The role of BK-type Ca^{2+}-dependent K^+ channels in spike broadening during repetitive firing in rat hippocampal pyramidal cells

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1. The role of large-conductance Ca^{2+}-dependent K^+ channels (BK-channels; also known as maxi-K- or *slo*-channels) in spike broadening during repetitive firing was studied in CA1 pyramidal cells, using sharp electrode intracellular recordings in rat hippocampal slices, and computer modelling.

2. Trains of action potentials elicited by depolarizing current pulses showed a progressive, frequency-dependent spike broadening, reflecting a reduced rate of repolarization. During a 50 ms long 5 spike train, the spike duration increased by 63·6 ± 3·4 % from the 1st to the 3rd spike. The amplitude of the fast after-hyperpolarization (fAHP) also rapidly declined during each train.

3. Suppression of BK-channel activity with (a) the selective BK-channel blocker iberiotoxin (IbTX, 60 nM), (b) the non-peptidergic BK-channel blocker paxilline (2–10 μM), or (c) calcium-free medium, broadened the 1st spike to a similar degree (~60 %). BK-channel suppression also caused a similar change in spike waveform as observed during repetitive firing, and eliminated (occluded) most of the spike broadening during repetitive firing.

4. Computer simulations using a reduced compartmental model with transient BK-channel current and 10 other active ionic currents, produced an activity-dependent spike broadening that was strongly reduced when the BK-channel inactivation mechanism was removed.

5. These results, which are supported by recent voltage-clamp data, strongly suggest that in CA1 pyramidal cells, fast inactivation of a transient BK-channel current (I_{CT}), substantially contributes to frequency-dependent spike broadening during repetitive firing.

Broadening of neuronal action potentials during repetitive firing is a widespread phenomenon in somata, dendrites and nerve terminals (e.g. Aldrich *et al.* 1979; Gainer *et al.* 1986; Jackson *et al.* 1991; Andreasen & Lambert, 1995; Ma & Koester, 1996). This phenomenon has also been recorded in the mammalian brain *in vivo*. Hippocampal pyramidal cells in behaving rats fire brief bursts of 2–10 action potentials at frequencies of 100–300 Hz, so-called 'complex spikes', during which the spikes typically become broader towards the end of the burst (Fox & Ranck, 1981). Intracellular recordings from hippocampal pyramidal cells in anaesthetized cats also showed spontaneous and evoked spike bursts with prominent spike broadening (Kandel & Spencer, 1961).

Since most of the influx of Ca^{2+} typically occurs during the late part of each action potential (Llinas *et al.* 1982), spike broadening is an efficient way of increasing Ca^{2+} influx, thus modulating intracellular Ca^{2+} signals and Ca^{2+}-dependent ion channels, enzymes, second messenger cascades, gene transcription, and release of transmitters or hormones (Jackson *et al.* 1991; Byrne & Kandel, 1996; Sabatini & Regehr, 1997).

Previous studies have indicated that spike broadening during repetitive firing in neurones is often due to cumulative inactivation of voltage-gated K^+ channels, including slowly inactivating 'delayed rectifier' K^+ channels (Aldrich *et al.* 1979) and fast-inactivating K^+ channels (A-channels) (Jackson *et al.* 1991; Ma & Koester, 1996). Cumulative inactivation during a spike train can cause a progressive decline in the K^+ current that is available for spike repolarization, thus causing spike broadening. This mechanism has been described in a variety of neurones, including molluscan somata (Aldrich *et al.* 1979; Ma & Koester, 1996), magnocellular hypothalamic neurones (Bourque & Renaud, 1985) and pituitary neurosecretory terminals (Jackson *et al.* 1991). An enhanced activation of voltage-gated Ca^{2+} channels late in the train, can also contribute to the spike broadening during repetitive firing in some cases (Aldrich *et al.* 1979).

Recently, evidence for an A-channel-dependent spike-broadening mechanism was found in mouse hippocampal CA1 pyramidal neurones. In these cells genetic deletion of the K^+ channel subunit Kvβ1.1, which mediates fast N-type inactivation, was accompanied by a reduction in the spike broadening during repetitive firing (Giese *et al.* 1998). The mutant mice also showed a reduction of the Ca^{2+}-dependent slow after-hyperpolarization (sAHP) following a spike train, suggesting that the intracellular Ca^{2+} influx was reduced, presumably due to the reduced spike broadening.

Since Ca^{2+}-activated K^+ channels of the BK type appear to play a major role in spike repolarization in hippocampal pyramidal cells (Storm, 1987*a*,*b*, 1990; Lancaster & Nicoll, 1987), it seems likely that these channels will modulate the spike broadening during high-frequency repetitive firing. If the BK-like channels show little or no inactivation, as reported in most studies of BK-channels (Blatz & Magleby, 1987), they would be expected to counteract the spike broadening through negative feedback: the broader the spike, the larger the Ca^{2+} influx and BK-channel activation, which in turn would limit spike broadening. In contrast, if the repolarizing BK-like channels inactivate rapidly, they could contribute to spike broadening during repetitive firing, in a manner similar to A-channels. Several lines of evidence support the latter idea. (1) The fast after-hyperpolarization (fAHP), which represents a continuation of the BK-dependent spike repolarization, is largest after the first spike and rapidly declines in amplitude during high-frequency firing (Lancaster & Nicoll, 1987; Borg-Graham, 1987; Storm, 1990; Borg-Graham, 1998). (2) Although the BK-like Ca^{2+}-dependent K^+ current in hippocampal pyramidal cells was initially described as a sustained current, I_C (Lancaster & Adams, 1986), some voltage-clamp and single-channel studies also reported evidence for a fast transient, tetraethylammonium-sensitive Ca^{2+}-dependent K^+ current in CA3 (Zbicz & Weight, 1985) and CA1 pyramidal cells (Storm, 1987*c*, 1990; McLarnon, 1995; Hicks & Marrion, 1998). This transient calcium-dependent potassium current, which was dubbed 'I_{CT}'; (Storm, 1987*c*, 1990), is reminiscent of the fast-inactivating BK-channel current found in adrenal chromaffin cells (Solaro & Lingle, 1992; Lingle *et al.* 1996). (3) Modelling studies suggest that a rapidly inactivating BK-current is required to account for the frequency-dependent decline of the fAHP (Borg-Graham, 1987, 1998; Warman *et al.* 1994). (4) Recently an accessory subunit (β2) conferring inactivation of BK-channels was discovered (Wallner *et al.* 1999). However, direct tests of the hypothesis that BK-like channels contribute to frequency-dependent spike broadening have so far been lacking.

In the present study we tested the hypothesis that BK-type K^+ channels, which are involved in spike repolarization, also contribute to the spike broadening during repetitive firing. The converging results from current-clamp experiments and computer modelling suggest that a fast transient BK-current underlies spike broadening in rat CA1 pyramidal cells. Three preliminary reports have been presented (Ramakers *et al.* 1998; Halvorsrud *et al.* 1999; Shao *et al.* 1999).

METHODS

Current-clamp experiments with sharp microelectrodes

Transverse hippocampal slices (400 μm thick) from adult male Wistar rats (150–300 g) were prepared after decapitation under deep halothane anaesthesia. During recording, at 29–31 °C, the slices were superfused with medium containing (mM): 125 NaCl, 25 $NaHCO_3$, 1·25 KCl, 1·25 KH_2PO_4, 2·0 $CaCl_2$, 1·5 $MgCl_2$, 16 glucose, and saturated with 95% O_2–5% CO_2. Bicuculline-free base (10 μM) was added to block inhibitory synaptic transmission.

Intracellular somatic recordings from CA1 pyramidal cells were obtained with sharp microelectrodes filled with 2 M potassium acetate (resistance, 60–100 MΩ), coupled to an Axoclamp-2A amplifier (Axon Instruments) in bridge current-clamp mode (3 kHz low-pass filter). The membrane potential was manually clamped at −60 mV. Action potentials were elicited by 50 ms depolarizing current pulses, once every 30 s. The current intensity was normally adjusted to trigger 5 spikes per pulse (Fig. 1*A*). Spike trains were elicited by a single long pulse, rather than by a train of brief pulses, to allow a more natural burst with spike frequency adaptation, resembling the bursts recorded from hippocampal pyramidal cells *in vivo* (Fox & Ranck, 1981), and to avoid contamination from capacitative transients at the beginning and end of each brief pulse. The action potential duration was measured at 1/3 of the total spike amplitude (measured from the threshold level) (Fig. 1*A* and *B*, dashed line). Since the 5th spike sometimes overlapped with the current turn-off transient, measurements of this spike was not included in the analysis.

Data acquisition, storage and analysis

The sharp electrode current-clamp data (Figs 1–5) were filtered at 3 kHz, digitized at 10 kHz and stored on video tape (Instrutec VR-10), and analysed and plotted using pCLAMP program version 7.0 (Axon Instruments) and Origin 5.0 (Microcal). Student's paired *t* test was used for statistical analysis. Numerical values are expressed as means ± S.E.M.

Drug application

Substances were applied extracellularly by adding them to the superfusing medium. Most of the iberiotoxin (IbTX) was made and generously provided by Dr Hans-Guenther Knaus, University of Innsbruck. Some IbTX was purchased from Peptide Institute (Tokyo, Japan) and Alomone Laboratories (Jerusalem, Israel). The remaining drugs were obtained from Sigma.

Computational methods

Computer simulations were performed using the simulator NEURON version 4.1 (Hines & Carnevale, 1997). Simulation results were cross-verified using the Surf-Hippo simulator, version 2.8 (Borg-Graham, 1998). Except for the kinetics of the CT current (see below), all the electrophysiological and morphological properties of the model cell were adapted from the 'Working model' of Borg-Graham (1998).

The cell was represented by six compartments consisting of an isopotential soma (surface area 3848 μm^2) and a passive dendrite (length 1200 μm and diameter 12 μm). The specific membrane resistances differed between the soma and the dendrite (2·5 and

40 kΩ cm^{-2}, respectively) to account for the somatic shunt introduced by sharp electrode recordings. A uniform intracellular resistivity of 200 Ω cm and a specific membrane capacitance of 0·7 μF cm^{-2} was assumed. The active conductances in the soma included a single Na^+ current (I_{Na}), three voltage-gated Ca^{2+} currents I_T, I_N, I_L, four voltage-gated K^+ currents I_A, I_{DR}, I_M, I_D, one Ca^{2+}-dependent K^+ current I_{AHP}, a K^+ current depending on both voltage and Ca^{2+}, I_{CT}, and a hyperpolarization-activated non-specific cation current I_H (Storm, 1990; Borg-Graham, 1991). Calculation of all currents was based on the Hodgkin-Huxley formalism with rate constants derived from the Boltzmann equation (Borg-Graham, 1991). Exceptions were I_{Na} and I_{CT}, which were calculated from a Markov model with four and three states, respectively. The rate constants of the original I_{CT} in the 'Working model' were modified (see Appendix) to obtain a fast transient I_{CT} similar to the one recorded in voltage-clamp experiments (Shao *et al.* 1999; Halvorsrud *et al.* 1999). This modification was needed because I_{CT} in the original 'Working model' (Borg-Graham, 1998) does not show inactivation during a sustained depolarizing step, although it inactivates during the intervals in a train of brief depolarizations.

The intracellular Ca^{2+} dynamics, which were confined to the soma, included Ca^{2+} influx, diffusion, instantaneous buffering, and an extrusion mechanism (Borg-Graham, 1987). A juxtamembrane shell (1 μm thick) was divided into two distinct diffusional compartments: one very small portion of the shell incorporating co-localized Ca^{2+} channels and I_{CT}, and the remaining shell volume whose [Ca^{2+}] activated I_{AHP}. A third diffusional core compartment consisted of the remainder of the cell volume, and communicated with the larger shell compartment. Co-localization of I_{CT} and Ca^{2+} channels (Borg-Graham, 1987; Gola & Crest, 1993; Marrion & Tavalin, 1998) could account in part for the rapid and transient activation of I_{CT} during the spike. This co-localization allows the kinetics of I_{AHP} which are more integrative, by assuming that I_{AHP} are not co-localized with Ca^{2+} channels. All Ca^{2+} compartments were initialized to 50 nM. The model cell was injected with 0·2 nA depolarizing current for 10 s, thus achieving a stable holding potential of −60 mV. The variable time-step methods in NEURON and Surf-Hippo were used to speed up the simulation during the long settling time.

The experimental procedures were approved by the responsible veterinarian of the Institute, in accordance with the Statute regulating animal experimentation, given by the Norwegian Ministry of Agriculture, 1996.

RESULTS

Spike broadening during repetitive firing under normal conditions

Stable intracellular recordings were obtained from 33 CA1 pyramidal cells. The cells were manually clamped at −60 mV by steady current injection, and spike trains were elicited by injecting 50 ms-long depolarizing current pulses, whose intensity was adjusted to trigger five spikes (Fig. 1*A*). The spike duration increased by 63·6 ± 3·4 % from the 1st spike (1·19 ± 0·05 ms) to the 3rd spike (1·96 ± 0·11 ms), but was slightly reduced for the 4th spike (1·78 ± 0·08 ms; Fig. 1*B* and *C*). Therefore, the spike broadening was measured from the 1st to the 3rd spike (Figs 2–5). Accompanying the broadening, there was also a change in the spike shape: a slowing of the rate of repolarization and development of a 'shoulder' (Fig. 1*B*) and the fast after-hyperpolarization (fAHP, arrow in *A*) was reduced. In contrast, there was little or no change in the spike amplitude or rate of depolarization (Fig. 1*B*). Thus, the broadening reflected a decrease in spike repolarization rate, suggesting a progressive decline in the repolarizing outward currents, and/or an increase in late inward current, during the spike train.

The frequency dependence of the spike broadening was investigated in the range between 10 and 150 Hz by injecting long current pulses of different intensities (Fig. 2). In each case, the spike broadening from the 1st to the 3rd spike was plotted as a function of the average discharge frequency (i.e. the mean of the two first interspike intervals). At frequencies below 20 Hz there was little

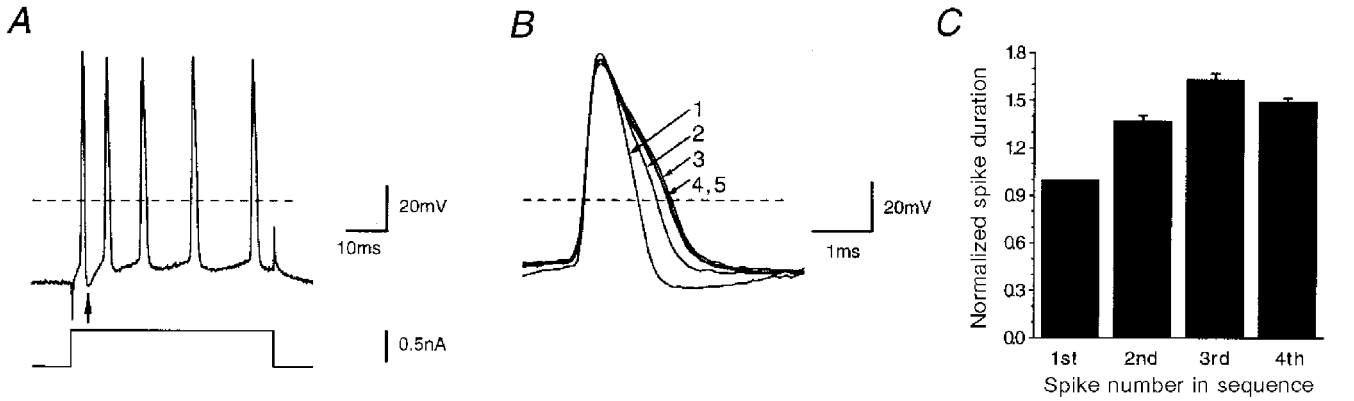

Figure 1. Action potential broadening during a 50 ms, 5 spike train

A, a train of 5 spikes in response to injection of a 50 ms-long depolarizing current pulse. Note the prominent fast after-hyperpolarization (fAHP, arrow) after the first spike. *B*, the 1st to the 5th spikes shown superimposed. *C*, summary of the normalized duration for spikes 1–4 during the trains (means ± S.E.M., $n = 16$). Here and in subsequent figures, the spike duration was measured at 1/3 of the full amplitude (dashed line in *A* and *B*).

broadening or decline in the fAHP (Fig. 2*A*), but the spike broadening and fAHP reduction increased with increasing frequency, reaching about 60% broadening at 100 Hz (Fig. 2*B–D*).

IbTX inhibited spike broadening during repetitive firing

Different blockers of BK-channels were used to test for their role in spike broadening. First, we used the highly specific BK-channel blocker iberiotoxin (IbTX; Galvez *et al.* 1990), at a dose of 60 nM, which was saturating under our experimental conditions, so that a doubling of the dose (120 nM) caused no further broadening of the 1st spike. Application of IbTX broadened all the spikes in the train and suppressed the fAHP of the first spike ($n = 16$; Fig. 3*A* and *B*). In parallel, the relative broadening from the 1st to the 3rd spike was substantially reduced, from 63·6 ± 3·4% in control medium to 25·4 ± 2·7% in IbTX (Fig. 3*C* and *D*, respectively; $n = 16$). Accordingly, IbTX broadened the 1st spike far more (by 61·3 ± 3·0%; Fig. 3*E*) than later spikes in the train (22·4 ± 2·2% broadening of the 3rd spike; Fig. 3*F*). The striking similarity between the IbTX-induced spike broadening and the activity-induced spike broadening within a train under control conditions (compare Fig. 3*C* *vs.* *E* and *D* *vs.* *F*) suggests a common underlying mechanism. In both cases, there was mainly a slowing of the final 2/3 of the repolarization phase and the development of a 'shoulder' on the falling phase (Fig. 3*C* and *E*), as previously seen with less selective BK-channel blockade (Storm, 1987*a*).

The effect of IbTX on the broadening from the 1st to the 3rd spike suggests that a large fraction of the channels that underlie the activity-dependent spike broadening had been blocked by IbTX. Given the selectivity of IbTX for BK-channels (Galvez *et al.* 1990), this result strongly suggests that BK-channels mediate spike broadening during repetitive firing in these cells. Still, the spike broadening during the train was not completely eliminated by IbTX (Fig. 3*D*, *G* and *H*), indicating that BK-channel-independent mechanisms also contribute to the spike broadening during repetitive firing (Ma & Koester, 1996; Giese *et al.* 1998).

In addition to affecting spike duration, IbTX also caused a reduction in the early spike frequency adaptation, which is thought to be partly due to the BK-current I_C (Lancaster & Nicoll, 1987; Storm, 1990). However, our data indicate that the effect of IbTX on the spike broadening could not be simply due to the change in discharge frequency, because the broadening during spike trains was significantly larger in control medium than in IbTX for the same frequency. In control medium, spike trains with a 1st interspike interval

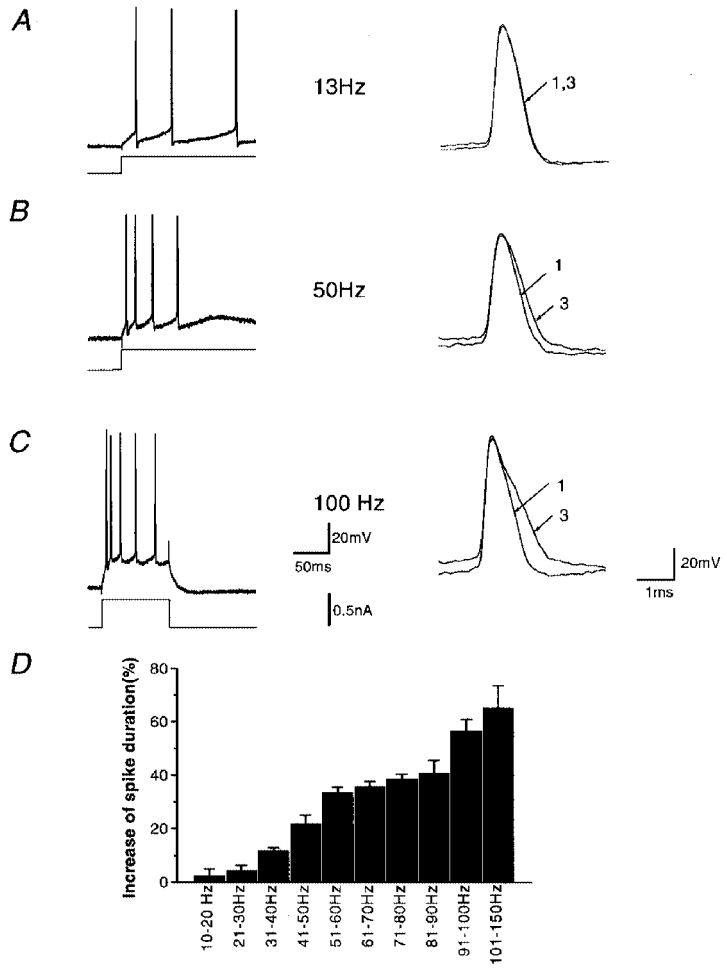

Figure 2. Frequency dependence of the spike broadening

A, during low-frequency repetitive firing (average frequency 13 Hz), there was no detectable broadening from the 1st spike to the 3rd (1 + 3) and no clear decline of the fAHP amplitude. *B* and *C*, during higher-frequency repetitive firing (average frequencies, from the 1st to the 3rd spike: 50 and 100 Hz), in response to stronger current injections, there was increasing broadening of the 3rd spikes and decline of the fAHPs. *A–C* are from the same cell. *D*, summary of the spike broadening at different average discharge frequencies (means ± S.E.M., $n = 10$).

instantaneous frequency of 133·6 ± 1·7 Hz showed a spike broadening from the 1st to the 2nd spike of 33·5 ± 1·7 % ($n = 6$). In contrast, after adding IbTX, and obtaining a matching (or even a slightly higher) frequency of 137·7 ± 5·6 % Hz by adjusting the injected current pulse intensity, the spike broadening was only 21·0 ± 3·3 % ($n = 6$). A similar difference was seen for the spike broadening from the 1st to the 3rd spike. This indicates that BK-channel blockade has an effect on the frequency-dependent spike broadening *per se*.

Paxilline inhibited spike broadening during repetitive firing

To further test the role of BK-channels in spike broadening, we used the tremorgenic indole terpene paxilline, another specific blocker of BK-channels (Knaus *et al.* 1994).

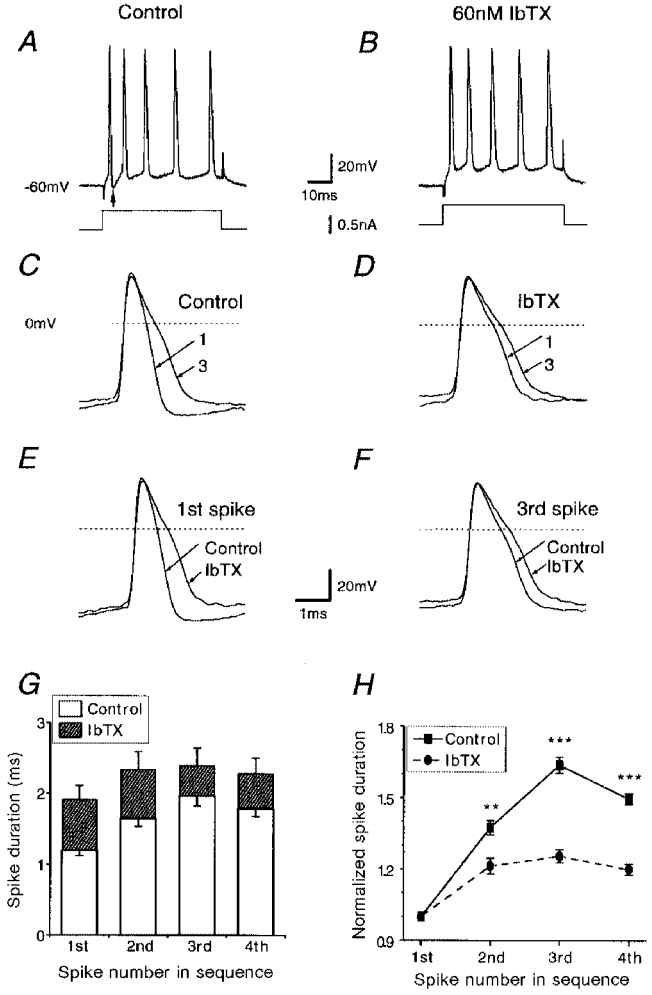

Figure 3. The BK-channel blocker iberiotoxin (IbTX) reduced the spike broadening during a burst

A and *B*, spike trains (50 ms, 5 spikes) in control and in the presence of IbTX (60 nM). *C* and *D*, spike broadening from the 1st to the 3rd spike within the trains was reduced by IbTX. *E* and *F*, IbTX broadened the 1st spike more than later spikes in the train. IbTX also reduced the fAHP (*A* (arrow) and *B*). *G* and *H*, summary of IbTX effects on the spike broadening during spike trains (means ± S.E.M., $n = 16$): *G*, on an absolute time scale (ms), shaded areas represent the increased spike duration by IbTX; *H*, on a normalized time scale (** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$, Student's two-tailed paired *t* test). *A*–*F* are from the same cell. In this and the two following figures (Figs 4 and 5) the membrane potential prior to activation was manually clamped at −60 mV, and the depolarizing pulse strength was adjusted to always elicit 4 or 5 spikes within 50 ms.

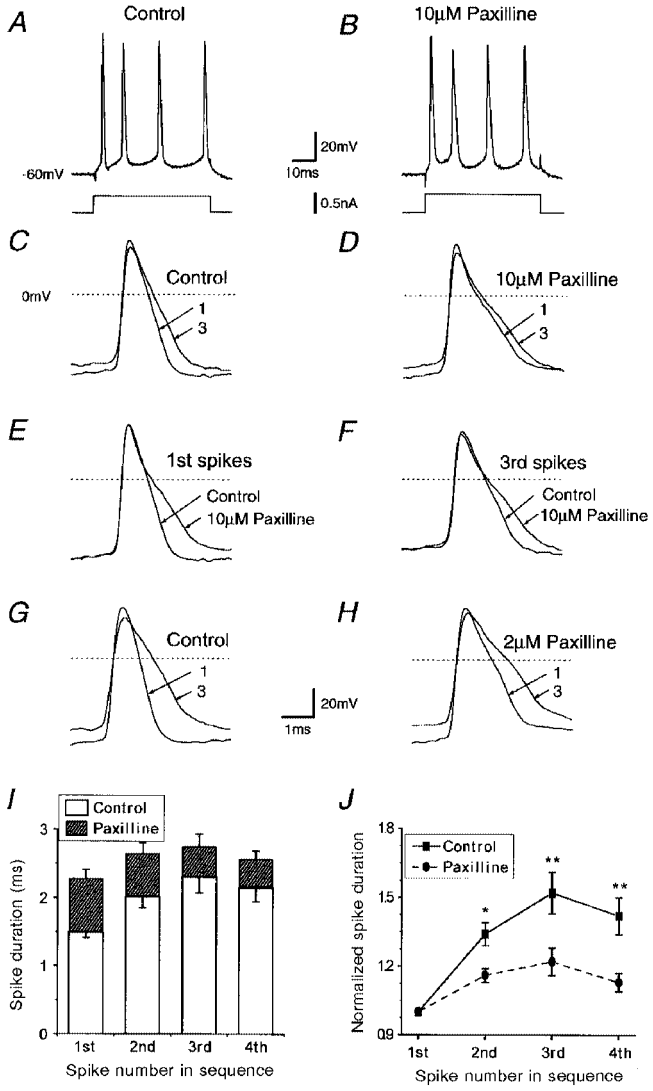

Figure 4. The BK-channel blocker paxilline reduced the spike broadening during a burst

A and *B*, spike trains (50 ms, 4 spikes) in control and in the presence of paxilline (10 μM). *C* and *D*, the spike broadening from the 1st to 3rd spike during the train was reduced by paxilline. *E* and *F*, paxilline broadened the 1st spike more than later spikes in the train. Paxilline also reduced the fAHP (*A* and *B*). *G* and *H*, a similar effect caused by a lower dose of paxilline (2 μM). *I*, summary of paxilline effects on the spike broadening during spike trains on an absolute time scale (ms) (means ± S.E.M., $n = 7$), shaded areas represent the increased spike duration by paxilline; *J*, the same data normalized (* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$, Student's two-tailed paired *t* test).

Paxilline (10 μM) blocked the fAHP (Fig. 4*A* and *B*) and slowed the last 2/3 of the spike repolarization (Fig. 4*E*). Like IbTX, paxilline also broadened all spikes in the train, and reduced the broadening from the 1st to the 3rd spike (Fig. 4*C* and *D*, *I* and *J*): from 52·2 ± 9·2% in normal medium to 21·7 ± 5·6% in 10 μM paxilline ($n = 7$). The paxilline-induced changes in spike waveform closely resembled the activity-induced endogenous spike broadening (compare Fig. 4*C* and *E*). A lower dose of paxilline (2 μM) had similar effects ($n = 2$, Fig. 4*G* and *H*). These results suggest that repetitive firing and paxilline suppressed the same repolarizing current component.

The remaining broadening of the later spikes in the presence of IbTX or paxilline (Figs 3*D*, *G* and *H* and 4*D*, *I* and *J*) suggests that channels other than BK also contribute to the activity-induced spike broadening.

Calcium-free medium reduced the spike broadening during repetitive firing

We next perfused the slices with $MnCl_2$-containing Ca^{2+}-free medium, to prevent BK-channel activation by eliminating the Ca^{2+} influx. As expected, the fAHP was blocked and the rate of spike repolarization was slowed (Fig. 5*A–D*). In addition, the broadening from the 1st to the 3rd spike was reduced from 49·4 ± 8·7% (control) to 22·5 ± 5·9% in Ca^{2+}-free medium ($n = 8$), and there was also a significant decrease in the relative spike broadening of the 2nd and 3rd spikes in Ca^{2+}-free medium (Fig. 5*E*).

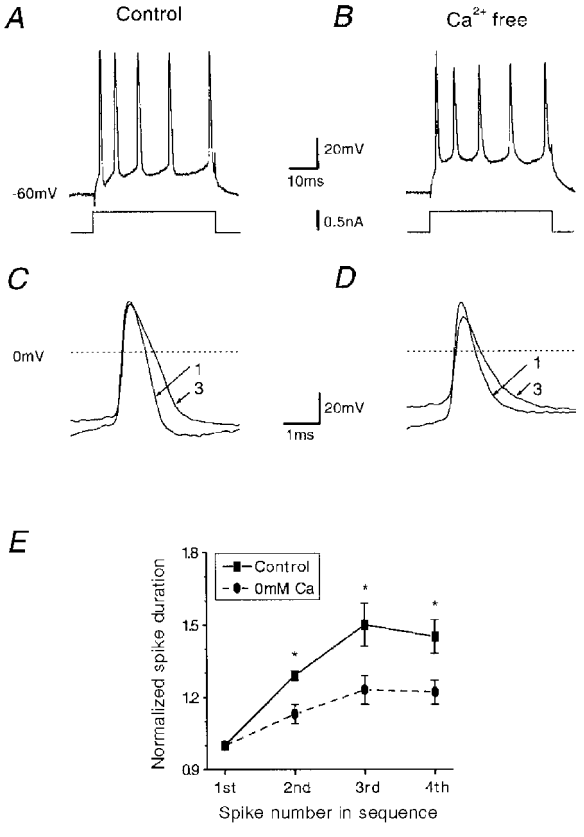

Figure 5. Calcium-free medium reduced the spike broadening during a burst

A and *B*, spike trains (50 ms, 5 spikes) in control and in Ca^{2+}-free medium. *C* and *D*, perfusion with Ca^{2+}-free medium with 2 mM manganese reduced the spike broadening from the 1st spike to the 3rd within a train. *E*, normalized spike duration in medium with and without Ca^{2+} (means ± S.E.M., $n = 8$; *$P < 0{\cdot}05$, Student's two-tailed paired t test).

Note that the Ca^{2+}-free medium, unlike the specific BK-channel blockers (Figs 3*E* and 4*E*), eliminated the 'shoulder' during the repolarization. This 'shoulder' is probably due to inward Ca^{2+} currents (Storm, 1987*a*,*b*; Lancaster & Nicoll, 1987), whose recruitment may contribute directly to the spike broadening (Aldrich *et al.* 1979).

Computer simulations

Since many hypotheses for the functions of ion channels cannot readily be tested experimentally with the available methods, computer simulations were performed to assess the contribution of various current components to spike broadening. We used the reduced compartmental model developed by Borg-Graham (1998), which incorporates 11 active currents and intracellular Ca^{2+} dynamics (see Methods). A 3-state Markov model represents I_{CT} and includes a voltage-dependent removal of inactivation in order to reproduce the transient presence of I_{CT} over the first few spikes in the train. Based on the experimental finding of a fast transient BK-like current elicited during depolarizing voltage steps (Zbicz & Weight, 1985; Storm, 1987*c*; Halvorsrud *et al.* 1999; Shao *et al.* 1999), the kinetics of I_{CT} were modified to inactivate in response to such voltage steps (see Appendix).

Using this modified model, we simulated spike trains in response to 50 ms current pulses, with the current intensities adjusted to elicit five spikes. Comparisons between simulations with transient BK-channels present (Fig. 6*A–C*), without any BK-channels (Fig. 6*D–F*), and with non-inactivating BK-channels (Fig. 6*G–I*) support the hypothesis that BK-channel inactivation is important for activity-dependent spike broadening.

Simulations with a transient BK-channel in the model produced a similar spike train as observed experimentally (compare Fig. 6*A*, with Fig. 1*A*). In particular, the fAHP (▲) was prominent after the 1st spike, but was strongly reduced after subsequent spikes, and there was substantial spike broadening during repetitive firing (Fig. 6*C* and *J* (□)). The model showed that the broadening was largely due to cumulative inactivation of I_{CT} during the spike train (Fig. 6*B*, continuous line). As I_{CT} declined, the A-current (dashed line) replaced I_{CT} as the main repolarizing current during the last three spikes.

When I_{CT} was removed from the model, the fAHP disappeared and the spike broadening was strongly reduced (Fig. 6*D*, *F* and *J* (▼)), qualitatively similar to the

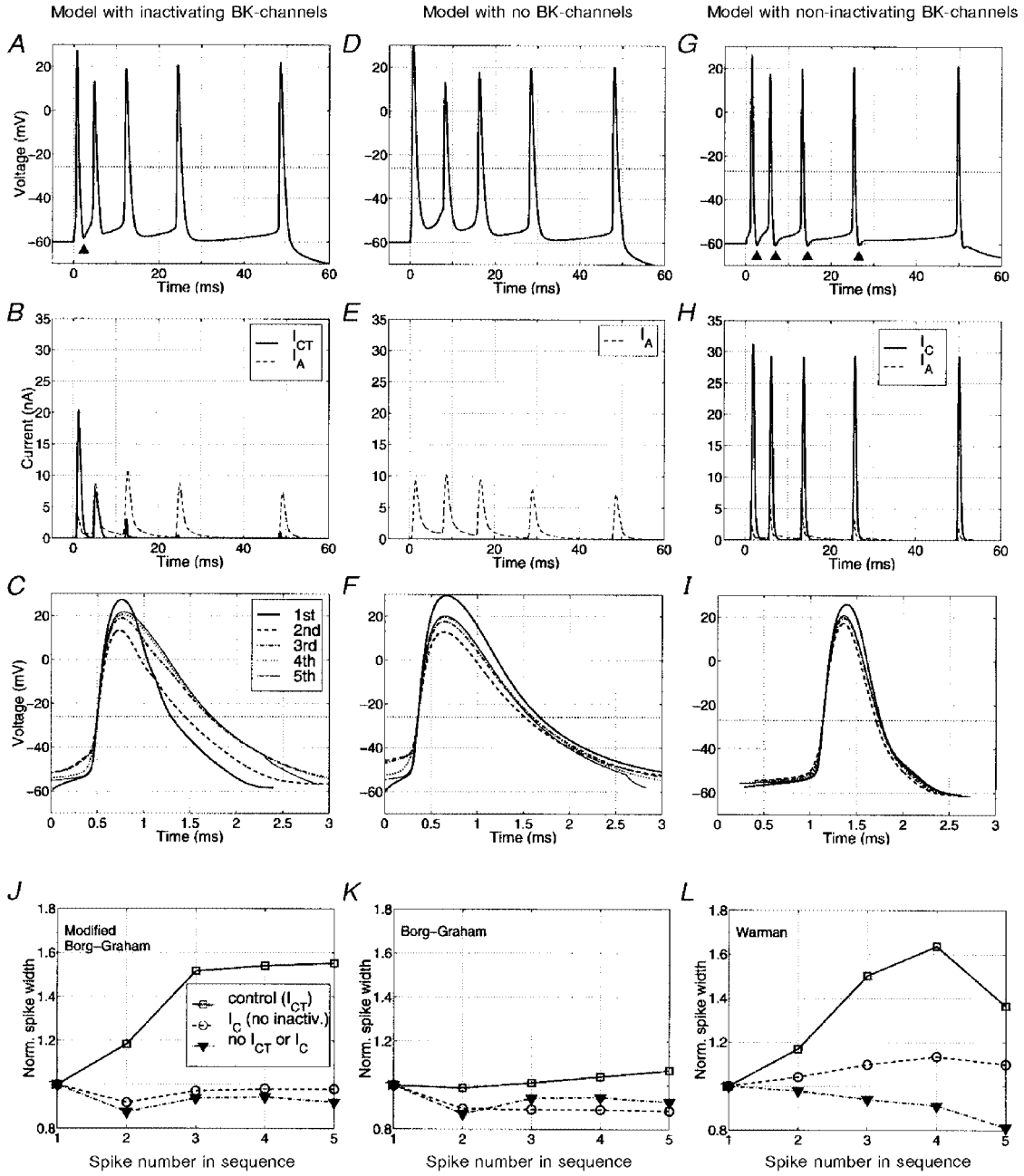

Figure 6. Computer simulations of spike trains in a model CA1 pyramidal cell

A–J show simulations based on our modified version of the Borg-Graham model (Borg-Graham, 1998). *A–C*, simulations under control conditions. *A*, a spike train in response to a 50 ms-long depolarizing current pulse, with a prominent fast AHP (▲) after the 1st spike. *B*, plots showing the cumulative inactivation of I_{CT} together with I_A during the spike train in *A*. *C*, the spikes from *A*, superimposed on an expanded time scale, to illustrate the spike broadening, which is also plotted in *J* (□). *D–F*, the BK-current I_{CT} has been omitted ('blocked'). *D*, spike train. *E*, A-current. *F*, superimposed spikes. Note the reduced spike broadening (plotted in *J*, ▼) and fAHP. *G–I*, the inactivation mechanism of the BK-current has been disabled, resulting in a non-inactivating BK-current (I_C). Note the uniformly narrow spikes (*I*), the reduced spike broadening, and the prominent fAHPs throughout the train (▲ in *G*). *J–L*, plots of normalized spike duration from 50 ms-long trains of 5 spikes, using three different models. *J*, our modified version of the 'Working model' of Borg-Graham. *K*, the original 'Working model' of Borg-Graham (Borg-Graham, 1998). *L*, the Warman model (Warman *et al.* 1994). With each model, a train of 5 spikes in response to a 50 ms current pulse was simulated, with a transient BK-current (□), without any BK-current (▼), and with a non-inactivating BK-current (○). Action potential trains were elicited by 50 ms somatic current injections of 1·1, 0·6 and 1·5 nA (see Fig. 6*A*, *D* and *G*, respectively), corresponding to the minimal current needed to elicit 5 spikes. Spike width was measured at 1/3 of total spike amplitude of the 1st spike.

experimental results with BK-channel blockers (Figs 3*B*, *D* and *H*, and 4*B*, *D* and *J*). Under these conditions, the A-current activated more during the first spike (Fig. 6*E*, dashed line) due to the spike broadening, thus partly substituting for the lost I_{CT}.

In contrast, when only the inactivation mechanism of I_{CT} was disabled, leaving a non-inactivating BK-current (dubbed I_C) the fAHPs were maintained throughout the train (Fig. 6*G* (▲)) and I_C showed essentially no decline (Fig. 6*H*). Under these conditions, the spike widths were strongly reduced and activity-dependent broadening was eliminated (Fig. 6*I* and *J* (○)). These simulations support the hypothesis that inactivation of the BK-channels is essential for spike broadening during repetitive firing.

To test for the theoretical alternative possibility that an increase in the cumulative activation of the slowly activating K^+ currents I_M and I_{DR} (rather than a reduction in I_{CT}) might underlie the observed reduction in spike broadening after BK-channel blockade (see Discussion), we looked specifically at these currents during the simulated spike trains shown in Fig. 6*A*, *D* and *G*. Contrary to this alternative hypothesis, we found that the peak value of I_{DR} during the spike was reduced from the 1st to the 2nd spike both under control conditions (spike train in Fig. 6*A*) and after BK blockade (spike train in Fig. 6*D*), and that the reduction was larger in the latter case (reduction: from 5·3 to 2·6 nA after BK blockade *vs.* from 3·5 to 2·5 nA in the control). In contrast, I_M increased both in control (from 0·3 to 0·9 nA) and after BK blockade (from 0·5 to 1·6 nA), but I_M is far too small to make up for the change in I_{DR}. Hence, the sum of the repolarizing voltage-gated K^+ currents I_{DR}, I_M and I_A showed virtually no increase from the 1st (14·1 nA) to the 2nd (14·3 nA) spike after BK blockade, although there was a large increase in the control (6·5 nA for the 1st, 12·0 nA for the 2nd spike). Therefore, cumulative activation of the slowly activating K^+ currents cannot explain the reduced frequency-dependent spike broadening after BK blockade. Rather, these currents tend to reduce the broadening effect of I_{CT} inactivation, and the effect of BK-channel blockade. This further strengthens our hypothesis that I_{CT} inactivation is crucial for frequency-dependent spike broadening.

To demonstrate that the simulation results are not specific to this particular model, we also performed simulations using both the unmodified Borg-Graham model (Borg-Graham, 1998) and the model by Warman *et al.* (1994) (Fig. 6*K* and *L*). The Borg-Graham model also exhibits cumulative inactivation of I_{CT} during the spike train. However, I_{CT} peaks at a late stage in the repolarization, and substantial broadening can be seen only when measured at voltage levels more negative than 1/3 of full spike amplitude. The Warman model (Fig. 6*L*) gives a broadening quite similar to our modified model.

DISCUSSION

Evidence for BK-channel-dependent spike broadening

The main result of the present study is that rat CA1 pyramidal cells show a frequency-dependent spike broadening that is substantially reduced when the BK-channels are suppressed. The change in spike shape during a spike train (in particular, the development of a 'shoulder') closely resembled that caused by BK-channel blockers (Figs 3*C* and *E*, and 4*C* and *E*). Furthermore, the effect of BK-channel blockers was much reduced late in the train (Figs 3*F* and 4*F*), suggesting that BK-channel activity declines during repetitive firing. Finally, the spike broadening during the train was similarly reduced by IbTX, paxilline, or Ca^{2+}-free Mn^{2+}-containing medium (Figs 3–5). These results support our hypothesis: that BK-channels mediate spike broadening during repetitive firing in CA1 pyramidal cells. It will be important to test whether this mechanism is important *in vivo*.

Mechanism of BK-dependent spike broadening during repetitive firing

Activity-dependent spike broadening may be due to cumulative inactivation of the outward currents contributing to spike repolarization. It therefore seems plausible that the fast-inactivating BK-like outward current I_{CT} (Zbicz & Weight, 1985; Storm, 1987*c*) generated by fast-inactivating BK-channels (Marrion & Tavalin, 1998) is responsible for the BK-dependent spike broadening during repetitive firing. We have started to characterize an IbTX-sensitive transient outward current, I_{CT}, by whole-cell voltage clamp in CA1 pyramidal cells (Shao *et al.* 1999; Halvorsrud *et al.* 1999). This current shows apparent fast inactivation during a train of brief depolarizing steps and a time course of recovery from inactivation compatible with the frequency dependence of spike broadening. These properties are as expected for a current causing activity-dependent spike broadening.

Possible alternative explanations for our results

Although the above conclusions seem most plausible, one should consider alternative interpretations. (1) Could the reduction in spike broadening by BK blockade be an indirect effect, rather than directly due to loss of a transient BK-current? In general, spike broadening caused by BK-channel blockade is expected to increase other K^+ currents during the spike (e.g. I_A, I_D, I_{DR}) because of the longer depolarization. Could this explain the reduced spike broadening after BK-channel blockade, even if the BK-channels do not inactivate? A fast transient voltage-gated K^+ current, such as I_A, could probably not mediate such an indirect effect, because broadening of the first spikes would tend to strengthen the cumulative inactivation of I_A, thus causing more spike broadening in IbTX, not less. Of course, if the spikes become so broad after BK-channel blockade that I_A inactivates fully during the 1st spike, the I_A-

dependent broadening would disappear, but this is not possible with an I_A inactivation time constant of >10 ms (Numann *et al.* 1987; Storm, 1990; Hoffman *et al.* 1997). This possibility is also contradicted by our simulations (Fig. 6*B–E*). Also a sustained K^+ current would fail to mediate any indirect IbTX effect on spike broadening, if the current is sufficiently fast to fully activate during a normal 1st spike.

It seems possible, however, that slowly activating K^+ currents, like the hippocampal slow delayed rectifier, I_{DR} (=I_K; Numann *et al.* 1987) and/or M-current, I_M, which normally contribute little to spike repolarization (Storm, 1987*a*, 1990), could be recruited by the IbTX-broadened spikes, and might even undergo cumulative activation. Such currents might then provide a stronger negative feedback on spike duration, activating more the broader the spike, thus limiting the broadening during the train. If so, the BK-channels would act only indirectly, by normally preventing the slow current from activating enough to strongly limit the broadening, which could primarily be due to other mechanisms, e.g. I_A inactivation. Thus, the reduction in spike broadening after IbTX might conceivably occur without any BK-channel inactivation, just because spikes broadened by IbTX would engage slow K^+ currents like I_{DR} or I_M.

Computer simulations

In order to test the above and other alternative hypotheses, which cannot readily be tested experimentally, we performed computer simulations. All the simulations, using three different models, consistently showed a substantial reduction in the spike broadening during repetitive firing when the inactivation of the BK-current was removed (Fig. 6*J–L*). However, the Borg-Graham model (Fig. 6*K*) showed clear broadening only close to the base of the spikes (not seen in Fig. 6*K*, since the spikes were always measured at 1/3 of the full amplitude). As described in Results, our simulations indicate that the reduction in spike broadening after BK-channel blockade was not due to recruitment of I_{DR} or I_M. This further supports the idea that BK-channel inactivation is essential for spike broadening during repetitive firing.

In the original model (Borg-Graham, 1998), the voltage-dependent inactivation of I_{CT} speeds up with hyperpolarization. Hence, it inactivates during interspike intervals but not during a sustained depolarizing step. Based on the available voltage-clamp data (Halvorsrud *et al.* 1999; Shao *et al.* 1999), the kinetics of I_{CT} were modified to inactivate in response to depolarizing steps (see Appendix). While the new version of I_{CT} quantitatively reproduces certain measures of broadening, the activity-dependent spike broadening after BK-channel 'blockade' was less than observed with IbTX or paxilline (compare Figs 6*J*, 3*H* and 4*J*), and the lack of a 'shoulder' in the original and revised models suggests that a kinetic component is missing. If so, a further revision of the I_{CT} model will be warranted. Nevertheless, since these models integrate much of the available quantitative data from CA1 pyramidal cells, and reproduce several major features of their response properties (Borg-Graham, 1987), they provide valuable quantitative tests of our hypothesis. The convergent results obtained with three different models indicate that the spike-broadening effect of I_{CT} is robust.

Comparison with previous reports

The conclusion that BK-channels produce frequency-dependent spike broadening may seem surprising in view of previous findings indicating that transient voltage-gated K^+ channels, in particular A-type channels, are responsible for this effect (Aldrich *et al.* 1979; Bourque & Renaud, 1985; Jackson *et al.* 1991; Ma & Koester, 1996). Also in mouse CA1 pyramidal cells, the frequency-dependent spike broadening was found to be partly dependent on A-current inactivation, mediated by Kvβ1.1 (Giese *et al.* 1998). However, the reduction in spike broadening obtained by deletion of the Kvβ1.1 gene (Giese *et al.* 1998) was smaller than the effect of BK-channel blockers reported here. It seems likely that a strong BK- and a weaker A-channel-dependent spike-broadening mechanism coexist in CA1 pyramidal cells.

To our knowledge, frequency-dependent spike-broadening due to BK-channels has not previously been reported in vertebrate or invertebrate neurones or other cells. Most BK-channel recordings have shown little or no inactivation (Blatz & Magleby, 1987; Marty, 1989). However, some reports have described inactivating BK-like current components: in frog sympathetic ganglia (MacDermott & Weight, 1982), *Drosophila* muscle (Elkins & Ganetzky, 1988), rat chromaffin cells (Ding *et al.* 1998) and in rat spinal motoneurones (Takahashi, 1990). These currents inactivated within 3–10 ms, i.e. roughly similar to I_{CT}.

In hippocampal pyramidal cells, early single-electrode voltage-clamp recordings suggested the existence of transient BK-like currents. In CA3 cells, a transient (5–20 ms duration) Ca^{2+}-dependent K^+ current was reduced by 10 mM TEA (Zbicz & Weight, 1985). In CA1 cells, a similar fast-inactivating, Ca^{2+}-dependent K^+ current, I_{CT}, was blocked by 1 mM TEA, like BK-channels (Storm, 1987*c*). However, the interpretation of these data was problematic (Storm, 1990). Recent recordings from inside-out patches from dissociated CA1 neurones showed transient BK-channels with a decay time constant of 3·8 ms (Marrion & Tavalin, 1998) comparable to our recent voltage-clamp recordings (Halvorsrud *et al.* 1999; Shao *et al.* 1999). More slowly inactivating BK-channels ($\tau = 32$ ms) were observed in cultured CA1 neurones (McLarnon, 1995).

In adrenal chromaffin cells, detailed analysis of inactivating BK-channels suggested that they arise from the assembly of inactivating and non-inactivating subunits (Ding *et al.*

Table 1. The allowed transitions and their corresponding rate parameters of the modified I_{CT} model

Transition	$1/\tau_{max}$ (ms^{-1})	k (mV)	$V_{½}$ (mV)	$1/\tau_{min}$ (ms^{-1})	α (mM^{-n})	n
O → I	0	1	−10	10	—	—
I → C	0	−10	−120	10	—	—
C → O	1	7	−20	1000	10^8	3
O → C	0	−5	−44	100	—	—

τ_{max}, k, $V_{½}$, τ_{min}, α and n are defined in the Appendix.

1998). Very recently, the first transmembrane subunits ($\beta 2$) conferring fast inactivation of BK-channels have been identified. (Wallner *et al.* 1999). The $\beta 2$ subunit is mainly expressed in fetal kidney and produces slower inactivation than that of I_{CT}, but it seems likely that related subunits, causing faster inactivation, may exist in the brain.

Functional implications

Because most of the influx of Ca^{2+} ions occurs late during each action potential (Llinas *et al.* 1982), spike broadening can efficiently modulate $[Ca^{2+}]_i$. Since spike broadening due to K^+ channel inactivation is such a widespread phenomenon (Aldrich *et al.* 1979; Bourque & Renaud, 1985; Jackson *et al.* 1991), it is tempting to speculate that it may be important for signalling, perhaps by increasing the gain of the $[Ca^{2+}]_i$ signal as a function of spike number and frequency. This may be a reason why fast inactivation mechanisms have become so elaborate and varied. By contributing to frequency-dependent modulation of $[Ca^{2+}]_i$ signals, spike broadening is potentially important for a range of Ca^{2+}-dependent processes (Gainer *et al.* 1986; Jackson *et al.* 1991; Hawkins *et al.* 1993). It was previously shown that the reduced spike broadening in Kvβ1.1-deficient CA1 cells was accompanied by a reduced Ca^{2+}-dependent sAHP, indicating reduced Ca^{2+} influx (Giese *et al.* 1998). Frequency-dependent spike-broadening also increases hormone release in the pituitary (Gainer *et al.* 1986), and a similar broadening in presynaptic terminals may cause frequency facilitation of transmitter release. However, such a mechanism does not seem to be important in the Schaffer–CA1 hippocampal synapses under normal conditions (Lærum *et al.* 1991).

Somatic spike broadening would be expected to enhance the dendritic depolarization provided by the somatic spike, and might therefore facilitate spike backpropagation into the dendrites, thus influencing dendritic $[Ca^{2+}]_i$, synaptic integration and plasticity (Spruston *et al.* 1995). Cumulative inactivation of the dendritic I_A (Hoffman *et al.* 1997) would be expected to increase this tendency. In spite of these factors, spikes late in a train fail to actively invade the dendrites (Callaway & Ross, 1995), suggesting that other factors dominate. Lack of BK-channels and fAHPs in the dendrites may promote inactivation of dendritic Na^+ channels, thus inhibiting backpropagation of late spikes (Poolos & Johnston, 1999). Nevertheless, the passive dendritic depolarization caused by somatic spikes may be enhanced by spike broadening, due to low-pass filtering by the dendritic cable.

The functions of transient BK-channels would be expected to differ from those of the voltage-gated A-channels. Being activated by both Ca^{2+} and voltage, BK-channels mediate feedback regulation of activity based on both of these parameters. Whereas A-channels can already activate below the spike threshold, and regulate subthreshold synaptic inputs and discharge latency, the BK-channels in CA1 cells seem to be activated primarily by action potentials (Storm, 1990), presumably because they depend on high-threshold Ca^{2+} channels (Marrion & Tavalin, 1998). Furthermore, I_{CT} inactivates faster than I_A in CA1 cells (half-decay time about 5 ms (Halvorsrud *et al.* 1999), *vs.* inactivation time constant 10–40 ms for I_A (Numann *et al.* 1987; Storm 1990; Hoffman *et al.* 1997)). We propose that this fast inactivation and activation enables I_{CT} to produce a brisk modulation of the first few spikes in a burst, with a rapid suppression of the fAHP, whereas I_A produces a slower broadening, like the one seen after BK-channel blockade (Figs 3 and 4). Since BK-channels are subject to modulation, BK-dependent spike broadening may also be altered by neuromodulators.

APPENDIX

The BK-channel in the 'Working model' (Borg-Graham, 1998) is a 3-state Markov model that can be in the open state (O), closed state (C) or inactivated state (I). The voltage dependence of the transition rates from states i to j is of the form:

$$r_{ij}(V) = (\tau_{min} + ((\tau_{max} - \tau_{min})^{-1} + \exp((V - V_{½})/k))^{-1})^{-1}.$$

Here, the inverse values of τ_{min} and τ_{max} represent the upper and lower bounds, respectively, of $r_{ij}(V)$. The parameters $V_{½}$ and k are similar to those used in the extended Hodgkin-Huxley model. The parameters (τ_{min}, τ_{max}, $V_{½}$, k) of the transition rates in the original model were modified according to Table 1, to make I_{CT} transient during depolarizing voltage-clamp steps. Note that the C → O rate transition depends on $[Ca^{2+}]$ and is given by the

product of $r_{ij}(V)$ and $\alpha[Ca^{2+}]^n$, where n is the number of bound Ca^{2+} ions and $[Ca^{2+}]$ is taken from a subdomain adjacent to the Ca^{2+} channels. See Borg-Graham (1998) for a more complete discussion of the model. The maximum (peak) conductance density was doubled to become $\bar{g}_{CT} = 20{\cdot}8$ mS cm^{-2}. In some of the simulations, the inactivation mechanism was disabled (Fig. 6*G*, *H* and *I*) by disregarding the O $\rightarrow$ I transition.

Note added in proof

A recent article by X. M. Xia, J. P. Ding & C. J. Lingle (*Journal of Neuroscience* **19**, 5255–5264 (1999)) describes a new BK-channel subunit, $\beta 3$, found in adrenal chromaffin and insulinoma cells, which produces relatively fast inactivation (time constant 25–30 ms) of BK-channels.

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