Calcium dynamics in hippocampal neurones
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Citation for published version (APA):
Borgdorff, J. A. (2002). Calcium dynamics in hippocampal neurones
Chapter VI

Calcium-dependent properties of a high-affinity Ca\(^{2+}\)-activated K\(^+\) current in acutely isolated CA1 pyramidal neurones

With:
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Summary

We describe here a Ca\textsuperscript{2+}-activated K\textsuperscript{+} current in isolated CA1 pyramidal neurones of the hippocampus, using whole-cell voltage-clamp recordings in combination with fura-2 [Ca\textsuperscript{2+}]\textsubscript{i} imaging and [Ca\textsuperscript{2+}]\textsubscript{i} manipulation between 0 and ~900 nM with the Ca\textsuperscript{2+}-ionophore ionomycin.

The current shows steep Ca\textsuperscript{2+} dependence in the sub-micromolar range of [Ca\textsuperscript{2+}]\textsubscript{i} that can be described with a Hill equation with a $K_d$ of 300 ± 27 nM (mean ± S.E.M.) and a Hill coefficient of 4.7 ± 0.5.

Raising [Ca\textsuperscript{2+}]\textsubscript{i} from 20 nM to 500 nM caused through activation of this current an eleven-fold increase in the membrane conductance, from 3.2 ± 0.8 nS to 37 ± 3 nS.

None of the Ca\textsuperscript{2+}-dependent current parameters exhibit dependence on membrane voltage.

Prolonged periods of high and stable [Ca\textsuperscript{2+}]\textsubscript{i} did not alter the current amplitude, indicating a lack of time-dependent (in)activation mechanisms on the seconds time scale.

Current-variance analysis in 140 mM symmetrical [K\textsuperscript{+}]\textsubscript{i};[K\textsuperscript{+}]\textsubscript{o} indicates a single-channel conductance of 2.3 ± 0.8 pS that underlies the current, with Ca\textsuperscript{2+} modulating the channel open probability $p_0$. At maximal current activation $p_0$ was estimated as 0.80 ± 0.1.

Comparing the current properties with the activation properties of the voltage-gated K\textsuperscript{+} currents $I_{KA}$ and $I_{KD}$, recorded from this preparation shows that this current has its most significant contribution as a hyperpolarising force at resting membrane potentials during modest elevations of [Ca\textsuperscript{2+}]\textsubscript{i} from [Ca\textsuperscript{2+}]\textsubscript{i} resting levels.
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Introduction

In hippocampal CA1 pyramidal cells, different types of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents are activated during and after action potential (AP) firing (Hotson and Prince, 1980; Brown and Griffith, 1983; Lancaster and Adams, 1986; Lancaster and Nicoll, 1987; Stocker et al., 1999). They contribute to spike repolarisation (Lancaster and Nicoll, 1987; Storm, 1987), spike-frequency adaptation (Madison and Nicoll, 1984; Stocker et al., 1999; Velumian and Carlen, 1999), the medium afterhyperpolarisation (mAHP) that lasts for about 100 ms (Storm, 1989; Stocker et al., 1999) and the slow afterhyperpolarisation (sAHP), that peaks 100-250 ms after AP firing has stopped and may last for several seconds (Hotson and Prince, 1980; Stanton et al., 1992). The voltage-operated calcium channels (VOCC’s) that open during the AP (Lancaster and Zucker, 1994) provide the rise in intracellular free calcium ([Ca\textsuperscript{2+}]), necessary for the activation of the Ca\textsuperscript{2+}-activated K\textsuperscript{+} current.

Two classes of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, called BK and SK, are thought to underlie these currents (Sah, 1996). These channel types distinguish themselves by specific voltage- and Ca\textsuperscript{2+}-dependent properties, single-channel conductance and pharmacological profile. Thus, it has been concluded that the BK-type channel plays a role in Ca\textsuperscript{2+}-dependent spike repolarisation (Shao et al., 1999), while the SK-type channel contributes - at least partially - to spike frequency adaptation and the mAHP (Stocker et al., 1999) and accounts fully for the sAHP (Sah and Isaacson, 1995). A further refinement suggests the recent cloning of SK channels (Kohler et al., 1996). All three subtypes found so far (SK1, SK2, SK3) are expressed in CA1 pyramidal cells (Stocker and Pedarzani, 2000) while different subtypes appear to be linked to the different AHPs (Kohler et al., 1996; Stocker et al., 1999).

SK-type Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels recorded from CA1 pyramidal cells have the following features: a small single-channel conductance (2-10 pS), a steep activation curve in the sub-micromolar range of [Ca\textsuperscript{2+}], lack of voltage- and time-dependent (in)activation kinetics and high rate constants for channel opening and closing (Lancaster et al., 1991; Marrion and Tavalin, 1998; Selyanko et al., 1998).

Voltage-clamp experiments in slice preparations have demonstrated for CA1 pyramidal cells that brief activation of VOCC’s evokes substantial current mediated by SK channels where channel open probabilities up to 0.4 can be reached (Sah and Isaacson, 1995; see also: Valiante et al., 1997). Given the modest increases in bulk [Ca\textsuperscript{2+}], reported for these protocols (Regehr and Tank, 1992) this underlines the functional importance of the high Ca\textsuperscript{2+}-affinity/stEEP activation curve of the SK channels. Also close proximity of SK channels to Ca\textsuperscript{2+} channels may contribute to efficient activation. In CA1 pyramidal cells this principle has been demonstrated for the L-type Ca\textsuperscript{2+} channel (Marrion and Tavalin, 1998), which locates at the soma and proximal dendrites (Westenbroek et al., 1990, Christie et al., 1995). Similar observations were made in CA3 neurones (Tanabe et al., 1998) and sympathetic neurones (Davies et al., 1996) but not in others (Martinez-Pinna et al., 2000). On the other hand are sAHP kinetics and amplitude easily disturbed by relatively low amounts of ‘slow’ Ca\textsuperscript{2+} chelators (Zhang et al., 1995; Spigelman et al., 1996). This indicates (Naraghi and Neher, 1997) a relatively large
average functional distance between VOCC and SK channel for the total SK channel population.

SK channel activation underlying the sAHP in CA1 cells does not reach equilibrium with the mean cytoplasmic [Ca\(^{2+}\)] response seen during and after AP activation. This follows from the fact that the temporal dynamics of this [Ca\(^{2+}\)] response and sAHP current amplitude do not match (Jahromi et al., 1999; Sah and Clements, 1999). This has been explained by slow SK channel kinetics upon Ca\(^{2+}\) (un)binding, shown in [Ca\(^{2+}\)] step protocols (but see: Lancaster and Zucker, 1994). The spatio-temporal characteristics of [Ca\(^{2+}\)] where the SK channels locate certainly deviates from the mean [Ca\(^{2+}\)], and may therefore also contribute to this observation (Eilers et al., 1995; Gil et al., 2000). The complexity of the relation between Ca\(^{2+}\) influx, cytoplasmic [Ca\(^{2+}\)], and SK current activation follows also from the fact that a change in the source of Ca\(^{2+}\) influx results in altered sAHP dynamics (Lancaster and Zucker, 1994). In contrast to this seems the apamin-sensitive fraction of the SK current to reach relatively rapidly equilibrium upon a [Ca\(^{2+}\)] rise. At least this what the fast kinetics of the mAHP (Stocker et al., 1999) and rapid activation in [Ca\(^{2+}\)] step protocols (Sah and Clements, 1999) suggest.

In this paper we describe the activation properties of a Ca\(^{2+}\)-dependent K\(^+\) current in freshly isolated hippocampal pyramidal CA1 neurones that has a high Ca\(^{2+}\) affinity. The whole-cell voltage-clamp approach allowed an estimate of the total available current and comparison with the major voltage-dependent (but Ca\(^{2+}\)-independent) K\(^+\) currents these cells posses.

Materials and methods

Cell preparation

Hippocampal CA1 pyramidal cells were isolated as follows. Male Wistar rats (80-120 grams, Harlan, Zeist, the Netherlands) were decapitated, the hippocampi were quickly isolated, put in cold standard ACSF and cut into 400 \(\mu\)m thick transversal slices. The CA1 region was removed from each slice and incubated for 90 minutes at 32\(^\circ\)C in an oxygenated solution containing (in mM): NaCl 120, PIPES (piperazine-N, \(N^\prime\)-bis[2-ethanesulfonic acid]) 10, KCl, 5, CaCl\(_2\) 1, MgCl\(_2\) 1, D-glucose 25 and 1 mg ml\(^{-1}\) bovine trypsin (Bovine, type XI) (pH = 7.0). Thereafter the medium was replaced by the same solution without the enzyme and kept at room temperature. Cells were mechanically dissociated from tissue chunks and the suspension was brought into the recording chamber mounted on an inverted microscope (Nikon Diaphot). Spindle-shaped cells that appeared smooth and bright, without visible organelles were selected for recording. The Dutch animal welfare committee approved the experimental protocols.

Voltage-clamp recordings

Whole-cell recording was performed in the following extracellular solution; (in mM) 80 NaCl, 40 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, 25 D-Glucose, 0.0005 TTX (pH = 7.4). Patch pipettes were filled with 140 KF, 20 HEPES, 10 EGTA, 2 MgCl\(_2\), 0.1 fura-2 (pH = 7.3). All chemicals were obtained from Sigma (Sigma St. Louis, MO) except fura-2
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(Molecular Probes, Eugene, OR) and TTX (Tocris Cookson Ltd, Bristol, UK). In the experiments where other values for [K$^+$]$_o$ were used, NaCl was replaced by an equimolar amount of KCl. The contribution of the voltage-activated calcium current was found to be negligible in our experiments and therefore not pharmacologically blocked. Just after attaining whole cell we did observe an increase in [Ca$^{2+}$]$_i$ during membrane depolarisations to 0 mV, presumable caused by calcium current activation, but the [Ca$^{2+}$]$_i$ response disappeared within a few minutes. Recordings were done at room temperature. Pipette resistance in the bath solution was 2-3 MΩ. Voltage clamp measurements were done with an Axopatch 200 amplifier (Axon Instruments, Burlingame, CA). After attaining the whole-cell mode [Ca$^{2+}$]$_i$ was allowed to decrease to a low and stable [Ca$^{2+}$]$_i$ level, which typically took several minutes. During this period the linear leak current amplitude showed clear relationship with [Ca$^{2+}$]$_i$ (not shown). During the recording the membrane potential was kept close to $E$ for the voltage-activated delayed rectifier current $I_{K_D}$, determined at the beginning of each experiment from the tail current of $I_{K_D}$ with a voltage step protocol. This was done to avoid a shift in [K$^+$]$_i$ during prolonged current activation. The signal was filtered at 5 kHz with the 4-pole Bessel filter of the amplifier and digitised at 10 kHz. Capacitive transients were compensated using the dials of the amplifier. No series resistance compensation was performed.

**Variance analysis and the Y-tube application system**

For variance analysis we created brief repetitive changes in $E_k$ with a home-made Y-tube application system filled with high [K$^+$]$_o$. The Y-tube position was adjusted at the start of the recording. This was done though the determination of $E_{rev}$ for $I_{K_D}$ (same protocol as given above) during application; when $E_{rev}$ was close to the theoretical values for $E_k$ and remained stable with repeated applications the experiment was started.

**Imaging and manipulation of [Ca$^{2+}$]$_i$**

The calcium-sensitive dye fura-2 was used to determine [Ca$^{2+}$]$_i$. The dye was excited pair wise at $\lambda = 340$ nm and $\lambda = 380$ nm (each exposure time 100 ms) with a custom-made wavelength switcher. Fluorescence for the two wavelengths ($F_{340}$, $F_{380}$) was collected at $\lambda = 510$ nm with a cooled CCD camera (CE200, Photometrics). For each pair we determined the ratio $R = F_{340}/F_{380}$ over the cell body and calculated [Ca$^{2+}$]$_i$ with the standard formula given by:

$$[\text{Ca}^{2+}]_i = K_d \cdot \beta \cdot \frac{R - R_{\text{min}}}{R_{\text{max}} - R} \quad (1)$$

The value for the calibration constant $K_d$ was taken from literature as 225 nM (Grynkievicz et al., 1985). $R_{\text{min}}$ was estimated from the experimental data. First we plotted the lowest value for $F_{340}/F_{380}$ for each cell recorded at the beginning of each experiment as a function of the experimental day. These data distributed around a horizontal line indicating that $R_{\text{min}}$ remained constant during the entire recording period. The lowest $R$ value of the population was taken as $R_{\text{min}}$. $R_{\text{max}}$ was determined in a separate set of experiment where we added the Ca$^{2+}$ ionophore ionomycin (1 μM) to the extracellular medium, while measuring $R = F_{340}/F_{380}$ as described above. For a large set of
these experiments we arrived at a mean ratio $R_{\text{max}}/R_{\text{min}}$ of 14 ($n = 24$). For the estimation of $\beta$, subsequent pairs of $F_{340}/F_{380}$ were taken from the presented experiments for those cases where $[Ca^{2+}]_i$ increased between the two time points. This resulted in a pair at low ($F_{340L}, F_{380L}$) and high $[Ca^{2+}]_i$ ($F_{340H}, F_{380H}$). For 188 of these double pairs recorded from 18 cells was calculated:

$$A = \frac{F_{340H}}{F_{380H}} - \frac{F_{340L}}{F_{380L}} \quad \text{and} \quad B = \frac{1}{F_{380H} - F_{380L}}$$

(2)

Plotting values for A against B for all estimates gave the distribution shown in Fig. 1. The estimate of the slope (S) of this plot is 0.58 (straight line in Fig. 1; linear fit through the origin). It can be proven that:

$$\beta = 1 + S \left[ \frac{R_{\text{max}}}{R_{\text{min}}} - 1 \right]$$

(3)

resulting in an estimate for $\beta$ of 8.5.

$[Ca^{2+}]_i$ manipulation in the experiments was done by drop application of ionomycin (1 $\mu$M). In this way we could slowly raise $[Ca^{2+}]_i$ at a rate of $5 \pm 7$ nM s$^{-1}$ (mean $\pm$ S.D. for the whole data set). $[Ca^{2+}]_i$ values varied between 0 and $\sim$900 nM. $[Ca^{2+}]_i$ measured immediately before and after each current measurement were almost identical (see also Fig 2C). The mean of the two values was used in the analysis. The slow rate of change of $[Ca^{2+}]_i$ and the large amount of mobile intracellular $Ca^{2+}$ buffer (10 mM EGTA) should prevent large $Ca^{2+}$ gradients around the point of $Ca^{2+}$ influx. Therefore we do not expect serious gradients between the mean cytoplasmic $[Ca^{2+}]_i$ recorded in this study and $[Ca^{2+}]_i$ directly under the cell membrane, which the $Ca^{2+}$ activated ion channels experience.

Data analysis
Current and variance analysis was done with custom-made software. Procedures are explained in the results section. A part of the data fitting was done with Kaleidograph 3.08 (Synergy software Inc, Reading USA). Data is expressed as mean $\pm$ S.E.M. unless stated differently. For statistical analysis we used Student’s T-test. $P < 0.05$ was considered significant.

Figure 1. Estimation of $\beta$ (eq. 1) for fura-2 calibration. For a full explication of the figure see ‘materials and methods’ section and the legend of Fig. 1 in chapter V.
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**Figure 2.** Activation of a Ca\textsuperscript{2+}-dependent current through the manipulation of [Ca\textsuperscript{2+}]\textsubscript{i}. A, Voltage and calcium imaging protocol. Isolated CA1 pyramidal cells were kept under whole-cell voltage clamp at a membrane potential of -30 mV (close to E\textsubscript{K} in this experiment). Membrane voltage was stepped to -60 mV for 3 s with 10 s intervals. Fura-2 fluorescence for the excitation wavelengths 340 and 380 nm was measured at the start (●) and end (○) of each voltage step (shutter scheme gives exposure intervals for the wavelengths) and used to calculate [Ca\textsuperscript{2+}]\textsubscript{i}. B, [Ca\textsuperscript{2+}]\textsubscript{i} was varied between 0 and ~900 nM through the application of ionomycin. Plotting start (●) versus end (○) [Ca\textsuperscript{2+}]\textsubscript{i} values (n = 529; whole data set) gives a straight line with slope of 1, indicating stable [Ca\textsuperscript{2+}]\textsubscript{i} levels during the voltage steps. C, A rise in [Ca\textsuperscript{2+}]\textsubscript{i} (mean value for a ●/○ pair) is matched by an increase in current amplitude at -60 mV. Note the lack of (de)activation kinetics. D, Same experiment as in C, but now for the complete recording. The mean current amplitude for each step to -60 mV and [Ca\textsuperscript{2+}]\textsubscript{i} (symbols as in A) are shown.
Results

Identification of a Ca\(^{2+}\)-activated outward current in isolated CA1 neurones

Raising the intracellular calcium concentration ([Ca\(^{2+}\)]\(_i\)) in freshly isolated CA1 hippocampal pyramidal neurones in the sub-micromolar range leads to a substantial increase in the membrane current (Fig. 2). The measuring protocol we used to demonstrate this is given in Fig. 2A. Cells were held under whole-cell voltage clamp with the membrane potential stepped for 3 s to -60 mV for every 10 s. In between, the membrane potential was kept close to the reversal potential for K\(^+\), here set to -30 mV for a [K\(^+\)]\(_i\) of 140 mM and a [K\(^+\)]\(_o\) of 40 mM. This was done to avoid loss of intracellular potassium during prolonged activation of the Ca\(^{2+}\)-activated current. Before and after each voltage step fura-2 fluorescence was measured over the cell soma for 340 and 380 nm wavelength excitation (respectively F\(_{340}\) and F\(_{380}\)) from which [Ca\(^{2+}\)]\(_i\) was calculated (described in the method section).

With drop application of a low dose of the Ca\(^{2+}\) ionophore ionomycin (1 μM) to the extracellular recording solution we could induce a slow rise in [Ca\(^{2+}\)], with an average of 5 ± 7 nM s\(^{-1}\) (mean ± S.D. for the whole data set). Figure 2B displays the [Ca\(^{2+}\)]\(_i\) value before each voltage step plotted against the corresponding [Ca\(^{2+}\)]\(_i\) value after the voltage step for all our experimental data. As can be seen vary [Ca\(^{2+}\)]\(_i\) values between 0 and ~900 nM and forms the data a straight line with a slope of 1. These observations implicate that we created different, but steady-state [Ca\(^{2+}\)]\(_i\) values for each voltage step.

Figures 2C and 2D present a cell recording in which [Ca\(^{2+}\)]\(_i\) was raised from ~100 to ~500 nM (voltage protocol as in Fig. 1A). Figure 2C shows the membrane current for the indicated [Ca\(^{2+}\)]\(_i\) levels. It is clear that from 100 nM onwards the current amplitude increases until it almost saturates at ~500 nM. Note also that the current amplitude follows instantaneously the voltage step, showing no activation or inactivation kinetics. Figure 2D plots the average current amplitude at -60 mV (mean current during the last 2 s of the voltage step) for the whole experiment as a function of time. The top trace demonstrates that the corresponding [Ca\(^{2+}\)]\(_i\) values before (●) and after (○) the voltage step overlap. For further analysis we used as an estimate for [Ca\(^{2+}\)]\(_i\) the average of the two values. We observed also (data not shown) that the Ca\(^{2+}\)-activated current reversed if [Ca\(^{2+}\)]\(_i\) relaxed back to low levels and that this current could be evoked repeatedly.

K\(^+\) is charge carrier of the current

The reversal potential (E\(_{\text{rev}}\)) for the Ca\(^{2+}\)-activated current follows the theoretical reversal potential for K\(^+\) ions (E\(_K\)), indicating that K\(^+\) is the main charge carrier of the current. Figure 3Aa-Ac show a recording with a protocol in which 100 ms voltage steps were made to -40, -30 and -20 mV, while raising [Ca\(^{2+}\)]\(_i\). E\(_K\) for this recording was calculated as -31.6 mV ([K\(^+\)]\(_i\):[K\(^+\)]\(_o\) = 140:40 mM). Figure 3Ab shows that E\(_{\text{rev}}\) indeed must be close to E\(_K\) as increased [Ca\(^{2+}\)]\(_i\) introduces an inward current at -40 mV, an outward current at -20 mV and almost no current at -30 mV. This is also easily seen in Fig. 3Ac which displays the relationship between current amplitude and membrane potential for all [Ca\(^{2+}\)]\(_i\) values. The point at which the I-V curves cross, indicated with an arrow, approximates E\(_{\text{rev}}\). Calculating the voltage at which two I-V curves at subsequent [Ca\(^{2+}\)]\(_i\) values cross
was done through linear interpolation between -20 and -30 mV. The mean value for all pairs was -29.5 mV for this cell. For a group of cells this was \(-31.5 \pm 0.5\) mV \((n = 11)\); a value close to \(E_K\).

Lowering \([K^+])o\) to 5 mM or 2.5 mM shifted the \(E_{rev}\) respectively to \(-79.0 \pm 1.5\) mV \((n = 7)\) and \(-95.5 \pm 0.6\) mV \((n = 10)\). These values match \(E_K\) (respectively \(-84.2\) mV and

**Figure 3.** \(K^+\) selectivity of the \(Ca^{2+}\)-activated current. \(Aa\), stepping the membrane potential around \(E_K\) (initial part of the voltage protocol in Fig. 1A) while raising \([Ca^{2+}]i\) shows current reversal close to \(E_K\) \((E_K = -31.6\) mV, \(K^+\) condition as indicated). The inset shows the voltage protocol. \(Ab\), Current versus \([Ca^{2+}]i\) for -20, -30 and -40 mV membrane potential for the same recording. \(Ac\), I-V plot constructed from \(Aa\) for each \([Ca^{2+}]i\). Estimated \(E_{rev}\) is -29.5 mV (average of the voltage at which two subsequent I-V curves cross, indicated by the arrow). The mean current amplitude at \(E_{rev}\) (vertical arrow) is treated as offset current and is subtracted from each current trace before data analysis. \(B\), \(E_{rev}\) as a function of \([K^+])o\) (black dots, mean \pm S.E.M. as in the other figures, cell numbers are indicated). The straight line indicates the theoretical value for \(E_K\).
-101.7 mV). These data are summarised in Fig. 3B. We conclude from these results that the observed Ca\(^{2+}\)-activated current is mainly carried by K\(^+\) ions. As also the single-channel and Ca\(^{2+}\)-activation properties of this current comply with the SK-type Ca\(^{2+}\)-activated K\(^+\) channel, as will be discussed below, we call the current in this study \(I_{SK}\).

**Other linear current components and current components at [Ca\(^{2+}\)] \(_r\) resting levels**

While raising [Ca\(^{2+}\)], we sometimes - but not always - observed a gradual leftward shift of \(E_{rev}\) that we interpreted as the appearance of a yet unspecified linear leak component not carried by K\(^+\) ions. We didn’t try to establish whether this component was related to ion channels, a-specific leak caused by the recording method or loss of [K\(^+\)]\(_i\) during the experiment. We present in this study data from 32 cells that showed negligible shifts of \(E_{rev}\) with respect to the first value in the recording (mean shift in \(E_{rev}\) of 1.6 mV during the recording).

From Fig. 3Ac it can be seen that at \(E_{rev}\) (arrow in the figure) a significant inward current exists. This current was treated as a recording offset current and its mean amplitude, estimated with the aforementioned interpolation procedure subtracted from the individual traces. An underlying assumption of this procedure is that the Ca\(^{2+}\)-independent part of the current that dominates at low [Ca\(^{2+}\)]\(_r\) levels is carried uniquely by K\(^+\) ions. In reality this applies only to \(~15\%\) of this current. This was concluded from experiments where we briefly changed [K\(^+\)]\(_o\) from 5 mM to 140 mM, while keeping the membrane potential at -80 mV and [Ca\(^{2+}\)]\(_i\) at low and stable levels. The resulting shift in \(E_k\) increased the slope conductance that consequentially relates to the fraction of current carried by K\(^+\) ions. This is shown for an example in Fig. 7A ([Ca\(^{2+}\)] = 20 nM). For seven of such recordings (mean [Ca\(^{2+}\)]\(_i\) of 50 ± 11 nM) we found through subtraction of a 1 s current trace at the 5 mM [K\(^+\)]\(_o\) condition from the 140 mM [K\(^+\)]\(_o\) condition, a 0.48 ± 0.08 nS K\(^+\) conductance. This value represents only 15% of the total slope conductance measured between -90 mV and -80 mV in 5 mM [K\(^+\)]\(_o\) for the same traces. The 85% that is left over remained undetermined in this study.

**Calcium-dependent properties of \(I_{SK}\)**

To establish the calcium-dependent properties of \(I_{SK}\) we calculated from the current at -60 mV the membrane conductance with the Goldman-Hodgkin-Katz (GHK) equation assuming K\(^+\) as the charge carrier and plotted the values against corresponding [Ca\(^{2+}\)]\(_i\) values. This relationship is shown in Fig. 4A for two individual examples. The steep activation curve in the sub-micromolar [Ca\(^{2+}\)]\(_i\) range is typical. The activation curve could be well described, as the fits (smooth lines) show, with a Hill equation of the form:

\[
g([\text{Ca}^{2+}]_i) = \frac{g_{\text{max}}}{1 + \left(\frac{K_d}{[\text{Ca}^{2+}]_i}\right)^h} + g_0
\]

Where \(K_d\) is the binding affinity of Ca\(^{2+}\), \(h\) the Hill coefficient, \(g_{\text{max}}\) the maximal conductance for the Ca\(^{2+}\)-sensitive part of the conductance (= \(I_{SK}\)) and \(g_0\) the Ca\(^{2+}\)-independent conductance.
We fitted eq. 4 to those individual recordings \((n = 15)\) that contained each 6 to 37 experimental values evenly distributed along the relevant \([Ca^{2+}]_i\) range. The membrane potential the cells were kept at ranged from -75 to -60 mV. Thus we estimated for \(I_{SK}\) a \(K_d\) of \(300 \pm 27\) nM, a Hill coefficient of \(4.7 \pm 0.5\) (range 3 to 8) and a \(g_{\text{max}}\) of \(37 \pm 3\) nS, while the mean value for \(g_0\) was \(3.2 \pm 0.8\) nS. Mean values for \(I_{SK}\) plotted against \([Ca^{2+}]_i\) are shown in Fig. 4B for these data. Individual values were calculated as \(\left(\frac{g_{(Ca^{2+})_i} - g_0}{g_{\text{max}}}\right)\), then sorted with respect to \([Ca^{2+}]_i\). Each data point represents the mean \pm S.D. of 10 subsequent values. The smooth line in the figure represents a Hill function using the mean estimates for the \(K_d\) (300 nM) and Hill coefficient (4.7). Boundaries for 10% and 90% current activation were estimated as respectively 190 nM and 500 nM, underlining the steep \(Ca^{2+}\)-dependence of the current.

**The threshold of \(Ca^{2+}\)-dependent current activation**

The membrane conductance appeared rather \(Ca^{2+}\) insensitive at \([Ca^{2+}]_i\) values below 100 nM. This was concluded from recordings with \([Ca^{2+}]_i\], sampled in its lower range. Fig. 5A shows the relationship between membrane conductance and \([Ca^{2+}]_i\) for such a recording. As can be seen remains the conductance between 15 and 100 nM \([Ca^{2+}]_i\), almost constant before it steeply increases above 100 nM. The size of \(Ca^{2+}\)-dependent current activation over the 0-100 nM \([Ca^{2+}]_i\) range was estimated for 9 cells that had at least 3 experimental values.

![Figure 4. The \(Ca^{2+}\)-dependence of \(I_{SK}\).](image)

From recordings as shown in Fig. 2 were selected those with \([Ca^{2+}]_i\) values evenly distributed over the relevant \([Ca^{2+}]_i\) range (6-32 \([Ca^{2+}]_i\) values per cell). The membrane conductance \(g\) was calculated from each current trace with the GHK equation. A, Two individual examples of the resulting \([Ca^{2+}]_i\) - \(g\) plots. Smooth line shows fit with eq. 4. Estimated values for the formula parameters are given in the panels. B, Average conductance for the \(Ca^{2+}\)-dependent part of the current calculated from all values from 15 cells (mean \pm S.D.; sorted data; 10 values per data point). The smooth curve gives a Hill function with as parameter values the mean of the individual estimates \((K_d = 300, h = 4.7; \text{see results})\).
values below 100 nM (voltage steps to -60 or -75 mV). From the individual recordings the mean slope dG/d[Ca^{2+}]_i was estimated as 1.5 ± 1.6 nS 100nM^{-1} (mean ± S.E.M.) which differs not significantly from zero. In line with this result one can calculate from the mean estimate of the K_d and Hill coefficient for I_{SK} (see above), that less than 1% of

Fig. 5B shows the average relationship between the membrane conductance and [Ca^{2+}]_i for these cells (5 values per point). The smooth line in the figure gives the distribution of cytoplasmic [Ca^{2+}], resting levels that we estimated in this cell preparation in a separate experiment as 75 ± 58 nM (mean ± S.D.; n = 15) indicating that the threshold for current activation lies just above [Ca^{2+}]_i resting values.

I_{SK} during prolonged activation
We investigated whether I_{SK} displayed changes in amplitude during prolonged periods of activation that were not related to [Ca^{2+}]_i. Therefore we selected 7 cells that had high and stable [Ca^{2+}]_i values for at least 1 minute and looked at the progression of the current amplitude. [Ca^{2+}]_i values for these cells fell between 340 and 950 nM with an average of 519 ± 72 nM for the first time point. With 10 s intervals 6 more values were recorded with an end value at t = 60 s of 521 ± 58 nM. No significant change in [Ca^{2+}]_i was found between any of the time points confirming the selection criteria.

The recorded current amplitudes remained stable under these conditions. Only a 7% decrease between the first and last time point was found that did not reach significance (p = 0.23). We conclude therefore that on the tens of seconds time scale I_{SK} current amplitude is uniquely related to [Ca^{2+}]_i with no other contributing factors.

**Figure 5.** I_{SK} activation below 100 nM [Ca^{2+}]_i is negligible. A, Individual example (plot as in Fig. 4A). Note that between 10 and 100 nM [Ca^{2+}]_i no current activation is seen. B, Averaged data from cells with at least 3 values below 100 nM [Ca^{2+}]_i (n = 9, mean ± S.D.; 5 values/point). The smooth curve represents the scaled distribution of [Ca^{2+}]_i resting levels found in this cell preparation (gaussian distribution estimated from mean ± S.D.).
**Figure 6.** Voltage dependence of the Hill coefficient and $K_d$ of $I_{SK}$. 

**A, Protocol.** The membrane potential was stepped from -50 mV to -110 mV while varying $[Ca^{2+}]_i$ (100 ms voltage steps except 200 ms for the step to -50 mV; see inset). The upper panel shows for an individual example the resulting current at different $[Ca^{2+}]_i$ as indicated. Note that at -50 mV a voltage-activated $K^+$ current superimposes on $I_{SK}$. The lower panel gives the $I - [Ca^{2+}]_i$ curves for each membrane potential for the same experiment. ($I = \text{mean current over the last 50 ms for each voltage step}$). Smooth lines show individual fits with eq. 4. 

**B, Plot of the mean values for the $K_d$ (upper panel) and the Hill coefficient (lower panel) against membrane voltage for four of such experiments (including the one in panel A).** Data close to $E_k$ (-84 mV) is not shown due to poor quality of fit. Straight lines indicate mean values of all plotted data. 

**C, Plotting the $K_d$ and Hill coefficient versus the membrane potential for all experimental data ($n = 61$, 3-5 voltage values per cell; straight lines as in B) confirms the results of panels A-B.**
while manipulating $[\text{Ca}^{2+}]$. In this way we could determine the relationship between current amplitude and $[\text{Ca}^{2+}]$, for a large set of membrane potentials as is shown for an example in the right panels. Note that at -50 mV a voltage-dependent potassium current is activated, creating an offset in the corresponding $I - [\text{Ca}^{2+}]$ curve (lower panel). Our results indicate that $I_{\text{SK}}$ activation properties are voltage independent. Figures 6A/B depict an experimental protocol we used in order to show this. The membrane potential was varied with 100 ms (with the exception of a 200 ms duration step to -50 ms) duration steps between -50 mV and -110 mV (left panel). The protocol was repeated every 10 s

To estimate the $K_d$, Hill coefficient and $I_{\text{max}}$ for the $\text{Ca}^{2+}$ activation process we fitted the $I - [\text{Ca}^{2+}]$ relationship with eq. 4 (g in the formula replaced by I). The smooth lines in the lower panel of Fig. 6A show these fits for the example. Fig. 6B demonstrates for four of these experiments that between -110 and -50 mV values of $K_d$ and Hill coefficient are independent from the membrane potential. This was also true for the $I_{\text{max}} - V$ relationship (not shown). We arrived at the same conclusion if we plotted $K_d$ and Hill coefficient estimates from all experiments against their respective membrane potential as is shown in Fig. 6C.

These data suggest that $I_{\text{SK}}$ activation is a purely $\text{Ca}^{2+}$-dependent process. Therefore we recalculated the average $K_d$ and Hill coefficient, including data from all voltage steps instead of the more limited voltage range (-60/-70 mV) used above. Thus we obtained 67 values for 21 cells (3 - 5 different voltages per cell), arriving at a final estimated $K_d$ of $313 \pm 20 \text{ nM}$ and a Hill coefficient of $4.7 \pm 0.3$ for $I_{\text{SK}}$, which is very close to the values presented above.

**Single-channel properties underlying $I_{\text{SK}}$**

To investigate single-channel properties underlying $I_{\text{SK}}$ we used current-variance analysis. The measurement protocol was designed to maximise and isolate the current variance ($\sigma^2$) of $I_{\text{SK}}$ and is presented in Figs. 7A/B. Cells were kept at a membrane potential of -80 mV (in some cases -90 mV). Every 20 seconds $[\text{K}^+]_0$, was briefly (1.5 s) raised from 5 mM to 140 mM with a Y-tube application system, shifting temporarily the theoretical value of $E_K$ from -84 mV to 0 mV. Consequently, the K$^+$ current amplitude is optimal during the 1.5 s K$^+$ concentration steps, where $[\text{K}^+]_0 = [\text{K}^+]_e = 140 \text{ mM}$ and the K$^+$ driving force is large, while in between these steps, close to $E_K$, it is small, preventing $[\text{K}^+]_e$ loss during sustained activation of $I_{\text{SK}}$. Also are the voltage-activated K$^+$ currents inactivated at this negative membrane potential, minimising their contribution to $\sigma^2$.

**Figure 7 (p. 151).** Single-channel properties underlying $I_{\text{SK}}$ using current variance analysis. Current mean amplitude ($I$) and variance ($\sigma^2$) were estimated at a membrane potential of -80 mV during brief changes of $[\text{K}^+]_0$ from 5 mM to 140 mM while $[\text{Ca}^{2+}]$, was varied. A, Example of the experimental protocol at low $[\text{Ca}^{2+}]$. Straight lines above the current traces indicate $[\text{K}^+]_0$ values (fat line indicates $[\text{K}^+]_0 = 140 \text{ mM}$, the thin line $[\text{K}^+]_0 = 5 \text{ mM}$). At 140 mM $[\text{K}^+]_0$ an inward (K$^+$) current appears. These traces were filtered with 5 Hz high pass gaussian filter (lower panel) in order to estimate $\sigma^2$. The current amplitude distributions over the indicated window of analysis (grey area) are shown right ($\sigma^2$ value indicated). Smooth lines give fit with a gaussian function. Note the increased $\sigma^2$ at 140 mM $[\text{K}^+]_0$. 
A high-affinity Ca\(^{2+}\)-activated K\(^+\) current in CA1 neurones

**Figure 7 (continued).**

**A**
- [Ca\(^{2+}\)]\(_i\) = 20 nM
- V\(_{\text{hold}}\) = -80 mV
- 5 mM [K\(^+\)]\(_o\)
- 140 mM [K\(^+\)]\(_o\)

- 5 Hz high pass
- \(\sigma^2 = 4 \, \text{pA}^2\)
- \(\sigma^2 = 14 \, \text{pA}^2\)

**B**
- [Ca\(^{2+}\)]\(_i\) (nM)
- [K\(^+\)]\(_o\)
- 0.5 nA
- 0.1 nA

- 0.5 nA
- 0.1 nA

**C**
- \(\sigma^2\) (pA\(^2\))
- \(i = 0.052 \, \text{pA}\)
- N = 43,262

**D**
- \(I\) (nA)
- \([\text{Ca}^{2+}]\) (nM)
- \(p_0\)
- hill = 5.7
- \(K_d = 222 \, \text{nM}\)
- \(P_{\text{max}} = 0.72\)

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**Figure 7 (continued).** **B**, Upper panel: current traces from the same recording at different [Ca\(^{2+}\)]\(_i\) values (right values) in order to activate I\(_{SK}\). Lower panels indicate the traces after the same filtering procedure shown for the data in **A** (symbols as in **A**). Note that \(\sigma^2\) (right values) varies with I (and [Ca\(^{2+}\)]\(_i\)). Note also that during this experiment [Ca\(^{2+}\)] decreased, giving slightly positive current slopes at [Ca\(^{2+}\)] values where I\(_{SK}\) has large Ca\(^{2+}\) sensitivity.

**C**, Plot of I versus \(\sigma^2\) for the complete experiment in **B** (I is mean current over the time window indicated for \(\sigma^2\)). Eq. 5 (smooth curve shows fit) was used to determine single channel current (i) and channels number (N) (values given in the panel). The value for I and \(\sigma^2\) at low [Ca\(^{2+}\)]\(_i\) (respectively 22 pA and 14 pA\(^2\) at 20 nM; determined as in **A** for 140 mM [K\(^+\)]\(_o\)) was subtracted from these data, in order to isolate the Ca\(^{2+}\)-dependent I - \(\sigma^2\) component.

**D**, [Ca\(^{2+}\)]\(_i\) - I relation for the same cell. Fit with eq. 4 (smooth line; g in the formula replaced by I) gives an I\(_{\text{max}}\) estimate of 1.62 nA. The maximum single channel open probability \(p_{\text{max}}\) for this cell is calculated as 0.72 from \(I_{\text{max}}/\{N*i\}\).
Fig. 7A shows an example of current traces for [K\textsuperscript{+}]\textsubscript{o} = 5 mM (top trace) and 140 mM. (2\textsuperscript{nd} trace) at the start of an experiment ([Ca\textsuperscript{2+}] is indicated). The change from 5 to 140 mM reveals an inward K\textsuperscript{+} current and increased current noise. To obtain a value for $\sigma^2$ the traces were filtered with a 5 Hz high-pass gaussian filter (bottom traces) and the current amplitude distribution determined (right panels) over a 1 second time interval, indicated by the grey area in the figure. Distributions were then fitted with a standard gaussian function (smooth lines in right panels) and $s^2$ used as the estimate of $\sigma^2$. As can be seen for the example introduces the K\textsuperscript{+} step an increase in $\sigma^2$ from 4 to 16 pA\textsuperscript{2}. Average values were respectively 2.2 ± 0.7 and 22 ± 4.7 pA\textsuperscript{2} for a [Ca\textsuperscript{2+}], level of 50 ± 11 nM ($n = 7$). The first value represents background $\sigma^2$, which is negligible in comparison to $\sigma^2$ related to the current carried by K\textsuperscript{+}. This also implicates, discussed already above, that at low [Ca\textsuperscript{2+}] a K\textsuperscript{+} current still exists.

Fig. 7B shows that raising [Ca\textsuperscript{2+}], activates $I_{SK}$ (upper panel) and its $\sigma^2$ (lower panels show filtered data as in Fig. 7A) during the shift in $E_K$. The recording is the sequel of the one shown in Fig. 7A. The [Ca\textsuperscript{2+}], and $\sigma^2$ values are indicated for each trace. We isolated $I_{SK}$ amplitude and $\sigma^2$ from the respective values at [Ca\textsuperscript{2+}], resting levels (Fig. 7A) through subtraction. Fig. 7C displays the resulting relationship between $I$ and $\sigma^2$. The data point could be well fitted (smooth line in Fig. 7C) with the parabolic equation:

$$\sigma^2 = i \times I - \frac{i^2}{N} \quad (5)$$

Where $I$ is the current amplitude of $I_{SK}$, $\sigma^2$ the current variance, $i$ the single channel current of the channels underlying $I_{SK}$ and $N$ the number channels.

If the single-channel open probability ($p_0$) becomes small then $i$ can be approximated by $\sigma^2/I$, according to eq. 5. We determined for our recordings the slope $\sigma^2/I$ for [Ca\textsuperscript{2+}], levels between 40 and 180 nM. Within this calcium range current activation is less than 10% of its maximum (calculated from the mean Hill function). We thus arrived at a value for $i$ of 2.3 ± 0.8 pS ($n = 7$; 4 - 13 data points per cell).

We wanted to know whether the K\textsuperscript{+} current at [Ca\textsuperscript{2+}], resting levels (see above) could be attributed to $I_{SK}$ as well. We therefore estimated $i$ at these levels as $[\sigma^2[I_\text{140mM} - \sigma^2[I_\text{5mM}]]/[I_\text{140mM} - I_\text{5mM}]$, where the subscripts refer to the [K\textsuperscript{+}], condition. The mean value for $i$ was 7.1 ± 1.0 pS ($n = 7$; 2-3 current traces per cell) which is significantly higher ($p = 0.001$) than what was found for $i_{SK}$, rejecting this idea. We arrived at the same conclusion for the macromscopic current. From the mean activation properties of $I_{SK}$ one can calculate that 0.02% of this current should be activated at 50 nM, while we find a much higher percentage (1.3%) for the actual available K\textsuperscript{+} current at this [Ca\textsuperscript{2+}], value (calculated as $[[I_\text{140mM} - I_\text{5mM}]/I_{SK_{max}}] \times 100 = 0.48/37\times100$).

For five cells (7-28 data points per cell) we could both adequately fit the $I_{SK}$ - [Ca\textsuperscript{2+}], relationship with the Hill equation (eq. 4, with g replaced by I) and the $I_{SK}$ - $\sigma^2$ relationship with eq. 5, like shown for the example in Figs. 7C/D. The mean value for the $K_d$ and Hill coefficient of $I_{SK}$ for this group of cells were respectively 313 nM and 4.5. From this we determined the single channel open probability at maximal $I_{SK}$ activation ($p_{omax}$) through the division of $I_{max}$ (from eq. 4) by $I$ at $p_0 = 1$, calculated as $i \times N$. The mean value of 0.80 ± 0.1 for $p_{omax}$ implicates optimal use of the available channel open...
probability range for Ca$^{2+}$ modulation.

Combining the experimental mean values for maximal $I_{SK}$ conductance (37 nS), maximal single-channel open probability (0.8) and single-channel conductance (2.3 pS), leads to an estimate of the total number of channels on the CA1 cell soma and proximal dendrites of about 20,000.

$I_{SK}$ in comparison with other potassium currents
To put the $I_{SK}$ activation properties into perspective we determined under specified [Ca$^{2+}]_i$ conditions the current activation properties of two important voltage-activated (but Ca$^{2+}$-independent) K$^+$ currents found in this preparation (Storm, 1990), the fast transient K$^+$ current, or A-current ($I_{KA}$) and the delayed rectifier current ($I_{KD}$). The two currents were isolated on behalf of their different biophysical properties (Storm, 1990) with the voltage protocols shown in Fig. 8A (left panels) for an example. Currents were evoked by 700 ms depolarising steps between -50 and -15 mV from a holding membrane potential ($V_{hold}$) of -60 mV. Then the protocol was repeated for a $V_{hold}$ of -130 mV (150 ms pre-pulse). $I_{KD}$ was estimated from the first protocol as the mean current at the end voltage step (time interval indicated with $O$). Subtraction of this value from the peak amplitude (●) of the second protocol gave the estimate for the peak of $I_{KA}$. We ran the protocols for 8 cells at start of the recordings described above at a mean [Ca$^{2+}]_i$ value of 19 ± 4 nM. The average relationship between conductance (calculated from I with GHK equation for $K^+$) and membrane voltage for $I_{KD}$ and $I_{KA}$ are shown in the right panel of Fig. 8A. The smooth lines in the plot present the fit with the Boltzmann equation we used to describe the relationship. Fits of the individual data gave values for $V_b$, $V_c$ and $g_{max}$ of respectively -32 ± 4 mV, -14 ± 1.6 mV and 18.1 ± 2.7 nS for the peak of $I_{KA}$ and -48 ± 2 mV, -8.3 ± 0.9 mV and 10.7 ± 2.1 nS for $I_{KD}$. Where $V_b$ is the voltage at which the current is half maximal, $V_c$ the current slope at this voltage and $g_{max}$ the maximal conductance. For these same cells we determined $g_{max}$ for $I_{SK}$ (same experimental procedure as described above) from $I_{SK}$ peak current (indicated with ● in Fig. 7A), that is composed of a mixture of $I_{KA}$ and $I_{KD}$, and calculated for these values the mean g-V relationship given in Fig. 8B. For comparison we subtracted from the data points at high [Ca$^{2+}]_i$ the mean difference in value between the two conditions. The smooth line in Fig. 8B gives the Boltzmann fit of the subtracted data. The lines overlap well with the data points measured at low [Ca$^{2+}]_i$. This indicates that $I_{SK}$ and the voltage-dependent currents $I_{KA}$ and $I_{KD}$ superimpose at different calcium levels (see also in the lower panel of Fig. 6A the trace for -50 mV) without affecting $I_{KA}$ and $I_{KD}$ activation properties.

For 5 of these cells we could measure the first voltage protocol with steps from a $V_{hold}$ of -60 mV at low (54 ± 18 nM) and high (351 ± 77 nM) [Ca$^{2+}]_i$. We determined the peak current (indicated with ● in Fig. 7A), that is composed of a mixture of $I_{KA}$ and $I_{KD}$, and calculated for these values the mean g-V relationship given in Fig. 8B. For comparison we subtracted from the data points at high [Ca$^{2+}]_i$ the mean difference in value between the two conditions. The smooth line in Fig. 8B gives the Boltzmann fit of the subtracted data. The lines overlap well with the data points measured at low [Ca$^{2+}]_i$. This indicates that $I_{SK}$ and the voltage-dependent currents $I_{KA}$ and $I_{KD}$ superimpose at different calcium levels (see also in the lower panel of Fig. 6A the trace for -50 mV) without affecting $I_{KA}$ and $I_{KD}$ activation properties.

The next step was to calculate the contribution of the different K$^+$ currents to the total K$^+$ conductance at different [Ca$^{2+}]_i$ values. For this we used the voltage- and/or Ca$^{2+}$-dependent properties of $I_{SK}$, $I_{KA}$ and $I_{KD}$ described in this article and the steady-state voltage- and Ca$^{2+}$-dependent properties of $I_{BK}$ which are described in chapter V for this
Figure 8. Comparison of $I_{SK}$ with voltage-activated potassium currents. A, Characterisation of the voltage-dependent $K^+$ currents $I_{KA}$ and $I_{KD}$ at low $[Ca^{2+}]_i$. Currents were evoked with two voltage protocols (left panels: voltage steps from -60 or -130 mV to values between -60 and +15 mV). Current amplitudes were measured at time points/inter vals (•/○) as indicated in the middle panels for an example. $I_{KA}$ peak amplitude was calculated as (• - ○) and for $I_{KD}$ was taken (○). Right panel: mean conductance for $I_{KA}$ (•) and $I_{KD}$ (○) plotted against membrane potential ($n = 8$; from current amplitude with GHK equation) at low $[Ca^{2+}]_i$ (value indicated). The Boltzmann equation (smooth lines) was used to estimate maximal conductance ($g_{max}$) for $I_{KA}$ and $I_{KD}$. After $I_{KA}$ and $I_{KD}$ were determined, $I_{SK}$ was activated for the same cell. The mean ratio for the $g_{max}$ for $I_{SK}$, $I_{KD}$ and $I_{KA}$ was $1 : 0.44 : 0.83$ ($g_{max}$ for $I_{SK}$ from fit with eq. 4). B, The peak conductance for the voltage protocol in $A$, upper panel (•) is a mixture of $I_{KD}$ and $I_{KA}$. The plot gives the peak conductance for low and high $[Ca^{2+}]_i$ ($n = 5$; part of the data in $A$; $[Ca^{2+}]_i$; as indicated). Subtracting the two curves (subtraction of the mean difference; smooth line gives Boltzmann fit of resulting curve) shows that the shape of the g-V curve is independent from $[Ca^{2+}]_i$. C, Predicted contribution of $g_{KA}$, $g_{KD}$, $g_{SK}$ and $g_{BK}$ as a function of membrane potential at 100 nM, 1 µM and 10 µM $[Ca^{2+}]_i$. Traces are calculated from the experimentally determined parameters (see results. For $I_{BK}$ see chapter V). D, Sum of all four $K^+$ conductances shown in the panels of C ($[Ca^{2+}]_i$ is indicated). 3 nS ‘resting conduct ance’ is added to each trace.
cell preparation. \([\text{Ca}^{2+}]_i\) values were: 100 nM, which is around resting levels; 1 \(\mu\)M with \(I_{SK}\) fully activated but not \(I_{BK}\); 10 \(\mu\)M, which is only observed after long membrane depolarisation (e.g. after tetanic stimulation: Petrozzino et al., 1995). Fig. 8C shows the calculated \(g - V\) relationship for each current for the three conditions. The sum of all four currents for the different \([\text{Ca}^{2+}]_i\) levels shown in Fig. 8D (logarithmic scaling, 3 nS resting conductance added). From these figures it becomes clear that \(I_{SK}\) has its most significant contribution to the total \(K^+\) conductance at the more negative membrane potentials and sub-micromolar \([\text{Ca}^{2+}]_i\) levels.

**Discussion**

The SK-like current (\(I_{SK}\)) is unique in the sense that it is one of the very few intrinsic \(K^+\) channels that shows no voltage dependence. Its analysis could therefore not rely on the usual voltage step protocols but had to be concentrated around its major modulator \(\text{Ca}^{2+}\). We made the current visible *in situ* for the CA1 pyramidal neurone by step changes in the driving force for \(K^+\) under whole-cell voltage-clamp conditions against a slowly varying background of \([\text{Ca}^{2+}]_i\). This allowed to determine the \(\text{Ca}^{2+}\) dependence in detail over the full physiological range of \([\text{Ca}^{2+}]_i\), and also confirmed the lack of voltage-dependent activation and deactivation. Finally, our approach made it possible to relate \(I_{SK}\) amplitude to other important \(K^+\) channels under different \([\text{Ca}^{2+}]_i\) conditions and deduce the functional importance of this current.

*The \(\text{Ca}^{2+}\)-dependent properties of \(I_{SK}\) compare well with those of \(SK\ 1-3\)*

All parameters that describe the SK current were independent of membrane voltage. Its conductance was under our steady-state conditions controlled by bulk \([\text{Ca}^{2+}]_i\), with a steep concentration dependence: The affinity for \(\text{Ca}^{2+}\)-dependent activation (\(K_d\)) was \(300 \pm 27\) nM, with a Hill factor of 4.7 \(\pm\) 0.3. The parameters fall well within the range determined for cloned SK channels (SK1 - SK2 - SK3). Their \(K_d\) is reported in the 300-750 nM range and their Hill coefficient between 4 and 5 (Kohler et al., 1996; Hirschberg et al., 1998; Xia et al., 1998b; Keen et al., 1999).

The \(\text{Ca}^{2+}\) sensitivity of the channel implies that 10% - 90% amplitude modulation of the current lies within a \([\text{Ca}^{2+}]_i\) range of 190 - 500 nM. This range suggests 1) that the contribution of SK to current at resting \([\text{Ca}^{2+}]_i\) (< 100 nM) is relatively small; and 2) one or a few action potentials typically raising \([\text{Ca}^{2+}]_i\) tens of nanomolars (Schiller et al., 1995; Spruston et al., 1995; Helmchen et al., 1999), are sufficient for substantial modulation of the current amplitude. The maximal attainable conductance is large (37 nS), which indicates that the current can considerably contribute to the cell’s firing pattern.

All three subtypes of SK channels are expressed in CA1 pyramidal cells (Stocker and Pedarzani, 2000) and their biophysical properties (\(K_d\), Hill, single-channel conductance) are not different. They match with the values that we recorded and our data gave no indication that more than one component is present.
Current-variance analysis suggested a single channel conductance of $2.3 \pm 0.8 \text{ pS}$ and a maximum open probability ($P_{\text{max}}$) of $0.8 \pm 0.1$. These values comply with numbers reported by others ($P_{\text{max}}$ for SK2 is 0.8 in Hirschberg et al., 1998). As expected for such a small channel we estimated that a total number of about 20,000 channels contribute to the current in a CA1 cell of a size best represented by 9 μF. Similar high numbers have been reported by others (Sah and Isaacson, 1995; Valiante et al., 1997).

It has further been demonstrated (Xia et al., 1998b; Keen et al., 1999) that co-assembly with the EF-hand $\text{Ca}^{2+}$-binding protein calmodulin lends $\text{Ca}^{2+}$ sensitivity to SK1-3. The calcium sensor of the SK channel is calmodulin. We therefore expect that calmodulin is interacting with the SK channels under whole-cell conditions, conform the constitutive binding of calmodulin for SK channel (Xia et al., 1998b).

Our methods exclude determination of the response of the current to fast changes in $[\text{Ca}^{2+}]_i$; according to the literature SK channels in CA1 open rapidly ($\tau$ Between 4 and 10 ms) and close much slower ($\tau$ Between 20 and 60 ms) upon a step to saturating $[\text{Ca}^{2+}]_i$ (Hirschberg et al., 1998; Xia et al., 1998b) in agreement with the relative high affinity that we report.

**$I_{SK}$ and afterhyperpolarisations**

SK channels underlie the intrinsic afterhyperpolarisation (AHP) that directly follows an action potential (AP) and is involved in defining the firing pattern. In CA1 pyramidal cells we distinguish the fast AHP that is carried by voltage-dependent BK channels and a slow AHP (sAHP), which lasts several hundred milliseconds. An intermediate component (mAHP) with a duration lying in between is also described and is sensitive to the bee venom apamin (Stocker et al., 1999). In terms of current amplitude, this mAHP is the largest SK current fraction (Sah and Clements, 1999; Stocker et al., 1999) and it influences the early AP firing frequency but not so much the late phase (Stocker et al., 1999). Activation of the channels underlying the apamin-insensitive slow AHP (sAHP) is less steep. As a result the peak amplitude of the sAHP increases almost linearly with the first seven AP's in a spike train and is responsible for late spike frequency adaptation (Madison and Nicoll, 1984; Storm, 1989; Stocker et al., 1999). A relation between AHP type and molecular constitution is emerging. The SK2 and SK3 channels are linked to the mAHP while the SK1 is linked to the sAHP (Sah and Clements, 1999; Stocker et al., 1999).

In addition to differences in biophysical properties of the underlying channels also the localisation of the channels over the cell surface can contribute to how channel activity shows up in membrane voltage changes in the AHP. The channels underlying the sAHP are predominantly localised in the proximal dendrites (Bekkers, 2000). The location of the mAHP is not known yet.

A comparison of our SK current with current amplitudes observed in voltage-clamp recordings in intact neurones in the slice suggests that we retained the majority of the current. Using voltage-operated calcium channels (VOCC's) to generate $\text{Ca}^{2+}$ influx and activation of the AHP, Stocker found that of the 174 pA AHP peak amplitude, 120 pA were attributed to the mAHP and 54 pA to the sAHP (Stocker et al., 1999) ($V_{\text{hold}}$ at -50 mV; $[\text{K}^+]_e:[\text{K}^+]_i$ is 2.5:145 mM). Similar values (183 ; 128 resp. 55 pA) were reported by
A high-affinity Ca\textsuperscript{2+}-activated K\textsuperscript{+} current in CA1 neurones

Sah and Clements who created [Ca\textsuperscript{2+}]; steps using flash photolysis (Sah and Clements, 1999). For our dissociated cells we estimate a maximal amplitude for \(I_{SK}\) of \(\sim 300\ pA\) (\(V_{\text{hold}}\) at -50 mV; \([K^+]_o;[K^+]_i\) is 2.5:140; using a conductance of 37 nS in the GHK current equation). This is larger than the sum of the sAHP and mAHP.

The maximum open probability that we report for \(I_{SK}\) (\(p_{\text{omax}} = 0.8\)) is higher than the ones reported during the sAHP (\(p_0 = 0.4\) in: Sah, 1995. See also: Valiante, 1997). This could be related to the fact that \(I_{SK}\) activation through VOCC's, as is the case for the sAHP, is less effective or is performed under different modulatory conditions (Knopfel et al., 1990). Our low intracellular ATP condition is likely maximising the open probability of the SK channels (Pedrazani et al., 1998). If we take into account the lower open probability for the sAHP we can still attribute at least 200 pA of \(I_{SK}\) to the mAHP. This indicates that it represents a considerable part of the SK current in the soma and the proximal dendrites which are retained in the dissociated cells.

The major modulator of the SK channels is [Ca\textsuperscript{2+}], and it determines the time course of the AHP. At saturating concentrations (> 600 nM) \(I_{SK}\) is maximal and will not reflect changes in [Ca\textsuperscript{2+}], but for lower values the steep [Ca\textsuperscript{2+}] dependence will result in a decay of \(I_{SK}\) that is 2-2.5 times faster than the [Ca\textsuperscript{2+}] relaxation. [Ca\textsuperscript{2+}] decay in the proximal dendrites is much faster than in the soma (280-615 ms compared to 1.5 seconds in: Sah and Clements, 1999) (see also Regehr and Tank, 1992; Schiller et al., 1995). In order to obtain relaxation times reported for the mAHP (~100 ms) it is almost inevitable that the channels are located in the (proximal) dendrites. Our experiments have well controlled uniform [Ca\textsuperscript{2+}], and they never revealed a fast-inactivating SK current.

**\(I_{SK}\) in relation to VOCC’s**

The close relation of Ca\textsuperscript{2+} influx and SK activation opens the possibility for almost direct (local) coupling. In fact experiments show that there exist L-type calcium channels, whose opening is directly associated with the activation of a single SK channel (Marrion and Tavalin, 1998). However, the addition of a slow Ca\textsuperscript{2+} buffer EGTA easily disrupts the coupling between calcium-channel activation and the \(I_{AHP}\) (Zhang et al., 1995; Spigelman et al., 1996). These experiments might indicate that the mean distance over which the interaction occurs is larger than the single-channel experiments suggest. This is in strong contrast with observations on interference with BK channels, also associated with HVA current, where only a fast chelator like BAPTA can disrupt the coupling (Velumian and Carlen, 1999).

The SK single-channel measurements are inevitably biased in favour of co-localised calcium channels but do not exclude the existence of a large additional pool. In fact, the channel numbers strongly suggest this. Density estimates of calcium channels indicate that there are about 5000 HVA calcium channels on the CA1 soma (Kay and Wong, 1987) of which two third is L-type (Fisher et al., 1990; Magee and Johnston, 1995). If 50% of the L-type channels is functionally coupled to a SK channel (Marrion and Tavalin, 1998) they will activate at most 1700 SK channels. Comparing this number to the estimate of the total available SK channels (20,000 channels) this strongly indicates that there is also a population of channels that is only loosely coupled to calcium channels.
These considerations suggest two pools of SK channels based on their location, one that is closely associated with the L-type channel and can respond fast, be it in an all or none fashion due to the narrow and steep $[Ca^{2+}]_i$ sensitivity range. The other pool has to follow bulk $[Ca^{2+}]_i$; for those channels the observed sensitivity range guarantees that they can operate in the physiological relevant range in a graded way. Depending on the details of $[Ca^{2+}]_i$ clearance we might speculate that the combination of both pools results in a graded AHP, with a start transient originating from the coupled channels.

$\textit{ISK and the resting membrane potential}$

In CA1 neurones at resting membrane potential (RMP) $[Ca^{2+}]_i$ lies around 50-70 nM (Regehr and Tank, 1992; Wadman and Connor, 1992). A small, sustained $K^+$ current has been observed under these conditions (Selyanko and Sim, 1998). Our data show a similar persistent current in the very low calcium range (20-80 nM), not related to the SK channels. For concentrations of 180 nM and higher the SK current is larger than this residual current but at resting $[Ca^{2+}]_i$, it does not contribute significantly. Similar observation were reported for cultured hippocampal neurones in which SK channel activation had a threshold of 175 nM $[Ca^{2+}]_i$ (Selyanko et al., 1998) and for the cloned SK channels, where at 100 nM $[Ca^{2+}]_i$, is very little activation is seen.

The complication of these relations is that the crucial factor is $[Ca^{2+}]_i$, which is at least correlated with membrane voltage, due to an inward leak of $Ca^{2+}$ through sustained activated calcium channels (Magee et al., 1996). As soon as $[Ca^{2+}]_i$ is above threshold the SK channels will constitute a sustained hyperpolarising force that functions as a feedback in a sense that hyperpolarisation will partially shut the calcium channels and reduce the inward leak. The gain of this system is determined by the voltage sensitivity of the $Ca^{2+}$ influx and by the conversion of $Ca^{2+}$ influx to SK activation, which has an integrating property. It will stop contributing as soon as $[Ca^{2+}]_i$ is below ~180 nM and can not set resting membrane potential. Therefore we prefer to state that the SK channels can strongly assist in repolarising the membrane without a direct contribution to the set point of resting membrane potential.

The reasoning given above holds for the pool of SK channels that senses bulk $[Ca^{2+}]_i$. For the other pool that is closely linked to L-type calcium channels, the same feedback mechanism holds, but determined by local $[Ca^{2+}]_i$, levels which are likely higher than bulk $[Ca^{2+}]_i$ and controlled by L-type channel open probability (Gil et al., 2000). This pool therefore might become activated even if bulk $[Ca^{2+}]_i$ has reached resting levels and may even have some voltage-dependent gain related to L-type calcium channel activation.

$\textit{ISK is an important contributor to the total K}^+\text{ conductance}$

The membrane potential is determined by the relative permeability of all contributing ion currents. Potassium currents are the most important ones and present in a large variety (Brown et al., 1990; Storm, 1990). We compared the SK conductance in the CA1 neurones with three other prominent $K^+$ conductances for the relevant voltage and $[Ca^{2+}]_i$ range: the delayed rectifier ($I_{KD}$), the transient A current ($I_{KA}$) and the voltage- and $Ca^{2+}$- dependent BK current ($I_{BK}$). The properties of $I_{KD}$ and $I_{KA}$ we report here are in good agreement with absolute numbers found earlier (Numann et al., 1987; Spigelman et al.,
1992; Vreugdenhil and Wadman, 1995). Our data also show that $I_{KD}$ and $I_{KA}$ amplitude are not prominently dependent on $[Ca^{2+}]_i$. Comparing the contribution of these four currents relies on the specific $[Ca^{2+}]_i$ and voltage condition. Furthermore there is also a dynamic component due to the time course of activation and inactivation. At 100 nM $[Ca^{2+}]_i$ or below there is hardly any contribution of $I_{SK}$ and $I_{BK}$ so that at this concentration the voltage-dependent currents determine the $K^+$ conductance. At the low voltage range the muscarinic $I_M$ and the inward rectifier $I_Q$ could also contribute (Storm, 1990). Above 600 nM $[Ca^{2+}]_i$, $I_{SK}$ has become the dominant current, about twice as large as the voltage-dependent ones, but $I_{BK}$ is not yet available: the two parameters voltage and $[Ca^{2+}]_i$ can recruit the same amount of $K^+$ conductance in an additive way.

Once $[Ca^{2+}]_i$ reaches a level high enough to fully activate $I_{BK}$ (10 μM) this current will provide the dominant $K^+$ conductance, again voltage and $[Ca^{2+}]_i$ dependent. It is however unlikely that such levels of $[Ca^{2+}]_i$ can be maintained for a long time in a large part of the neurone. In vivo the relative importance of the different $K^+$ conductances is subject to the modulation by several other cellular modulators (Storm, 1990).

Functionally, $Ca^{2+}$-dependent currents are involved in the control of action potential firing. The properties of $I_{BK}$ allow it to contribute in the shape of the action potential. In contrast, the $I_{SK}$ has no influence on the wave shape but it can strongly affect spike threshold and so determine firing frequency and adaptation. The feedback mechanism (described above) that involves accumulation of intracellular calcium provides control over $K^+$ conductance with an almost voltage-independent gain, unlike the other $K^+$ conductances, but ideally suited for this task.