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CONTROL OF THE REPETITIVE DISCHARGE OF RAT CA1 PYRAMIDAL NEURONES *IN VITRO*

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SUMMARY

1. Experiments using intracellular recording techniques were performed on rat hippocampal neurones *in vitro*, to study the discharge properties of these cells.

2. When CA1 pyramidal cells were excited by injecting long depolarizing current pulses (approximately 600–800 ms), they responded with an initial rapid action potential discharge which slowed, or accommodated, and then stopped after 200–300 ms. The train of action potentials was followed by a hyperpolarization which was due primarily to calcium-activated potassium conductance ($G_{K(Ca)}$). The amplitude of this hyperpolarization increased with an increasing number of action potentials in the initial discharge.

3. Blocking the calcium-activated potassium conductance, by injecting EGTA into the cell, by bathing the cell in cadmium, a calcium channel blocker, or by bathing the cell in calcium-free medium, reduced the after-hyperpolarization (a.h.p.) and accommodation such that the frequency of action potential discharge increased and the duration of this discharge was prolonged. Blocking the calcium-activated potassium conductance had a greater effect on discharge frequency later in the action potential train, as late interspike intervals were shortened more than early ones by the application of cadmium or of calcium-free medium. This was presumably because the calcium-activated potassium conductance was more developed later in the train.

4. Accommodation was not completely abolished in the absence of calcium and presence of cadmium, suggesting that other factors, in addition to calcium-activated potassium conductance, contributed to this process. This remaining accommodation was reduced by low doses of carbachol, suggesting that the M-current also plays a role in accommodation.

5. We conclude that accommodation of the action potential discharge of hippocampal pyramidal cells may be regulated by at least two potassium currents: the calcium-activated potassium current and the M-current. Both of these currents are turned on during excitation of the neurone and act in an inhibitory manner on that neurone to limit further action potential discharge.

INTRODUCTION

A characteristic feature of many neurones is that they adapt, or accommodate, their responses to impinging stimuli. In a number of cases, slow potassium conductances have been associated with adaptation (cf. Connor & Stevens, 1971). In frog sympathetic ganglia, the M-current, which is a non-inactivating, voltage-sensitive potassium current, has clearly been established to play a role in this phenomenon (Adams, Brown & Constanti, 1982). Additionally, a number of studies have suggested that calcium-activated potassium conductance ($G_{K(Ca)}$) may also contribute to accommodation (Meech, 1978; Partridge, 1982). Direct experimental support for the involvement of calcium-activated potassium conductance comes from studies on invertebrate neurones. In crustacean stretch receptors, accommodation is enhanced by intracellular calcium injection, and reduced by agents which block potassium conductances or block increases in intracellular calcium (Ottoson & Swerup, 1981, 1982). Lewis & Wilson (1982) have shown that early adaptation in *Aplysia* neurones is associated with calcium influx and with a potassium current. Conditions which prevented the rise in intracellular calcium blocked both the potassium current and the early adaptation.

In spinal motoneurones repetitive firing frequencies are related to the duration of after-hyperpolarizations (a.h.p.s) (Kernell, 1965; Calvin & Schwindt, 1972; Schwindt & Calvin, 1973), which are largely due to calcium-activated potassium conductance (Krnjevic, Puil & Werman, 1978). Mathematical models of motoneurone firing suggest that calcium-activated potassium conductance can account for much of the accommodation of cell discharge (Baldissera & Gustafsson, 1974*a*, *b*; Barrett, Barrett & Crill, 1980). However, a major limitation of these *in vivo* studies on motoneurones is the difficulty of selectively blocking the calcium-activated potassium conductance. It has recently been reported that application of calcium channel blockers to frog motoneurones *in vitro* (Buchert-Rau & Sonnhof, 1982), and to cells in the myenteric plexus (Grafe, Mayer & Wood, 1980), blocks a.h.p.s due to calcium-activated potassium conductance and increases repetitive firing.

In the present *in vitro* study, experiments were performed to determine the mechanisms which control repetitive firing in hippocampal pyramidal neurones. We have found that blocking calcium-activated potassium conductance with either cadmium, calcium-free medium or intracellular injection of EGTA markedly attenuates, but does not abolish, accommodation. This residual accommodation is reduced by the muscarinic agonist carbachol. Since carbachol is known to block the M-current in these cells (Halliwell & Adams, 1982), this current may also contribute to accommodation. Some preliminary results have appeared in previous reports (Madison & Nicoll, 1982, 1983).

METHODS

The methods used in these experiments have been described previously (Alger & Nicoll, 1982; Nicoll & Alger, 1981). Briefly, rat hippocampal slices were cut and placed in a holding chamber for at least 1 h. A single slice was then transferred to the recording chamber and held between two nylon nets, submerged beneath a continuously superfusing medium. The standard medium consisted of (mM): NaCl, 116·4; KCl, 5·4; $MgSO_4$, 1·3; $CaCl_2$, 2·5; NaH_2PO_4, 1·0; $NaHCO_3$, 26·2;

glucose, 11. For some experiments a calcium-free medium was used. The composition of this medium was the same as that of the standard medium except that $CaCl_2$ was omitted and the concentration of $MgSO_4$ was raised to 12 mM. Media containing higher concentrations of magnesium were tested, but these tended to render the neurones unresponsive, while in lower concentrations the membrane potentials of the neurones became unstable. The temperature of the bath was maintained between 29 and 31 °C unless otherwise noted. Drugs were applied either by addition to the bathing medium or by injection into the cells through the recording electrode. Solutions were prepared from concentrated stocks immediately before use. For bath application, a latching solenoid valve was used which allowed for rapid switching between superfusate solutions without disturbing the stability of the intracellular recording. Agents applied in this manner were: cadmium chloride, tetrodotoxin (TTX) and carbamylcholine chloride (carbachol). Ethyleneglycol-bis (β-aminoethyl-ether) *N*,*N*′-tetraacetic acid (EGTA) was added to the electrolyte in the recording electrode (0·2 M in 2 M-potassium methylsulphate) for intracellular injection. All drugs were obtained from Sigma Chemical Company.

Conventional intracellular recording techniques were used. Intracellular electrodes were pulled from 'omega dot' glass tubing (1·2 mm o.d., Glass Company of America), and filled with 2 M-potassium methylsulphate ($KMeSO_4$, ICN Pharmaceuticals). The resistance of these electrodes was 80–120 MΩ. Neurones were stimulated using a standard bridge circuit (WPI model 701) by passing step depolarizing current pulses. In some cells, direct current was applied through the micro-electrode to maintain the membrane potential at the control value. This procedure is referred to in the text as manual voltage clamp. Frequency measurements reported in this study were obtained by taking the reciprocals of the intervals between action potentials (interspike intervals). The results presented in this paper are based on data from recordings of over 140 neurones. Results were recorded on a chart recorder (Gould Instruments, model 2200), or were photographed directly from the oscilloscope screen (Grass Kymograph Camera).

RESULTS

CA 1 pyramidal cells responded to square depolarizing current pulses with a rapid action potential discharge, followed by a period of silence. This silent period was observed in the vast majority (135/141) of cells studied. Increasing the size of the depolarizing current from threshold increased the number of action potentials in this initial train until a maximum of six to ten was reached. The frequency of firing in this initial train declined, or accommodated, with each successive interspike interval until the discharge stopped completely. That the cell cannot maintain its firing rate to a steady depolarizing input would seem to indicate the presence of a factor or factors which serve to counteract the excitatory drive of the stimulus. Indeed, the accommodation of action potential discharge frequency was usually associated with a pronounced 'sag' in the voltage trace (Fig. 1*A*). By superimposing a long- and short-duration current pulse, it was evident that this 'sag' was due to an after-hyperpolarizing potential (a.h.p.) which developed during the repetitive discharge (Fig. 1*A*). This suggests that the current underlying the a.h.p. may be involved in producing the accommodation.

The origin of the a.h.p.

The a.h.p. that follows a depolarizing stimulus in pyramidal cells has been shown to be due largely to a calcium-activated potassium conductance (Alger & Nicoll, 1980; Hotson & Prince, 1980; Schwartzkroin & Stafstrom, 1980; Gustafsson & Wigstrom, 1981). It has been reported previously that a regenerative action potential is required for activation of the a.h.p. (Hotson & Prince, 1980; Gustafsson & Wigstrom, 1981). Our results are in agreement with this. Increasing the number of action potentials

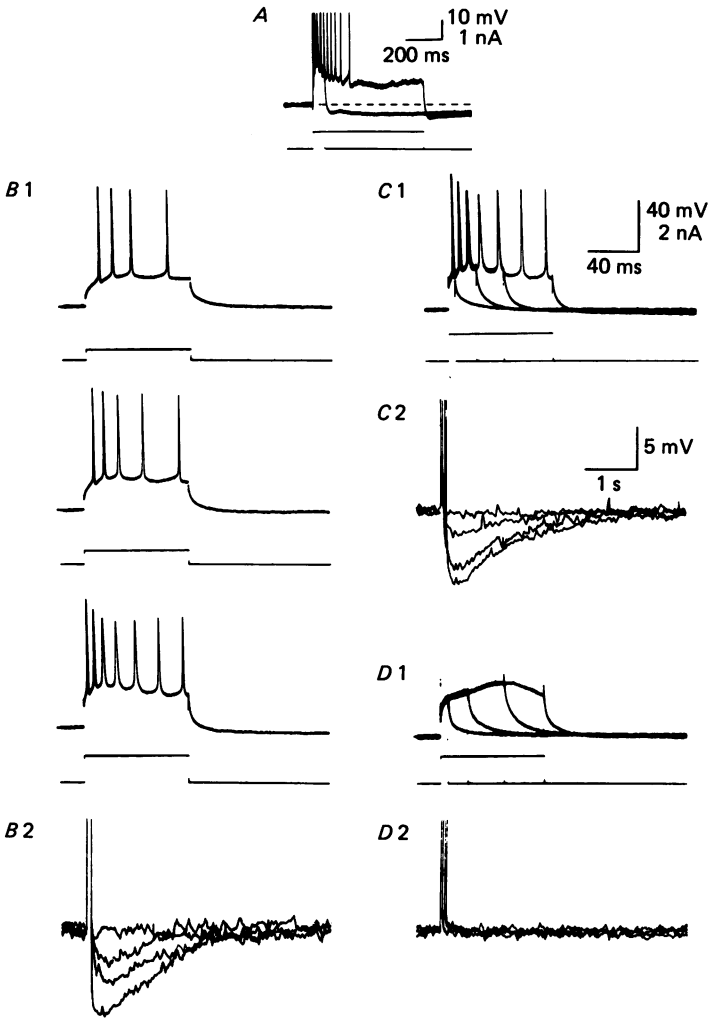

Fig. 1. Accommodation in hippocampal pyramidal cells is accompanied by a calcium-activated potassium a.h.p. *A*, film records of the response of a hippocampal pyramidal cell to depolarizing current passed through the recording electrode. Responses of the cell to a long (approximately 600 ms) and a short (approximately 60 ms) depolarizing current are shown superimposed. Action potentials in this record are truncated. Membrane potential −55 mV. *B*1, film records of the response of a pyramidal cell to depolarizing current pulses of increasing amplitude. Current trace is positioned below the voltage trace. *B*2, superimposed tracings of chart records corresponding to *B*1 (slower sweep) showing the a.h.p. that follows the current pulse. The smallest a.h.p. shown in the superimposed tracings is the response to a stimulus giving a single action potential (film record not shown). Membrane potential −56 mV. *C*1, film records of the response of another pyramidal cell to superimposed depolarizing current pulses of fixed amplitude and increasing duration. *C*2, tracings of chart records corresponding to *C*1. *D*, records from the same cell as in *C* taken 11 min after addition of TTX (1 μM) to the superfusing medium. *D*1, film records of the cell's response to current pulses of same amplitude and similar duration as those in *B*1. *D*2, tracings of chart records corresponding to *D*1. The amplitude of the current pulse was below threshold for calcium action potentials, so no a.h.p. was elicited. Membrane potential in *C* and *D* −56 mV. The calibrations in *B* and *D* are the same as in *C*. In all Figures, the current monitor record is positioned below the voltage record.

elicited by a stimulus, either by increasing the amplitude of the depolarizing pulse (Fig. 1*B*1), or by increasing the stimulus duration (Fig. 1*C*1), caused a corresponding increase in the size of the a.h.p. (Fig. 1*B*2 and *C*2). Addition of 1 μM-TTX, a sodium channel blocker, to the superfusing medium abolished the action potentials elicited by the depolarization and eliminated the a.h.p. (Fig. 1*D*).

Regulation of repetitive firing of pyramidal neurones

The slow time course of the a.h.p. and the fact that it summates with successive action potentials make the calcium-activated potassium conductance a prime candidate for a role in accommodation. Specifically, when the neurone is excited, each action potential adds more calcium-activated potassium conductance to the underlying membrane conductance, which acts to slow and eventually to stop further discharge. To test this possibility directly, we injected the calcium chelating agent EGTA (0·2 M in 2 M-$KMeSO_4$) into cells through the recording electrode. This procedure is known to reduce calcium-activated potassium a.h.p.s in these cells (Alger & Nicoll, 1980; Schwartzkroin & Stafstrom, 1980; Hablitz, 1981; Newberry & Nicoll, 1983), presumably by binding intracellular calcium. Fig. 2*A* compares the effect of EGTA on the a.h.p. and on action potential accommodation. Over a period of several minutes, EGTA leaked out of the electrode and the amplitude of the a.h.p. was reduced (Fig. 2*A*2). At the same time, action potential accommodation was decreased (Fig. 2*A*1). The cells injected with EGTA showed no silent period at the end of a long depolarizing current pulse, and the frequency of the early discharge was increased ($n = 10$).

Since EGTA leaked out of the electrode quite readily, it was often difficult to obtain control records before the EGTA had taken effect. Therefore, we attempted to block the calcium-activated potassium conductance selectively, by applying a calcium channel blocker to the solution, or by bathing the preparation in calcium-free medium, so that records before and after the blockade could be compared in the same cell. Cadmium chloride at a concentration of 100 μM was effective in blocking calcium entry into the cell, since it abolished the calcium action potential elicited in the presence of 1 μM-TTX and 5 mM-tetraethylammonium (not shown). Cadmium caused a marked and reversible decrease in the amplitude of the calcium-activated potassium a.h.p. and simultaneously reduced accommodation of action potential discharge (Fig. 2*B*1 and *B*2). Bathing the preparation in calcium-free medium produced results similar to cadmium (not shown).

The cadmium-induced increase in repetitive discharge was seen over a wide range of stimulus intensities, though the increase in firing was more pronounced at higher stimulus strengths (Fig. 3*A* and *B*). Cadmium abolished the silent period at the end of the stimulus and increased the frequency of discharge. This increase in frequency occurred mostly in the later interspike intervals. Indeed, the first interspike interval was little affected by cadmium over a large range of stimulus intensities, while the third interspike interval was markedly shortened (Fig. 3*C*). During the course of a single depolarizing stimulus, the difference between firing frequencies recorded in control conditions and those recorded in cadmium grew larger with successively later interspike intervals (Fig. 3*D*). This presumably occurred because the calcium-activated potassium conductance was more developed, and hence exerted a greater influence on discharge, later in the action potential train.

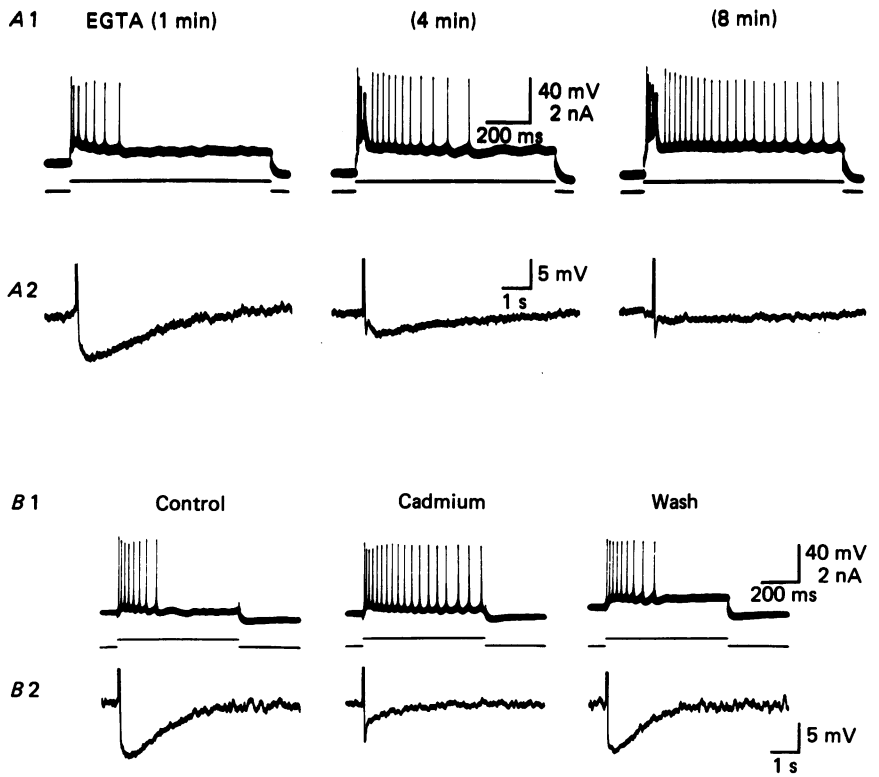

Fig. 2. Blockade of calcium-activated potassium conductance reduces accommodation of action potential discharge in pyramidal neurones. *A* 1, film records of the response of a pyramidal cell to a depolarizing current pulse, taken 1 min, 4 min and 8 min after impalement with an EGTA-filled (0·2 M in 2 M-$KMeSO_4$) micro-electrode. The 'hump' seen at the beginning of the current pulse was often seen in EGTA-injected cells and may be a calcium action potential induced by an increased driving force for calcium ions. *A* 2, chart records of the response of the pyramidal cell to a 60 ms long current pulse taken at a slower sweep to show the a.h.p. Records in *A* 2 were recorded within 15 s of those in *A* 1. Membrane potential −55 mV. *B* 1, film records of the response of a pyramidal cell to a depolarizing current pulse in control, after 14 min in 100 μM-cadmium, and 80 min after return to control solution. *B* 2, chart records of the response of the cell to a short (60 ms) current pulse, recorded within 15 s of the records in *B* 1. Membrane potential = −57 mV.

Other factors controlling accommodation

While most of the accommodation in CA 1 pyramidal cells appears to be caused by calcium-activated potassium conductance, there is still some residual slowing of action potential discharge, even after blockade of the calcium-activated potassium conductance by cadmium, calcium-free medium or EGTA. This suggests that some factor besides the calcium-activated potassium conductance may also contribute to the process of accommodation. Results shown in Fig. 4 provide some additional evidence for the existence of such a factor. If a long depolarizing current pulse was interrupted after the initial train of action potentials, the cell could be made to fire during the time when it normally would have been silent (Fig. 4*A* 1 and *A* 2). This occurred at a time when the calcium-activated potassium a.h.p. had decayed very

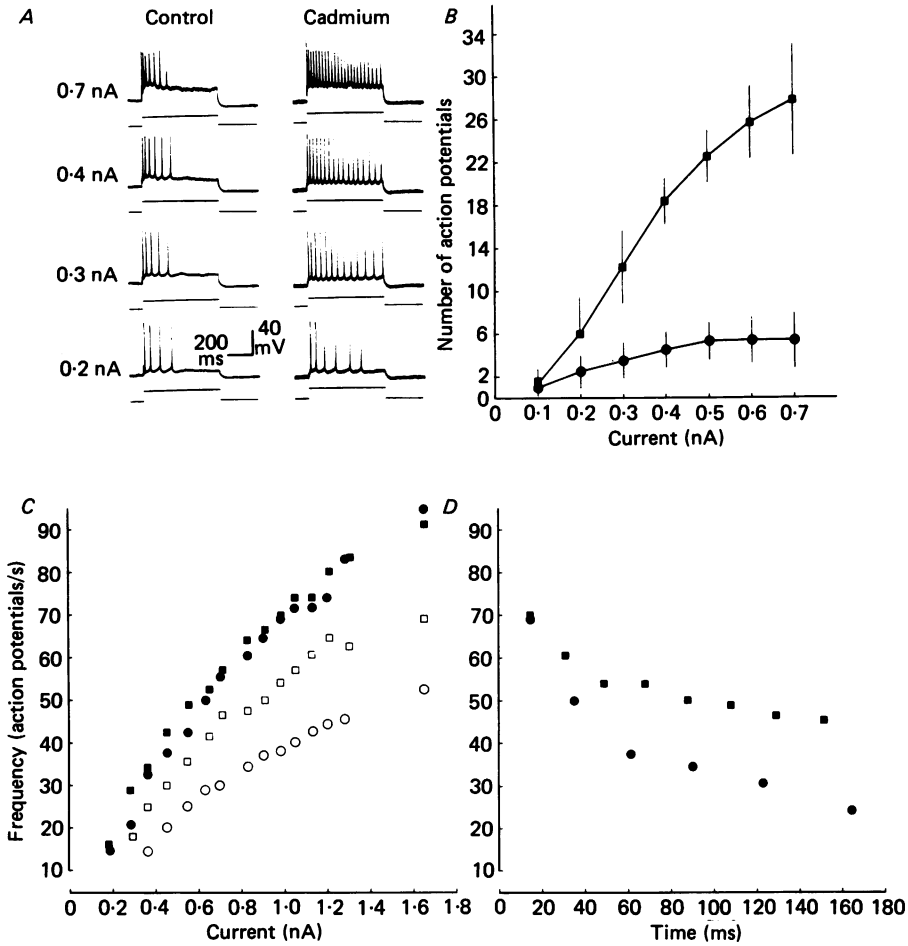

Fig. 3. Characterization of the effects of blocking calcium-activated potassium conductance on accommodation. *A*, film records of the response of a pyramidal cell to 600 ms depolarizing current pulses of increasing amplitude, in control, and 48 min after addition of cadmium (100 μM) to the superfusing medium. Membrane potential −67 mV. *B*, plot of the number of action potentials elicited by a 600 ms depolarizing pulse *vs.* stimulus current: in control (●), and in 100 μM-cadmium (■). Values shown are for nine cells (mean ± S.D.; including illustrated cell). *C*, plot of frequency *vs.* current, showing the frequency taken at the first (●) and third (○) interspike intervals in control, and after 72 min in 100 μM-cadmium (■), (□). *D*, plot of frequency *vs.* time for a stimulus current of 1 nA in control (●) and after 72 min in 100 μM-cadmium (■). The records in *C* and *D* are from the same pyramidal cell. The temperature of the bath was 25 °C in *C* and *D*. Membrane potential −61 mV.

little from its peak amplitude (Fig. 4*A*3). In the absence of the initial conditioning stimulus (Fig. 4*A*4), the first two short depolarizing pulses elicit two spikes instead of none. This illustrates the inhibitory effect of the calcium-activated potassium conductance which is produced by the initial conditioning stimulus. In Fig. 4*B*, a pyramidal cell was stimulated with an initial conditioning current pulse to activate the calcium-activated potassium conductance, and then was tested at varying times

with a single short current pulse. Ten of these test pulses are shown superimposed. This shows the approximate duration of repolarization that is required to allow the cell to begin firing action potentials again after the conditioning stimulus (approximately 200 ms). These results suggest that a voltage-sensitive (depolarization-activated) current may contribute to accommodation, and that such a current is turned off by repolarization of the membrane potential. One such current which has properties that could account for these data is the M-current.

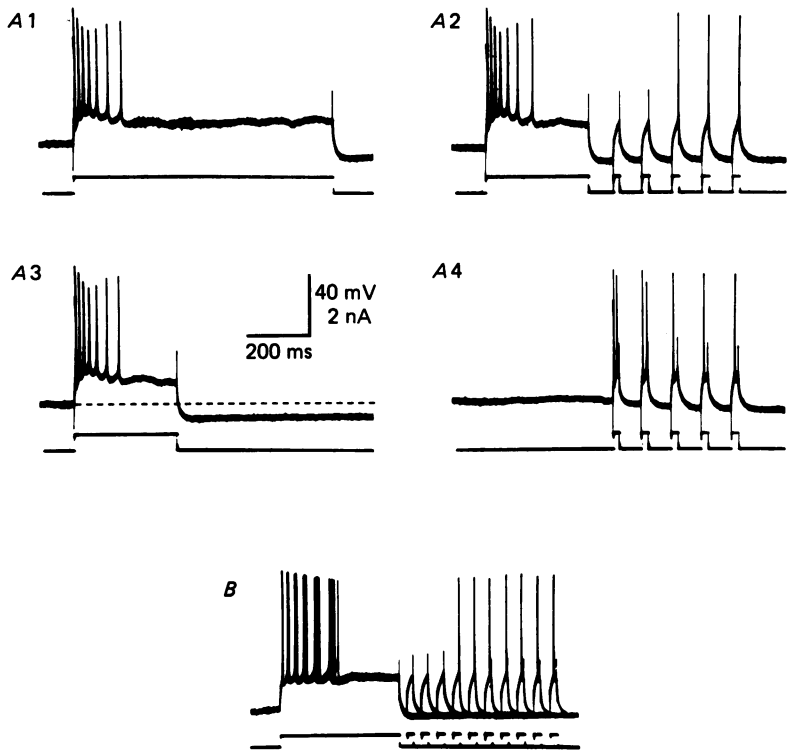

Fig. 4. A voltage-sensitive current may contribute to accommodation in pyramidal cells. Film records shown in *A* are all from the same pyramidal cell. Current traces are positioned below the voltage traces. Shown are responses of the pyramidal cell to a long depolarizing current pulse (*A*1); a brief conditioning current pulse, followed by a series of short test pulses (*A*2); the conditioning stimulus alone (*A*3); and a series of test pulses alone (*A*4). Membrane potential = −55 mV. *B*, superimposed film records from another cell showing the response to a conditioning stimulus followed by a single test pulse which was applied at different latencies. Membrane potential −66 mV.

The M-current in pyramidal cells (Halliwell & Adams, 1982), is a potassium current which is activated by depolarization, is non-inactivating, and has slow activation kinetics. To test for a contribution of the M-current in accommodation of pyramidal cell discharge, we applied the muscarinic agonist, carbachol, which antagonizes the M-current (Adams *et al.* 1982, Halliwell & Adams, 1982). Because muscarinic agonists have been shown to reduce the calcium-activated potassium conductance (Benardo & Prince, 1982; Cole & Nicoll, 1983; but see Brown & Griffith, 1983), calcium-free

medium containing 100 μM-cadmium was applied to the preparation first, in order to minimize the calcium-activated potassium conductance, so that possible effects of carbachol on the M-current could be observed. Application of the calcium-free/cadmium medium caused the usual increase in repetitive firing, which reached a maximum level after a few minutes. Addition of the muscarinic agonist, carbachol (1 μM) to the medium resulted in a further increase in the number of action potentials elicited by the stimulus (Fig. 5*A*) and an increase in the frequency of the action potential discharge (Fig. 5*B*; $n = 9$). During the application of carbachol the membrane potential depolarized with an increase in resistance. The membrane potential was manually voltage clamped (see bar labelled −d.c.) to return it to control level. We have found that the depolarizing action of carbachol is little affected by blockade of calcium currents, contrary to the observations of Benardo & Prince (1982).

With higher doses of carbachol, inactivation of the action potentials often occurred. That is, during the course of the depolarizing stimulus the action potentials became progressively shorter and wider, until the cell was unable to produce further action potentials. This was presumably due to the large depolarization of the membrane potential caused by higher doses of carbachol, and also to the underlying increase in membrane resistance, which resulted in a much larger voltage deflexion during the depolarizing current pulse.

DISCUSSION

We have shown in the present paper that pyramidal cell action potential discharge induced by a depolarizing stimulus accommodates during the course of the stimulus. The response of the pyramidal cell to such a depolarizing stimulus consists of an initial rapid train of action potentials which is followed by a silent period. Thus there appears to be some factor or factors which counteract the excitatory drive of the stimulus and cause the action potential discharge to slow and eventually to stop.

In addition to action potentials, the depolarizing stimulus also evokes an a.h.p. which is largely due to the calcium-activated potassium conductance (Alger & Nicoll, 1980; Hotson & Prince, 1980; Schwartzkroin & Stafstrom, 1980; Gustafsson & Wigstrom, 1981). This a.h.p. is presumably due to the development of an outward current during repetitive firing, such as has been demonstrated in hippocampal pyramidal cells (Bragdon & Wilson, 1982; Brown & Griffith, 1983). We believe that this outward current is reflected in the 'sag' of the membrane voltage that develops during a depolarizing current stimulus. In the present study we have performed experiments to determine the role that the current underlying the a.h.p. plays in regulating repetitive firing.

A number of studies have suggested a role for the calcium-activated potassium conductance in regulating repetitive firing (Kernell, 1965; Calvin & Schwindt, 1972; Schwindt & Calvin, 1973; Baldiserra & Gustafsson, 1970, 1974*a*, *b*; Meech, 1978; Barrett *et al.* 1980). We sought to demonstrate such a role directly by selectively blocking this conductance using intracellular injections of EGTA and by bath application of the calcium channel blocker, cadmium, or of calcium-free medium. Blocking the calcium-activated potassium conductance in hippocampal pyramidal

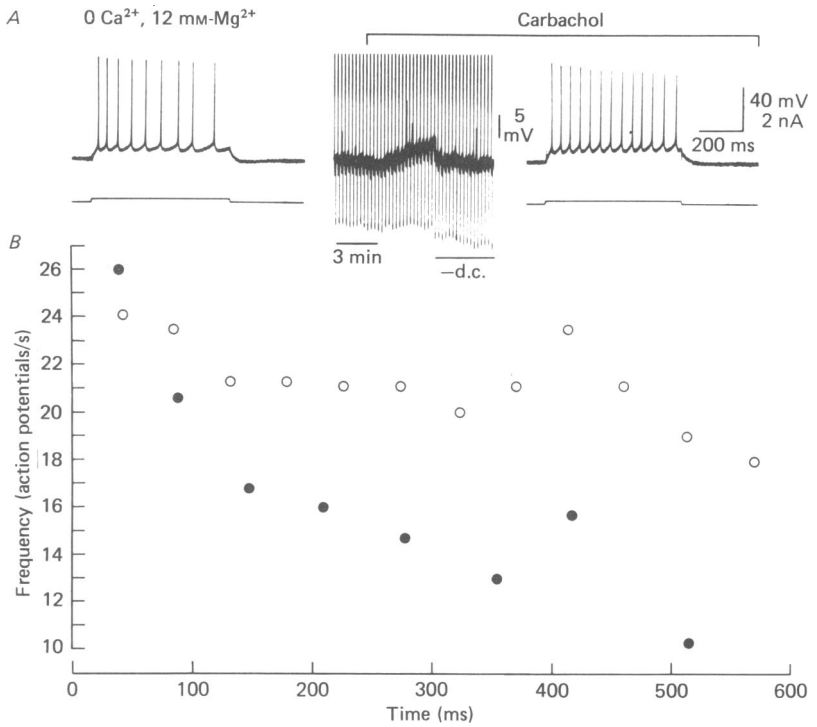

Fig. 5. A carbachol-sensitive component of accommodation of action potential discharge in pyramidal cells. All records taken from the same pyramidal cell. *A*, left record: film record of the response of a pyramidal cell to a depolarizing current stimulus. Cell had been bathed for 40 min in calcium-free/12 mM-magnesium medium containing 100 μM-cadmium chloride. Middle record: chart record showing the effects of addition of 1 μM-carbachol to the superfusing medium. The downward deflexions are constant-current hyperpolarizing pulses to measure membrane input resistance. The membrane potential was returned to control level by passing direct current through the recording electrode (see bar labelled −d.c.). This was done to demonstrate more clearly the resistance increase caused by carbachol. Upward deflexions are depolarizing current stimuli, such as those shown in the left- and right-hand record. Right record: film record, as in the control record, but 10 min after the application of 1 μM-carbachol. *B*, plot of the frequency of discharge in calcium-free/cadmium medium (●), and after addition of carbachol (○). Data in graph corresponds with records in *A*. To determine the frequency of discharge in carbachol, the resting membrane potential was returned to control levels with direct current passed through the recording electrode. Membrane potential −58 mV. An abrupt and transient increase in the firing frequency during depolarizing stimuli occurred in many cells tested (see part *B* of this Figure for example). Although this type of transient increase in firing frequency often occurred, it did not appear at a consistent latency in different cells.

cells results in a dramatic increase in the firing of the cell in response to a depolarizing stimulus. This block of accommodation and its recovery upon washing out cadmium followed the same time course as the block and recovery of the a.h.p. That accommodation is blocked by agents that selectively block the calcium-activated potassium conductance is strong evidence that repetitive spiking frequency is limited by this conductance. As has been shown previously (Hotson & Prince, 1980; Gustafsson & Wigstrom, 1981), and confirmed here, the a.h.p. is an action-potential-

dependent potential which markedly summates with successive action potentials. The build-up of the a.h.p. with successive action potentials is relatively slow (cf. Brown & Griffith, 1983), so its effects on discharge frequency are greater at later interspike intervals. Thus cadmium has little effect on the first interval, but substantially shortens the later intervals of the train.

Calcium-activated potassium conductance does not appear to be the only factor that controls accommodation in hippocampal pyramidal cells. After prolonged exposure to calcium-free medium and cadmium, and blockade of the calcium-activated potassium conductance, some residual accommodation was still present. In addition, pyramidal cells can be made to fire during their 'silent period' and at a time when calcium-activated potassium conductance is fully activated, simply by repolarizing the membrane potential briefly. We believe that this suggests a voltage-senstive component to accommodation. Previous studies have suggested that the voltage-sensitive M-current contributes to accommodation in other systems (Adams *et al.* 1982), and in the frog sympathetic ganglion the M-current is activated by excitatory synaptic potentials, and acts to curtail these same excitatory events (Tosaka, Tasaka, Miyazaki & Libet, 1983). The M-current in pyramidal cells is also activated by depolarizing stimuli (Halliwell & Adams, 1982) and could be acting in a similar manner to counteract excitatory stimuli, such as injected current, to cause accommodation of action potential discharge. We tested this possibility by applying carbachol after the calcium-activated potassium conductance had been blocked by application of calcium-free medium and cadmium. Carbachol increased the frequency of action potential discharge at a time when the membrane potential was manually voltage clamped at the resting level, suggesting that the M-current does play a role in accommodation.

In summary, we have shown that hippocampal CA1 pyramidal cells show action-potential frequency accommodation. This accommodation appears to be due to at least two potassium conductances: the calcium-activated potassium conductance and the M-current. These two conductances are activated during depolarization and exert a braking action on action potential discharge. Thus blocking these currents results in an increase in the frequency of action potential discharge, and an increase in the number of action potentials elicited by an excitatory stimulus.

These present findings are particularly interesting in light of the recent reports that a variety of neurotransmitters can block that calcium-activated potassium conductance (Wood & Mayer, 1979; Horn & McAffee, 1980; Cottrell, 1982; Benardo & Prince, 1982; Madison & Nicoll, 1982; Morita, North & Tokimasa, 1982; Aldenhoff, Gruol, Rivier, Vale & Siggins, 1983; Haas & Konnerth, 1983), and that acetylcholine mediates slow synaptic responses which may be due, in part, to blockade of the M-current (Adams & Brown, 1982; Cole & Nicoll, 1983). Thus the calcium-activated potassium conductance and the M-current provide mechanisms whereby these neurotransmitters can regulate the excitability and repetitive firing properties of central nervous system neurones.

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