Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurones

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1. The roles of multiple potassium conductances underlying action potential repolarization and after-hyperpolarization (AHP) in visually identified st. oriens–alveus (st. O–A) inhibitory interneurones of neonatal rat CA1 hippocampal slices were determined using whole-cell patch clamp techniques.

2. 4-Aminopyridine dose-dependently prolonged the action potential repolarization. The effects of 4-AP persisted in Ca^{2+}-free conditions. Action potentials evoked from hyperpolarized potentials possessed an increased rate of repolarization. These data suggest an involvement of the rapidly activating transient current, I_A, in spike repolarization.

3. Action potential duration was increased in the presence of Ca^{2+}-free, Cd^{2+}-containing solution, iberiotoxin or 1 mM TEA. The fast component of the AHP was attenuated by these agents suggesting that the Ca^{2+}-activated K^+ conductance, I_C, underlies both the spike repolarization and fast AHP.

4. In Ca^{2+}-free conditions, TEA (>1 mM) dose-dependently prolonged the action potential duration by blocking a late conductance in action potential repolarization, suggesting a role for the sustained current, I_K.

5. The slow AHP was attenuated by Ca^{2+}-free medium, apamin or the Ca^{2+} chelator EGTA, suggesting a role for the Ca^{2+}-activated K^+ conductance, I_{AHP}.

6. We conclude that action potential repolarization and AHP of st. O–A interneurones result from the activation of pharmacologically distinct, temporally overlapping potassium conductances. These findings are discussed with reference to the voltage clamp data presented in the preceding manuscript.

Action potentials are a fundamental property of excitable cells in the mammalian central nervous system. The arrival of action potentials at the presynaptic terminal initiates a sequence of events which lead to release of neurotransmitters influencing the excitability of the postsynaptic cells. In the hippocampus, the action potential properties of the principal neurones, the pyramidal cells, have been extensively characterized. A variety of potassium conductances are known to be involved in the repolarization and the after-hyperpolarization (AHP) of pyramidal neurone action potentials (for review see Storm, 1990; Halliwell, 1990). In contrast, nothing is known concerning potassium conductances underlying action potentials in the various types of interneurone in the hippocampus. The action potential phenotype of a variety of hippocampal inhibitory neurones are known to be distinct from those of the pyramidal cells (Lacaille, Kunkel & Schwartzkroin, 1989). Unlike pyramidal cells, they have a shorter duration action potential (~1 ms at 35 °C) followed by a prominent AHP without an obvious after-depolarization (Lacaille, Meuller, Kunkel & Schwartzkroin, 1987; Lacaille *et al.* 1989; Buhl, Han, Lörinczi, Stezhka, Karnup & Somogyi, 1994). The AHP of st. O–A interneurones is relatively brief (~200 ms duration) in comparison with its pyramidal neurone counterpart (McBain, 1994). In addition, inhibitory neurones of st. O–A demonstrate spontaneous action potential firing at the resting potential and sustained repetitive firing during depolarizing pulses with little accommodation (Lacaille *et al.* 1987; McBain, 1994). It is likely that the contrasting action potential firing rates and patterns observed in pyramidal neurones and interneurones are in part attributable to the different K^+ conductances expressed on these cells. In the accompanying manuscript (Zhang & McBain, 1995) we used voltage clamp techniques to characterize three potassium current phenotypes present

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Table 1. Action potential properties of st. oriens–alveus interneurones (means ± S.E.M. (n))

Action potential amplitude (mV)	84·5 ± 1·7 (48)
Action potential time to peak (ms)	1·5 ± 0·1 (48)
50% Repolarization time (ms)	2·5 ± 0·1 (48)
Action potential duration (ms)	3·8 ± 0·1 (48)
Fast AHP amplitude (mV)	14·1 ± 0·6 (30)
Fast AHP duration (ms)	2·5 ± 0·1 (30)
Slow AHP amplitude (mV)	16·6 ± 0·8 (40)
Slow AHP duration (ms)	226·9 ± 12·3 (40)

on st. O–A interneurones. All these currents were activated by voltage steps occurring within the range of the interneurone action potential voltage trajectory. In the present study, we examine the individual contributions of these and additional potassium currents in the repolarization and after-hyperpolarization of action potentials in inhibitory neurones of the stratum oriens–alveus of CA1 using whole-cell current clamp techniques.

METHODS

Membrane parameters and action potential measurement

Hippocampal slices were prepared as described previously (McBain, 1994). Briefly Sprague–Dawley rats (11–20 days) were killed by decapitation following deep anaesthesia using isoflurane. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) (mM): NaCl, 130; $NaHCO_3$, 24; KCl, 3·5; NaH_2PO_4, 1·25; $CaCl_2$, 1·5; $MgSO_4$, 1·5; glucose, 10; saturated with 95% O_2–5% CO_2 (pH 7·4, 307 mosmol l^{-1}). Using a Vibratome (Oxford series 1000; Polysciences, Warrington, PA, USA), 250–350 μm-thick sagittal slices were cut from the middle third of the hippocampus.

All electrophysiological measurements were made at room temperature (24–26 °C), unless otherwise stated, in order to permit a direct comparison with the data obtained from voltage clamp experiments presented in the accompanying manuscript (Zhang & McBain, 1995). To minimize the influence of excitatory synaptic input, the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM) was routinely added to the bathing solution; this allowed measurement of spontaneous intrinsic action potential activity free from excitatory synaptic events.

In all inhibitory neurones studied, spontaneous action potentials were observed immediately on breakthrough to whole-cell mode preventing an accurate estimation of the resting membrane potential. Injection of negative current was used to hold the cell at a slightly hyperpolarized potential throughout the experiment (mean, −59·8 ± 0·7 mV; $n = 41$). At this holding potential the frequency of action potential discharge decreased, permitting adequate separation of individual action potentials and their AHPs. Input resistances were determined by use of hyperpolarizing electrotonic current pulses (25–50 pA, 500 ms in duration) delivered at the experimental holding potential. Care was taken in all recordings to ensure that no 'sag' in the voltage trace, normally observed at extreme hyperpolarized potentials in these cells, was observed during this manipulation (McBain, 1994). At this potential interneurones had input resistances ranging from 200 to 600 MΩ (mean, 330·3 ± 22·0 MΩ; $n = 36$).

In order to determine the threshold for action potential firing, inhibitory neurones were held at −80 mV, and a 4 s depolarizing ramp to −40 mV was then delivered from the pCLAMP suite of programs (Axon instruments). Two to three measurements of the threshold potential were averaged for each neurone.

The following criteria were used to measure the parameters of the action potential (see Fig. 1*Bb*). The action potential amplitude was measured as the difference between the point of spike initiation and its peak amplitude. The action potential duration was measured from the voltage of spike initiation to the point at which the repolarization crossed the same voltage. The 50% repolarization time was measured from the action potential onset to the point at which the repolarization fell to half of the peak amplitude. After-hyperpolarization (AHP) amplitude was measured from the spike initiation to the maximal AHP amplitude, and AHP duration measured from the end of spike repolarization to the point at when the AHP decayed back to the holding potential.

RESULTS

Action potential properties of st. oriens–alveus interneurones

Current clamp recordings were made from a total of 119 interneurones of st. O–A. On breakthrough into whole-cell configuration, spontaneous action potentials were observed in all st. O–A interneurones studied, without the requirement for membrane depolarization. Recordings made at 35 °C revealed action potentials of short duration, with time courses similar to those described previously by Lacaille *et al.* (1989). The mean amplitude and duration of these action potentials were 74·5 ± 3·2 mV and 1·1 ± 0·1 ms, respectively ($n = 6$; Fig. 1*A*; cf. data in Table 1). No evidence for burst firing or rhythmical discharges were observed in any cell. At 24 °C st. O–A interneurones fired action potentials with the properties shown in Table 1 and Fig. 1*B–D*. Upon breakthrough into whole-cell mode, the frequency of action potential firing ranged from 9 to 31 Hz (mean, 21 ± 1 Hz; $n = 16$). Addition of the AMPA receptor antagonist DNQX (20 μM) was without effect on the action potential firing pattern or rate ($n = 6$, data not shown), confirming that intrinsic mechanisms determine the generation of the spontaneous activity (McBain, 1994). At this temperature the action potential firing pattern was identical to that observed in recordings made at 35 °C, albeit at a slower frequency. In all subsequent recordings the solution temperature was held at room temperature.

St. oriens–alveus interneurones possess 'calcium spikes'

Application of the voltage-dependent Na^+-channel blocker tetrodotoxin (TTX; 0·5–1 μM) abolished all spontaneous action potential firing. However, in response to a depolarizing pulse (20–100 pA), cells displayed a single long-duration spike (~10–40 ms; $n = 5$), which was subsequently abolished by 2 mM cobalt chloride (Co^{2+}), demonstrating that activation of voltage-gated Ca^{2+} channels can generate a prominent 'calcium-spike' during depolarizing pulses (Fig. 1*D*)

Fast and slow duration AHPs follow single action potentials

In st. O–A cells, the AHP following a single action potential displayed two major components (Fig. 1*Ab* and *Bb*). In the majority of cells (75%) the AHP was dominated by a 'slow' AHP with properties shown in Table 1. In these neurones, although a 'fast' component of the AHP was often discernible, it was impossible to make any accurate measurement. In the remaining 25% of cells a prominent fast AHP was observed (Table 1, Fig. 1). In these cells a slow AHP (16·2 ± 1·3 mV in amplitude and

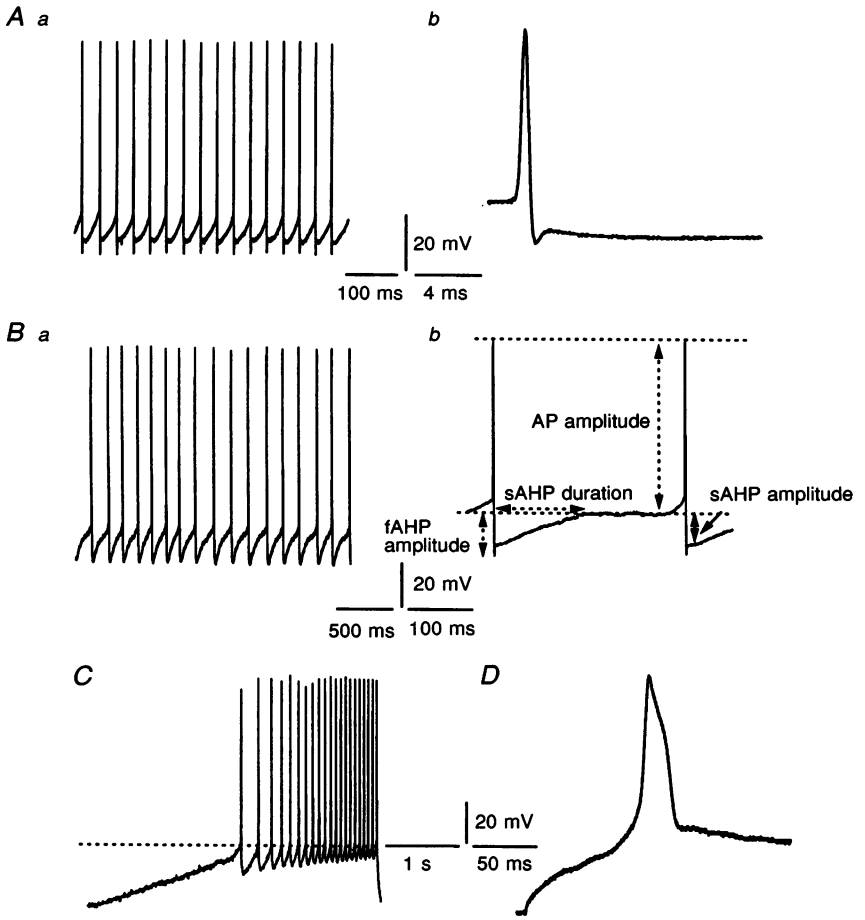

Figure 1. Action potential properties of st. O–A interneurones

All st. O–A cells fired spontaneous action potentials upon whole-cell breakthrough. At 35 °C (*Aa* and *Ab*) and 24 °C (*Ba* and *Bb*) the pattern of action potential firing is identical but slower at 24 °C (note the different calibration in *Aa* and *Ba*). At both temperatures the frequency of spontaneous action potential firing was usually determined by the rate of decay of the slow AHP of the previous action potential. *Bb*, membrane hyperpolarization by current injection attenuates the action potential firing frequency and permits the resolution of single action potentials and their associated AHPs ($V_{hold} = -61$ mV). The various parameters measured throughout the text are indicated in this panel. sAHP, slow AHP; fAHP, fast AHP; AP, action potential. *C*, the threshold for action potential firing was determined using a current injection ramp. Cells were held at −80 mV and a 2 s depolarizing ramp designed to cross threshold was delivered. The dashed line indicates action potential threshold (−55 mV). The rate of action potential firing is accelerated during the depolarizing ramp with no change in the threshold potential. *D*, st. O–A cells possessed a prominent calcium spike. In 1 μM TTX all spontaneous action potential firing was abolished. In response to a step depolarization (100 pA, 500 ms at $V_{hold} = -70$ mV) a prominent, slow 'calcium-spike' was observed.

210·8 ± 27·1 ms in duration, $n = 30$) immediately followed the fast component.

Repetitive firing properties

The repetitive firing properties of interneurones in response to long (500 ms) depolarizing pulses of increasing intensity was determined in six cells (Fig. 2). In order to determine the degree of spike firing adaptation or 'accommodation' in these interneurones we compared the spike frequency between the first and last interspike intervals (ISI) in each cell. The change in spike frequency between these two ISIs was 23·2 ± 7·3%. The frequency–current (f–I) relationship was then determined for different ISIs. In six of six cells the slope of the f–I curve for the first ISI gave a primary firing range of 626 ± 13 Hz nA^{-1} and a secondary firing range of 206 ± 18 Hz nA^{-1} (Fig. 2*B*). In the adapted state the slope of the f–I curve for ISI 22 was 413 ± 17 Hz nA^{-1} (Fig. 2*C*). These data confirm the findings of Lacaille & Williams (1990), who performed similar experiments using sharp microelectrode recordings from st. O–A interneurones.

In the next series of experiments we attempted to characterize pharmacologically the compliment of potassium currents responsible for both the spike repolarization and associated AHPs of st. O–A inhibitory neurones. The concentrations of pharmacological agents used were chosen to match the concentrations used in the accompanying voltage clamp study.

A 4-AP-sensitive conductance underlies a component of action potential repolarization

At low concentrations 4-AP (50 μM) prolonged the action potential duration of st. O–A inhibitory neurones by 53·3 ± 3·7% (control; 3·73 *versus* 5·72 ms, $n = 11$; in addition, the time to 50% repolarization was prolonged by 13·9 ± 0·8% (from 2·23 to 2·88 ms, $n = 11$; Fig. 3*A*). No significant changes were observed in the membrane potential (control, −60·6 mV, and 4-AP, −60·4 mV; $n = 11$), the slow AHP amplitude (18·1 *versus* 17·1 mV, $n = 11$), or the firing frequency (1·5 *versus* 1·5 Hz at −60 mV, $n = 11$). The spike broadening caused by 4-AP commenced at the peak of the action potential, suggesting

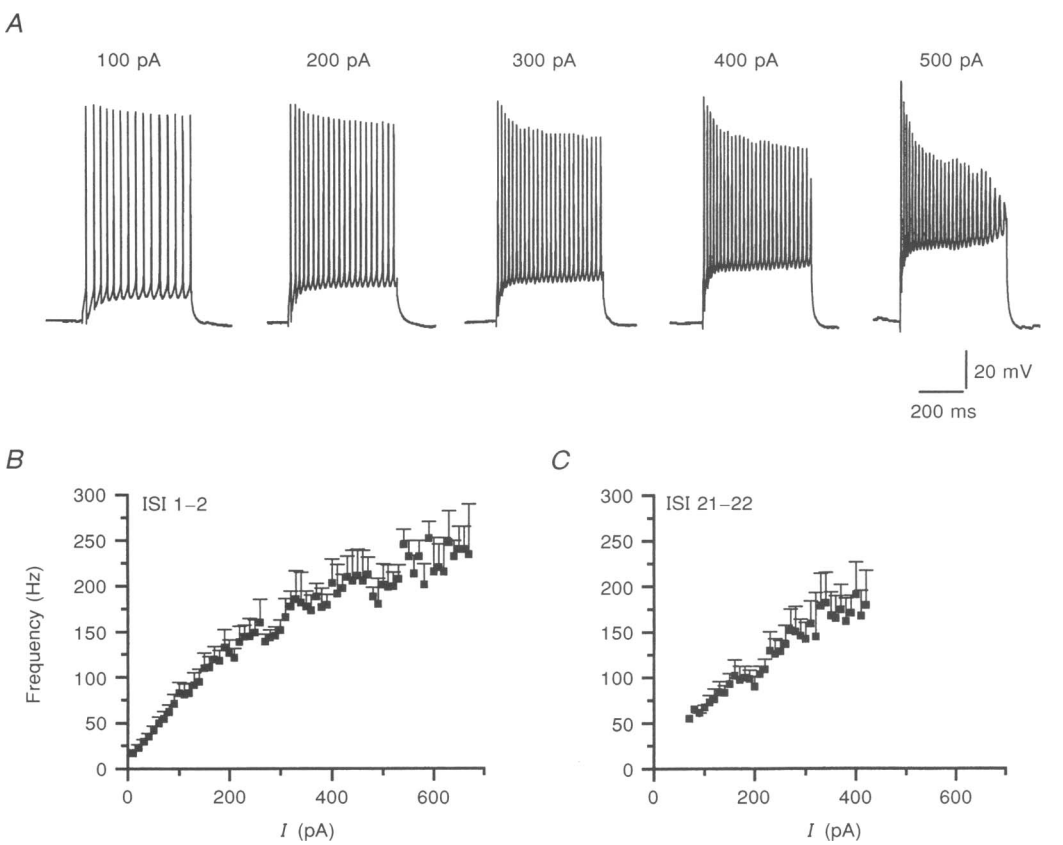

Figure 2. Repetitive firing properties of st. oriens–alveus interneurones

The extent of action potential accommodation and the frequency–current (f–I) relationship of st. O–A cells was determined by analysing the firing pattern in response to varying amplitude electrotonic pulses (10 pA increments, 500 ms in duration, 0·1 Hz). *A*, in this particular cell only modest action potential accommodation (<10%) was observed. *B* and *C* demonstrate the average f–I relationship determined for six st. O–A cells. The f–I relationship for ISI 1–2 was best fitted by two linear equations, suggesting a primary and secondary firing frequency (see text for details). ISI 21–22 had only a primary firing frequency and was best fitted by a single linear equation.

that the current blocked has a major role in spike repolarization. The effects of 4-AP were fully reversed on washout.

Data from our voltage clamp study demonstrated that low concentrations of 4-AP, in addition to their effect on the transient current, reduced a fraction of the sustained current, I_K. We next determined whether the effects of low concentrations of 4-AP on spike duration block a similar conductance to that blocked by low concentrations of TEA, as seen under voltage clamp conditions.

Following addition of 1 mM TEA the action potential duration was prolonged by 61 ± 3% over control. In the presence of 1 mM TEA, 50 μM 4-AP was without further effect on the action potential duration (63 ± 5% over control, $n = 4$; Fig. 3*B*). The difference between the two treatments was not significant ($P > 0{\cdot}05$), suggesting that a similar conductance involved in action potential repolarization is blocked by both low concentrations of 4-AP and TEA.

4-AP applied in the millimolar range has been used as a defining criterion for I_A in many types of neurones. In the accompanying study, we demonstrated a transient current in these interneurones selectively blocked by 4-AP in the millimolar concentration range ($IC_{50} = 1{\cdot}8$ mM). In the next series of experiments we set out to determine what action potential component is determined by activation of this transient current using concentrations of 4-AP shown to block ~50 and 80% of the transient current in these cells.

Application of 4-AP (2 mM), produced a membrane depolarization of 3·2 ± 0·2 mV ($n = 9$) concomitant with an increase in the spontaneous action potential firing rate (increased by 30·5 ± 3·7%, $n = 9$). Measurement of action potentials properties was determined following the restoration of the pre-drug holding potential by injection of hyperpolarizing current. In the presence of 2 mM 4-AP, the spike duration was 183% of the control (control, 3·6 ± 0·3 *versus* 6·9 ± 0·5 ms in 2 mM 4-AP; $n = 9$), and the time for 50% repolarization was increased by 57·8 ± 1·1% ($n = 9$; Fig. 3*A*). In addition, the peak spike amplitude was augmented by addition of 4-AP. At a concentration of 10 mM 4-AP the action potential duration was 221% that of control (control, 2·9 ± 0·3 *versus*

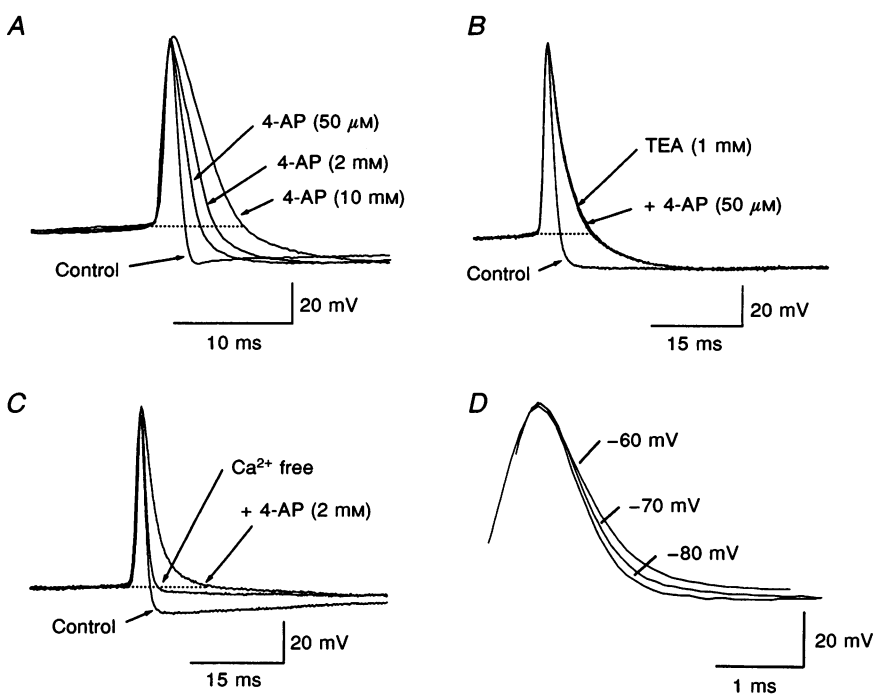

Figure 3. The effects of 4-AP on the repolarization of action potentials

A, superimposed records of a spontaneous action potential before (Control), 5 min after 50 μM 4-AP application, and 10 min in 2 mM and 10 mM 4-AP. The spike duration was prolonged by addition of 4-AP without affecting the AHP. *B*, the effects of low concentrations of 4-AP (50 μM) were prevented by the prior application of TEA (1 mM). *C*, in a different cell, perfusion with Ca^{2+}-free, Cd^{2+}-containing (100 μM) solution prolonged the action potential duration and eliminated the AHP. In the continued presence of Ca^{2+}-free, Cd^{2+}-containing solution, the effects of 4-AP on the spike duration were still observed. *D*, action potentials elicited from a variety of holding potentials show that the rate of repolarization was accelerated at more negative holding potentials. In this experiment a single cell was held at the three membrane potentials indicated, for a period of 5 s prior to a step depolarization to −55 mV. Alignment of the repolarization phase of the first action potential reveals an augmentation in the repolarization rate.

8·3 ± 1·2 ms in 10 mM 4-AP; $n = 3$) and the time for 50% repolarization was increased by 124 ± 0·9% ($n = 3$; Fig. 3*A*).

To determine whether 4-AP antagonized a Ca^{2+}-dependent component of spike repolarization, the above experiments were repeated in the presence of a nominally Ca^{2+}-free, Cd^{2+} (0·1 mM)-containing solution (subsequently referred to as 'Ca^{2+}-free'). In Ca^{2+}-free conditions the effects of 4-AP (2 mM, $n = 6$) were identical to those described above in the presence of Ca^{2+} (Fig. 3*C*), suggesting that 4-AP blocks a Ca^{2+}-independent conductance responsible for the repolarization of the action potential, presumably the transient current, I_A.

A transient current in hippocampal pyramidal neurones has also been shown to be blocked by dendrotoxin (Halliwell, Othman, Pelchen-Mathews & Dolly, 1986). In the present set of experiments, however, dendrotoxin (500 nM, $n = 5$) was without effect on action potential properties (data not shown), consistent with our voltage clamp data. Prior treatment of the perfusion system and recording chamber with 0·1% BSA in order to avoid unspecific adsorption of dendrotoxin yielded identical data ($n = 3$).

Our voltage clamp data showed that the transient current was partially inactivated at holding potentials close to the resting membrane potential. Conditioning steps to more negative membrane potentials, therefore, should deinactivate I_A channels, increasing the fraction of channels available for subsequent activation during an action potential. We measured the rate of spike repolarization following a period of hyperpolarization ($V_{hold} = -60$ to -80 for 5 s). In all cells tested the repolarization rate increased in action potentials generated from hyperpolarized levels ($V_{hold} = -60$ mV, 40·1 ± 3·4 mV ms^{-1} *versus* $V_{hold} = -80$ mV, 44·2 ± 3·8 mV ms^{-1}; $n = 10$, $P > 0·05$, Student's paired t test, Fig. 3*D*), further suggesting the involvement of I_A in spike repolarization.

A role for Ca^{2+}-activated K^+ currents in action potential repolarization

Large-conductance Ca^{2+}-activated K^+ channels (termed BK or I_C) have been identified in various neurones (reviews by Rudy, 1988, and Storm, 1990). Currents through these channels increase with a rise in $[Ca^{2+}]_i$ and membrane depolarization and have a role in spike repolarization in pyramidal neurones (Storm, 1990). To determine the contribution of I_C to the action potential repolarization and AHP in st. O–A cells, we tested the effects of iberiotoxin (IBTX), low concentrations of TEA and the Ca^{2+} channel blocker Co^{2+} on the action potential. IBTX is a potent and selective blocker of I_C (or BK) channels, with an inhibition constant (K_i) of ~250 pM (Galvez *et al.* 1990). Importantly, IBTX does not affect the cloned voltage-gated K^+ channels

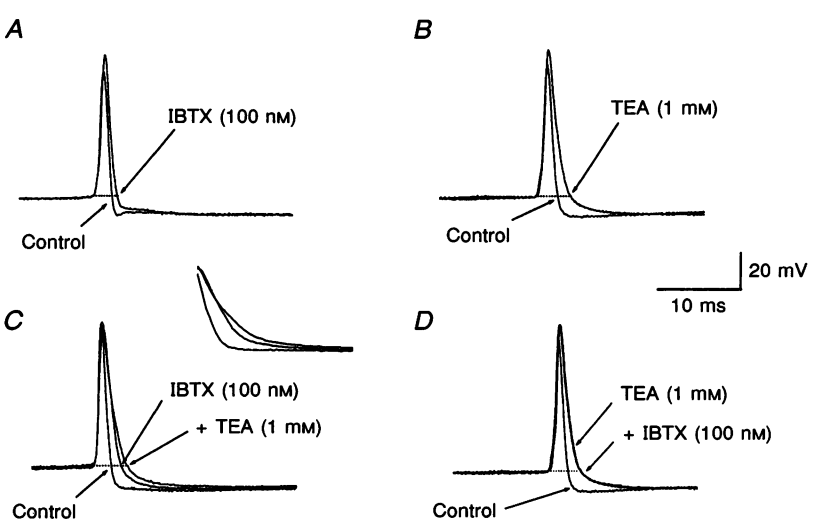

Figure 4. I_C underlies spike repolarization and the fast AHP

A and *B*, application of IBTX (100 nM) and TEA (1 mM) slowed the spike repolarization and in *A* the prominent fast AHP was eliminated by IBTX. The block by IBTX was uniform throughout the repolarization phase. In contrast, although TEA caused a similar degree of block at the 50% repolarization time point as IBTX, TEA caused a fractionally larger block on the total spike duration, suggesting an additional component was blocked by 1 mM TEA. Note the increase in the spike amplitude observed in the presence of both agents. *C*, in the presence of a maximal concentration of IBTX, TEA (1 mM) caused a further broadening of the action potential, without a significant change in the time to 50% repolarization. The additional component blocked by TEA results in the development of a distinct 'shoulder' in the spike repolarization (inset). In contrast IBTX applied after inclusion of TEA in the perfusate was without effect on the spike repolarization (*D*), confirming that TEA blocks an additional conductance to that blocked by IBTX.

sensitive to the related toxin charybdotoxin (Garcia & Kaczorowski, 1992). Likewise externally applied TEA at low concentrations ($K_i = 200\ \mu M$) effectively blocks channels with a similar conductance to those blocked by IBTX (Blatz & Magleby, 1987; Lancaster, Nicoll & Perkel, 1991).

In the presence of IBTX (100 nM), the 50% repolarization time and the spike duration were prolonged by 25·1 ± 3·0 and 37·8 ± 5·2% ($n = 18$), respectively (Fig. 4*A*). In addition, the amplitude of the spike increased moderately but significantly by 4·2 ± 0·9% ($n = 18$), suggesting a role for this conductance early in spike repolarization. No significant changes in the resting membrane potential, the slow AHP amplitude or duration were observed. Higher concentrations of IBTX (500 nM) were without further effect. The effects of IBTX were not reversed on washing with drug-free ACSF. Likewise, TEA (1·0 mM) prolonged the action potential 50% repolarization time by 23 ± 1·9% and the spike duration by 65·7 ± 6·8%, and increased the amplitude of the spike by 4·4 ± 0·4% ($n = 10$; Fig. 4*B*), but in a reversible manner. Like IBTX, 1 mM TEA did not significantly affect the membrane potential nor the amplitude and duration of the slow AHP. The degree of block by TEA (1 mM) on the spike 50% repolarization time was identical to the fraction blocked by IBTX. In contrast, the effects of these agents on the spike duration were quite different. The increase in duration caused by TEA was significantly greater than the block by IBTX, suggesting that at 1 mM, TEA may inhibit additional conductances to the current blocked by IBTX. To address this question, we first applied IBTX (100 nM) followed by simultaneous application of IBTX plus 1 mM TEA. IBTX (100 nM) alone increased the duration of spike by 40·4 ± 9·3% ($n = 4$), while IBTX plus 1 mM TEA further prolonged the spike duration by an additional 23·1 ± 5·8% without an effect on the spike 50% repolarization time ($n = 4$; Fig. 4*C*). In contrast, when 1 mM TEA was added first, the duration of the spike increased by 55·7 ± 7·0% ($n = 4$); however, no further increase in spike duration was observed by the combination of 1 mM TEA plus IBTX (5·03–5·10 ms, $n = 4$; Fig. 4*D*). These data suggest that, in addition to blocking the same conductance as IBTX, TEA blocks a conductance underlying the late component of spike repolarization.

Further evidence for a role of I_C in spike repolarization was obtained from experiments using Ca^{2+}-free, Co^{2+} (1 mM)-containing external solution. When the cells were bathed in this solution, the action potential duration was increased 39·2 ± 4·2% ($n = 4$). No further broadening of the action potential was observed when IBTX (100 nM) was added to the Ca^{2+}-free, Co^{2+}-containing solution (data not shown). In contrast, 4-AP (2 mM) further prolonged the action potential in the Ca^{2+}-free medium by 130 ± 10·7% ($n = 4$; Fig. 3*C*), suggesting that both a 4-AP-sensitive and Ca^{2+}-activated K^+ currents mediate the repolarization phase of the action potential.

Effects of TEA

In order to examine the role of the TEA-sensitive, sustained outward current, I_K, identified in the previous paper, we applied TEA (1–30 mM) to interneurones under conditions which minimized the influence of Ca^{2+}-activated K^+ conductances (0 mM Ca^{2+}, 0·1 mM Cd^{2+}-containing solution). TEA (1–30 mM) dose-dependently prolonged the action potential duration (Fig. 5). In contrast to the effects

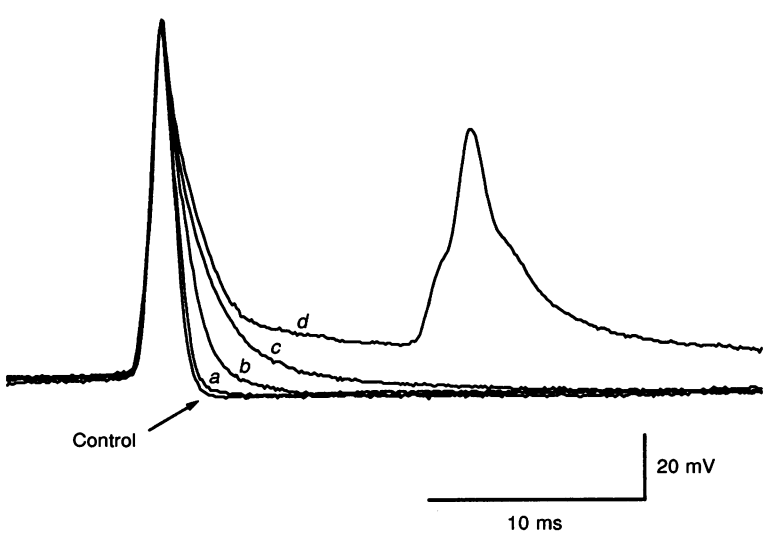

Figure 5. TEA blocks a late component of spike repolarization

In a Ca^{2+}-free solution, addition of TEA (1–30 mM) causes a progressive prolongation of the action potential duration. The action potential component blocked by TEA commences at the action potential peak; however, maximal block is seen late in the spike repolarization, resulting in a shoulder in the repolarization phase. In 30 mM TEA an aborted spike was observed on the slow repolarization phase. *a*, 1 mM TEA; *b*, 5 mM TEA; *c*, 15 mM TEA; *d*, 30 mM TEA.

of IBTX and 4-AP, the conductance blocked by higher concentrations of TEA resulted in the appearance of a distinct shoulder on the spike repolarization (Fig. 5). Analysis of both the spike duration and the times to 50% spike repolarization revealed the time-dependent effects of TEA. In control conditions and in the presence of 1, 5, 15 and 30 mM TEA the times to 50% spike repolarization were, 2·1 ± 0·05, 2·3 ± 0·03 (6·2% increase), 3·3 ± 0·2 (58·6%), 4·2 ± 0·1 (100%) and 4·8 ± 0·1 ms (127%) ($n = 3$), respectively. In contrast, the duration of the action potential under the same conditions were, 3·1 ± 0·05, 3·4 ± 0·1 (10% increase), 6·9 ± 0·4 (122%), 7·8 ± 0·6 (152%) and 20·2 ± 4·8 ms (860%) ($n = 3$). In one additional cell, application of TEA resulted in a prolonged after-depolarization, which precluded an accurate estimation of the times to 50% repolarization or the duration of the action potential. TEA was without any effect on any of the other action potential parameters measured. The effects of TEA were fully reversed on wash with drug-free saline.

These data demonstrate that, in addition to I_A, spike repolarization is mediated by conductances sensitive to IBTX and low concentrations of TEA, possibly I_C and a third, relatively slowly developing, TEA-sensitive conductance, presumably the sustained outward current, I_K.

Fast and slow duration after-hyperpolarizations

We next performed a series of experiments aimed at determining what conductances underlie the AHP of action potentials in st. O–A interneurones. Addition of nominally Ca^{2+}-free, Cd^{2+}-containing (0·1 mM) medium, resulted in a marked alteration of the action potential firing pattern. Under these conditions, cells tended to fire in short trains of action potential punctuated by periods of relative inactivity (Fig. 6*Bb*). Ca^{2+}-free medium reduced the amplitude of the fast AHP by 92·4 ± 5·9% ($n = 4$) and the slow AHP by 76·3 ± 4·6% ($n = 14$) concomitant with the increase in the spike duration (40·1 ± 4·6%, $n = 18$; Fig. 6*B*). In addition the duration of the slow AHP was attenuated by 28·6 ± 6·2% ($n = 18$). In st. O–A inter-

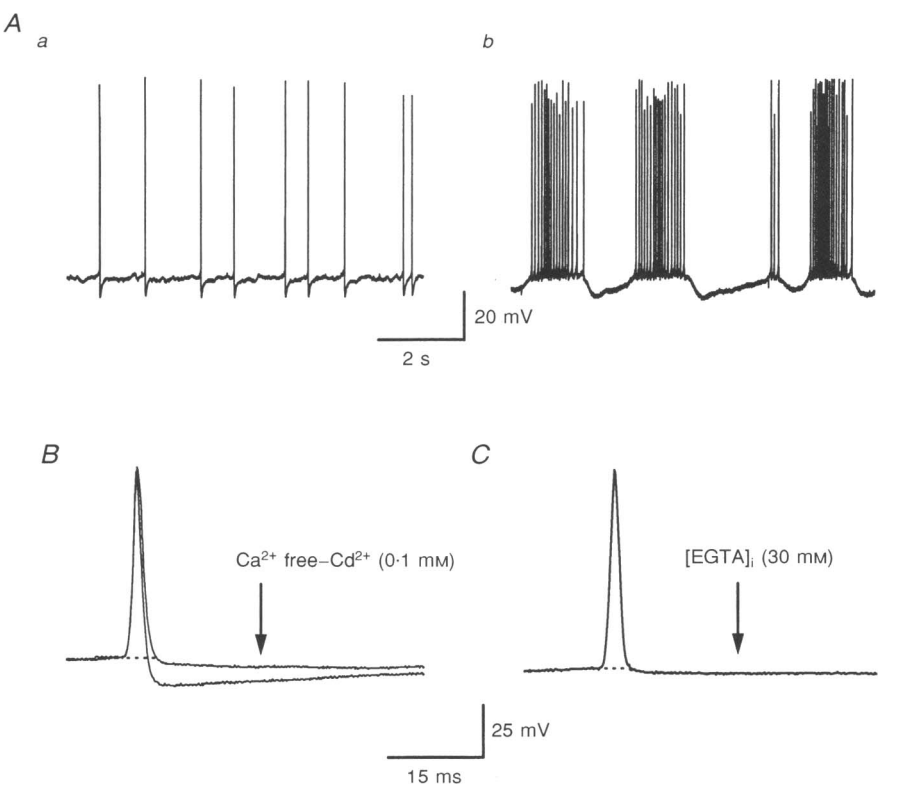

Figure 6. The spike AHP is determined by Ca^{2+}-dependent components

At a holding potential of −60 mV, irregular, single action potential firing was observed under control conditions (*A a*). On introduction of Ca^{2+}-free, Cd^{2+}-containing (0·1 mM) solution cells commenced burst firing of prolonged trains of action potentials (*A b*). Bursts of action potentials were of varying duration. Note the lack of any prominent AHP in the action potentials in Ca^{2+}-free conditions. *B*, single action potentials obtained from the same cell as shown in *A* and *B* reveal the marked attenuation of the AHP in Ca^{2+}-free conditions. *D*, in a separate cell, the calcium ion chelator, EGTA (30 mM), was included in the recording pipette solution. Under these conditions no prominent AHP was observed following single action potentials demonstrating the calcium dependence of the AHP. The external solution contained 1·5 mM Ca^{2+}.

neurones the presence of a slowly depolarizing pacemaking potential makes an accurate determination of the AHP properties difficult. Therefore we are likely to have underestimated the degree of block in Ca^{2+}-free solutions.

Further evidence for a role of Ca^{2+}-dependent K^+ conductances in the AHP came from experiments using electrodes containing high concentrations of the Ca^{2+} chelator EGTA (30 mM). In these experiments the AHP was markedly attenuated (Fig. 6*C*). The average amplitude and duration of the slow AHP in these EGTA-filled cells were 5·4 ± 2·1 mV and 150 ± 12 ms, respectively ($n = 3$; cf. AHP properties in Table 1). In none of the three cells tested was there any evidence of a fast component of the AHP. Therefore, EGTA appears to be able to adequately buffer $[Ca^{2+}]_i$ to block generation of the AHP.

To characterize further the specific conductances underlying the AHP, we examined the effects of a variety of agents known to block Ca^{2+}-activated K^+ conductances. Initial experiments were performed with IBTX and apamin, each of which is specific in blocking large and small conductance Ca^{2+}-activated K^+ channels, respectively. IBTX (100 nM) selectively reduced the amplitude of fast AHP from an average of −14·1 mV to −5·8 mV (59% reduction, $n = 6$; Fig. 4*A*). The effect of IBTX on both the repolarization and the fast AHP suggests that a common conductance underlies both events, namely I_C. In contrast, IBTX reduced the slow AHP amplitude by only 17·7% concomitant with a slight reduction in the AHP duration (12·5%; $n = 11$). Similar results were also observed with 1 mM TEA. The amplitude of the fast AHP was reduced by 93·2 ± 10·4% by TEA ($n = 7$; see Fig. 4*B*). Like IBTX, the slow AHP was not significantly affected by 1 mM TEA ($n = 7$; Fig. 5*B*). These experiments suggest that the fast AHP is mediated by the IBTX-sensitive conductance also involved in spike repolarization, presumably I_C.

Apamin reduces a component of the slow AHP

Removal of extracellular Ca^{2+} reduced the amplitude of the slow AHP by ~76%. However, this slow AHP was largely unaffected by IBTX and low concentrations of TEA, suggesting the participation of another Ca^{2+}-activated K^+ conductance. To determine whether the low conductance Ca^{2+}-activated K^+ channels which underlie I_{AHP} were involved in the generation of the slow AHP, we tested the effects of apamin, a selective antagonist of these channels. Apamin (100 nM) reduced the slow AHP amplitude and duration by 26·4 and 33·8%, respectively (Fig. 7*A*, $n = 8$). At a concentration of 1 μM, apamin reduced the amplitude of the slow AHP by 50% ($n = 3$). No significant changes were observed in action potential duration or the resting membrane potential in the presence of apamin, suggesting a specific role for apamin-sensitive channels in the slow AHP. On occasion, when the fast component of the AHP was present, apamin caused an apparent enhancement of this fast AHP, which presumably results from the attenuation of the slow AHP conductance, usually masking this AHP component (data not shown). In all but one cell the effects of apamin were not reversed on washout of the drug.

An increase in cell excitability was observed following apamin application, despite the lack of effect of apamin on the resting membrane potential. During a sustained depolarization pulse (500 ms, 50 pA), the number of spikes increased by 65% in the presence of 100 nM apamin (Fig. 7*B*; $n = 6$). In some neurones (3/7), we also observed an increase in the rate of spontaneous action potential firing without a detectable change in the membrane potential. These experiments indicate that apamin-sensitive I_{AHP} not only participates in the termination of single action potential firing but also plays a role in the frequency of rapid discharges of action potentials following a small depolarizing step.

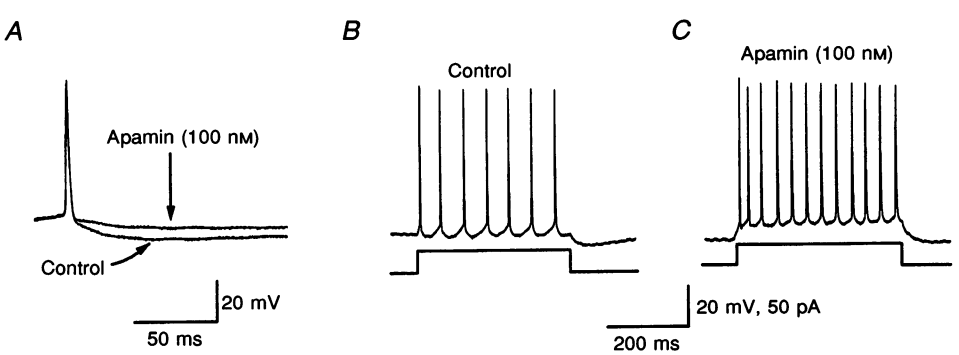

Figure 7. Apamin selectively attenuated the slow AHP and augmented the firing frequency

A, superimposed action potentials before and after addition of apamin (100 nM). Apamin reduced the amplitude of the slow AHP without affecting the duration of the spike or the initial component of the AHP. *B* and *C*, apamin enhanced the action potential firing frequency during a step depolarization. The interspike interval was reduced following the application of apamin suggesting that the rate of repetitive firing was determined in part by the properties of the slow AHP.

The effects of carbachol, monoamines and 8-bromo-cAMP on the slow AHP

We next examined the effects of the muscarinic receptor agonist carbachol on the slow AHP. Carbachol is known to affect selectively the medium duration AHP (50–100 ms) of CA1 pyramidal neurones by blocking I_M (Storm, 1989). Carbachol (10 μM) depolarized the membrane potential by $2{\cdot}4 \pm 0{\cdot}3$ mV ($n = 8$) concomitant with an increase in the firing rate ($1{\cdot}2 \pm 0{\cdot}1$ Hz at a V_{hold} of -65 mV; $3{\cdot}4 \pm 0{\cdot}2$ Hz in carbachol; $n = 8$). Hyperpolarizing current injection was then applied to restore the membrane potential to the control value. The amplitude and duration of the slow AHP were reduced by $20{\cdot}0 \pm 3{\cdot}4$ and $21{\cdot}3 \pm 2{\cdot}2\%$ ($n = 8$), respectively. No significant change was observed in the spike duration or the amplitude of the fast AHP ($n = 4$). The effects of carbachol were reversibly blocked by prior treatment with the muscarinic receptor blocker atropine (100 μM, $n = 4$). In order to determine whether carbachol blocked another component in addition to the slow AHP, the above experiments were repeated in nominally Ca^{2+}-free medium. When the cells were bathed in Ca^{2+}-free solution, the amplitude of the slow AHP was reduced by 77% ($n = 4$). No additional reduction in the AHP or effect on any spike parameter was observed when carbachol (up to 50 μM) was added (data not shown, $n = 4$).

In hippocampal pyramidal cells, noradrenaline and other monoamines block a major component of the slow duration AHP (Storm, 1987) through a protein kinase A-mediated mechanism (Pedarzani & Storm, 1993). Perfusion with histamine (10 μM) reduced the amplitude of the slow AHP by $17 \pm 1{\cdot}2\%$ in st. O–A interneurones ($n = 5$). Similar results were obtained with the cAMP analogue 8-bromo cAMP ($n = 4$) and noradrenaline ($n = 5$, data not shown).

DISCUSSION

As described for most central neurones, the temporal overlap of a variety of K^+ conductances shapes the repolarization and AHPs of identified st. O–A inhibitory interneurones. St. O–A cells, however, displayed only two temporally distinct AHPs, in contrast to the four after-potentials recorded from CA1 pyramidal cells (Storm, 1987). In addition, no evidence for a prominent after-depolarization was observed in these cells. The underlying K^+ conductances are active within a brief temporal 'window' and the entire trajectory of a single action potential and associated AHPs is complete in ~200 ms compared with >1 s in CA1 pyramidal cells (Storm, 1987). The kinetics of this action potential in st. O–A cells undoubtedly is determined by the types of K^+ channel subunit expression and distribution on these cells. These two studies (see also Zhang & McBain, 1995) are to our knowledge the first analysis of K^+ currents and conductances expressed by an identified population of hippocampal interneurones.

The duration of the action potential was determined by the combination of three independent conductances, the IBTX-sensitive, Ca^{2+}-dependent K^+ conductance, I_C, a 4-AP-sensitive conductance, I_A, and a TEA-sensitive, slowly activating sustained conductance, I_K. I_A and I_C were both significantly activated at the action potential peak. The action potential amplitude was often augmented in the presence of these channel blockers, suggesting that the action potential amplitude is normally attenuated, by the rapid activation of these conductances.

The transient A-current has been described as having a central role in spike repolarization in a variety of central neurones (Storm, 1990; Halliwell, 1990). In our voltage clamp experiments the transient current of st. O–A cells was activated with a time to peak of ~2·0–3·0 ms at room temperature. In the present experiments, the action potential time to peak was ~1·5 ms, suggesting that an appreciable fraction of the transient current could be activated during a single spike. The rapid and near complete activation of this current in this time frame underscores the important role played by I_A in action potential repolarization in st. O–A cells. Our voltage clamp data showed that the time for recovery of inactivation of the transient current had a time constant of ~140 ms. The AHP duration of action potentials in this study was in excess of 220 ms. This would suggest that an adequate recovery of I_A occurs between each action potential to permit near complete activation in subsequent spikes. It should be noted that the kinetic and voltage-dependent properties of I_A, are remarkably similar to those of the transient current described in acutely dissociated pyramidal cells (Numann, Wadman & Wong, 1987), suggesting that the brevity of the action potential in st. O–A cells cannot arise from I_A alone.

The sustained outward current, I_K, recorded under voltage clamp, activated rapidly, reaching ~80% of its peak at 1·5 ms and reaching maximal amplitude by ~80 ms. It is likely that a significant fraction of the sustained current will be available during a single action potential in st. O–A cells. TEA blocked the spike repolarization at concentrations identical to those which selectively block the sustained current in the accompanying paper. Consistent with a block of the sustained current by TEA, the component of the action potential blocked by TEA occurred late in the repolarization phase, suggesting that the sustained component is not involved in the initiation of spike repolarization, but rather in the control of repetitive firing and the action potential frequency.

Both the action potential repolarization and the fast AHP in st. O–A interneurones were sensitive to low concentrations of TEA ($\leqslant 1$ mM), IBTX and the removal of Ca^{2+}_o. Therefore, action potential repolarization and the generation of the fast AHP in st. O–A cells are mediated predominantly by the Ca^{2+}-activated K^+ current (I_C).

Antagonism of this conductance significantly augmented the action potential amplitude, suggesting appreciable activation of I_C at the action potential peak. Our voltage clamp data show that the Ca^{2+}-dependent component of the outward current activates rapidly, consistent with a role for this current early in spike repolarization. This is in marked contrast to the role of I_C in action potential repolarization in pyramidal neurones. In these cells the conductance mediated by I_C is responsible for repolarization of only the last two-thirds of the action potential (Storm, 1987). The lack of time- or voltage-dependent inactivation of I_K, in these st. O–A cells, and a rapid rate of activation are likely to be very important determinants of st. O–A interneurone action potential phenotype and their repetitive firing properties. Since I_C is rapidly activated by both membrane potential depolarization and an increase in intracellular Ca^{2+} concentration, it is likely to serve as an effective brake to the overexcitation of st. O–A cells.

The block of both the fast and slow AHPs in Ca^{2+}-free conditions suggests that the activation of the underlying K^+ conductances depends on $[Ca^{2+}]_i$ elevation, presumably through voltage-dependent Ca^{2+} channels and the observed calcium spike. In st. O–A cells a two component AHP was observed; however, the relative proportions of these two AHP components differed from cell to cell and was not consistently observed in one morphological cell type over the other. The temporal and pharmacological properties of the slow AHP component were identical, irrespective of the presence or absence of the fast AHP, suggesting that the overall properties of this AHP are independent of the fast AHP component.

The sensitivity of the slow AHP to apamin and monoamines, and its insensitivity to block by IBTX and low concentrations of TEA, indicates that distinct conductances underlie both AHP events. The slow AHP of st. O–A cells is temporally distinct from both the medium duration and late slow AHPs described in pyramidal cells (Storm, 1987, 1989). In pyramidal and GH3 cells, the conductance underlying the slow AHP results from the Ca^{2+}-activated K^+ current, I_{AHP} (Storm, 1987; Lang & Ritchie, 1988). This current is slowly activating and TEA resistant, and displays little voltage dependence. I_{AHP} in bullfrog ganglion cells is maximally activated immediately following a single spike, with a time course similar to the slow AHP observed in the st. O–A interneurones. In hippocampal pyramidal neurones, the amplitude of the slow AHP is proportional to the amount of Ca^{2+} entering during an action potential discharge, such that the AHP is proportional in size to the frequency and duration of action potential firing (Lancaster & Adams, 1986). Thus, the short duration of this event in st. O–A cells may simply be determined by the relative brevity of the action potential and consequently the limited amount of Ca^{2+} entry occurring during a single spike. Consistent with this hypothesis is the observation that, following a high frequency burst of action potentials, a slow AHP of several seconds in duration was observed (L. Zhang & C. J. McBain, unpublished observation). In our cells, a considerable fraction of the slow AHP was blocked by apamin, similar to the effect of apamin on the slow AHP of GH3 cells (Lang & Ritchie, 1988) but in contrast to that seen in pyramidal neurones (Lancaster & Nicoll, 1987). Apamin has been shown to block I_{AHP} current in neocortical cells (Schwindt *et al.* 1988), in spinal motoneurones and in hypothalamic magnocellular neurosecretory cells (Zhang & Krnjevic, 1987; Bourque & Brown, 1987), but not in hippocampal pyramidal cells (Lancaster & Nicoll, 1987). Interestingly, autoradiography studies of K^+ channel binding in the rat brain have demonstrated the presence of [^{125}I]-apamin-sensitive binding sites in cells of st. O–A (Gehlert & Gackenheimer, 1993) supporting a role for an apamin sensitive conductance in these cells. In the presence of apamin the action potential frequency during a sustained depolarization was increased, consistent with a role for I_{AHP} in determining the rate of repetitive action potential firing. In addition, following the block of the slow AHP, many cells fired in rhythmical bursts of action potentials, suggesting a prominent role for these Ca^{2+}-dependent conductances in determining the firing pattern phenotype.

Finally, these data will be of considerable use in establishing reliable models of interneurone and hippocampal physiology. Previous models have modified data obtained from pyramidal cells to obtain spontaneous action potential firing properties reminiscent of interneurones, in the absence of sufficient voltage clamp data (e.g. Traub, Miles & Buzsaki, 1992). In the study of Traub & Miles (1991) interneurones were modelled by removing both calcium and calcium-dependent currents from their pyramidal cell model. While both models have provided useful information on the role of inhibitory cells in the hippocampal network, the absence of adequate voltage and current clamp data from specific interneurone populations makes the role of interneurones in these models unreliable. It is hoped that this characterization of the role of voltage-dependent currents in specific interneurone populations will facilitate the incorporation of these cell properties into existing models.

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