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LONG-LASTING FACILITATION OF EXCITATORY POSTSYNAPTIC POTENTIALS IN THE RAT HIPPOCAMPUS BY ACETYLCHOLINE

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SUMMARY

1. The effects of acetylcholine (ACh) on excitatory postsynaptic potentials (EPSPs) evoked by stimulating Schaffer–commissural afferents and on ionophoretically applied L-glutamate ligands, were investigated in CA1 neurones of hippocampal slices using current- and voltage-clamp techniques.

2. ACh produced a transient suppression followed by a long-lasting facilitation of EPSPs. The facilitation was also seen in Cs^+-filled cells under voltage-clamp conditions. Both suppressing and facilitating effects were blocked by atropine.

3. All components of the EPSP were reduced in the initial phase of ACh action, while only the slow component was enhanced during the later phase. The facilitation was blocked by an *N*-methyl-D-aspartate (NMDA) receptor antagonist, *d*-2-amino-5-phosphonovalerate (2-APV) and by hyperpolarization.

4. ACh also facilitated responses to ionophoretically applied NMDA in voltage-clamped, Cs^+-filled cells in Ba^{2+}-treated slices. ACh facilitated responses to L-glutamate which was blocked by 2-APV. ACh failed to affect responses to kainate or quisqualate.

5. We conclude that ACh, acting on muscarinic receptors, exerts a primary effect in the hippocampus to specifically amplify NMDA receptor-mediated synaptic responses and thereby facilitate EPSPs.

INTRODUCTION

The importance of ACh in learning and memory has long been recognized (Buresova, Bures, Bohdanecky & Weiss, 1964; Deutch, 1971; Drachman & Leavitt, 1974; Bartus, Reginald, Dean, Beer & Lippa, 1982) and has stimulated extensive investigations into the actions of ACh on neuronal functions. Despite these investigations, the modes by which ACh operates in memory have remained elusive. As synaptic plasticity has been proposed to underlie learning and memory processes (Hebb, 1949; Teyler & DiScenna, 1984), the study of cholinergic effects on synaptic transmission, particularly long-lasting effects, may be extremely important in elucidating the role of ACh in learning and memory.

The hippocampus is a model region for the study of learning and memory processes as well as synaptic plasticity (see Swanson, Teyler & Thompson, 1982; Teyler &

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DiScenna, 1985). The hippocampus receives a massive cholinergic innervation from the medial septum-diagonal band (Lewis, Shute & Silver, 1967), which is essential for a variety of mnemonic functions associated with the hippocampus (see Kesner, 1988; Ordy, Thomas, Volpe, Dunlap & Colombo, 1988). Cholinergic neurotransmission underlies a slow synaptic potential in hippocampal neurones (Cole & Nicoll, 1983) which results in a gradual and long-lasting increase in cellular excitability secondary to depolarization, increased input resistance and reduced accommodation caused by ACh (Benardo & Prince, 1981; Segal, 1988). The effects are mediated by the blockade of at least four types of K^+ conductances (Segal, 1982; Halliwell & Adams, 1982; Benardo & Prince, 1982; Cole & Nicoll, 1983; Nakajima, Nakajima, Leonard & Yamaguchi, 1986; Madison, Lancaster & Nicoll, 1987).

In addition, ACh exerts a number of effects on synaptic transmission. ACh markedly reduces evoked EPSPs and increases spontaneous ones in slices and in culture (Yamomoto & Kwai, 1967; Dodd, Dingledine & Kelly, 1981; Segal, 1982). In the intact brain, medial septal stimulation as well as ionophoretic application of ACh, facilitates population spikes (PSs) evoked by commissural and perforant path stimulation, respectively (Krnjevic & Ropert, 1982; Ropert & Krnjevic, 1982). These effects on synaptic transmission, unlike those on general membrane properties, are of short duration (milliseconds to seconds) and do not conform to the notion of ACh as a long term modulatory neurotransmitter (Benardo & Prince, 1981).

The aim of the present study was to determine whether ACh exerts a long-lasting effect on EPSPs, and if so, to determine whether the effect is primary and therefore due to a specific action of ACh on synaptic responses, or secondary to changes in passive membrane properties or blockade of potassium currents.

METHODS

Hippocampal slices were taken from rapidly decapitated adult (150–200 g) Wistar rats. Slices were prepared by standard methods (Segal, 1982) and incubated in an interface slice chamber perfused with warmed (32–34 °C) artificial cerebrospinal fluid containing (mM): NaCl, 124; KCl, 4; $NaHCO_3$, 26; NaH_2PO_4, 1·25; $CaCl_2$, 2·5; $MgSO_4$, 1·5; D-glucose, 10; pH = 7·4, in an oxygen-rich atmosphere (95% O_2, 5% CO_2).

Signals were recorded from cells in CA1 pyramidal layer. Signals were amplified with an Axoclamp-2 amplifier (Axon Instruments) and plotted continuously on a chart recorder. Fast events were also captured on computer for off-line analysis; averaging of five to ten traces and subtracting one trace from another. Current clamp experiments were performed using potassium acetate (4 M, 50–80 MΩ) and CsCl (3 M, 20–40 M) containing micropipettes. Single electrode voltage-clamp experiments were performed at a discontinuous sampling frequency of 3–4 kHz, in cells with high input resistances (more than 100 MΩ) using low-resistance micropipettes containing KCl (4 M, 30–40 MΩ) or CsCl (4 M, 20–40 MΩ).

Synaptic responses were recorded in slices pre-incubated and perfused with 300 μM-picrotoxin to study excitatory postsynaptic potentials (EPSPs) in isolation. In some cases a Krebs solution containing 4 mM-Ca^{2+} and 4 mM-Mg^{2+} was used to suppress hyperexcitability, while in other cases a low concentration of kynurenic acid (100 μM), a broad spectrum excitatory amino acid antagonist, which had little effect on EPSPs, was used to suppress hyperexcitability. The results are presented separately. EPSPs and excitatory postsynaptic currents (EPSCs) were evoked at a rate of 0·1 Hz and 0·3 Hz by 0·5 ms monophasic pulse stimulation of stratum radiatum some 2–3 mm away from the recording site.

Responses to ionophoretically applied drugs were examined in tetrodotoxin (TTX, 10 μM)-treated slices. Responses were quantified by measuring the area below the voltage–current

response. Changes in responses are represented as percentage change of the area below the response as this accounted for a change in response amplitude as well as duration. Seven-barrelled ionophoretic micropipettes were filled with 2 M-NaCl, 1 M-ACh chloride (pH = 7·0), 100 mM-NMDA and 100 mM-kainate or 100 mM-quisqualate or 1 M-glutamate (pH = 8·0; Sigma) and applied by current-balanced microionophoresis (pipette resistances 30–100 MΩ). Atropine (100 μM), oxotremorine-M (100 μM) and 2-APV (5 mM) (Sigma) were applied by microdrop application in nanolitre volumes.

Some cells were filled with Cs^+, using depolarizing current pulses, in order to block outward K^+ conductances that may have been evoked by ACh. Cs^+-filled cells had broad spikes generated from about −70 mV, high input resistances (60–120 MΩ) and depolarized membrane potentials, about −25 mV. Adequate blockade of K^+ conductance was further assessed by the failure of topical application of ACh to depolarize the cell or to increase its input resistance. In some cases slices were also treated with Ba^{2+} (1 mM) to further ensure the blockade K^+ conductances. The blockade of Ca^{2+}-dependent K^+ conductances by Ba^{2+}, was judged by the absence of slow after-hyperpolarizations and by the appearance of long-lasting Ba^{2+} spikes. Action potentials were inactivatable at the depolarized potentials in Cs^+-filled cells and were therefore not generated by EPSPs. The initial amplitude of EPSPs and EPSCs was set to 30–50% of the maximum evoked response. Cells recorded with potassium acetate and KCl had stable resting membrane potentials, around −70 mV and, action potentials of 90–110 mV. Cells included in this study had stable synaptic responses and membrane potentials for 5–30 min prior to applying drugs.

RESULTS

Acetylcholine facilitates EPSPs

Topical application of ACh in amounts that did not produce significant changes in passive membrane properties, either by ionophoresis or by microdrop, caused an initial reduction in EPSP amplitude followed by a gradual and long-lasting facilitation (Fig. 1). ACh applied to picrotoxin-treated slices incubated in a Krebs solution containing 4 mM-Ca^{2+} and 4 mM-Mg^{2+}, caused an increase of the amplitude of EPSPs by 28±8% and of the duration by 21±7% (mean±S.D.; $n = 5$; maximal effect). In these cases, the peak increase in the EPSP was reached within 20 min after the application of ACh and recovery was recorded within 1 h (Fig. 1). The facilitation of EPSPs was not associated with a change in input resistance (Fig. 1). In slices where the picrotoxin-induced hyperexcitability was suppressed with a low concentration (100 μM) of kynurenic acid, ACh caused a 25±8% increase in EPSP amplitude and a 17±6% increase in EPSP duration ($n = 4$). The remaining cells presented in this study were recorded in kynurenic acid-treated slices.

Bath application of atropine, a muscarinic receptor antagonist, before applying ACh prevented both the suppressing and facilitating effects of ACh (Fig. 2*A*; $n = 4$). Atropine in itself had no effect on EPSPs in two of the cells and caused a small (5–10%) reduction of the EPSP over 10 min in the other two cells tested. When applied after ACh had already facilitated EPSPs, atropine had no effect on the facilitated EPSPs in three cells and caused a gradual reduction in the EPSPs of one cell (data not shown). In slices that were not pre-treated with picrotoxin, ACh caused variable effects on EPSPs. Of ten cells studied the EPSPs of only three cells were increased (data not shown).

The effects of ACh on EPSPs were further studied under current- and voltage-clamp conditions, in Cs^+-filled cells in order to prevent outward K^+ conductances caused by ACh ($n = 12$). An application of ACh facilitated EPSPs (by 35·4±11·0%) in ten of the twelve cells studied (Fig. 2*B*). Under voltage-clamp conditions, ACh

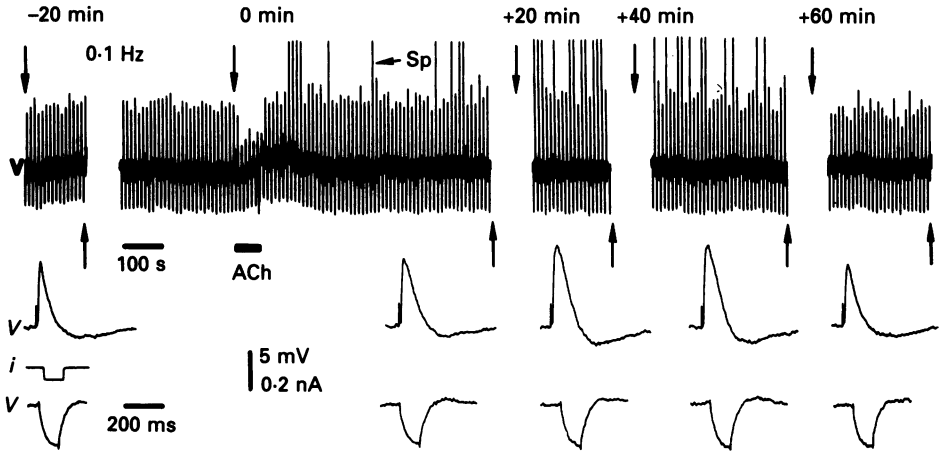

Fig. 1. ACh causes long-lasting facilitation of EPSPs: ACh (application indicated by the bar; 50 nA) initially suppresses EPSPs evoked at a rate of 0·1 Hz compared to control EPSPs recorded for 20 min (−20 min). A 2·5 mV depolarization is caused by the application of ACh. The initial effect of ACh is followed by a long-lasting facilitation of the EPSP generating, in some cases, spikes (Sp). The peak facilitation is reached about 20 min (+20 min) after the application of ACh and the EPSP amplitude and duration return to control values after 1 h (+60 min). The expanded traces below represent individual EPSPs at indicated (arrows) intervals of the recording. The voltage (V) response to a 100 ms current (i) pulse (left) are shown for selected intervals of the recording.

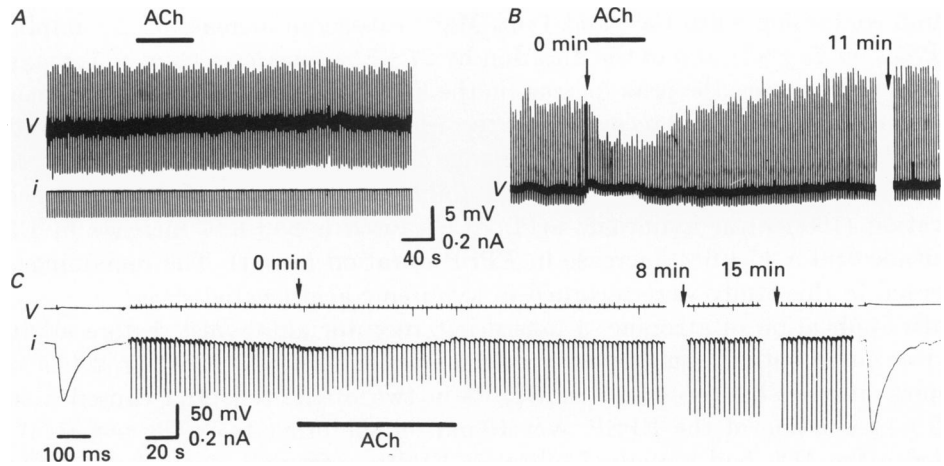

Fig. 2. Muscarinic receptor-mediated facilitation of EPSPs is independent of changes in voltage and K^+ conductances caused by ACh: bath application of atropine prevents both initial suppressing and facilitating effects of ACh (A). ACh initially suppresses and later facilitates EPSPs evoked in a Cs^+-filled cell (B). ACh initially suppresses and later facilitates EPSCs in a Cs^+-filled cell under voltage-clamp conditions (C). Bar indicates the duration of ACh application (50 nA). V, voltage; and i, current; stimulation rate of 0·3 Hz.

increased the amplitude of EPSCs by 31·7 ± 9·0 % and duration by 19 ± 7 % (Fig. 2*C*; $n = 4$). The effect of ACh on synaptic responses was cumulative. An additional application of ACh after EPSPs or EPSCs were already facilitated once, resulted in a further increase as high as 55 % above control pre-drug values (data not shown; $n = 4$).

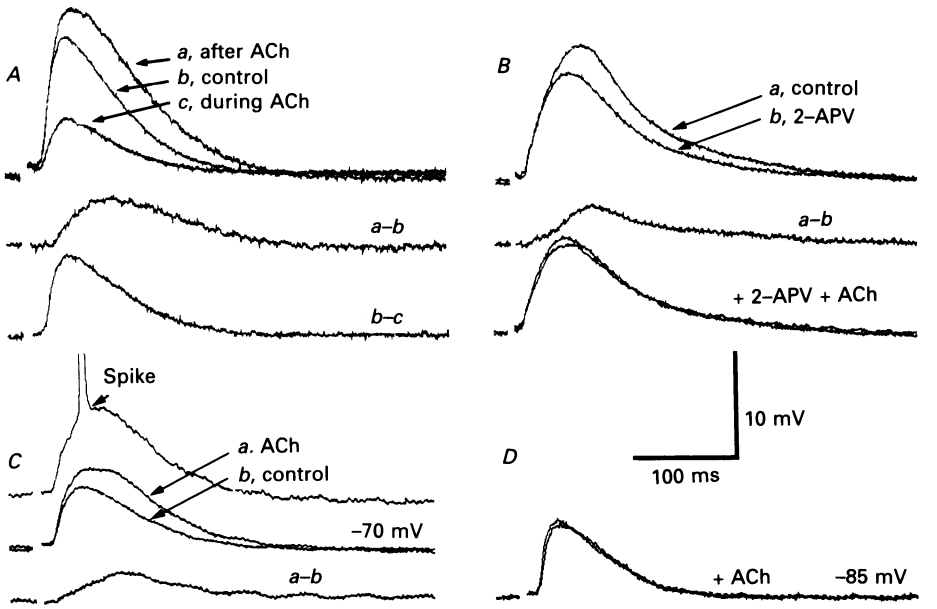

Fig. 3. ACh facilitates the slow NMDA receptor-mediated component of the EPSP: EPSPs presented here represent computer generated averages of five EPSPs and in *A*, *B* and *C* the differences in EPSPs are shown. *A*, the initial suppression caused by ACh is of all the components of the EPSP ($b-c$), while the late facilitation is of a slow component ($a-b$). *B*, 2-APV blocks a slow component of the EPSP ($a-b$) and blocks the facilitatory effect of ACh on the EPSP (below). *C*, ACh facilitates a slow component of the EPSP recorded at −70 mV in potassium acetate ($a-b$). Action potentials were frequently generated following ACh (top). *D*, hyperpolarizing a cell to −85 mV reduces the slow component and blocks the facilitatory effect of ACh.

EPSPs recorded in Cs^+-filled cells at about −25 mV membrane potential, reached a peak within 30 ms and decayed within 200 ms. When the cell was hyperpolarized to −85 mV, the EPSP peaked within 15 ms and repolarized within 100 ms; the subtraction of the latter EPSP from the one recorded at rest revealed a slow component of the EPSP which peaked within 60 ms and decayed within 200 ms. The initial suppression of EPSP amplitude by ACh was expressed as a reduction of both fast and slow components of the EPSP, while the long-lasting facilitation was of the slow component only (Fig. 3*A*; $n = 10$). An NMDA receptor antagonist, 2-APV, reduced a slow component of the EPSP and blocked the facilitatory effect of ACh (Fig. 3*B*; $n = 4$). 2-APV did not affect the ACh-induced initial reduction of EPSP amplitude (data not shown). In potassium acetate recordings, the facilitation of EPSPs was detected at a membrane potential of −70 mV (Fig. 3*C*; $n = 9$) and was not detected when the cell was hyperpolarized to −85 mV where the slow component

was absent (Fig. 3*D*; $n = 3$). The initial reduction in EPSP caused by ACh was unaffected by hyperpolarization.

Acetylcholine facilitates responses to NMDA

To examine the possibility that ACh exerts its effect postsynaptically and specifically on NMDA receptors, we applied NMDA via ionophoresis onto TTX-

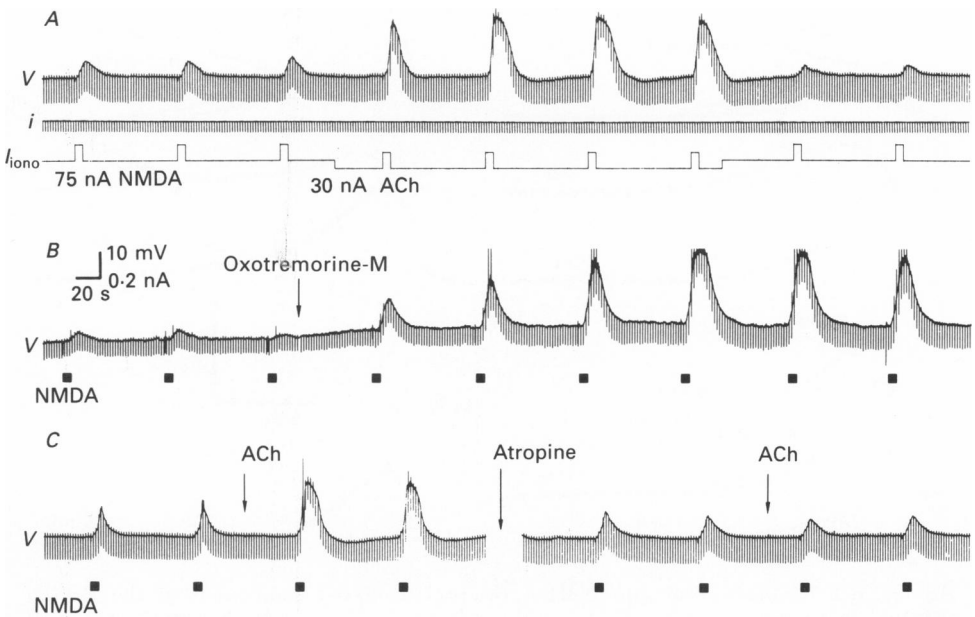

Fig. 4. ACh facilitates NMDA-evoked responses: in this and all subsequent figures recording of cells made in TTX-treated slices. *A*, the voltage (*V*) response to 75 nA pulses of NMDA (1·5 min intervals) are facilitated by 30 nA application of ACh (indicated in the ionophoretic trace below (i_{iono})). Recovery is associated with suppression of the NMDA response (i = current). *B*, a nanolitre drop application (arrow) of oxotremorine-M (1×10^{-4} M) facilitates voltage responses evoked by pulses of NMDA (■). *C*, atropine (1×10^{-4} M) prevents facilitation of responses to pulses of NMDA (■) caused by a nanolitre drop application of ACh (10 mM).

treated slices. NMDA produced a characteristic slow depolarization resulting in a regenerative response when the membrane potential reached 10–20 mV above rest. Topical application of ACh produced a potent facilitation of responses to NMDA in seventeen of eighteen cells examined (Fig. 4*A*). The area below the voltage response to NMDA was increased by $213 \pm 110\%$ (mean $\pm$ S.D.; $n = 17$) of pre-drug values. The large standard deviation was mostly due to different doses of ACh applied; by applying progressively larger amounts of ACh the potentiation could be increased from 0 to over 400% (data not shown; $n = 5$). In twelve of the seventeen cells, the facilitation was of short duration, recovering within 1·5 min after termination of ACh current. The recovery time appeared to be dependent on the initial amplitude of the NMDA response and the degree of its facilitation by ACh (data not shown); thus the larger the response, the slower the recovery. The longest recovery time recorded in

these cells was 10 min ($n = 2$). Recovery was associated with a suppression of the NMDA response as compared to control in eleven of seventeen cells (see Fig. 4*A*).

To verify that the facilitatory effect of ACh was not an artifact of the ionophoretic mode of application, we used the microperfusion technique as well; nanolitre

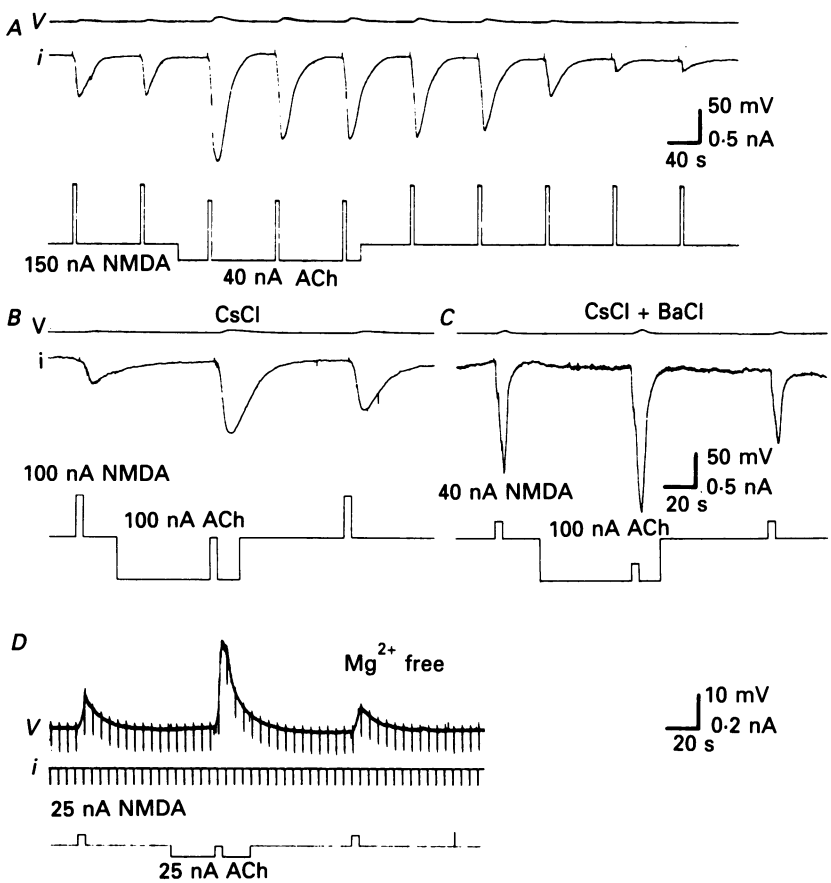

Fig. 5. ACh-induced facilitation is independent of changes in voltage or K^+ conductances: in each figure the voltage (V) trace is above, the current (i) trace in the middle and the ionophoretic trace is below. *A*, application of a small amount of ACh (40 nA) fails to evoke any current directly, but facilitates NMDA-evoked currents by > 200% in a cell recorded with a KCl micropipette and voltage clamped at resting potential. Post-facilitation suppression is also illustrated in the last two responses. *B*, ACh facilitates NMDA-evoked currents in a Cs^+-filled cell. *C*, ACh facilitates NMDA-evoked currents in a Cs^+-filled cell in Ba^{2+}-treated (1 mM) slices. Barium typically caused sharp 'jagged' response to NMDA. *D*, ACh facilitates NMDA responses in an external medium free of Mg^{2+}.

microdrop application of ACh (10 mM) to the slice facilitated NMDA responses for 1·5–3 min ($n = 5$). Facilitation of NMDA responses was also produced by microdrop application of oxotremorine-M, an M2 muscarinic agonist (Fig. 4*B*; $n = 4$) and the facilitation caused by either microdrop or ionophoretic application of ACh, was prevented by atropine, a muscarinic receptor antagonist (Fig. 4*C*; $n = 6$) but not by the M1 receptor antagonist, pirenzepine (data not shown; $n = 4$).

We further examined the possibility that changes in membrane potential caused by ACh-induced blockade of K^+ conductances, may underly the facilitation of NMDA-evoked responses. In these experiments the ionophoretic pipette was placed near the cell body to allow better control of NMDA currents and to partially

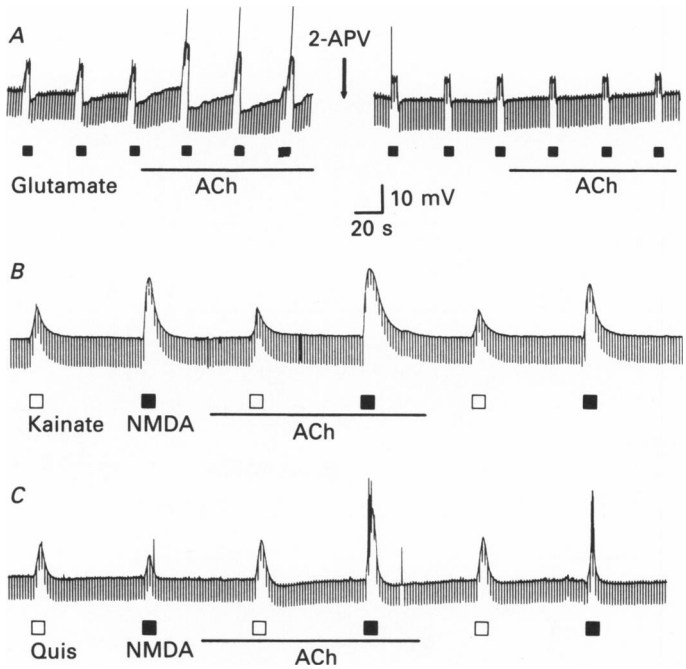

Fig. 6. ACh facilitates the NMDA subclass of L-glutamate receptor. *A*, bath application of 2-APV (5 mM) prevents the facilitation of responses to pulses of L-glutamate (■; 45 s intervals) caused by ACh (bars; 50 nA). *B*, ACh (bar) fails to effect the response to a pulse of kainate (□) while facilitating the response to a pulse of NMDA (■). *C*, ACh (bar) causes a potent facilitation of the response to a pulse of NMDA (■) while only slightly increasing the response to a pulse of quisqualate (Quis, □).

circumvent the difficulty of voltage clamping remote dendrites. NMDA evoked a slow transient inward current under voltage-clamp conditions ($n = 10$). ACh increased the current evoked by NMDA by $217 \pm 88\%$ of control, as recorded with KCl-containing micropipettes (Fig. 5*A*; $n = 4$). ACh also increased NMDA-evoked currents in Cs^+-filled cells by $193 \pm 75\%$ (Fig. 5*B*; $n = 3$) and by $178 \pm 97\%$ in Cs^+-filled cells in Ba^{2+}-treated slices (Fig. 5*C*; $n = 3$). One unique property of the NMDA receptor-mediated response that may underly the effect of ACh is its voltage sensitivity (Nowak, Bregestovski, Ascher, Herbert & Prochiantz, 1984). Thus, the NMDA response is larger at depolarized potentials. Magnesium has been shown to mediate this voltage sensitivity in that it blocks the response to the drug at polarized potentials (Nowak *et al.* 1984). The voltage sensitivity is therefore absent when Mg^{2+} is removed from the bathing medium. In five cells examined in Mg^{2+}-free medium, the responses to NMDA were still potentiated by ACh indicating that the enhanced response to NMDA is not due to a mere putative depolarizing effect of ACh (Fig. 5*D*).

Finally, we examined the specificity of the ACh effect by using L-glutamate, kainate and quisqualate in addition to NMDA. ACh facilitated responses evoked by L-glutamate ($68 \pm 11\%$) and this facilitation was prevented by the NMDA receptor antagonist 2-APV (Fig. 6*A*; $n = 3$). Furthermore, while ACh facilitated responses to NMDA ($180 \pm 95\%$), it failed to affect responses to kainate (Fig. 6*B*; $n = 5$). In a further five cells ACh increased the response to NMDA by $210 \pm 87\%$ while causing only a small increase ($18 \pm 8\%$) in responses to quisqualate (Fig. 6*C*).

DISCUSSION

Acetylcholine, acting on muscarinic receptors, produced an initial suppression of EPSPs, which was followed by a long-lasting facilitation of a slow component of the EPSP. This slow component of the EPSP is likely to be mediated by the activation of NMDA receptors (Collingridge, Herron & Lester, 1988). The facilitation of the EPSP was blocked by an NMDA receptor antagonist and by hyperpolarization which suppresses NMDA receptor activation (Nowak *et al.* 1984). It is therefore likely that ACh facilitates an NMDA receptor-mediated component of the EPSP. Indeed, ACh produced a potent facilitation of responses to ionophoretically applied NMDA but not kainate or quisqualate.

The initial suppression of EPSPs by ACh has been previously described and is thought to involve presynaptic elements (Yamomoto & Kwai, 1967; Dodd *et al.* 1981; Krnjevic, Reiffenstein & Ropert, 1981; Segal, 1982). Indeed, M2-receptor activation mediates inhibition of glutamate release in the hippocampus (Marchi & Raiteri, 1989) which may account for a reduction by ACh of both fast and slow components of the EPSP observed here and may mask a fast initial facilitation of the NMDA receptor-mediated component of the EPSP, as seen in response to topical application of NMDA.

Suppression of responses to NMDA was often seen following facilitation by ACh (e.g. Figs 4*A* and 5*A*), but was not seen following facilitation of EPSPs. A phenomenon of 'inactivation' of NMDA receptor-mediated responses has been reported (see Ascher & Nowak, 1987) and has also been seen following facilitation of responses to NMDA by GABA in the cortex (Walden, Speckman & Bingmann, 1989). Suppression is therefore probably a property relevant to the activation of NMDA receptors and is not a selective effect of ACh. This apparent use-dependent reduction of NMDA reactivity may therefore be more readily detected when responses to prolonged (5–15 s), exogenously applied NMDA are examined as opposed to fast (millisecond) synaptically released L-glutamate.

The mechanism by which ACh facilitates NMDA responses is not completely clear. The facilitation was detected in voltage-clamped, Cs^+-filled cells in Ba^{2+}-treated slices and in Mg^{2+}-free medium, suggesting that the effect of ACh is independent of changes in voltage and K^+ conductances. The specificity of the facilitatory effect for the NMDA subclass of L-glutamate receptor also suggests that the mechanism is independent of changes in passive membrane properties caused by ACh. One possible mechanism is the interaction between ACh and Ca^{2+} conductances. Both muscarinic receptor-mediated decreases and increases in Ca^{2+} currents have been reported (Gahweiler & Dreifuss, 1981; Gahweiler & Brown, 1987; Pitler, McCarren &

Alger, 1988). Since a major component of the response to NMDA involves inward Ca^{2+} currents (MacDermott, Meyer, Westbrook, Smith & Barker, 1986; Ascher & Nowak, 1986), it is possible that ACh facilitates this component. ACh may cause an increase in the Ca^{2+} component of NMDA responses or may activate second messenger systems which regulate intracellular Ca^{2+} which in turn modifies responses to NMDA. Possible interactions of ACh with factors which regulate NMDA receptors are currently being investigated.

ACh produced a facilitation of the NMDA receptor-mediated component of the synaptic response which peaked within 20 min and recovered within 1 h. The long-lasting effect of ACh on EPSPs was not due to the continued presence of ACh as atropine had no significant effect on already potentiated EPSPs. It may be possible that ACh exerts an initial effect on the postsynaptic cell which causes a long-lasting enhancement of the NMDA-mediated response and that this effect of ACh is maintained by synaptic activation. This long-lasting effect of ACh on the slow component of the EPSP is different from the tetanus-induced long-term potentiation (LTP) which is expressed primarily in the fast, kainate/quisqualate component (Muller, Joly & Lynch, 1988).

The facilitation of NMDA receptor-mediated responses by ACh may have marked physiological significance. An obligatory requirement for NMDA receptor activation in the generation of LTP in some synapses, has been demonstrated (Collingridge, Kehl & McLennan, 1983; Harris, Ganong & Cotman, 1984). ACh may therefore be involved not only in increasing synaptic efficacy *per se*, but also in increasing the probability for synaptic drive to generate NMDA-mediated LTP. Indeed, inhibiting the break-down of ACh with physostigmine or with HP029 causes a significant enhancement of LTP generated in CA1 of hippocampal slices and *in vivo* (Ito, Miura & Kadokawa, 1988; Tanaka, Sakurai & Hayashi, 1989). However, the effects of blocking muscarinic receptor activation with scopolamine on LTP, vary from decreases (Ito, Miura and Kadokawa, 1988; Tanaka, Sakurai and Hayashi, 1989; Hirotsu, Hori, Katsuba & Ishihara, 1989) to no effects (Stringer, Greenfield, Hackett & Guyenet, 1983; Tanaka *et al.* 1989). This variability may be due to differences in intrinsic levels of ACh.

We observed consistent facilitatory effects of ACh on EPSPs when the inhibitory synaptic drive was reduced. While this is an artificial condition, it is interesting to note that GABAergic fibres from the septum preferentially innervate GABAergic interneurones in the hippocampus (Freund & Antal, 1988). The application of picrotoxin may thus serve a similar function as the activation of this GABAergic pathway; i.e. to ensure the facilitation of NMDA receptor-mediated responses by ACh by blocking local inhibition. Synchronized cholinergic–GABAergic activity arising from the septum, may therefore result in amplified NMDA receptor-mediated synaptic responses. Indeed, medial septal stimulation significantly enhances LTP generated in the dentate gyrus by perforant path tetanization (Robinson, 1986; Robinson & Racine, 1986). Preliminary studies have shown that serotonin, by inhibiting GABAergic interneurones in the hippocampus (Segal, 1989), also enables long-lasting facilitation of EPSPs by ACh (H. Markram and M. Segal, unpublished observation).

The significance of ACh-induced facilitation of NMDA receptor-mediated responses

may also extend to other physiological phenomena in the hippocampus. As NMDA receptors have been implicated in a variety of seizure models in the hippocampus (Croucher, Collins & Meldrum, 1982; Dingledine, Hynes & King, 1986; Hwa & Avoli, 1989) the facilitatory effect of ACh on NMDA responses may underlie muscarinic receptor-mediated generation of seizures in the hippocampus (see Davis & Hatoum, 1980; Jope, Morrisett & Snead, 1986; Jope, Simonato & Lally, 1987). The amplification of NMDA responses by ACh may also underly the muscarinic receptor-mediated generation of rhythmic slow activity in the hippocampus which results from the propogation of action potentials through a local circuit (see Bland, 1986; Bland, Colom, Konopacki & Roth, 1988; Tse & MacVicar, 1989).

The present study provides the first direct evidence that ACh induces long-lasting changes in synaptic efficacy. This long-lasting facilitation is, furthermore, specific to responses mediated by the NMDA subclass of L-glutamate receptor. The action of ACh on NMDA responses could have at least two important physiological effects, namely, amplification of synaptic efficacy and increased probability of NMDA receptor-mediated generation of LTP. This facilitatory action could therefore provide a link between the known effects of ACh on memory and learning processes and the known cellular mechanisms of plasticity.

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