sensitivity. The BK channels have a large unit conductance (250–300 pS in 150 mM symmetrical KCl), and are highly voltage sensitive with the open probability in the presence of Ca^{2+} increasing as the patch is depolarized. These properties are similar to those of BK channels found in rat myotubes (Barrett et al. 1982). In addition, BK channels from both preparations are blocked by external TEA (Blatz and Magleby 1984; Lang, unpub-

lished observation). In cell-attached patches of spontaneously firing cells, BK channels are active during the repolarization and for 30 ms after the peak of the patch action current. This shows that BK channels are involved in repolarization of the action potential but do not contribute to the slow afterhyperpolarization. A similar function for BK channels has been proposed for rat myotubes (Romey and Lazdunski 1984), bullfrog sympathetic ganglion cells (Pennefather et al. 1985) and hippocampal pyramidal cells (Lancaster et al. 1986). In these preparations, applications of inhibitors of the BK channel (TEA or charybdotoxin) increase action potential duration and reduce the fast hyperpolarization following the spike but do not affect the slow afterhyperpolarization.

The SK channels, in contrast to BK channels, have a much smaller unit conductance (9–14 pS in 150 mM symmetrical KCl), are not strongly voltage sensitive and are more sensitive to Ca^{2+} at negative membrane potentials. We have shown that SK channel activity in voltage clamped GH_3 cells coincides in time with a slowly decaying Ca^{2+}-activated K^+ tail current. In addition, SK channels can be activated by TRH-induced mobilization of intracellular Ca^{2+} and thus contribute to the TRH-induced hyperpolarization. Finally SK channels were demonstrated to be active at negative membrane potentials during periods of high action potential firing rates and to contribute to the slow afterhyperpolarization of prolonged, single action potentials. Thus, SK channels contribute to the membrane conductance at potentials near rest during periods of elevated cytosolic Ca^{2+} concentrations.

Studies of whole-cell currents (Ritchie 1987a, b) show that GH_3 cells have an apamin-sensitive Ca^{2+}-activated K^+ conductance that is sensitive to elevations in cytosolic Ca^{2+} at membrane potentials near rest. The SK channels in GH_3 cells exhibit single channel characteristics that are nearly identical to the apamin sensitive SK channels that have been described in cultured rat myotubes (Blatz and Magleby 1986). It is thus very likely that the SK channel in GH_3 cells represents the apamin sensitive component of the Ca^{2+}-activated K^+ conductance. Under certain circumstances, SK channels were shown to contribute to the slow afterhyperpolarization in GH_3 cells. Consistent with this observation are studies which show that apamin inhibits a slow afterhyperpolarization in myotubes (Romey and Lazdunski 1984) and sympathetic ganglion cells (Pennefather et al. 1985; Kawai and Watanabe 1986). Unlike sympathetic ganglion cells and rat myotubes, GH_3 cells have an additional Ca^{2+}-activated K^+ conductance that is apamin-resistant, but TEA sensitive, and also activated by Ca^{2+} at potentials near rest. Two other channels, with unit conductances of 4 pS and 38 pS in 150 mM symmetrical KCl, were seen at potentials near rest under conditions of increased intracellular Ca^{2+}. However, their Ca^{2+} dependency, ionic selectivity, and pharmacology have not yet been determined.

In order to estimate the possible contribution of SK channel activity to the resting membrane potential, we have estimated the minimal number of SK channels likely to be present in GH_3 cells. The mean apamin sensitive whole-cell current at 0 mV is about 130 pA (Ritchie 1987a), and the unitary SK current under a physiological K^+ gradient is about 0.22 pA at 0 mV. Therefore, a minimum of 590 SK channels are likely to be present per cell. Under physiological conditions, the slope conductance of SK channels in the voltage range of −80 to −30 mV is about 3 pS. For a typical cell with resting membrane potential of −42 mV, an average input resistance of 2 GΩ, and an apamin sensitive current reversal potential of −75 mV (Ritchie 1987a), activation of 10% of the SK channel population could hyperpolarize the cell by about 8 mV[2].

In conclusion, at least two types of Ca^{2+}-activated K^+ channels exist in GH_3 cell membranes. These channel types have different properties which appear to be tailored for different functional roles in the cell. The BK channels with their large conductance and steep voltage dependency contribute to the rapid repolarizaiton of the action potential. The SK channels, with their higher Ca^{2+} sensitivity at negative membrane potentials, influence cell excitability by contributing to the slow afterhyperpolarization when the duration of the Ca^{2+} action potential is slightly prolonged or to the background membrane conductance during periods of rapid firing.

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[2] The membrane potential (V) during SK channel activation is given by the equation $V = [G_m V_m + G_{sk} V_{sk}] / [G_m + G_{sk}]$ where G_m and V_m are the resting membrane conductance and potential, respectively, G_{sk} is the conductance contributed by the SK channels and V_{sk} is the reversal potential for SK channels

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