Cholinergic modulation of synaptic transmission and plasticity in entorhinal cortex and hippocampus of the rat

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Abstract—Effects of cholinergic agents on synaptic transmission and plasticity were examined in entorhinal cortex and hippocampus. Bath application of carbachol (0.25–0.75 μM) induced transient depression of field potential responses in all cases tested (24/24 in layer III of medial entorhinal cortex slices and 24/24 in CA1 of hippocampal slices; 11.0 ± 1.9% and 7.8 ± 2.5%, respectively) and long-lasting potentiation in some cases (4/24 in entorhinal cortex and 12/24 in hippocampus; 33.7 ± 3.7% and 32.1 ± 9.9%, respectively, in successful cases). Carbachol (0.5 μM) induced transient depression, but not long-lasting potentiation, of N-methyl-D-aspartate receptor-mediated responses in entorhinal cortex. At 5 μM, carbachol induced transient depression only (55.9 ± 4.7% in entorhinal cortex and 41.4 ± 2.9% in hippocampus), which was blocked by atropine. Paired-pulse facilitation was not altered during carbachol-induced potentiation but enhanced during carbachol-induced depression. These results suggest that the mechanism of carbachol-induced depression and potentiation are decreased transmitter release and selective enhancement of non-N-methyl-D-aspartate receptor-mediated responses, respectively. Long-term potentiation could be induced in the presence of 10 μM atropine by theta burst stimulation. The magnitude was significantly lower (15.2 ± 5.2%, n = 9) compared with control (37.2 ± 6.1%, n = 8) in entorhinal cortex, however.

These results demonstrate similar, but not identical, cholinergic modulation of synaptic transmission and plasticity in entorhinal cortex and hippocampus. © 2000 IBRO. Published by Elsevier Science Ltd.

Key words: acetylcholine, long-term potentiation, muscarinic receptor, brain slice, carbachol, memory.

Acetylcholine (ACh) is known to play an important role in learning and memory.6 It is quite conceivable that ACh modulates learning and memory through one or more of its known physiological effects. ACh depolarizes cells, facilitates burst firing and induces slow excitatory postsynaptic potential (EPSP) by blocking potassium channels.7,12,17,28,29,32 In contrast, ACh suppresses neurotransmitter release18 and excites inhibitory neurons.31 In addition, cholinergic activation promotes development of theta rhythm.8,20 Thus ACh exerts both excitatory and inhibitory physiological effects and modulates theta rhythm, all of which can potentially modulate learning and memory.

ACh is also known to influence synaptic plasticity. Cholinergic stimulation increased long-term potentiation (LTP) in CA1,9,20 and enabled associative LTP in piriform cortex,33 but muscarinic decreased LTP in CA3.39 Cholinergic neurotoxin AF64A suppressed LTP induction in dentate gyrus in vivo1 and in mossy fiber–CA3 synapses in vitro,39 while cholinergic neurotoxin 192 immunoglobulin G (IgG)-saporin had no effect on LTP induction in CA1 in vitro.22 Scopolamine also suppressed potentiation of the population spike in CA1.19,21 In a different study, however, scopolamine had no effect on LTP induction in CA1 but suppressed LTP in CA3.37

Finally, effects of cholinergic antagonists on LTP induction were different according to tetanic stimulation patterns.24,36 Thus, while reports are somewhat conflicting, plenty of evidence indicates that LTP induction is modulated by ACh. This is not surprising considering that ACh modulates excitability of neurons (see above). In fact, most of the cholinergic effects on LTP induction could be explained by the effects of ACh on cellular excitability. Furthermore, recent studies have shown that carbachol, an ACh receptor agonist, can induce long-lasting potentiation of synaptic transmission in hippocampus.4 Thus ACh is capable of modulating as well as inducing synaptic plasticity in hippocampus.

Previous studies have shown that various physiological changes, such as oscillatory firing, could be induced by cholinergic activation in entorhinal cortex (EC).14–16,25–27,34,38 EC is the major locus of information exchange between hippocampus and other cortical areas.40 This anatomical feature suggests EC as a critical structure for proper operation of hippocampus. Like hippocampus, EC receives a profuse cholinergic innervation from the basal forebrain.2,3,13 Thus it is highly likely that cholinergic influences on EC physiology play an important role in modulating hippocampal learning and memory. In the present study, we investigated cholinergic modulation of synaptic transmission and plasticity in EC and compared the results with those in hippocampus. The results indicate similar, but not identical, cholinergic effects in EC and hippocampus.

EXPERIMENTAL PROCEDURES

Hippocampal and EC slices were prepared as described previously.25,31 In brief, transversely and horizontally sectioned 400-μm-thick hippocampal and medial EC slices were prepared, respectively, from adult male Sprague–Dawley rats (150–200 g). Handling...
of animals was performed following the NIH guidelines. All efforts were made to minimize the number of animals used and their suffering. Slices were constantly perfused with oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (ACSF) in an interface chamber and maintained at 35 ± 1°C. Monosynaptic field potentials were recorded in the stratum radiatum of the CA1 area in hippocampus in response to electrical stimulation of the Schaffer collateral/commissural fibers by two stimulating electrodes. In medial EC, field potentials were recorded in layer III in response to electrical stimulation of layers II/III by two stimulating electrodes. Stimulating electrodes were constructed of twisted strands of stainless steel wires (113 µm O.D.) and recording electrodes were glass micropipettes filled with 2 M NaCl (1–5 MΩ). Stimulation pulses (0.1 ms) were delivered every 20 s at currents ranging from 50 to 150 µA to obtain baseline responses (10–20 min) that were about half-maximal. Responses were amplified ×100, filtered at band pass 1–3 kHz, and digitized by a personal computer at 10 kHz. N-Methyl-d-aspartate (NMDA) receptor-mediated responses were assessed by measuring peak amplitude, and non-NMDA responses were assessed by measuring initial slope of field EPSPs.

LTP was induced by applying theta burst stimulation (TBS) that consisted of 10 high frequency bursts (four pulses, 100 Hz) repeated at 5 Hz. During TBS, stimulus duration was increased to 1.5–2 times the baseline stimulation. Magnitude of LTP or carbachol-induced potentiation was assessed by measuring the % increase of averaged responses over baseline between 20 and 30 min following TBS or drug washout. Magnitude of carbachol-induced depression was assessed by measuring the % decrease of averaged responses between 10 and 20 min following treatment of carbachol from the baseline. The NAC program (Eclectek, Irvine, CA, U.S.A.) was used for collection and analysis of data. Student’s t-test was used to compare the magnitudes of LTP induced in control vs atropine-treated slices, and chi-square test was used to compare the probability of inducing long-lasting potentiation by carbachol in EC vs hippocampus (alpha level 0.05). Result from each slice was counted as one experiment (n) and each slice was obtained from a separate animal. Results are expressed as mean ± S.E.M.

6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was prepared as stock solution of 100 mM in 100% dimethyl sulfoxide (DMSO) and diluted to 10 µM in ACSF. Thus the final concentration of DMSO was 0.01%, which did not affect field potential responses. CNQX was purchased from Tocris Cookson (Buckhurst Hill, U.K.), and atropine and carbachol were obtained from Sigma (St Louis, MO, U.S.A.). All drugs were delivered to the perfusion medium using a 22-gauge needle syringe that was driven by a syringe driver. This arrangement did not induce an artefactual response change, which is sometimes observed when switching between two perfusion bottles, as verified by delivering ACSF while monitoring field potential responses.

RESULTS

Effect of low dose of carbachol

Auerbach and Segal4,5 have shown that low doses (<1 µM) of carbachol induced long-lasting potentiation of synaptic responses in hippocampus, whereas high doses (>5 µM) of carbachol induced depression only. We accordingly tested effects of carbachol at low and high concentrations separately in EC and hippocampus. At low concentrations (0.25–0.75 µM), carbachol induced short-lasting, reversible depression of field EPSPs in all cases tested (24/24, 11.0 ± 1.9% depression) and gradually developing, long-lasting enhancement of synaptic responses in some cases (4/24) in EC (Fig. 1A, B). Interestingly, carbachol induced long-lasting potentiation in an all-or-none manner in both EC and hippocampus. We therefore report successful and unsuccessful cases separately. The average degree of potentiation in successful cases

![Fig. 1. Effects of low doses of carbachol on synaptic transmission. Bath application of 0.25–0.75 µM carbachol induced short-lasting depression and sometimes long-lasting potentiation of synaptic field potential responses. (A) The graph shows cases (n = 4) in which carbachol induced more than 20% increase in the initial slope of synaptic responses in EC. Field potential responses (average of five) before and after application of carbachol in one experiment are shown at the top. Calibration: 10 ms and 1 mV. (B) Experiments (n = 20) in which carbachol induced depression without long-lasting potentiation in EC. (C, D) Same as A and B except that experiments were performed in hippocampal slices. n = 12 and 12 for C and D, respectively.](image-url)
was 33.7 ± 3.7% in EC. Similar results were obtained in hippocampus. Low concentrations of carbachol (0.25–0.75 mM) initially depressed field potentials in all cases tested (24/24, 7.8 ± 2.5% depression) and induced long-lasting potentiation in some cases (12/24, Fig. 1C, D). The average magnitude of potentiation in successful cases was 32.1 ± 9.9% in hippocampus. Chi-square analysis indicated a significant \((P < 0.05)\) difference between EC and hippocampus in the probability of inducing long-lasting potentiation. Carbachol-induced long-lasting potentiation did not change the degree of PPF at the 50-ms interval in EC (25.3 ± 12.2% and 25.2 ± 14.8% during baseline and potentiation, respectively, \(n = 2\)) or hippocampus (91.1 ± 14.6% and 88.9 ± 16.8% during baseline and potentiation, respectively, \(n = 4\)).

### Effect of low dose of carbachol on N-methyl-D-aspartate receptor-mediated responses

NMDA receptors do not contribute to field potential responses under the current experimental condition (2.5 mM Mg\(^{2+}\)). In order to test the effects of carbachol on NMDA receptor-mediated responses, experiments were performed in EC in the medium containing low magnesium (0.5 mM) and 10 μM CNQX. Evoked synaptic responses consist entirely of the NMDA receptor component under this condition.\(^{41}\) A low dose (0.5 μM) of carbachol induced transient depression of NMDA receptor-mediated responses in all cases tested (15/15), but long-lasting potentiation was never observed. Rather, a low degree of short-lasting depression was observed (9.8 ± 4.2%, Fig. 2). The degree of short-lasting depression was 23.5 ± 3.3% of the baseline, which is comparable to that of non-NMDA receptor-mediated responses.

### Effect of high dose of carbachol

Bath application of 5 μM carbachol induced reversible depression of evoked field EPSP in all cases tested (15/15) in EC (Fig. 3A). The magnitude of depression was 55.9 ± 4.7% of the baseline, which is considerably higher than the depression induced by a low dose of carbachol. Long-lasting potentiation was not observed with 5 μM carbachol. In hippocampus, like in EC, 5 μM carbachol induced

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**Fig. 2.** Effect of a low dose of carbachol on NMDA receptor-mediated responses. NMDA receptor-mediated responses in EC were measured in medium containing low magnesium (0.5 μM) and CNQX (10 μM). Carbachol (0.5 μM) induced short-lasting depression without long-lasting potentiation \((n = 15)\). Records on top show examples of NMDA responses (average of five) before and after application of carbachol. Calibration: 20 ms and 0.3 mV.

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**Fig. 3.** Effect of a high dose of carbachol. Same as in Fig. 1 except that a high dose (5 μM) of carbachol was used. (A) Carbachol, at 5 μM, induced short-lasting depression of synaptic responses without long-lasting potentiation in EC \((n = 15)\). Records show field potential responses before and after treatment of carbachol. Calibration: 20 ms and 2 mV. (B) The effect of carbachol was completely blocked by 10 μM atropine \((n = 6)\). (C, D) Same as in A and B except that experiments were performed in hippocampus \((n = 15\) and \(n = 6\), respectively).
Effect of atropine on carbachol-induced depression

To address which type of ACh receptors is involved in mediating carbachol’s effects, we tested the effect of atropine, a muscarinic ACh receptor antagonist. Application of 10 μM atropine 10 min before addition of carbachol (5 μM) completely blocked carbachol-induced depression in both EC (n = 6; Fig. 3C) and hippocampus (n = 6; Fig. 3D).

Effect of atropine on long-term potentiation induction

LTP was reliably induced by TBS in EC and hippocampus under the control condition. In the presence of 10 μM atropine, LTP was still reliably induced by TBS in EC and hippocampus. Because the induction of LTP was not an all-or-none phenomenon as carbachol-induced long-lasting potentiation, all slices were included in calculating the average magnitude of LTP without dividing them into potentiated vs non-potentiated groups. The average magnitudes of potentiation were 37.2 ± 6.1% (n = 8) and 15.2 ± 5.2% (n = 9) for control and atropine group, respectively, in EC, and 64.6 ± 14.5% (n = 13) and 81.8 ± 16.7% (n = 13), respectively, in hippocampus (Fig. 4). Atropine had no significant effect on the magnitude of LTP in hippocampus. In EC, however, the magnitude was significantly lower under atropine treatment when compared with control (t-test, P < 0.05).

DISCUSSION

The primary goal of the present study was to examine cholinergic modulation of synaptic transmission and plasticity in EC. The secondary goal was to compare these with cholinergic effects in hippocampus under the identical condition. Low doses of carbachol (0.25–0.75 μM) reliably induced short-lasting, reversible depression of synaptic responses both in EC and hippocampus. This effect was followed by slowly developing, long-lasting potentiation in some cases. In contrast, a high dose (5 μM) of carbachol induced larger magnitude of short-lasting depression without long-lasting potentiation in EC and hippocampus. These results are consistent with previously reported results. A muscarinic antagonist, atropine, completely blocked carbachol-induced depression in EC, indicating that the carbachol effect is mediated by muscarinic ACh receptors. This is also in agreement with the previous study. Atropine had no significant effect on the magnitude of LTP (n = 13 for both groups). Records show superimposed examples of field potential responses (average of five) before and after LTP induction for control and atropine treatment group. Calibration: 10 ms and 1 mV.

short-lasting depression (41.4 ± 2.9% of baseline) without long-lasting potentiation in all cases tested (15/15, Fig. 3B). Paired-pulse facilitation (PPF) at 50-ms interval was increased from 25.3 ± 5.2% to 83.4 ± 8.8% in EC and from 80.8 ± 3.3% to 115.9 ± 5.4% in hippocampus following carbachol treatment.

Effect of atropine on carbachol-induced depression

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size of baseline responses for a given stimulation intensity, between potentiated and non-potentiated slices. Each slice was obtained from a separate animal. Thus it is not likely that long-lasting potentiation was obtained from a small group of particular animals. Employment of intracellular recording may reveal the factors that determine induction of long-lasting potentiation by carbachol.

Previous studies have provided conflicting results regarding cholinergic modulation of LTP induction in hippocampus. Some reported cholinergic facilitation, while others suggested cholinergic inhibition of LTP induction. Some studies even reported absence of ACh effect on LTP induction. The weight of current evidence appears to indicate, however, that cholinergic input is not essential for induction of LTP, but ACh modulates LTP induction in a facilitatory way. In EC, delivery of TBS resulted in induction of LTP even after blocking of muscarinic ACh receptors by atropine. The magnitude of LTP was significantly lower compared with control, however, in hippocampus, similar degrees of LTP were induced with and without atropine treatment. Thus, our results indicate that cholinergic input is not essential for induction of LTP, but it facilitates induction of LTP in EC under the current stimulation protocol. Considering that ACh is not essential for LTP induction and that conflicting results have been reported, its modulatory effects on LTP induction probably depend on experimental conditions. It is entirely possible that atropine modulates induction of hippocampal LTP but does not affect EC LTP under different experimental conditions. Thus, future investigations should be focused on finding conditions under which ACh modulates induction of LTP.

Overall, the present results demonstrate cholinergic modulation of synaptic transmission and plasticity in EC and indicate that cholinergic modulation is generally similar, but somewhat different, in EC and hippocampus. These effects may contribute to cholinergic modulation of hippocampal learning and memory.

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