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CHARACTERIZATION OF A SLOW CHOLINERGIC POST-SYNAPTIC POTENTIAL RECORDED *IN VITRO* FROM RAT HIPPOCAMPAL PYRAMIDAL CELLS

BY ALISON E. COLE* AND R. A. NICOLL

From the Departments of Pharmacology and Physiology, University of California, San Francisco, CA 94143, U.S.A.

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SUMMARY

1. Intracellular recording from CA1 pyramidal cells in the hippocampal slice preparation was used to compare the action of exogenously applied acetylcholine (ACh) and cholinomimetics to the effect of electrically stimulating sites in the slice known to contain cholinergic fibres.

2. ACh depolarized pyramidal cells with an associated increase in input resistance, blocked a calcium-activated potassium conductance ($G_{K(Ca)}$), and blocked accommodation of action potential discharge. All of these actions were blocked by the muscarinic antagonist, atropine.

3. Repetitive electrical stimulation of stratum (s.) oriens evoked a series of fast excitatory post-synaptic potentials (e.p.s.p.s) followed by an inhibitory post-synaptic potential. These potentials were followed by a slow e.p.s.p. that lasted 20–30 s.

4. The slow e.p.s.p. was selectively enhanced by eserine and blocked by atropine. Ionophoretic application of ACh closely mimicked the time course of the slow e.p.s.p.

5. The slow e.p.s.p. was blocked by tetrodotoxin and cadmium, indicating that it was dependent on propagated action potentials and on calcium.

6. Considerably higher stimulus strengths were needed to elicit a slow e.p.s.p. than to elicit the earlier synaptic potentials. The size of the slow e.p.s.p. was markedly increased by repetitive stimulation.

7. Stimulation of the alveus, s. oriens, s. pyramidale and fimbria all evoked a slow e.p.s.p., while stimulation of s. radiatum was relatively ineffective.

8. The input resistance of the cell increased during the slow e.p.s.p. Hyperpolarizing the cell decreased the size of the slow e.p.s.p. and at membrane potentials of -70 mV or greater, little response was recorded.

9. Stimulation of s. oriens blocked $G_{K(Ca)}$ and accommodation of action potential discharge. These effects, which could be seen in the absence of any change in membrane potential, were enhanced by eserine and blocked by atropine.

10. The present electrophysiological results establish that CA1 pyramidal cells receive a cholinergic input and demonstrate that this input can dramatically alter the firing properties of these neurones for tens of seconds in the absence of any marked

* Present address: Laboratory of Neurophysiology, NIH-NINCDS, 9000 Rockville Pike, Bethesda, MD 20205, U.S.A.

effect on membrane potential. Such an action contrasts with previously characterized synaptic potentials in this region of the brain.

INTRODUCTION

The existence of a projection from cells in the medial septal nucleus to the hippocampus was first suggested by anatomists near the turn of the century (Elliot Smith, 1910; Herrick, 1910). An abundance of more recent neuroanatomical studies using degeneration techniques (Morin, 1950; McLardy, 1955; Raisman, 1966) and retrograde and anterograde transport techniques (Mosco, Lynch & Cotman, 1973; Rose, Hattori & Fibiger, 1976; Meibach & Siegel, 1977; Lynch, Rose & Gall, 1978; Crutcher, Madison & Davis, 1981) have firmly established the presence of a septo-hippocampal pathway. However, it was not until the work of Lewis & Shute (1967) that the cholinergic nature of the septo-hippocampal projection was demonstrated, based on the distribution of cholinesterase and choline acetyltransferase in these areas (Shute & Lewis, 1966; Lewis & Shute, 1967; Lewis, Shute & Silver, 1967). More recently, the release of acetylcholine (ACh) from the hippocampus has been shown to be increased during septal stimulation (Smith, 1974; Dudar, 1975).

Many investigations have found that application of ACh to hippocampal pyramidal cells causes a slow muscarinic excitation (Ben-Ari, Krnjevic, Reinhardt & Ropert, 1981; Dodd, Dingledine & Kelly, 1981; Halliwell & Adams, 1982; Benardo & Prince, 1982*b*). Krnjevic & Ropert (1982) have reported that stimulation of the medial septum facilitates the synchronized firing of hippocampal pyramidal cells in response to commissural inputs and that this effect is enhanced by anticholinesterase and reduced by muscarinic antagonists. However, the post-synaptic effects of stimulating cholinergic fibres have not been characterized with intracellular recording.

In the present study, using the hippocampal slice preparation, we have characterized responses recorded intracellularly from pyramidal cells that result from the activation of cholinergic fibres and we have compared these effects to those of ACh application. A preliminary account of some of these results has appeared (Cole & Nicoll, 1983).

METHODS

The methods for the rat hippocampal slice preparation used in the present paper are similar to those used in other studies from this laboratory (cf. Nicoll & Alger, 1981; Alger & Nicoll, 1982). Our standard bathing medium consists 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. Intracellular recording electrodes were pulled from 'omegadot' glass tubing and filled with 2 M-potassium methylsulphate (80–120 MΩ). The membrane potential of the impaled cell could be changed by passing current through the recording electrode using a bridge circuit (WPI 701). Only cells with stable membrane potentials of greater than −55 mV were used in this study. This study is based on recordings of over 140 cells obtained in approximately fifty slices. Bipolar stimulating electrodes were constructed from stainless-steel micro-electrodes (Haer). The two poles of the electrode were staggered so that when the cathode was inserted into the slice the anode was just above the slice. The stimulus consisted of a 0·02 ms pulse with an intensity of 15–30 V. The responses were all obtained from CA1 pyramidal cells.

Drugs were applied by addition to the superfusing medium or, in a few cases, by ionophoresis. For ionophoresis single-barrelled electrodes were filled with either 1 M-acetylcholine chloride or 1 M-carbamylcholine chloride (carbachol) and were ejected with positive current. The drugs used

were eserine (0·1–10 μM), acetylcholine chloride (10–100 μM), carbachol (1–20 μM), tetrodotoxin (1 μM), cadmium chloride (100 μM), and atropine methyl nitrate (0·01–1 μM). All of these were purchased from Sigma. Tetraethylammonium chloride (5 mM) was purchased from Eastman Kodak.

RESULTS

Effects of acetylcholine and carbachol

Bath application of either ACh or carbachol had dramatic effects on CA1 pyramidal cell excitability. The effects of carbachol could be seen at concentrations as low as 20 nM. In contrast, concentrations of ACh greater than 100 μM were usually required to elicit effects. This relative insensitivity to ACh was due, in part, to the action of cholinesterase in the tissue, because in the presence of eserine (0·1–10 μM), effects could be seen at 10-fold lower concentrations. Fig. 1*A* shows that ACh had two distinct actions on pyramidal cells. First, it depolarized the membrane potential and induced repetitive firing of the cell (Fig. 1*A*2). This depolarization was associated with an increase in the input resistance of the cell, which was determined by passing constant-current hyperpolarizing pulses through the recording electrode. To see this increase in resistance more clearly, a steady hyperpolarizing current was applied to return the membrane back to the resting level (see bar labelled −d.c. beneath trace). Secondly, the hyperpolarization that followed a series of action potentials evoked by a short (60–100 ms) depolarizing current pulse was severely reduced (Fig. 1*A*1 to *A*3). This after-hyperpolarization (a.h.p.) 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). In some instances concentrations of carbachol or ACh that produced little depolarization nevertheless reduced the a.h.p. The muscarinic antagonist, atropine, completely blocked both actions of ACh (Fig. 1*B*) at concentrations as low as 0·01 μM, which was the lowest concentration tested.

In response to long-duration current pulses (600–800 ms) the discharge of pyramidal cells adapts rapidly, and typically after six to eight spikes the cell falls silent for the duration of the pulse (cf. Madison & Nicoll, 1982). Carbachol (1–20 μM) and acetylcholine (10 μM–1 mM) markedly attenuated this spike frequency adaptation. In Fig. 2 an ionophoretic pulse of carbachol (5 nA for 5 s) depolarized the cell by approximately 5 mV (Fig. 2*B*) and dramatically increased the number of spikes evoked by the depolarizing pulse (Fig. 2*A* and *C*). This effect was not duplicated in this cell by depolarizing the cell to a similar extent with current (see record labelled +d.c. in Fig. 2*A*) or by increasing the size of the depolarizing pulse in order to control for the increase in input resistance (not shown). In addition to the change in membrane potential, the blockade of spike frequency adaptation was associated with the blockade of the a.h.p., which is seen as a small downward deflexion in Fig. 2*B*. With low concentrations of agonist it was possible in some cells to see a reduction in spike frequency adaptation in the absence of membrane depolarization. The blockade of spike frequency adaptation was prevented by low concentrations of atropine (not shown).

We have examined the effect of carbachol on calcium spikes to determine if the block of the a.h.p. is due to a block of calcium entry into the cell. Calcium spikes were evoked in the presence of tetrodotoxin (1 μM) and tetraethylammonium (5 mM).

At a time when carbachol had completely blocked the a.h.p. the calcium spike was still present and, in fact, was prolonged and the cell fired multiple spikes in response to a single depolarizing pulse (Fig. 3). This suggests that carbachol is blocking the a.h.p. at a step subsequent to calcium entry. However, since carbachol slightly reduced the peak of the calcium spike in approximately half of the cells, a direct action on calcium entry (cf. Giles & Noble, 1976) cannot be ruled out.

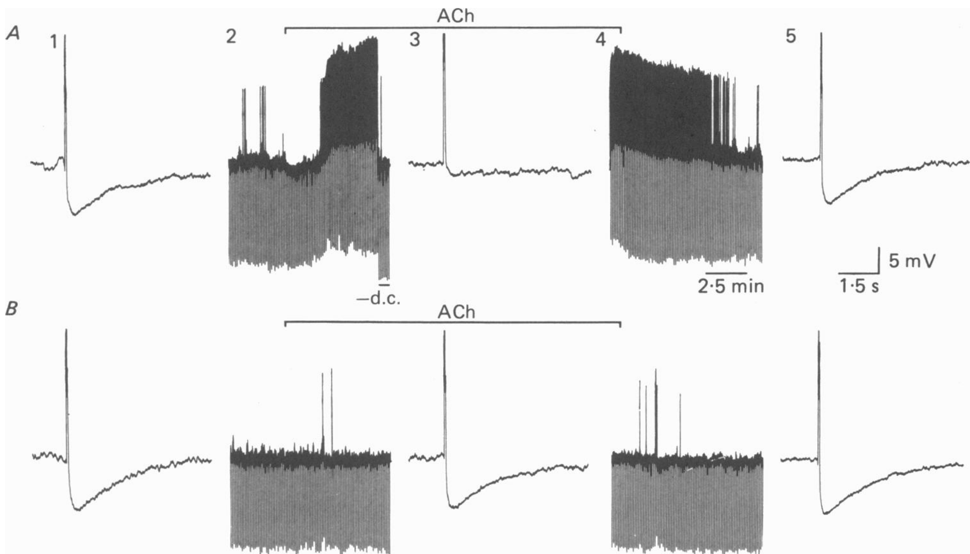

Fig. 1. Effects of ACh on a hippocampal CA1 pyramidal cell. *A*, chart records of responses in normal physiological solution. *A*1 shows the control a.h.p. following a 60 ms direct depolarizing current pulse; application of ACh (0·5 mM; *A*2) depolarized the membrane, increased cell firing and increased the cell's input resistance; the membrane was returned to resting potential with d.c. hyperpolarizing current (see bar labelled −d.c.); *A*3 shows the blockade of the a.h.p. in the presence of ACh. When ACh was washed out (*A*4) the membrane repolarized and the a.h.p. returned to control amplitude (*A*5). *B*, the same cell, but with atropine (1·0 μM) added to the superfusate. All actions of ACh on membrane potential and a.h.p. were completely blocked. Time calibration in *A*4 also applies to *A*2, *B*2 and *B*4. Calibrations in *A*5 are the same for all a.h.p. records. Membrane potential −62 mV.

Electrical stimulation of cholinergic fibres

Histochemical studies have localized the enzyme cholinesterase to fibres found primarily in the alveus, s. oriens and s. pyramidale (Shute & Lewis, 1966; Lewis & Shute, 1967; Lewis *et al.* 1967; Storm-Mathisen, 1970; Mosco *et al.* 1973; Lynch *et al.* 1978). These fibres enter the hippocampus via both the fornix and fimbria. The effects of electrical stimulation of these regions in the hippocampal slice have been examined in ninety-seven cells. For most of our studies we placed the stimulating electrode in s. oriens; repetitive stimulation at this site (Fig. 4*A*) produced short-latency fast excitatory post-synaptic potentials (e.p.s.p.s) followed by an inhibitory post-synaptic potential (i.p.s.p). In the majority of cells a small slow e.p.s.p. occurred after the i.p.s.p. This slow e.p.s.p. was markedly enhanced by eserine (0·1–10 μM;

$n = 39$), resulting in intense discharge during the early phase of the response. Atropine (0·1–1 μM; $n = 21$) entirely blocked the slow e.p.s.p. without affecting the fast e.p.s.p.s or i.p.s.p.

We have compared the effects of ionophoretically applied ACh to pathway stimulation. As shown in Fig. 4*B*, an ionophoretic pulse of ACh (15 nA for 0·5 s) of the same duration as the train of stimuli evoked a slow depolarization with a similar time course to the slow e.p.s.p.; both responses are blocked by atropine.

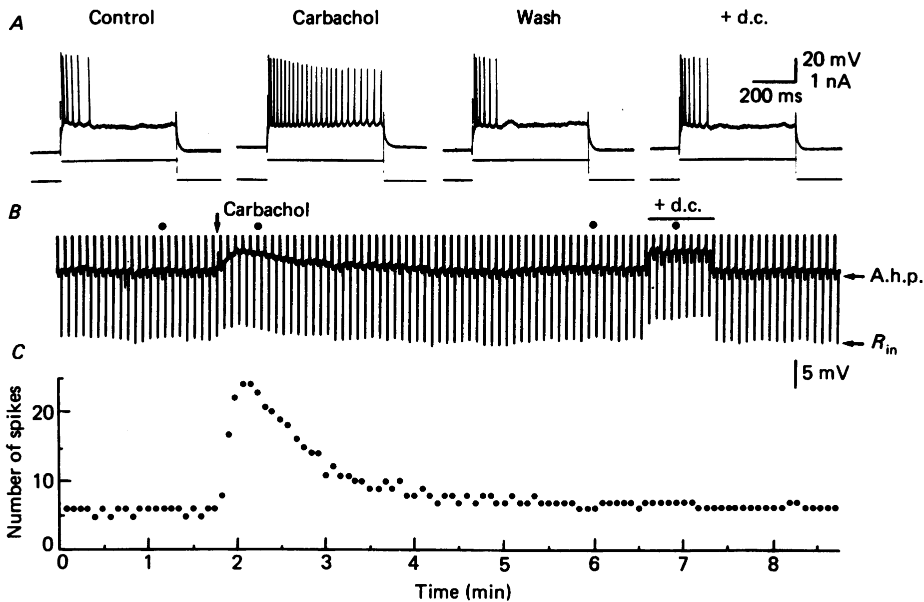

Fig. 2. Effect of carbachol on accommodation of cell discharge. *A*, response of cell to depolarizing current pulse (approximately 600 ms duration) at four different times, corresponding to the filled circles above the trace in *B*. Initially, the cell fired six spikes then fell silent; after an ionophoretic pulse of carbachol (5 nA, 5 s; see arrow in *B*) the cell depolarized and the number of spikes increased (2nd trace in *A*; *C*). During wash, the cell repolarized and the number of spikes returned to control level. When the cell was depolarized to a similar degree with direct current (+d.c. on *A* and *B*) there was no comparable effect on accommodation. The large downward deflexions in *B* are constant-current hyperpolarizing pulses for measuring the input resistance (R_{in}). *C*, plot of number of spikes *vs.* time, before, during and after ionophoresis of carbachol.

Since the distance between the stimulating and recording site was usually at least 1 mm, it seemed unlikely that the ACh was being released directly by the stimulating current. The finding that tetrodotoxin (TTX) blocked the slow e.p.s.p. as well as the preceding potentials (Fig. 5*A*) confirmed that the slow e.p.s.p. was dependent on propagated action potentials ($n = 5$). The slow e.p.s.p. was also calcium dependent, as expected for a synaptic potential dependent on the release of transmitter. This was shown by adding the calcium channel blocker, cadmium (100 μM), to the superfusing medium which blocked the slow e.p.s.p., as well as the earlier responses (Fig. 5*B*; $n = 5$).

We have compared the stimulus strength necessary for evoking the early fast e.p.s.p. and i.p.s.p. to the stimulus strength necessary for evoking the slow e.p.s.p. As

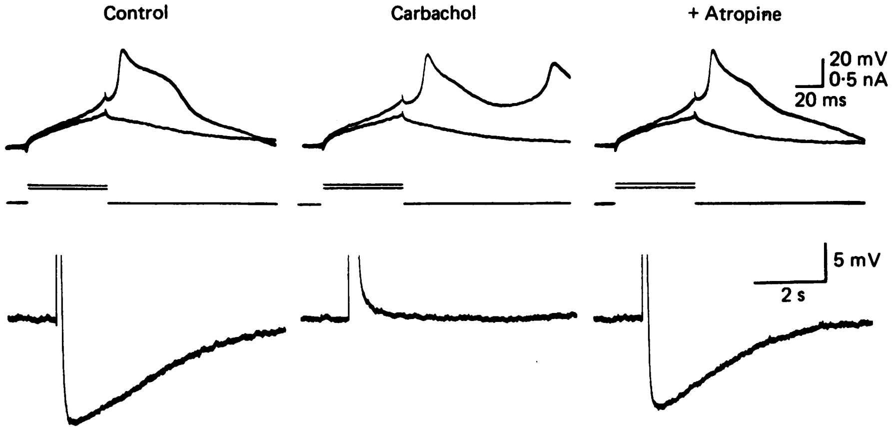

Fig. 3. Carbachol blocks a calcium-activated potassium response without blocking calcium spikes in CA1 pyramidal cells. Upper traces are film records of superimposed subthreshold and suprathreshold calcium spikes evoked in a pyramidal cell in the presence of 1 μM-TTX and 5 mM-TEA. Current traces are below the voltage records. The lower traces are simultaneously recorded chart records of the a.h.p., a calcium-activated potassium response. The responses in the middle column were recorded 8·5 min after 10 μM-carbachol was added to the superfusate; the record in the last column is 23 min after atropine (1 μM) was added. Membrane potential -56 mV.

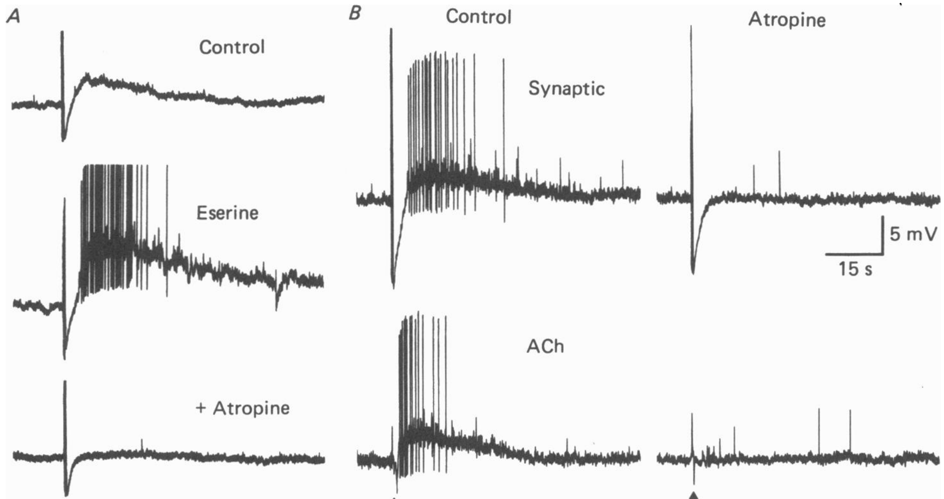

Fig. 4. Electrical stimulation of the hippocampal slice mimics the effects of exogenous ACh. *A*, repetitive stimulation (20 Hz for 0·5 s) in s. oriens (upper trace) elicited short-latency fast e.p.s.p.s, followed by an i.p.s.p. and the slow e.p.s.p. Eserine (2 μM; middle trace) greatly increased the slow e.p.s.p. amplitude and generated intense action potential firing. The slow e.p.s.p. was completely and selectively eliminated by atropine (0·1 μM; lower trace). Membrane potential -60 mV. *B*, upper traces show the synaptic response (slow e.p.s.p.) elicited by electrical stimulation (30 Hz for 0·5 s) in control solution and its blockade by atropine. Lower traces show the depolarization produced by an ionophoretic pulse of ACh (arrow) which mimics the synaptic response and is also blocked by atropine. All records in *B* obtained in the presence of 1 μM-eserine. Calibration in *B* applies to all records. Membrane potential -57 mV.

shown in Fig. 6*A* the fast e.p.s.p.s and i.p.s.p. were evoked at stimulus strengths considerably below those required for evoking a slow e.p.s.p. This suggests that the slow e.p.s.p. is produced by a separate group of fibres from those producing the fast synaptic potentials. Although, in the presence of eserine, a single stimulus could usually evoke a small slow e.p.s.p., the response showed marked summation as the frequency of stimulation was increased (Fig. 6*B*; $n = 11$) and approached a maximum at about 20–50 Hz. For most of the experiments we used a 0·5 s stimulus train at 20–30 Hz.

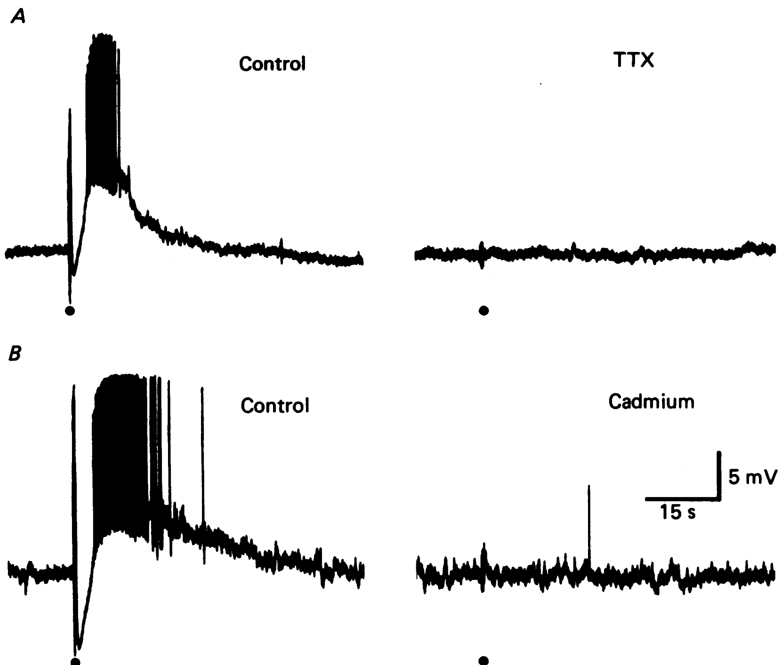

Fig. 5. Blockade of slow e.p.s.p. by TTX and cadmium. *A*, chart records of slow e.p.s.p. elicited by electrical stimulation (at filled circle) in s. oriens (20 Hz, 0·5 s) in control solution and blocked after TTX (1 μM) was added to the superfusate. Membrane potential −57 mV. *B*, same as in *A* but with cadmium (100 μM) added to superfusate. Records in *A* and *B* were obtained in the presence of 1 μM-eserine. Calibration in *B* applies to *A*. Membrane potential −55 mV.

We attempted to evoke slow e.p.s.p.s by stimulating various sites in the slice. The size of the responses varied considerably in different preparations, but in general, as illustrated in Fig. 7, stimulation of the alveus, s. oriens, and s. pyramidale were all effective in evoking a slow e.p.s.p., while stimulation of s. radiatum was relatively ineffective. In a few cases it was even possible to evoke a slow e.p.s.p. by stimulating the fimbria (Fig. 7).

In approximately two-thirds of the cells tested a small increase in input resistance could be detected during the slow e.p.s.p. This can be seen in Fig. 8*A* by comparing the amplitude of the membrane potential deflexions caused by constant-current hyperpolarizing pulses during the slow e.p.s.p. and during the passage of a steady depolarizing current (+d.c.) which depolarized the cell to the same extent. The size

of the slow e.p.s.p. was very sensitive to changes of the membrane potential. As the membrane was hyperpolarized the size of the slow e.p.s.p. was reduced and was virtually abolished at membrane potentials of −70 mV or greater (Fig. 8*B*). In two cells the conductance change associated with the slow e.p.s.p. was measured during d.c. hyperpolarization of the membrane potential. At membrane potentials that

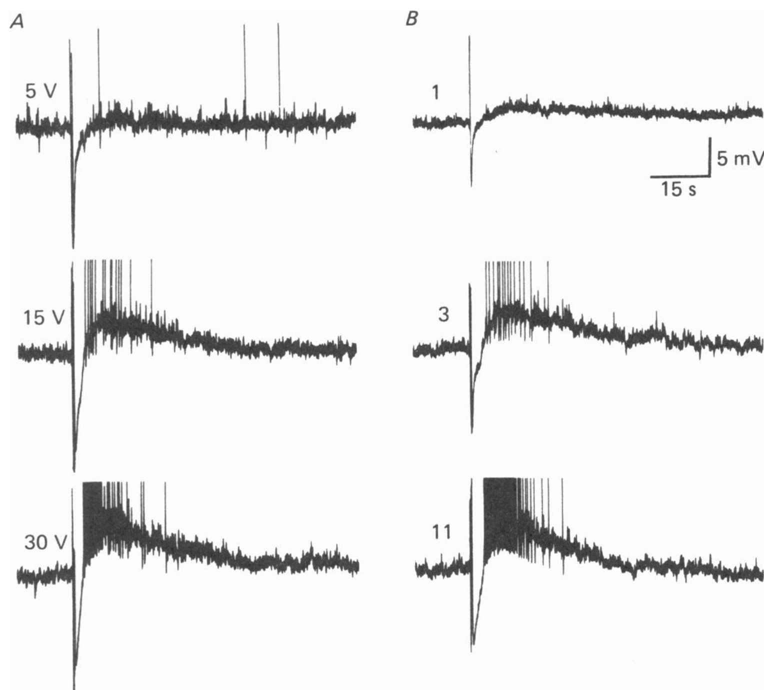

Fig. 6. Effect of stimulus intensity and frequency on the evoked slow e.p.s.p. in CA1 pyramidal cells. *A* shows the response to 5, 15 and 30 V stimulation for 0·5 s. Membrane potential −58 mV. *B* shows responses to one pulse, three pulses (6 Hz), and eleven pulses (22 Hz) in a stimulus train lasting 0·5 s. Records in *A* and *B* were obtained in the presence of 1 μM-eserine. Calibration applies to *A* and *B*. Stimulating electrodes in *A* and *B* placed in s. oriens. Membrane potential −55 mV.

evoked little slow e.p.s.p., little change in conductance was seen. This finding, along with the observation that little slow e.p.s.p. can be evoked at membrane potentials considerably less than the potassium equilibrium potential, which is approximately −85 mV (Alger & Nicoll, 1980), strongly supports the proposal that ACh blocks a voltage-sensitive potassium conductance (Halliwall & Adams, 1982). The spontaneous responses in records of Fig. 8*B* represent synchronous epileptiform bursts discharges which presumably originated in the CA2/CA3 region. This activity was seen in approximately 20–30 % of the preparations that had been exposed to eserine for over 30 min and was completely blocked by atropine.

Since exogenously applied ACh reduces the a.h.p. and accommodation in addition to directly depolarizing the membrane, we examined the possibility that repetitive stimulation of s. oriens might also produce similar effects. In Fig. 9*A* the stimulus

strength to s. oriens was adjusted so that the effect on the a.h.p. evoked by depolarizing current pulses was just detectable. In the presence of eserine (0·1–10 μM; $n = 18$) a slow e.p.s.p. appeared in response to repetitive stimulation and the a.h.p. was severely depressed during this potential. In the experiment of Fig. 9*B* the stimulus intensity was increased to produce a substantial reduction in the a.h.p. This

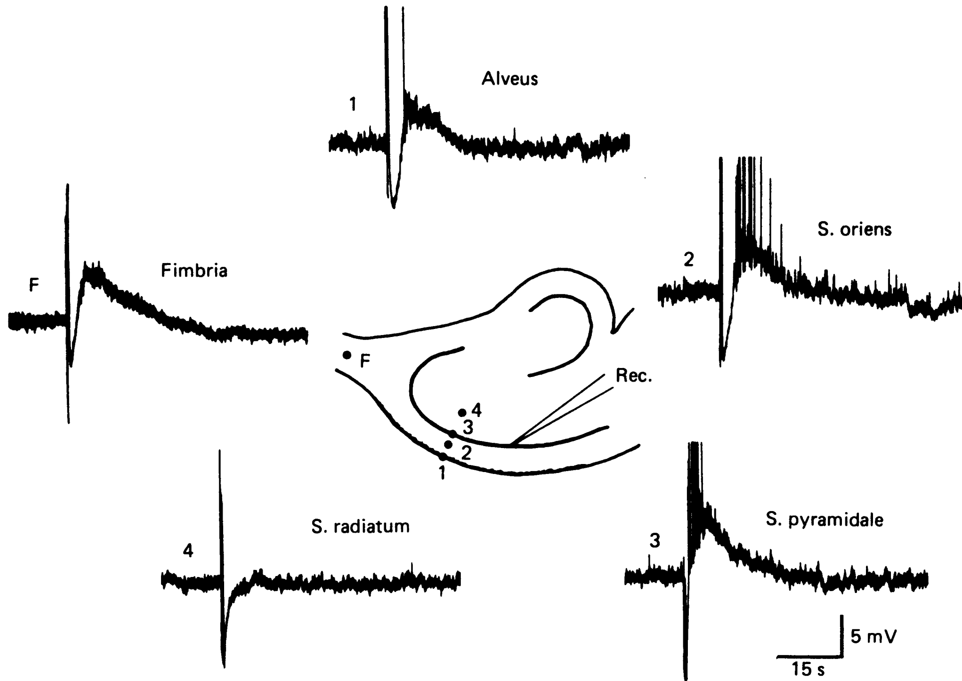

Fig. 7. Slow e.p.s.p. evoked in CA1 cell by stimulating various sites in the hippocampal slice. Diagram of hippocampal slice preparation illustrates placement of recording electrode (Rec.) in CA1, and placement of stimulating electrode in four different strata (1–4) and the fimbria (F). The records (starting at top and proceeding clockwise) show slow e.p.s.p.s evoked by stimulating in alveus (1), s. oriens (2) and s. pyramidale (3); stimulating s. radiatum (4) rarely produced a slow e.p.s.p.; stimulating the fimbria (F) also elicited a substantial slow e.p.s.p. In records 1–4, which are from the same cell, identical stimulus parameters (20 Hz for 0·5 s) were used and the stimulating electrode was moved to each of the sites indicated. The fimbrial record is from a different slice. All records were obtained in the presence of 1 μM-eserine. Membrane potentials −55 mV (1–4) and −60 mV (F).

reduction was prevented by adding atropine (0·1 μM; $n = 16$) to the superfusate. It can also be seen in Fig. 9 that eserine caused a reduction in the a.h.p. in the absence of pathway stimulation and that atropine caused a small increase in the a.h.p. in the absence of stimulation. This effect of eserine was seen in the majority of cells and increased with the time of exposure. The effect of atropine on the a.h.p. in the absence of pathway stimulation was much more variable and was seen in only a small proportion of cells.

Stimulation of s. oriens also had profound effects on accommodation of spike discharge during a long depolarizing current pulse ($n = 26$) even in the absence of

eserine. Fig. 10*A* shows that before pathway stimulation the cell fired five spikes, whereas immediately after the stimulus the cell fired thirteen spikes. This occurred in the absence of a slow depolarization. The stimulus-induced block of accommodation was prevented by atropine ($n = 16$; Fig. 10*B*).

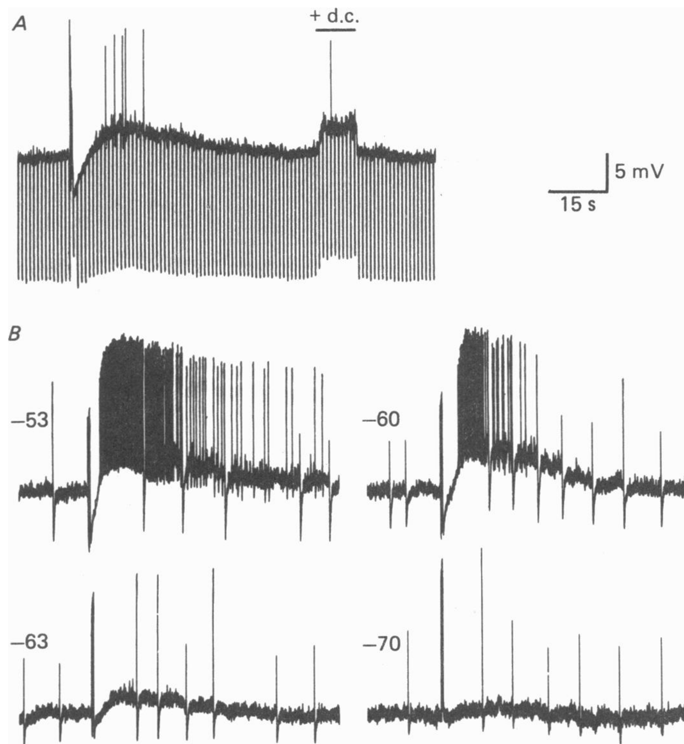

Fig. 8. Increased input resistance during the slow e.p.s.p. and effect of membrane potential on the slow e.p.s.p. In *A*, a small increase in input resistance during the slow e.p.s.p. is reflected in the increased amplitude of constant-current hyperpolarizing pulses (downward deflexions), as compared with the pulse amplitude during membrane depolarization of the same amplitude with direct current (see bar labelled +d.c.). Membrane potential −62 mV. As shown in *B*, the amplitude of the slow e.p.s.p. decreases as the cell is hyperpolarized with increasing direct current. At a membrane potential of −70 mV the response is almost completely eliminated. Records in *A* and *B* were obtained in the presence of 1 μM-eserine. Calibration in *A* applies to *B*. Spontaneous firing in *B* reflects synchronous epileptiform burst discharges from CA2/CA3. Membrane potential −60 mV.

DISCUSSION

In the present experiments we have compared the actions of exogenously applied ACh and carbachol on CA1 pyramidal cells to the responses evoked by stimulating sites in the slice known to contain cholinergic fibres. We have confirmed many of the actions of ACh reported by others. Thus, ACh depolarized pyramidal cells and

induced repetitive discharge. This depolarization was associated with an increase in the input resistance of the cell (cf. Ben-Ari *et al.* 1981; Dodd *et al.* 1981; Benardo & Prince, 1982*b*), which has been attributed to blockade of the M-current (Halliwell & Adams, 1982).

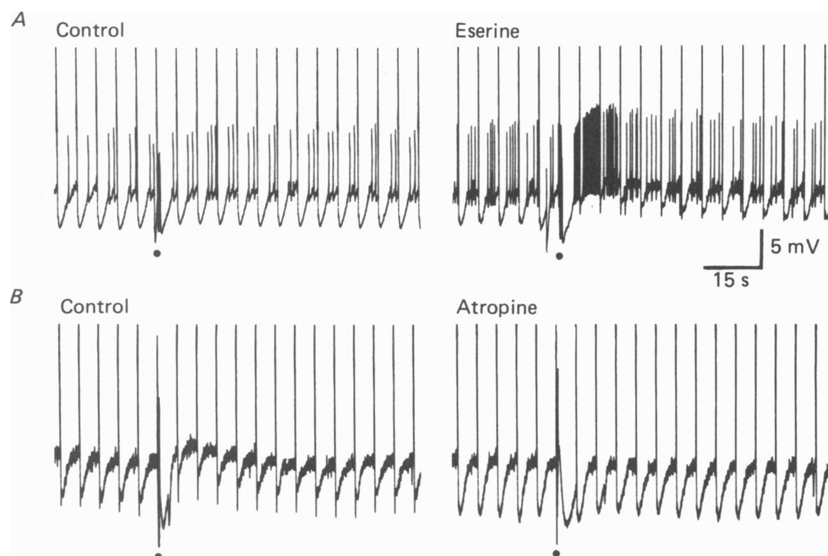

Fig. 9. Effect of train stimulation in s. oriens on the a.h.p.s recorded in CA1 cells. A.h.p.s were recorded following 100 ms direct depolarizing current pulses applied every 5 s. In *A*, train stimulation (see filled circle below trace; 30 Hz, 0·5 s) in control solution was adjusted so that a slight depression of the a.h.p.s following the train was just detectable. In the presence of eserine (0·3 μM) an identical stimulus evoked a large slow e.p.s.p., induced intense discharge and greatly reduced the a.h.p. In *B*, the train stimulation (filled circle) in control solution without eserine present was increased to evoke a slow e.p.s.p. and significant depression of the a.h.p.; both effects were blocked by atropine (1 μM). Calibration in *A* also applies to *B*. Membrane potential −56 mV.

ACh also severely depressed the slow after-hyperpolarization that follows a series of spikes evoked by a depolarizing current pulse. This potential is due in large part to an increase in calcium-activated potassium conductance ($G_{K(Ca)}$), since it is blocked by calcium antagonists (Alger & Nicoll, 1980; Hotson & Prince, 1980; Gustafsson & Wigstrom, 1981) and by intracellular injection of the calcium chelator, EGTA (Alger & Nicoll, 1980; Schwartzkroin & Stafstrom, 1980). ACh may be blocking $G_{K(Ca)}$ at a step subsequent to calcium entry because calcium spikes were prolonged and often multiple spikes were evoked in the presence of ACh. A depressant action of ACh on $G_{K(Ca)}$ has been reported in neurones of the myenteric plexus (Morita, North & Tokimasa, 1982) and in CA1 pyramidal cells (Benardo & Prince, 1982*b*). On the other hand, $G_{K(Ca)}$ does not appear to be affected by ACh in frog sympathetic ganglion cells (Adams, Brown & Constanti, 1982) and in a recent voltage-clamp study of hippocampal pyramidal cells (Brown & Griffith, 1983) muscarine had little effect on a slow outward current thought to be mediated by $G_{K(Ca)}$. If ACh were to reduce

$G_{K(Ca)}$ by enhanced calcium sequestration it is possible that during the relatively short voltage-clamp steps used in this study the effects on $G_{K(Ca)}$ would be small. Reduction of $G_{K(Ca)}$ has been observed during the action of other transmitters including serotonin on myenteric neurones (Wood & Mayer, 1979), noradrenaline on sympathetic (Horn & McAfee, 1980) and hippocampal pyramidal neurones (Madison & Nicoll, 1982; Haas & Konnerth, 1983) and histamine on hippocampal pyramidal neurones (Haas & Konnerth, 1983).

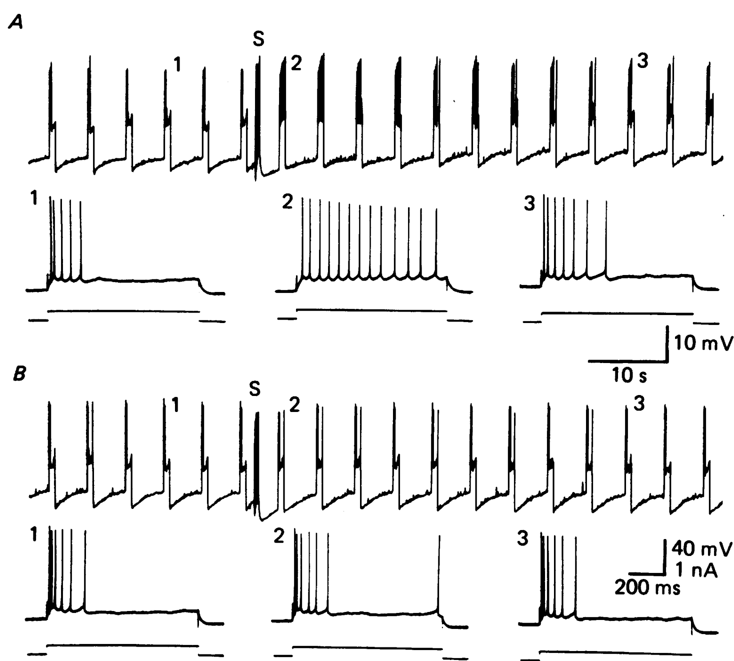

Fig. 10. Train stimulation in s. oriens blocks accommodation of cell discharge in CA1 pyramidal cells. *A*, upper trace is a chart record showing long (800 ms) depolarizing pulses applied every 5 s. Traces below are simultaneously recorded film records of the 800 ms pulse showing accommodation of cell firing. Current monitor is displayed immediately below. 1, 2 and 3 in upper and lower records correspond to the same 800 ms pulses before (1), immediately after (2) and 90 s after (3) the train of stimuli (40 Hz for 0·5 s; S above trace). *B*, same experiment as in *A*, but with atropine (1 μM) added to the superfusate. The block of accommodation and increased cell firing seen in *A*2 after the stimulus is completely antagonized by atropine (*B*2). Calibration in *A* is for both chart records; calibration in *B* is for all film records. Membrane potential -61 mV.

Finally, ACh and carbachol reduced the accommodation of action potential discharge which occurs during long depolarizing current pulses. This effect was observed at concentrations of ACh that had little effect on membrane potential and was due, in part, to the blockade of $G_{K(Ca)}$, since similar results were seen after selective blockade of $G_{K(Ca)}$ by cadmium (Madison & Nicoll, 1982). Blockade of the M-current would also be expected to reduce accommodation (Adams *et al.* 1982; Halliwell & Adams, 1982). However, we have not yet determined the relative

contribution of these two actions of ACh. All of the actions of ACh that we have observed in pyramidal cells are enhanced by eserine and completely blocked by low concentrations of the muscarinic antagonist, atropine.

There was a progressive decrease in the size of the a.h.p. in most cells when slices had been exposed to eserine for more than 30 min (cf. Benardo & Prince, 1982*a*), and in approximately 20% of the slices spontaneous epileptiform bursts appeared. These effects were blocked by atropine. Atropine, in the absence of eserine, usually had no effect on the a.h.p. but in a few cells caused a small increase. These findings suggest that there is a tonic release of ACh, but that the cholinesterase in the slice prevents this ACh from exerting a post-synaptic effect.

The facilitation of repetitive spike discharge by neurotransmitters has been seen in other neurones. This includes the muscarinic action of ACh on neocortical cells (Krnjevic, Pumain & Renaud, 1971) and sympathetic ganglion cells (Adams *et al.* 1982). Similar effects have been reported for serotonin on neurones in the myenteric plexus (Wood & Mayer, 1979), *Helix* ganglion (Cottrell, 1982) and rat facial nucleus (Vander Maelen & Aghajanian, 1980). Finally, in hippocampal pyramidal cells noradrenaline attenuates accommodation of spike discharge (Madison & Nicoll, 1982).

Our evidence indicating that we have activated a cholinergic pathway to CA1 pyramidal cells falls into three categories. First, electrical stimulation of the slice can evoke post-synaptic responses that exactly mimic all of the actions of exogenously applied ACh. Thus, the depolarizations elicited by both procedures followed the same time course and were both associated with an increase in input resistance of the cell. In addition, $G_{K(Ca)}$ and accommodation of action potential discharge were blocked. Secondly, the stimulation sites where these responses were evoked coincide with the known anatomical distribution of cholinergic fibres. These fibres, which originate mainly in the medial septal nucleus and enter the hippocampus via the fornix and fimbria, are concentrated in the alveus, s. oriens and immediately adjacent to s. pyramidale (Shute & Lewis, 1966; Lewis & Shute, 1967; Lewis *et al.* 1967; Storm-Mathisen, 1970; Mosco *et al.* 1973; Lynch *et al.* 1978; Kimura *et al.* 1981; Houser, Crawford, Barber, Salvaterra & Vaughn, 1983). The much higher stimulus intensity required to elicit a slow e.p.s.p. compared to the fast e.p.s.p.s and i.p.s.p. is consistent with the established small diameter of the cholinergic afferents (Shute & Lewis, 1966). Thirdly, all of the responses were selectively enhanced by the cholinesterase inhibitor, eserine, and blocked by the muscarinic antagonist, atropine. The fact that we have been able to exactly mimic all of the post-synaptic actions of ACh by electrically stimulating sites in the hippocampal slice that are known to contain cholinergic fibres provides strong electrophysiological support for a neurotransmitter role for ACh.

It is of interest to compare our results to those of Krnjevic & Ropert (1982) who studied the facilitation of pyramidal cell discharge by stimulating the medial septum. First, the dependence of this facilitation on frequency of tetanic stimulation is similar to the frequency dependence of the slow e.p.s.p. seen in the present study. Secondly, although the effects of brief (about 100 ms) tetani to the medial septum in their study only lasted for 0·3 s, successive brief tetani produced a depression lasting a few seconds followed by a facilitation lasting 20–30 s. This is remarkably similar to the time course of the i.p.s.p./slow e.p.s.p. sequence seen in the present study.

An important issue concerns the localization of the cholinergic input onto

pyramidal cells. The processes involved in accommodation of spike discharge to a somatic depolarizing stimulus are presumably located close to the initial segment, which is the normal site for impulse initiation. Anatomical studies suggest that the large majority of the cholinergic input is in the infrapyramidal layer in s. oriens adjacent to s. pyramidale, with a less dense input to the suprapyramidal layer at the base of s. radiatum (Shute & Lewis, 1966; Lewis & Shute, 1967; Fonnum, 1970; Storm-Mathisen, 1970). The fibres synapse primarily onto dendritic spines in these regions, and only rare axosomatic synapses were found (Shute & Lewis, 1966). Although this input is to some extent remote, recent work has suggested that hippocampal pyramidal cells are electrically compact (Brown, Fricke & Perkel, 1980; Turner & Schwartzkroin, 1980; Johnston, 1981). In addition, it is possible that ACh and/or a second messenger may diffuse away from the synaptic sites. However, it should be emphasized that all of the cholinergic synaptic responses reported here have been observed in the absence of eserine and therefore are not simply a result of grossly elevated levels of ACh that can diffuse to sites which are normally inaccessible.

Most synaptic potentials which have been analysed in the mammalian C.N.S. result from an increase in ionic conductance and have brief time courses, usually lasting no more than a second (Eccles, 1964; Krnjevic, 1974; Nicoll, 1982). Since the reversal potential for classical excitatory synaptic potentials is far removed from the resting potential, these synapses exert their effects primarily through a change in membrane potential. The cholinergic synaptic responses characterized in the present study are fundamentally different from classical synaptic excitation for two reasons. First, the cholinergic synaptic response studied here has a long duration, lasting tens of seconds. Similar long-lasting excitatory potentials have been described in peripheral vertebrate ganglia. These include both cholinergic (Nishi & Koketsu, 1968; Libet, 1970; Weight & Votava, 1970; Adams & Brown, 1982; North & Tokimasa, 1982) and non-cholinergic slow synaptic responses (Grafe, Mayer & Wood, 1980; Johnson, Katayama, Morita & North, 1981; Dun & Jiang, 1982; Jan & Jan, 1982; Tsumoo, Konishi & Otsuka, 1982). A slow e.p.s.p., possibly mediated by ACh, has briefly been described in neurones of the interpeduncular nucleus (Sastry, 1980). Secondly, no change in membrane potential was required for the transmitter to profoundly affect the firing pattern of the post-synaptic cell. This is because ACh blocks two intrinsic potassium currents whose primary function involves control of repetitive firing of the neurone and not resting membrane potential. Thus, the physiological role for the septo-hippocampal cholinergic pathway may not be to initiate cell discharge but rather to regulate and sustain the firing generated by other inputs. As techniques become more refined, synaptic responses similar to those reported here may be found to be widespread in the C.N.S.

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