Distribution of Slow AHP Channels on Hippocampal CA1 Pyramidal Neurons

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INTRODUCTION

Action potentials in neurons are often followed by a hyperpolarization that may exhibit several distinct phases. Three of these phases are thought to be mediated by three different kinds of Ca\(^{2+}\)-activated K\(^+\) channels: 1) high conductance (BK) channels that contribute to the rapid phase of action potential repolarization; 2) small conductance (SK) channels that mediate a faster phase of the afterhyperpolarization (AHP), which lasts up to several hundred milliseconds; and 3) channels that underlie a very slow phase of the AHP (sAHP), which may last for several seconds (Sah 1996).

Both BK and SK channels have been studied directly using single-channel recording techniques (Blatz and Magleby 1987; Marrion and Tavalin 1998; Park 1994). In contrast, single sAHP channels have remained elusive. Estimates of their unitary properties have been provided by noise analysis (Sah and Isaacson 1995; Valiante et al. 1997); in CA1 pyramidal cells these channels have a single-channel conductance of \(\approx 5\) pS. Thus single sAHP channels should be resolvable in patch-clamp recordings, provided a region of membrane is found in which the channels are present at sufficiently high density. Such a region has been suggested by experiments that used a voltage clamp “switchoff” technique to study the location of sAHP currents in CA1 pyramidal cells (Sah and Bekkers 1996). This work concluded that sAHP channels are concentrated in the proximal apical dendrites (< approximately 100 \(\mu m\) from the soma) at a density of roughly one channel per \(\mu m^2\) of membrane, assuming a uniform distribution.

The present experiments were designed to resolve individual sAHP currents in the proximal apical dendrites of CA1 pyramidal cells. Several approaches were taken in an effort to map the distribution of these channels. It is concluded that many sAHP channels may be found in the basal dendrites of these neurons.

METHODS

Transverse hippocampal slices (250 \(\mu m\) thick) were prepared from the brains of 2–3 wk old Wistar rats using standard techniques (e.g., Sah and Bekkers 1996). CA1 pyramidal neurons were identified and patch electrodes were positioned using infrared videomicroscopy. Recordings were made with two EPC-7 patch-clamp amplifiers. Patch electrodes had resistances of 3–6 M\(\Omega\) (whole cell) or 15–20 M\(\Omega\) (cell-attached). sAHP currents were evoked by a 200-ms voltage step to \(-10\) or \(0\) mV from a holding potential of \(-50\) or \(-60\) mV. The bath solution was composed of (in mM) 125 NaCl, 25 NaHCO\(_3\), 2.5 KCl, 1.25 Na\(_2\)HPO\(_4\), 1 MgCl\(_2\), 2 CaCl\(_2\), and 25 glucose (pH 7.4 when saturated with 95% O\(_2\)-5% CO\(_2\)). A HEPES-buffered version of this solution, used in cell-attached patch electrodes, substituted 25 mM HEPES for the NaHCO\(_3\) and NaH\(_2\)PO\(_4\). Internal solution was composed of (in mM) 115 potassium methylsulfate, 20 KCl, 10 phosphocreatine, 4 MgATP, 0.3 GTP, and 10 HEPES (pH 7.2 adjusted with KOH). All experiments were done at room temperature (22–25°C).

RESULTS

Dendritic cell-attached patch experiments

The first series of experiments sought to resolve single sAHP channels in the proximal 100 \(\mu m\) of the apical dendrites of CA1 pyramidal neurons (Fig. 1). A patch electrode in whole-cell mode at the soma was used to elicit a sAHP current (Fig. 1A, left). A second patch electrode in cell-attached mode was placed on the apical dendrite of the same cell 40–100 \(\mu m\) from the soma (70 \(\mu m\) in Fig. 1A) to look for single sAHP channels. The dendritic electrode contained HEPES-buffered external solution and its interior was voltage clamped at \(-80\) mV. Hence, assuming \(E_K \approx -90\) mV and a single-channel conductance for sAHP channels of 5 pS (obtained from noise analysis; Sah and Isaacson 1995), the expected single-channel current was about 0.6 pA (dashed horizontal lines in Fig. 1A, right), which is likely to be a lower limit (Valiante et al. 1997). No channel activity is apparent in the patch (Fig. 1A, right). Averages of 10 episodes did not reveal any mean current in the patch with the timecourse of the sAHP (Fig. 1A, bottom traces; \(n = 6\) cells).

In a slightly different version of this experiment, the somatic electrode was omitted and an extracellular stimulating elec-
trode was used to apply a tetanus to the alveus. The antidromic action potentials generated a sAHP in the cell, which was confirmed by a whole-cell recording from the dendritic electrode at the end of the experiment. In these experiments the dendritic electrode contained high-potassium internal solution and its interior was voltage clamped at $0 \text{ mV}$ in cell-attached mode. Again, no suggestion of sAHP channels was found ($n = 9$ cells; not illustrated).

A total of 15 experiments revealed no sign of single sAHP channels in the proximal apical dendrites of CA1 pyramidal cells. This was not a result of an inability to resolve channels of any sort; outward channels (presumably K$^+$ channels) were often seen after the stimulus with a brief latency (Fig. 1B). These did not have the slow kinetics expected of sAHP channels and were not blocked by bath application of 4 mM isoprenaline (not illustrated), which is known to inhibit sAHP channels (Sah 1996).

**Dendrotozy experiments**

It is possible that sAHP channels are damaged in some way by a patch electrode or are present at too low a density to be found in at least one of only 15 patches. To circumvent these problems, a different approach was taken (Fig. 2). While recording in whole-cell mode from the soma of a CA1 pyramidal cell, two additional patch pipettes were positioned $\sim 30 \mu\text{m}$ from the soma, the tip of one pipette just above and the other just below the primary apical dendrite of the same cell (Fig. 2, top). At this point little branching of the dendrite had occurred. By slowly lifting the lower of the two pipettes (over 10 min) it was possible to stretch and finally to break the apical dendrite at the location of these pincer pipettes. This could be done without dislodging the somatic electrode.

This maneuver reduced the amplitude of sAHP current by $44 \pm 7\%$ (mean $\pm$ SE, $n = 6$ cells, averaging over the final 10 min; Fig. 2A, filled symbols). Control measurements in which the dendrite was not cut gave a mean reduction of $13 \pm 10\%$ ($n = 3$ cells; Fig. 2A, open symbols; mean amplitude significantly reduced, $P < 0.02$, unpaired t-test). These results suggest that dendrotozy reduced the sAHP by $\sim 30\%$ compared with control. Illustrative recordings from one cell before and after dendrotozy are shown in Fig. 2B. The input resistance of the cell was increased by a factor $1.6 \pm 0.1$ ($n = 6$; Fig. 2C), as expected if a large part of the dendritic tree has been removed (cf. $1.0 \pm 0.03$, $n = 3$, for control experiments). The cut was also confirmed for each cell by re-patching with Lucifer yellow that was included in the internal solution; the cell was brightly fluorescent only to the end of the apical stump (not illustrated).

**FIG. 1.** Single slow afterhyperpolarization (sAHP) channels are not detected in cell-attached patches on the proximal apical dendrites of CA1 pyramidal cells, but voltage-gated K$^+$ channels are observed. A: single episodes (top) and averages of 10 episodes (bottom) from a whole cell (left) and cell-attached (right) recording from the same cell. Horizontal dashed lines (top right) indicate the minimum expected sAHP single-channel current. B: putative voltage-gated K$^+$ channels observed in a cell-attached dendritic patch from another CA1 pyramid. In both A and B the dendritic patch was 70 $\mu\text{m}$ from the soma.

**FIG. 2.** Cutting off the apical dendrite $\sim 30 \mu\text{m}$ from the soma only partially reduces the somatic sAHP current. A: normalized sAHP current amplitude versus time, recorded while slowly raising the lower pincer pipette to cut the dendrite (filled symbols; mean $\pm$ SE, $n = 6$ cells) or raising the upper pincer to leave the dendrite uncut (open symbols; $n = 3$ cells). B: example sAHP currents recorded in the same cell before and 24 min after cutting the apical dendrite. C: input resistance ($R_{in}$) of the same cell measured using a 5-mV hyperpolarizing step from $-70 \text{ mV}$ holding potential. In this case $R_{in}$ increased from 173 $\text{M}\Omega$ before to 281 $\text{M}\Omega$ after dendrotozy.
Somatic experiments

The dendrotomy experiments suggest that the majority (~70%) of sAHP channels may be located on the proximal 30 μm of apical dendrite, on the axon or basal dendrites, or on the soma. The next experiments focused on the soma. Dual whole-cell/cell-attached patch recordings were made from the same soma, the protocol otherwise being the same as that for the dendritic experiments described earlier. As in the case of those experiments, no single sAHP channels were seen under the tip of the cell-attached somatic electrode while a sAHP was being evoked via the whole-cell electrode (n = 10 cells; not illustrated).

Again, this negative result may follow from the low density of sAHP channels. Thus an attempt was made to assay a larger area of the somatic membrane for sAHP channels while excluding contributions from other parts of the cell. This was done by pulling a nucleated outside-out patch while continuously recording the sAHP current (Fig. 3). The sAHP steadily decreased in size as the patch was pulled and was absent from fully isolated nucleated patches (Fig. 3, trace 4; n = 5 cells). These patches had resting potentials similar to those of the intact cell (~60 mV) and fired action potentials.

Axotomy experiments

An attempt was made to patch clamp or cut off the axon or basal dendrites to determine whether the sAHP channels are concentrated there, but this was not possible in CA1 pyramids because of the small size and extensive branching of these processes. However, the axon was able to be cut in Layer V cortical pyramidal neurons where the axon is prominent (Stuart et al. 1997). Cutting the axon ~20 μm from the soma had no effect on the sAHP measured at the soma (not illustrated; n = 3 cortical cells). Whereas this result is only suggestive, it does raise the possibility that sAHP channels are not found in the basal dendrites of CA1 pyramidal cells.

DISCUSSION

Prompted by earlier work suggesting that sAHP channels are concentrated in the proximal apical dendrites of CA1 pyramidal cells (Sah and Bekkers 1996), this study sought unsuccessfully to resolve single sAHP channels in this region (Fig. 1). Amputation of the primary apical dendrite ~30 μm from the soma reduced by only about 30% the sAHP recorded at the soma, compared with control (Fig. 2), suggesting that many sAHP channels must be located in the soma or very proximal apical dendrite, the axon, or the basal dendrites.

The soma might be excluded because of the absence of sAHP channels in cell-attached somatic patches and the finding that a sAHP was not present in nucleated patches (Fig. 3). However, these experiments were not conclusive. The channels might have been missed in the cell-attached patches and, although the nucleated patches contained calcium currents (unpublished observations), these may not have been large enough to admit sufficient calcium to activate sAHP channels, even if they were present. It is also possible that the act of forming a nucleated patch somehow interfered with the activation of sAHP channels. Despite these uncertainties, it is likely from earlier work (Sah and Bekkers 1996) that few sAHP channels are present on or close to the soma. In those experiments the switchoff current after a somatic voltage clamp step was very fast for IPSCs known to have a somatic localization, but was relatively slow for the sAHP current. This result, which was model-independent and highly reproducible, is difficult to reconcile with a significant presence of sAHP channels on the soma or on very proximal dendrites.

An axonal localization of these channels remains possible. The axotomy experiments with Layer V cortical pyramidal cells are only suggestive, but indicate that, if CA1 and cortical pyramids are similar in this regard, the sAHP channels would have to be concentrated in the proximal axon less than ~20 μm from the soma.

Finally, the basal dendrites might harbor the majority of sAHP channels. This conclusion is compatible with all of the results presented here, as well as those of the switchoff experiments, which could only establish that the channels were remote from the soma and provided no information about their apical or basal localization (Sah and Bekkers 1996). However, this conclusion does appear to conflict with the results of an experiment that used the shunting of EPSPs by the sAHP to conclude that these channels were not present in the basal dendrites (Sah and Bekkers 1996). Recent findings on the boosting of EPSPs by the subthreshold activation of a persistent sodium current (Andreasen and Lambert 1999) suggest however that the shunting experiment of Sah and Bekkers (1996) may need to be reinterpreted.

In conclusion, a number of lines of evidence suggest that the majority of sAHP channels are not located in the distal apical dendrites, in the axon, or on the soma of CA1 pyramidal cells. By this process of elimination, it is suggested that the basal dendrites may be enriched in sAHP channels. Future work would need to confirm this suggestion directly.

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