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CHOLINERGIC EXCITATION OF GABAergic INTERNEURONS IN THE RAT HIPPOCAMPAL SLICE

BY T. A. PITLER AND B. E. ALGER

From the Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

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SUMMARY

1. Intracellular recordings were made from CA1 pyramidal cells in the rat hippocampal slice to study the cholinergic modulation of GABAergic inhibition. The cholinergic receptor agonist, carbamylcholine (carbachol), depressed evoked excitatory postsynaptic potentials (EPSPs) and evoked inhibitory postsynaptic potentials (IPSPs), but enhanced small spontaneously occurring membrane potential fluctuations that resembled IPSPs. Both atropine (1 μM) and picrotoxin (25–60 μM) abolished the small fluctuations.

2. Recording from cells with potassium or caesium chloride (KCl or CsCl)-filled microelectrodes enhanced and inverted spontaneous Cl^--dependent $GABA_A$-mediated IPSPs. These events appeared to result from the spontaneous firing of GABAergic interneurons since they could be inhibited by picrotoxin or bicuculline and nearly eliminated by tetrodotoxin.

3. Muscarinic acetylcholine (ACh) receptor activation significantly increased the frequency of spontaneous-activity-dependent IPSPs from $1{\cdot}7 \pm 0{\cdot}4$ s (mean $\pm$ S.E.M.) in control saline to $7{\cdot}0 \pm 1{\cdot}1$ s in carbachol (10–50 μM)-containing saline, although evoked IPSPs were inhibited. All effects of carbachol were completely reversed by atropine.

4. The increase in frequency of spontaneous IPSPs observed in carbachol was not secondary to changes in the postsynaptic cell and was not blocked by high doses of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5–10 μM) and 2-amino-5-phosphonovaleric acid (APV, 10–20 μM), which abolished evoked excitatory transmission. Amplitude histograms showed an increase in mean size as well as of frequency of spontaneous IPSCs in carbachol.

5. Stimulation of cholinergic afferents in stratum oriens in the presence of the acetylcholinesterase inhibitor eserine (1 μM) also increased spontaneous IPSP frequency, and the time course of this response was similar to that of the muscarinic slow EPSP. Postsynaptic factors or the activation of glutamatergic excitatory pathways could not account for this effect.

6. Evoked monosynaptic IPSCs in CNQX and APV were diminished by carbachol.

7. We conclude that GABAergic inhibitory interneurons possess muscarinic receptors, that activation of these receptors increases the excitability of the interneurons and that synaptically released ACh increases interneuronal activity.

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Cholinergic reduction of the monosynaptic IPSC may point to additional complexity in cholinergic regulation of the GABA system.

INTRODUCTION

Acetylcholine (ACh) is a ubiquitous neurotransmitter that has powerful modulatory influences on neuronal excitability in the mammalian brain through the activation of muscarinic receptors. There are numerous reports of muscarinic modulation of intrinsic ionic conductances, including the inhibition of several classes of potassium (Krnjevic, Pumain & Renaud, 1971; Dodd, Dingledine & Kelly, 1981; Benardo & Prince, 1982*a*; Halliwell & Adams, 1982; Cole & Nicoll, 1984; Madison, Lancaster & Nicoll, 1987; Alger, Pitler & Williamson, 1990) and calcium channels (Gahwiler & Brown, 1987). There is also evidence that ACh is capable of regulating neuronal excitability through the modulation of synaptically activated conductances, e.g. activation of muscarinic receptors depresses excitatory transmission via a presynaptic inhibitory mechanism (Hounsgaard, 1978; Valentino & Dingledine, 1981; Dutar & Nicoll, 1988; Williams & Johnston, 1990).

A particularly interesting problem involves the interaction of cholinergic and GABAergic systems. For example, in several models of the hippocampal θ-rhythm joint action of these systems is postulated (reviewed in Stewart & Fox, 1990). The experimental evidence for interaction is not strong however, and the actual relationship between the systems is not known. Several groups have shown that application of exogenous cholinergic agonists depresses the evoked GABAergic IPSP (Krnjevic, Reiffenstein & Ropert, 1981; Valentino & Dingledine, 1981; Haas, 1982; Muller & Misgeld, 1989); one suggested mechanism is presynaptic inhibition of GABA release, although depression of GABAergic interneuron excitability also seemed possible. On the other hand, studies have suggested that in certain cortical brain regions ACh enhances GABA release (McCormick & Prince, 1985). Observations that application of ACh caused a transient hyperpolarization and increase in small potentials presumed to be IPSPs suggested the possibility of cholinergic excitation of GABAergic interneurons in the hippocampus (Benardo & Prince, 1982*b*; Haas, 1982), although the hyperpolarization has also been attributed to an increase in K^+ conductance (Segal, 1982). Anatomical data in the hippocampus have revealed a likely cholinergic input directly to interneurons (Lynch, Rose & Gall, 1978), but the physiological significance of this pathway is not yet known.

A number of questions remain unanswered by previous studies. For instance, it is not clear if the hyperpolarization or small potentials initiated by ACh are actually GABAergic; no pharmacological tests were done. Also, the paradoxical finding that the evoked IPSP was depressed while the excitability of the interneurons was evidently enhanced has not been resolved; the suggestion that ACh increases and subsequently decreases interneuron excitability (Haas, 1982) needs to be tested. Lacking too is evidence that the cholinergic effects on the interneurons are direct, as they could conceivably be mediated via some other synaptic system. Finally, there is no evidence that the putative cholinergic receptors on GABAergic interneurons can be activated by synaptically released ACh; previous studies on this topic have been done using direct application of cholinergic agonists. We have undertaken the following study to address these issues.

METHODS

Male Sprague–Dawley rats (200–300 g) were anaesthetized with ether and decapitated. The brain was removed rapidly and the hippocampus dissected free. For most experiments, done with high-resistance intracellular recording pipettes, transverse sections 400 μm thick were cut on a tissue chopper and placed in a holding chamber at the interface of physiological saline and humidified atmosphere (95% O_2, 5% CO_2) where they were maintained at room temperature. Slices were transferred to a recording chamber as needed where they were held submerged between two nylon nets (Nicoll & Alger, 1981). The recording chamber was continually perfused with warm (31 °C) bubbled (95% O_2, 5% CO_2) saline that was comprised of (in mM): NaCl, 124; KCl, 3·5; NaH_2PO_4, 1·25; $NaHCO_3$, 26; $CaCl_2$, 2·5; MgCl, 3·5; glucose, 10.

Standard intracellular recording techniques were used in most experiments. Intracellular recordings were obtained from the CA1 region using 50–120 MΩ microelectrodes filled with either 3 M-KCl or 2 M-CsCl, buffered with 10 mM-HEPES, unless otherwise noted. Diffusion of Cl^- from the electrode raised the intracellular Cl^- sufficiently to invert the normally hyperpolarizing IPSPs to depolarizations. In some cases, microelectrodes also contained 100 mM-QX-314 to suppress action potentials. An Axoclamp-2 amplifier was used in the bridge mode for current-clamp recordings. In some experiments, a thick-slice, whole-cell voltage-clamp technique was used (Blanton, Lo Turco & Kriegstein, 1989; Mody & Otis, 1989). In these experiments, slices of the usual thickness were cut on a vibratome. Low-resistance electrodes (2–6 MΩ) were filled with (in mM): KCl or KCH_3SO_3, 130; MgATP, 5; $CaCl_2$, 1; EGTA, 11 or BAPTA, 10; HEPES, 10; pH 7·4. In some experiments QX-314, 5–10 mM, was also present in the electrode. Electrodes were passed through the CA1 pyramidal cell layer of the slice until small changes in resistance were seen. Suction was applied by syringe until > 1 GΩ seals formed. The patch was then ruptured by mouth suction. Recordings were quite stable, with resting membrane potentials of −60 to −70 mV and input resistances between 60 and 200 MΩ.

Only cells with resting potentials greater than −55 mV and input resistances greater than 30 MΩ were used for analysis. All drugs were administered via the perfusion saline. Values given are means ± S.E.M.

Data were stored on a VCR-based tape-recorder system (Neurocorder) and played back on a rectilinear chart recorder (Gould) or digital storage oscilloscope (Nicolet) for subsequent analysis. The criteria used in counting spontaneous IPSPs were amplitudes greater than 2 mV and a rise time of the IPSP depolarization greater than 250 μV/ms. For exogenous carbachol application, 16 or 20 s of continuous data were measured to determine mean spontaneous IPSP frequency. Cells were manually voltage clamped at their original resting potentials when IPSPs were counted. For measurements of IPSP frequency following stimulation protocols, 5 s bins were measured.

Bipolar stimulating electrodes were made from insulated stainless steel. EPSP–IPSP responses were evoked when the electrode was placed in stratum radiatum. For stimulation of cholinergic afferents, the electrodes were placed in stratum oriens (cf. Cole & Nicoll, 1984). Stimulation consisted of a 20 Hz train of pulses lasting for 0·5 s; pulses were approximately 100 μA and were 0·1 ms in duration.

DL-2-Amino-5-phosphonovaleric acid (APV), picrotoxin, carbamylcholine chloride (carbachol), atropine sulphate and eserine sulphate were obtained from Sigma Chemical Corp. (St Louis, MO, USA). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was obtained from Research Biochemicals, Inc. (Natick, MA, USA). Tetrodotoxin (TTX) was purchased from Calbiochem (San Diego, CA, USA). QX-314 was a gift from Astra Alab AB (Södertälje, Sweden).

RESULTS

Current-clamp data were obtained from forty-one cells that had a mean resting membrane potential of −64·2 ± 0·9 mV and input resistance of 67·0 ± 3·2 MΩ. Bath application of carbachol (10–25 μM) depolarized pyramidal cells and increased their input resistance, as reported by others.

Muscarinic effects on inhibitory synaptic transmission

Stimulation of the Schaffer–collateral pathway produces an EPSP–IPSP complex in hippocampal CA1 pyramidal cells recorded with 3 M-KCH_3SO_3-filled microelectrodes. The IPSP is comprised of both Cl^--, and K^+-dependent components that

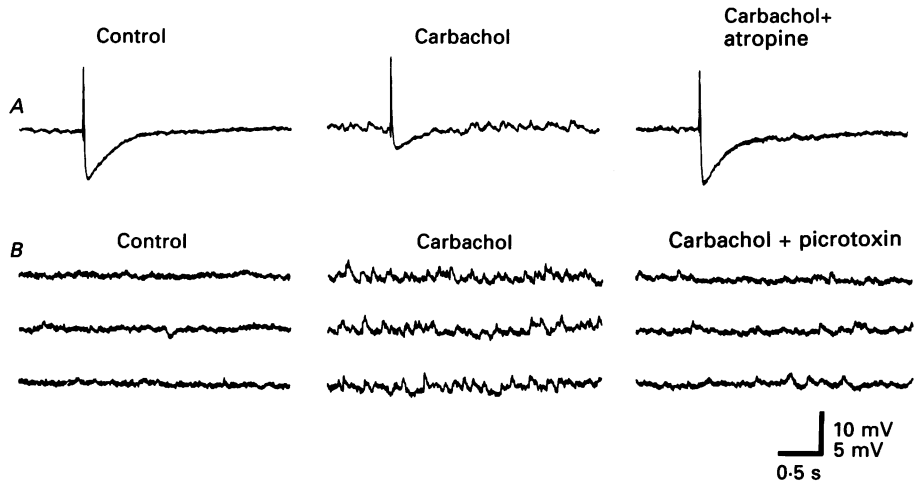

Fig. 1. Muscarinic receptor influence on evoked IPSPs and spontaneous membrane potential fluctuations. *A*, evoked synaptic potentials recorded from a CA1 pyramidal cell with a KCH_3SO_3-filled electrode. Stimulation was delivered to the Schaffer–collateral pathway. The response is a mixed excitatory and inhibitory postsynaptic potential with, in this example, an action potential activated by the EPSP (action potentials in this and subsequent figures are truncated). Application of 25 μM-carbachol resulted in a significant depression of the IPSP, which completely reversed with the addition of 1 μM-atropine within 5 min. Note that in the presence of carbachol the baseline showed an obvious increase in membrane potential fluctuations that were reduced by atropine. *B*, the membrane potential fluctuations induced by carbachol were sensitive to picrotoxin (60 μM), suggesting that they were largely the result of GABA release. Resting membrane potentials (RMPs) of cells shown in *A* and *B* were -57 and -62 mV, respectively.

are produced by $GABA_A$ and $GABA_B$ receptors, respectively, although the late, K^+-dependent phase is not obvious in the cell shown in Fig. 1. Muscarinic agonists decrease the amplitude of the entire IPSP (cf. Muller & Misgeld, 1989), an effect that can be completely reversed by atropine. In this paper we have focused on the early $GABA_A$ inhibition. A supramaximal stimulus was used for the experiment in Fig. 1 to emphasize the block of the evoked IPSP; as shown by a number of other groups, EPSPs elicited by somewhat weaker stimuli are also reduced by muscarinic agonists due to a presynaptic inhibitory mechanism (Yamamoto & Kawai, 1967; Valentino & Dingledine, 1981; Haas, 1982; Dutar & Nicoll, 1988).

We noted in addition, however, that in many cases during the period of depression of evoked IPSPs by carbachol, recordings of the membrane potential showed hyperpolarizing membrane potential fluctuations of 1–3 mV that were abolished by atropine (1 μM). These fluctuations were largely blocked by the $GABA_A$ antagonist picrotoxin (25–60 μM; e.g. Fig. 1), suggesting that they represented in large part

$GABA_A$ responses. We therefore note a seemingly paradoxical effect of carbachol: an apparent enhancement of spontaneous GABA release while there is an inhibition of the evoked GABAergic IPSP.

Carbachol-induced increase in spontaneous IPSPs

In order to resolve the spontaneous IPSPs more clearly we used intracellular electrodes in which Cl^- was the predominant anion in the filling solution. Diffusion

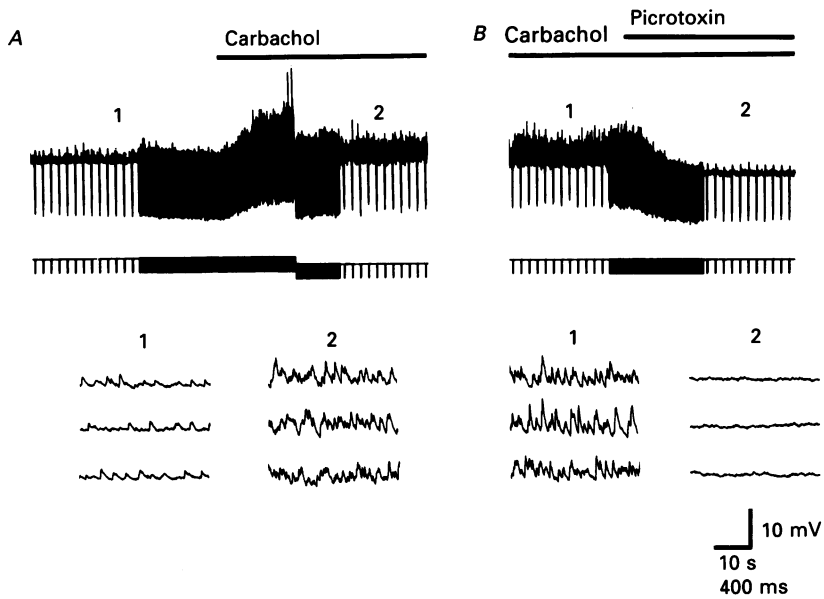

Fig. 2. Carbachol increases the frequency of spontaneous IPSPs. Cells in this figure were recorded with 3 M-KCl electrodes causing spontaneous IPSPs to appear as 1–10 mV rapidly depolarizing deflections. Top traces are voltage records; directly below are the records of current injection. Lowest sets of traces show segments of voltage records on an expanded time base taken at points indicated by the numbers in the top traces. Membrane resistance was monitored by 0·2 nA, 200 ms hyperpolarizing current pulses given at 0·5 Hz. *A*, bath application of 25 μM-carbachol resulted in a depolarization and simultaneous increase in frequency and amplitude of spontaneous IPSPs. Following the initial phase of the depolarization, the cell was manually voltage clamped at its original resting potential (RMP = −70 mV; the time scale as carbachol was added initially is compressed by a factor of 10; otherwise the time base corresponds to the 10 s calibration). The lower traces on an expanded time base more clearly show the frequency of spontaneous IPSPs in control (1) and in the presence of carbachol (2). *B*, spontaneous depolarizing IPSPs recorded in the presence of carbachol (> 30 min) were completely blocked by 60 μM-picrotoxin (RMP = −75 mV). Lower traces again show voltage records at an expanded time base taken in carbachol (1) and following addition of picrotoxin (2).

of Cl^- from these pipettes into the cells results in the appearance of numerous 1–10 mV rapidly depolarizing events that are picrotoxin (Figs 2 and 3) and bicuculline sensitive. The events most often occurred randomly, although at times they appeared in bursts. These large spontaneous synaptic events are not true miniature postsynaptic potentials reflecting quantal release, but in fact correspond

to synaptic release caused by the firing of GABAergic interneurons (Alger & Nicoll, 1980). This view is supported by the fact that the great majority of these spontaneous IPSPs can be blocked by addition of TTX to the bathing medium (see Fig. 3*C*). The term 'spontaneous' thus means simply that they are not evoked via

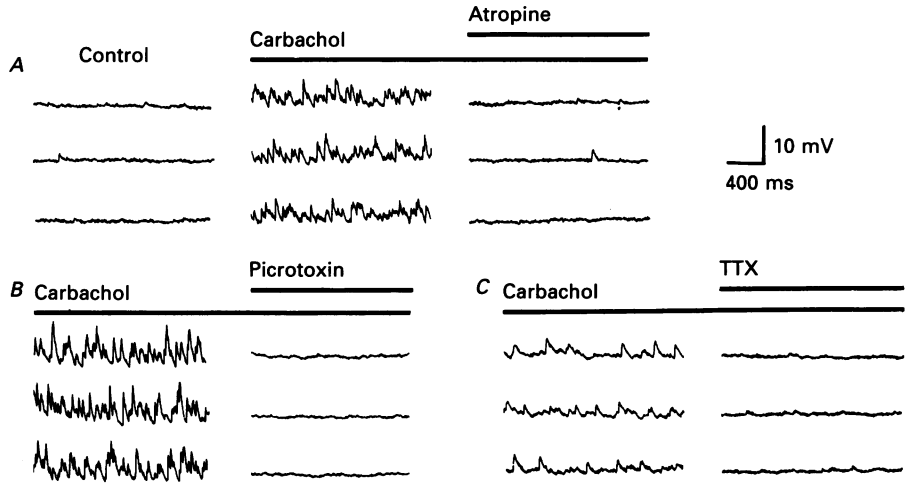

Fig. 3. Atropine, picrotoxin and tetrodotoxin block carbachol-induced spontaneous IPSPs. Bars above the traces signify the presence of agents listed. Carbachol was present in the bathing medium for at least 20 min prior to application of various antagonists. Records showing block of IPSPs were taken between 5 and 10 min after addition of indicated antagonist. *A*, increase in IPSPs caused by carbachol (25 μM) was reversed by 1 μM-atropine (RMP = −72 mV). *B*, IPSPs induced by carbachol were completely blocked by 60 μM-picrotoxin (RMP = −75 mV), and 0·3 μM-TTX blocked the largest events (*C*), although a few very small events persisted (RMP = −65 mV).

exogenous electrical stimulation, and is used to denote this distinction. Clearly any treatment that alters the excitability of the interneurons will alter spontaneous activity. The frequency of spontaneous IPSPs in control saline varied greatly from cell to cell (0 -> 10/s) with a mean of $1{\cdot}7 \pm 0{\cdot}4$/s ($n = 20$). Carbachol increased the frequency of spontaneous IPSPs in all cells in which they were observed in control saline and induced IPSPs to occur in many cells where none were present before application of carbachol. In a small percentage of cells, spontaneous IPSPs were never observed, and these cells were not studied further. In carbachol the cells were manually voltage clamped to their original resting potential so that the driving force on the IPSPs was essentially unchanged when compared to control. The mean frequency of spontaneous IPSPs measured 10–15 min after carbachol application (10–50 μM) was $7{\cdot}0 \pm 1{\cdot}1$/s. Addition of 1 μM-atropine after the carbachol-induced increase in IPSPs rapidly reduced the frequency of IPSPs to the previous control levels ($n = 3$). In all cases tested ($n = 7$), the spontaneous depolarizing IPSPs recorded in the presence of carbachol were abolished by the addition of picrotoxin (25–60 μM) to the bathing medium (Fig. 3*B*). Typically the effect of carbachol in increasing IPSP frequency was quite persistent, showing no apparent signs of decreasing for up to 30 min of application. In a few cases IPSP frequency did slowly

decline somewhat despite the continued presence of the cholinergic agonist, as noted by others (Haas, 1982). Nevertheless, the effects of atropine or of $GABA_A$ antagonists were obvious, as they occurred in a rapid and thorough way, clearly distinct from the occasional slow fading.

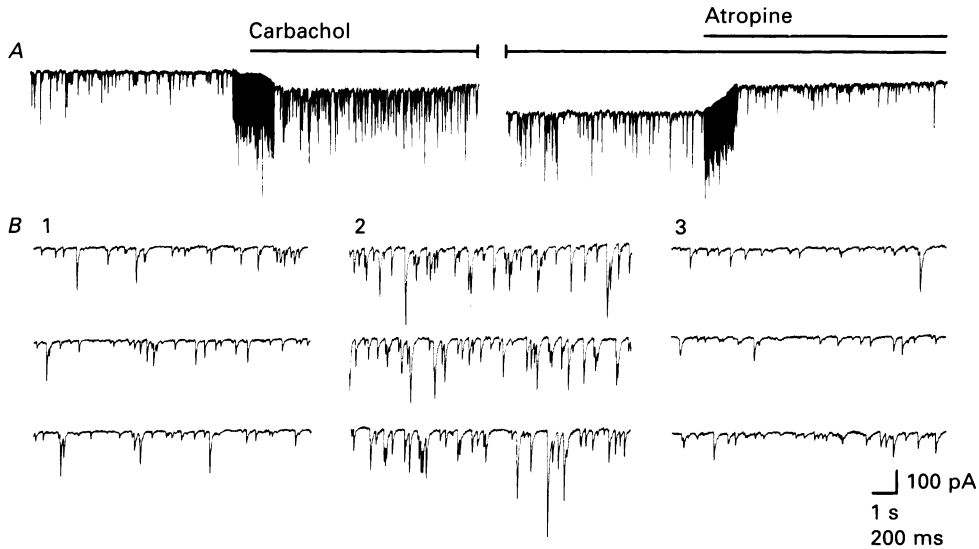

Fig. 4. Whole-cell voltage clamp of carbachol-induced increase in IPSCs and reversal with atropine. *A*, continuous current record of a CA1 cell held at −60 mV. The recording electrode contained 130 mM-Cl^-, which caused spontaneous $GABA_A$-mediated currents to be inward. Bath application of 25 μM-carbachol resulted in an inward current and a dramatic increase in spontaneous IPSCs. Both these effects were reversed by 1 μM-atropine applied 8 min after initial introduction of carbachol. Time bases are compressed by a factor of 10 during the initial addition of carbachol and atropine; otherwise the time base is given by the 1 s calibration. *B*, the time base of the above cell is expanded (200 ms calibration) showing occurrence of IPSCs in control (*B*1), in 25 μM carbachol (*B*2) and in carbachol plus 1 μM-atropine (*B*3).

The increase in spontaneous IPSPs is not secondary to carbachol-induced postsynaptic effects

Spontaneous IPSPs seen in the presence of carbachol often appear larger in amplitude than those in control saline. This could be the result of carbachol-induced increase in input resistance in the postsynaptic cell. Conceivably, if the increase in resistance were sufficiently great it could potentiate a population of subliminal IPSPs to an extent that they would become visible, which would increase apparent IPSP frequency. We do not believe this effect can explain our results, however. Typically the increase in input resistance was 20–30% measured at the control resting potential, and IPSP frequency increased over fivefold. Furthermore, we recorded from some cells with CsCl-filled electrodes. These electrodes prevent the large increase in resistance caused by carbachol that is seen with KCl electrodes, presumably because Cs^+ blocks some of the K^+ conductance whose inhibition by carbachol leads to the increase in resistance, i.e. the leak conductance (Krnjevic *et al.*

1971; Madison *et al.* 1987). Even with CsCl-filled electrodes, however, we saw a very similar increase in spontaneous IPSP frequency with carbachol. QX-314 also occluded the carbachol-induced membrane effects probably because it too blocks ion channels (see below), and yet increases in IPSP frequency were observed when QX-314 was present in the recording electrode.

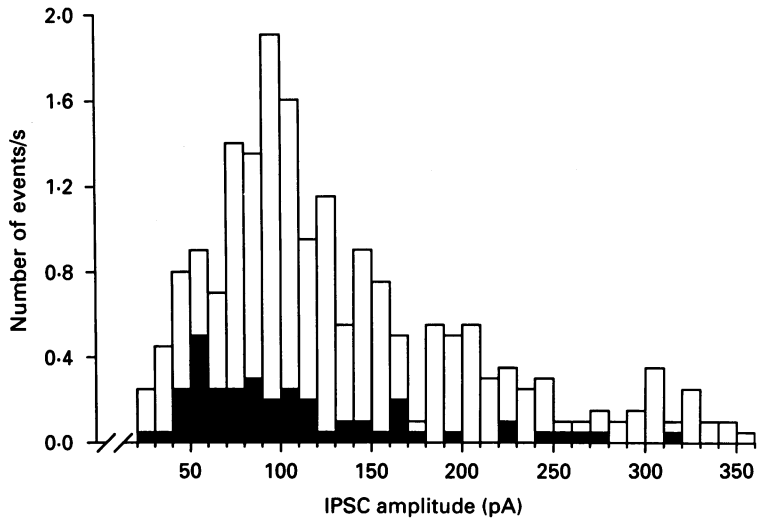

Fig. 5. Carbachol (25 μM) increases both the frequency and mean amplitude of IPSCs. Data for the amplitude histogram were analysed from a continuous 20 s period just prior to carbachol application and a 20 s period 4 min after switching to carbachol-containing medium. In this example the total number of events increased 578%, and the mean amplitude increased 14·8%. ■, control medium; □, 25 μM-carbachol.

In order to test the influence of postsynaptic, i.e. pyramidal, cell properties on the increase in IPSP frequency more directly, however, we employed gigohm-seal whole-cell voltage-clamp recordings from neurons in the slice preparation. Voltage-clamp recordings of inhibitory currents will not be complicated by changes in postsynaptic resistance or membrane potential. The low-resistance electrodes (2–6 MΩ) contained 130 mM-KCl (as well as other salts; see Methods) and spontaneous inward IPSCs could readily be recorded (Fig. 4). Even gigohm-seal methods cannot provide perfect space clamp of our cells. Nevertheless, space-clamp error cannot explain our results since an incompletely clamped dendritic depolarization would decrease, not increase, the inward IPSCs caused by elevated $[Cl^-]_i$. In seventeen cells in normal saline, 25 μM-carbachol induced an inward current and a marked increase in the frequency of spontaneous IPSCs. Although there was a slight decline in IPSC frequency in the illustrated cell during maintained carbachol perfusion, both the inward current and increase in IPSCs were readily reversed by atropine.

Amplitude histograms were constructed for four cells under the above conditions (an example is shown in Fig. 5). The increase in frequency of events with 25 μM-carbachol ranged from 251 to 642%, while the mean amplitude of the IPSCs

increased from 14 to 97%. The fact that spontaneous IPSCs increase in amplitude with carbachol suggests that GABA release is not inhibited at the presynaptic terminal.

Carbachol-induced increase in spontaneous IPSPs is not mediated by excitatory pathways

It is conceivable that carbachol increases spontaneous IPSPs indirectly by the muscarinic excitation of pyramidal cells and subsequent activation of feedforward

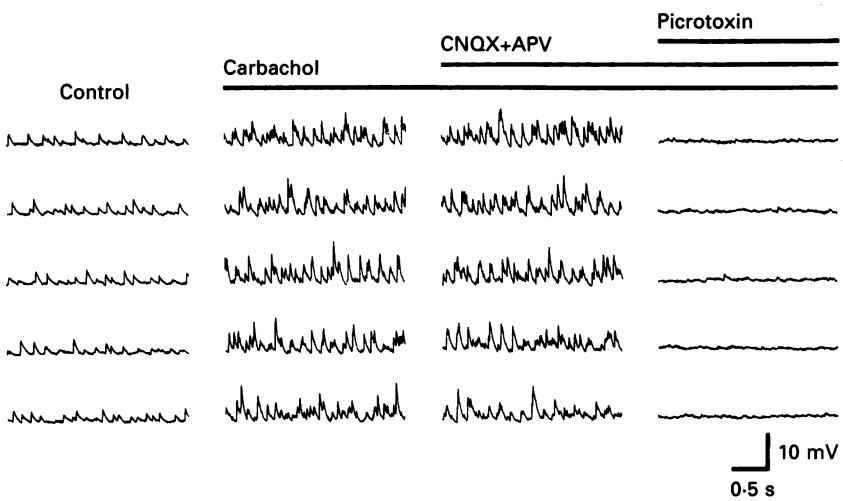

Fig. 6. Carbachol-induced increase in spontaneous IPSPs is not blocked by pharmacological inhibition of excitatory transmission. Bars above the traces signify the presence of the drugs indicated. The carbachol (25 μM)-induced increase in frequency of IPSPs was not overtly affected by addition of 10 μM-CNQX and 20 μM-APV, concentrations of glutamate antagonists that block excitatory transmission in the hippocampus. IPSPs are shown again to be sensitive to picrotoxin (60 μM). Traces shown are from a 12·5 s continuous record taken 10 min after the addition of the pharmacological agents indicated. All records taken from the same cell (RMP = −63 mV).

and recurrent pathways to GABAergic inhibitory interneurons. Because pyramidal cells utilize the excitatory transmitter glutamate (Cotman, Monaghan, Ottersen & Storm-Mathisen, 1987; Collingridge & Lester, 1989), we used a combination of the glutamate receptor antagonists CNQX and APV to block excitatory transmission in the slice and, hence, polysynaptic inhibition (Davies, Davies & Collingridge, 1990). Doses of CNQX (5–10 μM) and APV (10–20 μM) that completely blocked excitatory synaptic transmission did not substantially affect the resting frequency of IPSPs (2·1 ± 2·4/s in control *versus* 2·4 ± 1·9/s in CNQX + APV, $n = 4$) and did not prevent an increase in frequency induced by carbachol (6·4 ± 4·2/s). In two cells in which CNQX (10 μM) and APV (20 μM) were added after the addition of carbachol (25 μM) (Fig. 6), the frequency of spontaneous IPSPs decreased slightly from a mean of 8·3/s to 6·6/s, but remained much greater than the 1·2/s spontaneous IPSP frequency seen in control medium. Thus, it is very likely that there is a direct activation of GABAergic interneurons by muscarinic receptors.

Brief, high-frequency (20 Hz, 0·5 s) trains of stimulation from a stimulation electrode placed in stratum oriens result in a slow depolarizing response ('slow EPSP') in CA1 pyramidal neurons that typically lasts 20–60 s in the presence of the anticholinesterase inhibitor eserine and is blocked by muscarinic antagonists (Cole &

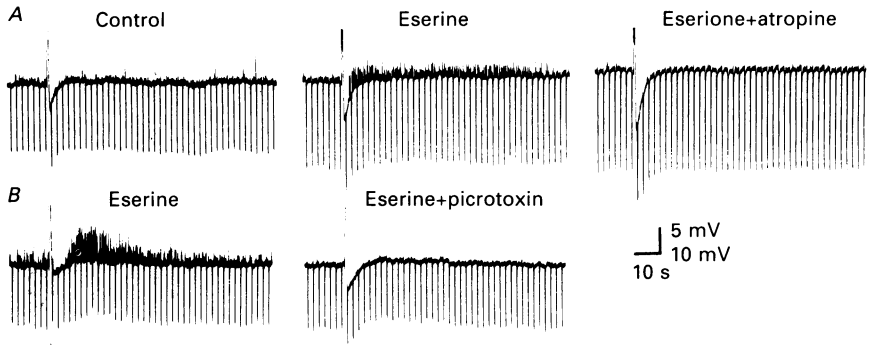

Fig. 7. Stimulation of cholinergic afferents in the presence of eserine increases spontaneous IPSP frequency. Cells shown were recorded with KCl-filled electrodes also containing 100 mM-QX-314 to block action potentials. *A*, a 50 Hz train of stimulation for 0·5 s produced no change in IPSP frequency in control medium, but in the presence of 1 μM-eserine resulted in an increase in spontaneous IPSPs lasting up to 2 min post-stimulation. Atropine (1 μM) blocked this effect (RMP = −62 mV). *B*, in another cell, the IPSPs were blocked by picrotoxin (RMP = −71 mV). Effects of both atropine and picrotoxin occurred within 5 min of application. Calibration for the top set of traces, 5 mV; bottom set of traces, 10 mV. Sharp downward deflections are due to 0·2 nA hyperpolarizing current pulses. The increase in the amplitude of the hyperpolarization that appears in the traces with atropine (top) or picrotoxin (bottom) plus eserine is probably due to two factors: (1) the block of the depolarizing IPSPs, which had overlapped and reduced it, and (2) the gradual increase in input resistance caused by QX-314 that increased membrane potential responses.

Nicoll, 1983, 1984; Muller & Misgeld, 1986; Segal, 1988; Pitler & Alger, 1990). While the slow EPSP is due to synaptic connections between cholinergic afferents and the pyramidal cells themselves, there is anatomical evidence of a cholinergic projection to interneurons as well (Lynch *et al.* 1978). This, together with our evidence for muscarinic receptors on the interneurons, suggests that stimulation of cholinergic afferents should increase the frequency of spontaneous IPSPs.

When recording with KCH_3SO_3-filled electrodes in the presence of the acetylcholinesterase inhibitor eserine, we noted an increase in membrane noise during the slow EPSP, much like what is seen with carbachol application (not shown). Typically, however, action potentials triggered during the peak of the slow EPSP obscure the small events, preventing a more detailed investigation. Therefore we recorded from cells with 3 M-KCl or 2 M-CsCl electrodes that also contained 100 mM-QX-314 to suppress the action potentials. Aside from blocking spikes in the postsynaptic cell, QX-314 appeared to depress the amplitude of the slow EPSP and to increase input resistance and membrane time constant gradually throughout the experiment. In cells recorded with CsCl electrodes, the slow EPSP was also predominantly blocked, presumably because Cs^+ blocks the K^+ channels responsible

for its generation (Madison *et al.* 1987). After a train of stimuli in stratum oriens we often saw a pronounced increase in IPSPs that had a time course very similar to a slow EPSP (Fig. 7). The effect was slow in onset, but was clearly noticeable 3–15 s following the train of stimulation. For this group of cells, the control frequency of

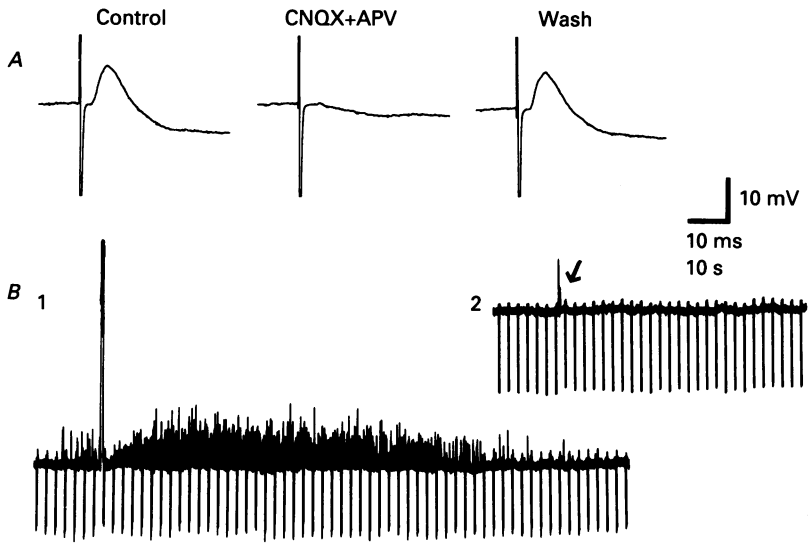

Fig. 8. Pharmacological blockade of excitatory transmission does not block the increase in spontaneous IPSPs due to stimulation of cholinergic afferents. *A*, in a cell recorded with a KCH_3SO_3-filled electrode, 5 μM-CNQX and 10 μM-APV abolished the EPSP (RMP = −69 mV). *B*1, cell recorded with a CsCl electrode in the presence of the same concentrations of CNQX and APV and 1 μM eserine (RMP = −72 mV). Stimulation of cholinergic afferents continued to cause an increase in spontaneous IPSPs. *B*2, the initial large depolarizing response to stimulation shown in *B*1 is due to a reversed IPSP activated by stimulation and not an EPSP as it was blocked by picrotoxin (arrow). Large downward deflections are responses to constant-current pulses.

spontaneous EPSPs was $1{\cdot}0 \pm 0{\cdot}3$/s ($n = 10$). A 5 s interval taken 8–20 s following the train (at which time IPSPs began to appear) showed an increase in frequency of IPSPs to $4{\cdot}2 \pm 0{\cdot}8$/s. The effect often persisted for more than 1 min and eventually returned to near-baseline levels in all cells. Significantly, the increased IPSP frequency induced by trains of stimulation could be abolished by atropine (Fig. 7*A*), indicating that cholinergic fibres were involved. These events were confirmed to be IPSPs by their sensitivity to picrotoxin (Fig. 7*B*). We do not believe that the QX-314 had a confounding effect on our results since we saw a similar increase in spontaneous IPSPs during the slow EPSP in two cells in which we did not include QX-314 in the recording pipette.

Finally, the increase in IPSP frequency produced by trains of stimulation in stratum radiatum was not blocked by CNQX and APV in the bathing medium at concentrations that suppressed the orthodromically activated EPSP (Fig. 8), suggesting that synaptically released ACh acted directly on the interneurons ($n = 4$).

Carbachol inhibits the evoked monosynaptic IPSC

It is established that carbachol inhibits the polysynaptically activated IPSP (e.g. Fig. 1) and presynaptically inhibits the glutamatergic EPSPs. Thus block of the evoked polysynaptic IPSP could be explained as a secondary consequence of EPSP

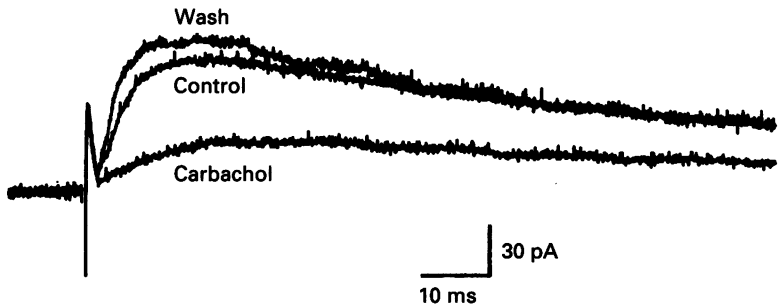

Fig. 9. Carbachol (25 μM) reduces the stimulus-evoked monosynaptic IPSC. The IPSC was evoked by a bipolar stimulator placed within 200 μm of the cell in the presence of both 10 μM-CNQX and 20 μM-APV. The whole-cell recording electrode contained KCH_3SO_3, causing the response to be a purely outward current following block of the inward EPSC by glutamate antagonists.

inhibition. Our data thus far would suggest that transmitter release from the $GABA_A$ interneurons *per se* would be unaltered or even enhanced. By using a combination of glutamate antagonists (as above) and by close positioning of a stimulation electrode, we could directly activate GABAergic pathways and evoke presumed monosynaptic IPSCs. For this experiment we used whole-cell recording techniques; most cells were recorded with KCH_3SO_3-containing electrodes so that the IPSC would appear outward and the block of the inward glutamatergic EPSC could be monitored. Surprisingly, in all five cells tested the 'monosynaptic' IPSC was substantially decreased ($70{\cdot}8 \pm 3{\cdot}8$ %; Fig. 9). Implications of this observation are discussed below.

DISCUSSION

Our results provide physiological support for the following conclusions. (1) Muscarinic receptors are located on hippocampal GABAergic interneurons. (2) These neurons are innervated by cholinergic fibres presumably arising from the medial septum. (3) The consequence of activating muscarinic receptors on GABAergic interneurons is an increase in interneuron excitability. Figure 10 summarizes these conclusions schematically. In this investigation we have focused on $GABA_A$-mediated responses. Although the $GABA_B$ response is reportedly also reduced by cholinergic agonists, additional experiments will be necessary to determine the cause of this effect.

Anatomical evidence indicates that cholinergic fibres terminate in the zone populated by interneurons and that muscarinic receptors are located in that zone, suggesting that the interneurons receive muscarinic innervation (Lynch *et al.* 1978). To date there has been little physiological evidence to support this hypothesis,

however. In this study, we found that carbachol potently alters the excitability of GABAergic neurons. Because the muscarinic effect was not blocked by the glutamatergic receptor antagonists CNQX and APV, it appeared independent of excitatory synaptic influences, implying that a direct excitatory influence on the inhibitory interneurons is involved.

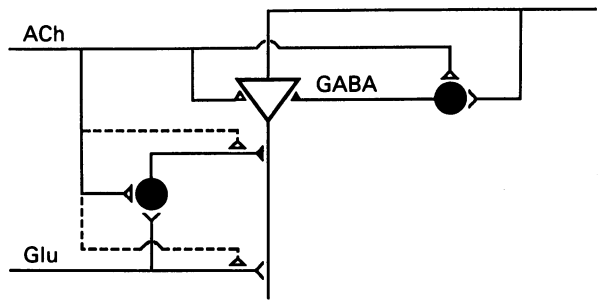

Fig. 10. Schematic diagram summarizing evidence about the termination of cholinergic fibres in the CA1 region. The large open triangle represents a pyramidal cell, and the large filled circles feedforward and feedback GABAergic interneurons that contact the pyramidal cell soma and dendrites (filled triangles). Glutamatergic afferent fibres are shown with forked endings. Cholinergic synapses (small open triangles) are made onto the pyramidal cell and interneurons. Dashed lines represent postulated cholinergic projections to the terminals of both glutamatergic and GABAergic fibres.

Previous studies showed biphasic (hyperpolarizing–depolarizing) actions of applied muscarinic agonists on pyramidal cells, which was interpreted as a modulatory effect of ACh on GABA release (Benardo & Prince, 1982*b*; Haas, 1982; McCormick & Prince, 1985). Cholinergic excitation of interneurons has been thought to be 'faster' than that of pyramidal cells and to involve a fundamentally different form of excitation, perhaps one resembling a 'conventional' neurotransmitter (i.e. one involving the direct ligand gating of an ion channel) rather than the slower muscarinic actions that result from the closing of ion channels (Brown, 1988). However, our data indicate that the duration of IPSP enhancement mimics that of the muscarinic EPSP and is blocked by atropine. It is important that most work on the slow EPSP, including our own, was done in eserine. Hence the duration of the phenomenon may have been artificially enhanced; nevertheless this experiment does establish that synaptically released ACh can enhance IPSP frequency. The experiment also suggests that excitation of interneurons may occur via the same mechanisms as those that increase pyramidal cell excitation.

The medial septal region, which provides the cholinergic input to the hippocampus (Nicoll, 1985), has been identified as the generator for a component of hippocampal θ-rhythm. Rhythmic firing of septal cells may serve as a pacemaker for θ. However, the actual effect of rhythmic cholinergic input is not yet certain. Possibilities include a role for monosynaptic cholinergic connections to hippocampal GABAergic interneurons (Stewart & Fox, 1990).

Krnjevic *et al.* (1981) have emphasized the effect of a cholinergic 'disinhibition' in exciting the pyramidal cells. The observations by several groups of a decrease in the evoked IPSP apparently provided a basis for functional disinhibition (Valentino &

Dingledine, 1981; Haas, 1982; Muller & Misgeld, 1989). Our data argue strongly that cholinergic input to GABA neurons is excitatory. The mechanism of disinhibition of the evoked EPSP remains unresolved; it could represent a secondary effect of presynaptic inhibition of excitatory fibres, a specific effect on a subpopulation of GABAergic terminals or an indirect effect on GABAergic interneurons. Carbachol-induced block of the 'monosynaptic' IPSC suggests that presynaptic inhibition of excitatory fibres may not be the only factor. It is possible that carbachol depolarizes GABAergic interneurons such that they fire rapidly and release transmitter ('spontaneous IPSPs') but become refractory to additional stimulation. Alternatively, carbachol could cause presynaptic inhibition of some GABAergic interneurons as well as activation of a different pool of interneurons; different classes of interneurons do exist in CA1 (e.g. Lacaille & Schwartzkroin, 1988). The increase in spontaneous IPSC amplitude indicates that GABA release is not inhibited at all presynaptic terminals, and, indeed, the increase in IPSC amplitude is probably a presynaptic effect since postsynaptic changes will not increase the amplitude of voltage-clamped IPSCs under our conditions. Finally, it may be that the monosynaptic IPSC isolated in the presence of glutamate antagonists is not solely monosynaptic, but is activated polysynaptically by other non-glutamatergic pathways, which are subject to cholinergic presynaptic inhibition. Direct recording from GABAergic interneurons will be necessary to distinguish among these possibilities.

Nevertheless, while the functional significance of the complex cholinergic interactions with the hippocampal GABAergic system is not entirely clear, it is of interest that the system provides for wide divergence of effects, given the ramification of the interneuron collateral system, and that pyramidal cells will be bombarded by IPSPs initiated by activity in the cholinergic pathway. These features seem well suited to a role for the septal inputs in regulating θ-rhythm in CA1.

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