An acetylcholinesterase inhibitor, eserine, induces long-term depression at CA3-CA1 synapses in the hippocampus of adult rats

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Mans RA, Warmus BA, Smith CC, McMahon LL. An acetylcholinesterase inhibitor, eserine, induces long-term depression at CA3-CA1 synapses in the hippocampus of adult rats. J Neurophysiol 112: 2388–2397, 2014. First published August 20, 2014; doi:10.1152/jn.00048.2014.—Studies in humans and rodents support a role for muscarinic ACh receptor (mAChR) and nicotinic AChR in learning and memory, and both regulate hippocampal synaptic plasticity using complex and often times opposing mechanisms. Acetylcholinesterase (AChE) inhibitors are commonly prescribed to enhance cholinergic signaling in Alzheimer’s disease in hopes of rescuing cognitive function, caused, in part, by degeneration of cholinergic innervation to the hippocampus and cortex. Unfortunately, therapeutic efficacy is moderate and inconsistent, perhaps due to unanticipated mechanisms. M1 mAChRs bidirectionally control synaptic strength at CA3-CA1 synapses; weak pharmacological activation using carbachol (CCh) facilitates potentiation, whereas strong agonism induces muscarinic long-term depression (mLTD) via an ERK-dependent mechanism. Here, we tested the prediction that accumulation of extracellular ACh via inhibition of AChE is sufficient to induce LTD at CA3-CA1 synapses in hippocampal slices from adult rats. Although AChE inhibition with eserine induces LTD, it unexpectedly does not share properties with mLTD induced by CCh, as reported previously. Eserine-LTD was prevented by the M1 mAChR-prefering antagonist 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), and pharmacological inhibition of MEK was completely ineffective. Additionally, pharmacological inhibition of p38 MAPK prevents mLTD but has no effect on eserine-LTD. Finally, long-term expression of eserine-LTD is partially dependent on a decrease in presynaptic release probability, likely caused by tonic activation of mAChRs by the sustained increase in extracellular ACh. Thus these findings extend current literature by showing that pharmacological AChE inhibition causes a prolonged decrease in presynaptic glutamate release at CA3-CA1 synapses, in addition to inducing a likely postsynaptic form of LTD.

acetylcholinesterase inhibitor; Alzheimer’s disease; hippocampus; long-term depression; muscarinic AChR

CHOLINERGIC AFFERENTS TO THE hippocampus, projecting from the medial septum and diagonal band of Broca, modulate multiple synaptic properties that can be observed across excitatory and inhibitory circuits and encompass cell excitability, long-term plasticity, or network oscillations. Current understanding of how the cholinergic system impacts hippocampal synaptic function is derived from pharmacological manipulation of nicotinic ACh receptors (nAChRs) and muscarinic AChRs (mAChRs), lesions of cholinergic afferents, and genetic approaches. Whereas a prominent role for cholinergic receptors in hippocampal function is evident, knowledge of the exact mechanisms by which they exert their effects remains incomplete.

In humans, nonspecific cholinergic antagonists disrupt memory (Lasser et al. 1989; Little et al. 1998; Preston et al. 1989), and degeneration of cholinergic input from the basal forebrain to the hippocampus is a hallmark of Alzheimer’s disease (AD) (Collerton 1986; Kasa et al. 1997; McKinney and Jacksonville 2005; Schliebs 2005). In rodent models, bilateral injection of scopolamine, a nonspecific muscarinic antagonist, into the dorsal hippocampus impairs spatial learning in rodents (Herrera-Morales et al. 2007). In accord with these observations, cholinergic lesion of the medial septum using 192-saporin has been shown to impair long-term potentiation (LTP), a putative cellular correlate of learning and memory, although some studies report no effect of septal lesion on LTP magnitude (Scheiderer et al. 2006). Also, 192-saporin-induced septal lesion reduces glutamatergic synaptic currents in CA1 pyramidal cells (Kanju et al. 2012), and other septohippocampal lesion studies in rodent brain produce memory and attentional deficits (Bartus et al. 1982; Berger-Sweeney et al. 2001; Callahan et al. 1993; Dekker et al. 1991; Drachman and Leavitt 1974; Fibiger 1991; Gold 2003; Nilsson et al. 1992; Perry et al. 1999; Sarter and Parikh 2005; Wrenn et al. 1999). In genetically engineered animals lacking M1 mAChRs, impaired LTP is evident (Seeger et al. 2004; Shinoe et al. 2005), as are impairments in spatial memory and consolidation, although deficits in these animals appear to encompass specific tasks rather than broadly disrupting all hippocampus-dependent learning (Anagnostaras et al. 2003; Hamilton et al. 2001; Miyakawa et al. 2001). This finding highlights the level of complexity in mAChR modulation of hippocampal function, and discrepancies within the learning and memory literature do exist. For instance, Sheffler and colleagues (2009) report no impairment in hippocampus-dependent learning, due to injection of a highly specific M1 mAChR antagonist, VU-022035, but allosteric M1 mAChR agonists enhance acquisition of hippocampus-dependent cognition in another report (Digby et al. 2012). Also, whereas disruption of the M2 gene causes clear behavioral deficits in spatial learning and hippocampal memory tasks (Bainbridge et al. 2008; Seeger et al. 2004), the injection of M2/M4-favoring antagonists actually improves cognitive performance in some studies (Aura et al. 1997; Baratti et al. 1993; Carey et al. 2001; Galli et al. 2000; Hamm et al. 1995; Kopf et al. 1998; Ohno et al. 1994; Packard et al. 1990; Vannucchi et al. 1997).
As suggested by the findings described above, the effects of activation of M1-4 mAChRs within area CA1 are particularly complex and often times opposing, with the overall effect of their activation depending on several factors, including the dose of agonist used and the timing of activation. Presynaptically, it is known that the M3 mAChR-prefering antagonist, the 1,1-dimethyl-4-diphenylacyetoxypropenidium iodide (4-DAMP)-sensitive receptor, depresses excitatory synapses by reducing the probability of neurotransmitter release, and a postsynaptically expressed form of muscarinic long-term depression (mLTD) is induced with a high dose of carbachol (CCh), using a M1-dependent mechanism at CA3-CAL synapses (Scheiderer et al. 2006, 2008; Volk et al. 2007) and in the visual cortex (McCoy and McMahon 2007). Conversely, an M2 mAChR-mediated potentiation of glutamatergic synapses, termed LTPm, can be induced by low-dose CCh application in vitro (Auerbach and Segal 1994). Interestingly, LTP has also been observed in vivo after an intracerebroventricular injection of the M2/M4 receptor-favoring antagonist methoctramine (Li et al. 2007).

In addition to evoking changes directly in synaptic strength, numerous studies show that mAChRs activated with an agonist or synthetically released ACh can modulate (in most cases, enhance) neuronal activity depending on several factors, including their activation depending on several factors, including the dose of agonist used and the timing of activation. Presynaptically, it is known that the M3 mAChR-prefering antagonist, the 1,1-dimethyl-4-diphenylacyetoxypropidium iodide (4-DAMP)-sensitive receptor, depresses excitatory synapses by reducing the probability of neurotransmitter release, and a postsynaptically expressed form of muscarinic long-term depression (mLTD) is induced with a high dose of carbachol (CCh), using a M1-dependent mechanism at CA3-CAL synapses (Scheiderer et al. 2006, 2008; Volk et al. 2007) and in the visual cortex (McCoy and McMahon 2007). Conversely, an M2 mAChR-mediated potentiation of glutamatergic synapses, termed LTPm, can be induced by low-dose CCh application in vitro (Auerbach and Segal 1994). Interestingly, LTP has also been observed in vivo after an intracerebroventricular injection of the M2/M4 receptor-favoring antagonist methoctramine (Li et al. 2007).
and stored at 4°C. A 1-mM stock solution was prepared in ddH2O, aliquoted, and stored at -20°C. On the day of use, stock solution was diluted in aCSF to a final concentration of 1 μM.

CGP 52432. CGP 52432 (CGP) was purchased from Tocris (cat. #1246). A 1-mM stock solution was prepared in ddH2O, aliquoted, and stored at -20°C. On the day of use, stock solution was diluted in aCSF to a final concentration of 1 μM.

Data Analysis

Data were expressed as mean ± SE. Comparison of data from different treatment groups was performed by Student’s t-test, and P < 0.05 was considered statistically significant. Data from electrophysiology experiments were filtered at 3 kHz, digitized at 10 kHz, and acquired using LabVIEW data acquisition software. The slope of the rising phase of fEPSP was measured and plotted vs. time. Each point represents the average of five raw data points. To determine the magnitude of LTD, the slopes of the rising phase of fEPSPs were normalized to baseline, and 5 min of raw fEPSPs was averaged. In the majority of experiments, the magnitude of LTD was measured 40 min

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postdrug (eserine or CCh) application. Exceptions occurred (see Fig. 1A; analysis at 65 min posteserine), because a lower concentration of eserine was used and (see Fig. 7A; analysis at 20 min posteserine application) because atropine was applied during the expression phase of the LTD.

RESULTS

Pharmacological Blockade of AChE Induces a Long-Lasting Synaptic Depression Requiring mAChR Activation

To test the effect of AChE inhibition on synaptic transmission, hippocampal slices from adult male rats (3–4 mo) were treated with eserine (100 nM) for 10 min during extracellular dendritic field potential recordings. We find this acute eserine treatment sufficient to induce a long-lasting depression, which we term eserine-LTD, at CA3-CA1 synapses (Fig. 1A; LTD: 83 ± 8% of fEPSP baseline slope; 85–90 min, n = 4). To test if a higher dose of eserine could accelerate the time course of LTD expression, we applied 10 μM eserine for 10 min. Compared with our initial experiments using 100 nM eserine, in which a clear depression of fEPSP slope was not observed consistently until 35–40 min after eserine washout, slices treated with 10 μM eserine displayed a stable depression more rapidly; a clear decrease in fEPSP slope consistently occurred as soon as 5 min after the start of eserine washout (Fig. 1B; LTD: 80 ± 3% of fEPSP baseline slope; 60–65 min, n = 6).

To ensure the effects of eserine are indeed a consequence of AChE inhibition and accumulation of extracellular ACh, we found this acute eserine treatment sufficient to induce a long-lasting depression, which we term eserine-LTD, at CA3-CA1 synapses (mLTD) (McCutchen et al. 2006; Scheiderer et al. 2006, 2008), and 4-DAMP-sensitive receptors, likely M3, mediating presynaptic depression during CCh application, we asked if eserine-LTD also requires M1 and/or M3 mAChR activation. To this end, the mAChR antagonist pirenzepine was bath applied at 75 nM, a dose highly selective for M1 mAChRs (Marino et al. 1998), in conjunction with 4-DAMP (100 nM) before the application of eserine. We found this combination of inhibitors capable of completely blocking eserine-LTD [Fig. 2A; LTD: 74 ± 5% fEPSP baseline slope in control solution (n = 5) vs. 1.02 ± 5% in pirenzepine + 4-DAMP (n = 5); P = 0.002, Student’s t-test]. To elucidate further the receptors necessary for eserine-LTD, we treated slices with pirenzepine alone and found that the eserine-LTD magnitude was not affected [Fig. 2B; LTD: 77 ± 3% fEPSP baseline slope in control solution (n = 3) vs. 72 ± 4% in pirenzepine (n = 7); P > 0.05 between groups]. In contrast to pirenzepine treatment alone, we found 4-DAMP (100 nM) to be sufficient in blocking eserine-LTD [Fig. 2C; LTD: 80 ± 2% of fEPSP baseline slope in control solution (n = 5) vs. 1.05 ± 5% in 4-DAMP (n = 5); P = 0.001, Student’s t-test]. This finding indicates a form of LTD induced by 4-DAMP-sensitive receptors.

Eserine-LTD Does Not Require pERK or p38 MAPK

Because mLTD induced by CCh requires activation of the ERK1/2 signaling pathway (Scheiderer et al. 2008; Volk et al. 2007), we next tested whether eserine-LTD shares this mech-

![Normalized fEPSP slope vs. Time (min)](http://jn.physiology.org/)

**Fig. 3.** Eserine-LTD does not depend on ERK1/2 activity. A: U0126 treatment for 20 min before carbachol (CCh) application completely blocks CCh-induced muscarinic LTD (mLTD) in 3–5 wk-old rats. LTD: 86 ± 3% of baseline fEPSP slope in control solution (n = 8) vs. 99 ± 5% of fEPSP slope in U0126 (n = 9). B: the blocking of mLTD in adult rats (age 3–4 mo) requires pretreatment with U0126, lasting at least 1 h. LTD: 76 ± 5% of baseline fEPSP slope in control solution (n = 6) vs. 76 ± 4% of fEPSP slope in U0126 < 1 h (n = 5). C: LTD: 76 ± 7% of baseline fEPSP slope in control solution vs. 1.05 ± 4% of baseline fEPSP slope in U0126 > 1 h (n = 6); P = 0.002, Student’s t-test. D: pretreatment with U0126 ≥ 1 h does not block eserine-LTD (10 μM); 77 ± 6% of baseline fEPSP slope in control solution (n = 5) vs. 75 ± 2% of baseline fEPSP slope in U0126 > 1 h (n = 4). *P ≤ 0.05; **P ≤ 0.01.

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Eserine induces LTD in the hippocampus. We first performed positive control experiments in young rats, aged 3–5 wk, an age at which a 20-min bath application of the MEK inhibitor U0126 (20 μM) reliably blocks mLTD (Scheiderer et al. 2008). As shown in Fig. 3A, U0126 completely blocked CCh-induced mLTD (86 ± 3% of fEPSP baseline slope in control solution (n = 8) vs. 99 ± 5% of fEPSP slope in U0126 (n = 9); P < 0.05 between groups). However, to block mLTD in adult rats, U0126 pretreatment, lasting at least 1 h, preceding CCh application, was required (Fig. 3B; LTD: 76 ± 3% of baseline fEPSP slope in control solution (n = 6) vs. 76 ± 4% of fEPSP slope in U0126 < 1 h (n = 5); Fig. 3C; LTD: 76 ± 7% of fEPSP slope in control solution vs. 1.05 ± 4% of fEPSP slope in U0126 > 1 h (n = 6); P = 0.002, Student’s t-test). With the use of a 1-h preincubation protocol, we found no effect of MEK inhibition on the magnitude of eserine-LTD (Fig. 3D; LTD: 77 ± 6% of baseline fEPSP slope in control solution (n = 5) vs. 75 ± 2% of baseline fEPSP slope in U0126 > 1 h (n = 4); P > 0.05 between groups), indicating that the cellular mechanisms of CCh mLTD and eserine-LTD do not share the requirement of ERK1/2 activation. We next tested for involvement of p38 MAPK, a signaling enzyme activated by metabotropic glutamate receptors (mGluRs) that share Gq coupling with M1 mAChRs and induce mGluR-LTD (Moult et al. 2008) in the mechanism of eserine-LTD. Similar to mGluR-LTD, we find that blocking p38 MAPK, using SB203580 (5 μM; for at least 1 h before agonist application), completely blocks mLTD induced by CCh in adult rats (Fig. 4A; LTD: 86 ± 2% of fEPSP slope in control solution (n = 6) vs. 97 ± 3% of fEPSP slope in SB203580 (n = 7); P = 0.003 between groups, Student’s t-test). However, eserine-LTD remained intact, despite p38 MAPK blockade (Fig. 4B; LTD: 83 ± 3% of baseline fEPSP slope in control solution (n = 4) vs. 85 ± 10% of baseline fEPSP slope in SB203580 (n = 5); P > 0.05 between groups). Taken as a whole, these data demonstrate that induction of eserine-LTD is independent of ERK1/2 or p38 MAPK signaling.

The expression profile of mAChRs within the hippocampus spans both excitatory and inhibitory neurons, and published findings show that an AChE inhibitor can selectively enhance the activity of GABAergic interneurons expressing M1 mAChRs (Cea-del-Rio et al. 2010). To eliminate the possibility that eserine induces LTD due to a potentiation of inhibitory GABAAR transmission, experiments were performed in the presence of 100 μM picrotoxin. Whereas it was evident from the observed population spikes that picrotoxin effectively blocked GABAAR transmission, the ability of eserine to induce LTD was unaffected (Fig. 5A; LTD: 86 ± 7% of baseline fEPSP slope (n = 6)). We also tested for a role of GABAARs in eserine-LTD by performing experiments in the presence of CGP. Similar to results in picrotoxin, CGP did not affect the magnitude of LTD induced by eserine (Fig. 5B; LTD: 86 ± 3% of baseline fEPSP slope in control solution (n = 4) vs. 89 ± 6% of fEPSP slope in CGP (n = 5); P > 0.05 between groups, Student’s t-test).

We next assessed the effect of eserine on presynaptic activity by analyzing the paired-pulse facilitation ratios before and during expression of eserine-LTD. We combined control experiments from three data sets (eserine ± U0126, SB203580, or CGP; Figs. 2C, 3, and 4) to increase the statistical power of our paired-pulse ratio (PPR) analysis. In the event that eserine-
LTD is due to a decrease in the probability of neurotransmitter release, we expected any decrease in fEPSP slope after eserine application to be accompanied by an increase in PPR (slope of fEPSP 2/slope of fEPSP 1). By normalizing the PPRs to the baseline PPRs of each experiment, we were able to overlay a plot of PPR on the same time course as the fEPSP slope. Consistent with a presynaptic mechanism of expression, changes in fEPSP slope during eserine-LTD experiments are accompanied by temporally matched fluctuations in PPR (Fig. 6A; n = 16). To test the strength of this correlation, a linear regression was performed, plotting fEPSP slope as a function of PPR over samples collected between 25 and 65 min of the LTD experiments. The results of this analysis indicate a very strongly negative correlation [Fig. 6B; linear regression: slope = −0.97, R² = 0.46, P = 0.0 × 10⁻³⁰ (n = 4,231 sweeps)] between PPR and fEPSP slope during eserine-LTD expression and strongly support a presynaptic component to the expression mechanisms of eserine-LTD.

Because AChE inhibition likely outlasts the 10-min bath application of eserine, we also asked if continued accumulation of extracellular ACh and constant activation of presynaptic mACHRs, which are known to depress synaptic transmission at CA3-CA1 synapses, account for the depression observed. To this end, we attempted to reverse eserine-LTD expression using a nonselective muscarinic receptor antagonist, atropine (1 µM), during 45–75 min of LTD expression. As shown in Fig. 7A, the synaptic depression is partially but significantly reversed by atropine [LTD: 78 ± 5% of baseline fEPSP slope pretatropine (40–45 min, n = 6) vs. 86 ± 6% of baseline fEPSP slope postatropine (70–75 min, n = 6); P = 0.02, Student’s paired t-test]. Importantly, control experiments show atropine treatment alone does not increase fEPSP slope (Fig. 7B; postatropine slope: 1.01% of baseline (40–45 min, n = 7, P > 0.05)]. We also analyzed PPRs during the atropine experiments and plotted them with respect to fEPSP slope (Fig. 7C). Consistent with results shown in Fig. 6A, eserine-LTD was accompanied by an increase in PPR. Strikingly, application of atropine resulted in PPR returning to the level of baseline on the same time course as the attenuation of LTD magnitude (Fig. 7C). It should be noted that fEPSP slope remained significantly depressed after atropine treatment (between 70 and 75 min; time point “2”), despite the return of PPR to baseline levels [Fig. 7, C and D; PPR: 1.08 ± 3% of baseline PPR pretatropine (time point “1”) vs. 1.01 ± 2% of baseline PPR postatropine (time point 2), n = 6; P = 0.002, Student’s paired t-test]. We interpret these results as evidence for a dual mechanism underlying eserine-LTD: a presynaptic mechanism dependent on constant activation of mACHRs and a mechanism independent of changes in release probability that likely occur at the postsynaptic site.

**DISCUSSION**

M₁ mACHRs are required for normal hippocampal-dependent learning and memory and bidirectionally modulate synaptic efficacy at hippocampal CA3-CA1 synapses (Anagnostaras et al. 2003; Hamilton et al. 2001; Miyakawa et al. 2001; Scheiderer et al. 2006, 2008; Seeger et al. 2004; Shinoe et al. 2005; Suzuki and Okada 2012). Previous work from our lab shows that pharmacologically activating M₁ mACHRs, using CCh, induce a Src kinase and phosphorylated ERK (pERK)-dependent form of LTD (mLTD) at CA3-CA1 synapses that are independent of the canonical M₃-Gqα-PLC signaling pathway (Scheiderer et al. 2006, 2008). Because inhibitors of AChE are used clinically to increase extracellular ACh levels in the treatment of dementia in AD, we tested the hypothesis that LTD is induced as a consequence of AChE inhibition and that it is mechanistically similar to that induced by CCh. Here, we report that a 10-min application of eserine induces LTD (eserine-LTD) but with properties not shared by mLTD. Inhibition of M₃ mACHRs, but not M₁ mACHRs, was required to prevent eserine-LTD, and pharmacological inhibition of MEK, which prevents the increase in pERK, was completely ineffective. Furthermore, we find that mLTD requires p38 MAPK activation, a signaling enzyme previously implicated in mGluR-LTD, but eserine-LTD does not share this property. Finally, whereas previous reports suggest that mLTD expression requires internalization of AMPA receptors (Volk et al. 2007), long-term expression of eserine-LTD is partially dependent on a decrease in presynaptic release probability, caused by tonic activation of presynaptic mACHRs by the sustained increase in extracellular ACh.

Given published literature showing that CCh-induced LTD at synapses in the hippocampus and various areas of cortex...
Atropine partially attenuates eserine-LTD but fully reverses an eserine-induced increase in PPR. A: atropine (1 μM), applied between 45 and 75 min of eserine-LTD (10 μM) expression. LTD: 78 ± 5% of baseline fEPSP slope preatropine (time point 1) vs. 86 ± 6% of baseline fEPSP slope postatropine (time point 2), n = 6; P = 0.02, Student’s paired t-test. B: 30 min treatment with atropine alone. Postatropine slope: 1.01% of baseline (40–45 min, n = 7). C: normalized PPR and paired-pulse facilitation ratios during atropine experiments shown in A, plotted with respect to fEPSP slope. Eserine-LTD and atropine-mediated attenuation of LTD are accompanied by an increase and normalization of PPR, respectively. PPR: 1.08 ± 3% of baseline PPR preatropine (time point 1) vs. 1.01 ± 2% of baseline PPR postatropine (time point 2), n = 6; P = 0.002, Student’s paired t-test. D: LTD and PPR data from time points 1 and 2 (pre- and postatropine, respectively), presented in a bar chart. fEPSP slope remains significantly depressed, despite normalization of PPR by atropine. *P ≤ 0.05; **P ≤ 0.01.

Fig. 7. Atropine partially attenuates eserine-LTD but fully reverses an eserine-induced increase in PPR. A: atropine (1 μM), applied between 45 and 75 min of eserine-LTD (10 μM) expression. LTD: 78 ± 5% of baseline fEPSP slope preatropine (time point 1) vs. 86 ± 6% of baseline fEPSP slope postatropine (time point 2), n = 6; P = 0.02, Student’s paired t-test. B: 30 min treatment with atropine alone. Postatropine slope: 1.01% of baseline (40–45 min, n = 7). C: normalized PPR and paired-pulse facilitation ratios during atropine experiments shown in A, plotted with respect to fEPSP slope. Eserine-LTD and atropine-mediated attenuation of LTD are accompanied by an increase and normalization of PPR, respectively. PPR: 1.08 ± 3% of baseline PPR preatropine (time point 1) vs. 1.01 ± 2% of baseline PPR postatropine (time point 2), n = 6; P = 0.002, Student’s paired t-test. D: LTD and PPR data from time points 1 and 2 (pre- and postatropine, respectively), presented in a bar chart. fEPSP slope remains significantly depressed, despite normalization of PPR by atropine. *P ≤ 0.05; **P ≤ 0.01.

requires M₁ mAChRs and an increase in pERK (McCoy and McMahon 2007; Scheiderer et al. 2008), it was surprising that eserine-LTD was not prevented by the M₁-favoring antagonist, pirenzepine alone, but instead was prevented by the M₃ mAChR-preferring antagonist 4-DAMP. It was also surprising that eserine-LTD and CCh-induced mLTD are mechanistically different. This prompted us to consider that synaptic depression induced by eserine might be a consequence of sustained activation of mAChRs caused by continued elevation of ACh from persistent AChE inhibition, even after washout of eserine from the chamber. This concept is supported by the partial reversal of eserine-LTD by the nonselective mAChR antagonist atropine. Persistent antagonism of AChE would require a slowly dissociating eserine-AChE complex. Through characterization of eserine pharmacology support this notion; the dissociation constant has been determined to be 7.1 μM [reviewed in Triggle et al. (1998)], and careful kinetic analysis of the three-step carbamylenzyme mechanism shows a notably slow dissociation (Stojan and Zorko 1997). It is likely that eserine remains bound to AChE for the duration of the expression phase of eserine-LTD, causing persistent elevation of synaptic ACh and concomitant activation of presynaptic mAChRs that are well known to decrease release probability at hippocampal synapses (Fernandez de Sevilla and Buno 2003; Fernandez de Sevilla et al. 2002; Valentino and Dingledine 1981). Unfortunately, the identity of the presynaptic mAChRs remains controversial. With the use of mAChR knockout mice, Dasari and Gulledge (2011) suggest that it is M₄, not M₁ or M₂, mAChRs; however, the sensitivity of eserine-induced presynaptic depression to 4-DAMP suggests a role for M₃ mAChRs.

A presynaptic expression mechanism for eserine-LTD should be revealed by analysis of the PPR. An accepted indicator of alterations in presynaptic release probability (Dobrunz 2002; Kamiya and Zucker 1994). Indeed, our analysis of PPR during the time course of eserine-LTD shows a very strongly negative correlation between fEPSP slope and PPR, supporting the interpretation that a decrease in presynaptic neurotransmitter release may partially contribute to eserine-LTD, and a body of literature supports this notion. Namely, agonism of presynaptic mAChRs with CCh triggers a rapid synaptic depression at CA3-CA1 synapses that is associated with an increase in PPR but does not persist after CCh washout (Fernandez de Sevilla et al. 2002; Scheiderer et al. 2006; Valentino and Dingledine 1981). Importantly, the increase in PPR induced by eserine is reversed completely by atropine treatment, supporting the concept that a presynaptic mechanism underlies eserine-LTD expression that is dependent on sustained elevation of synaptic ACh, likely due to slow dissociation of eserine from AChE. Importantly, the lack of effect of CGP on eserine LTD indicates that this presynaptic depression is independent of GABAA_Rs, consistent with a previous report by Kremin et al.
(2006), showing that presynaptic mAChR suppression is not dependent on these receptors.

A significant reduction in fEPSP slope was still evident, despite normalization of PPR by atropine to baseline, an observation that we interpret as evidence for a second, likely postsynaptic, mechanism of e elusive-LTD expression. Although an increase in pERK signaling is required for other forms of LTD, including CCh-induced mLTD (Scheideler et al. 2008), eusive-LTD persisted, despite blockade of MEK, the kinase that phosphorylates and activates ERK1/2, using U0126. In pursuing the signaling mechanism underlying eulsive-LTD, downstream of M1 mAChR activation, we also tested for a role of p38 MAPK, an enzyme known to mediate hippocampal mGlur-LTD, which requires activation of Gq-coupled mGlur5 during induction (Moul et al. 2008). We report that CCh-induced mLTD requires p38 MAPK activation, a new but anticipated finding, as both mGlur5 and M1/M3 are similarly coupled to Gq-coupled signaling pathways. However, eulsive-LTD was insensitive to p38 MAPK inhibition. Further experimentation will be necessary to determine the intracellular signaling cascades initiated during eulsive-LTD induction.

A multitude of complex and sometimes competing mechanisms of synaptic modulation has been described in the context of cholinergic innervation in the hippocampus (Auerbach and Segal 1996). It is generally supported that AChE inhibitors facilitate synaptic function and plastic mechanisms required for learning and memory by elevating extracellular synaptic ACh concentration. In the present study, we demonstrate that AChE inhibition and the consequent increase in extracellular ACh modulate synaptic efficacy at CA3-CA1 synapses through several complicated mechanisms, likely involving M3 mAChRs that could be located at pre- and postsynaptic sites. Future studies are needed to identify the location of the M1 mAChRs and the downstream signaling mechanisms invoked, which could include postsynaptically released endocannabinoids that retrogradely depress presynaptic glutamate release via activation of presynaptic CB1 receptors (Ohno-Shosaku et al. 2003). Our findings add to the body of literature on cholinergic modulation of synaptic function in the hippocampus by showing that AChE inhibition causes a prolonged decrease in presynaptic glutamate release, in addition to inducing a likely postsynaptic form of LTD. The prolonged decrease in presynaptic glutamate release in the presence of AChE inhibitors, which display tight binding affinity to AChE and slow dissociation, may have the unintended consequence of decreasing synaptic flexibility or induction of other forms of activity-dependent plasticity, potentially explaining why this class of drugs is not always beneficial for treating cognitive deficits in AD. Clearly more work is needed to understand fully the complex role of AChE inhibitors and the cholinergic system in modulating hippocampal function.

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DISCLOSURES

The authors declare that there are no conflicts of interest, financial or otherwise, associated with this project or publication.

AUTHOR CONTRIBUTIONS


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