Long-lasting facilitation by dehydroevodiamine · HCl of synaptic responses evoked in the CA1 region of rat hippocampal slices

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We tested the effects of dehydroevodiamine · Cl (DHED) on field excitatory postsynaptic potentials (fEPSPs) evoked by the electrical stimulation of Schaffer collaterals–commissural fibres in the CA1 region of rat hippocampal slices. Bath applications of 10 μM DHED for 20 or 40 min induced long-lasting facilitation of fEPSPs, which outlasted the presence of DHED. A 10 min treatment with a higher concentration (100 μM) also induced long-lasting facilitation. The long-lasting facilitation was blocked either by 10 μM atropine, the muscarinic receptor antagonist, or by 50 μM D-2-amino-5-phosphonopentanoic acid (D-AP5), an NMDA receptor antagonist. These results show that DHED produces long-lasting facilitation of synaptic transmission, and that this facilitation depends upon the activation of both the muscarinic and NMDA receptors. *NeuroReport* 14:399–403 © 2003 Lippincott Williams & Wilkins.

Key words: Acetylcholinesterase inhibitor; Dehydroevodiamine · HCl; Hippocampus; Long-lasting facilitation

INTRODUCTION

Loss of cholinergic function in the neocortex and hippocampus arising from degeneration or atrophy of the basal forebrain cholinergic neurons and the presence of neuritic plaques composed of amyloid-β peptide are consistent pathological features of the brain in Alzheimer's disease (AD) [1]. Therefore, current pharmacological strategies to attenuate the impaired memory ability of AD patients, are targeted towards the supplementation of acetylcholine (ACh) levels in synaptic sites, by using cholinomimetics, such as acetylcholinesterase (AChE) inhibitors, cholinergic agonists, and ACh precursors. For example, tacrine, an AChE inhibitor, has been shown to slow the decline of cognitive function and memory, and it is widely marketed for the treatment memory loss and intellectual decline in AD patients [2].

Recently, dehydroevodiamine · HCl (DHED) isolated from *Evodia rutaecarpa* Bentham, was found to have novel antiacetylcholinesterase and antiamnesic activities in a scopolamine-induced amnesia model, and to reduce neuronal loss in the hippocampal area of ischemic rats. These findings suggest beneficial effects of DHED on AD dementia [3,4]. While DHED is known to have various effects on CNS physiology, such as the suppression of Ca^{2+} channels [5] and the enhancement of cerebral blood flow [6], its molecular physiological actions related to the alleviation of memory loss have not been well documented. Since cholinergic agonists have been reported to modify synaptic transmission and plasticity [7–10], we were particularly interested to determine whether DHED also modifies synaptic transmission in the hippocampus, an important region for learning and memory. In the present study, we investigated the effects of DHED on synaptic responses in the CA1 region of rat hippocampal slices using extracellular field potential recordings.

MATERIALS AND METHODS

Recording of field excitatory postsynaptic potentials: Rats (Sprague–Dawley, 4–6 weeks old) were decapitated under halothane anesthesia. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.4 KH_2PO_4, 1.8 $CaCl_2.2H_2O$, 1.3 $MgSO_4$, 23 $NaHCO_3$, 10 glucose, continuously bubbled with 95% O_2/5% CO_2. Transverse hippocampal slices (400 μm) were prepared with a McIlwain tissue chopper (Hugo Sachs Electronik, March, Germany), and then incubated for $\geq$1 h in a chamber containing oxygenated ACSF before recording. A slice was transferred to a submerged recording chamber at 30°C that was perfused with ACSF saturated with 95% CO_2/5% O_2 at a flow rate of 1–2 ml/min. Field excitatory postsynaptic

potentials (fEPSPs) were evoked by stimulating Schaffer collaterals-commissural fibres with monophasic square wave pulses (100 μs duration). Recordings were made with a glass electrode (2–5 MΩ resistance) filled with ACSF using a DAM80 differential amplifier (World Precision Instruments, Sarasota, FL, USA). For baseline recording, fEPSPs were evoked every 30 s, and the stimulus intensity was adjusted to give similarly sized responses in each slice tested. Responses were acquired on-line and analyzed using the NAC software package (Electek, Irvine, CA, USA). All results are quoted as mean ± s.e.m. Statistical analysis was performed using Student's *t*-test with significance accepted at $p < 0.05$.

Drugs: DHED was synthesized and supplied by the Jeil Pharmaceutical Company (Seoul, Korea). All other chemicals were purchased from Sigma (St. Louis, MO, USA). DHED was dissolved in 0.2% dimethyl sulfoxide solution and prepared immediately prior to use.

RESULTS

Effects of DHED on field excitatory postsynaptic potentials: Prior to drug application, stable fEPSPs were maintained for ≥ 20 min. The application of DHED for 10 min produced increases in the initial slope of fEPSPs in a concentration-dependent manner (5 μM; 101.1 ± 3.1% of control, $n = 10$, $p > 0.1$; 10 μM; 116.9 ± 4.2% of control, $n = 10$, $p < 0.05$; 100 μM; 121.9 ± 1.5% of control, $n = 24$, $p < 0.01$, measured 50 min after washing out DHED; Fig. 1a). Prolonged treatments (20 and 40 min) with 10 μM of DHED also induced significant increases in the slope of fEPSPs, which plateaued ~15 min after DHED application, and these increased fEPSPs outlasted the presence of DHED. The average degrees of facilitation 50 min after washing out DHED were 127.4 ± 3.1% of control ($n = 17$, $p < 0.01$) for 20 min of treatment, and 122.5 ± 2.8% of control ($n = 18$, $p < 0.01$) for 40 min of treatment (Fig. 1b,c). By contrast, a prolonged treatment with a higher dose of DHED failed to induce long-lasting facilitation of fEPSPs. Instead, it initially produced a transient facilitation in fEPSPs, and showed sign of suppression 10–15 min after the start of DHED applica-

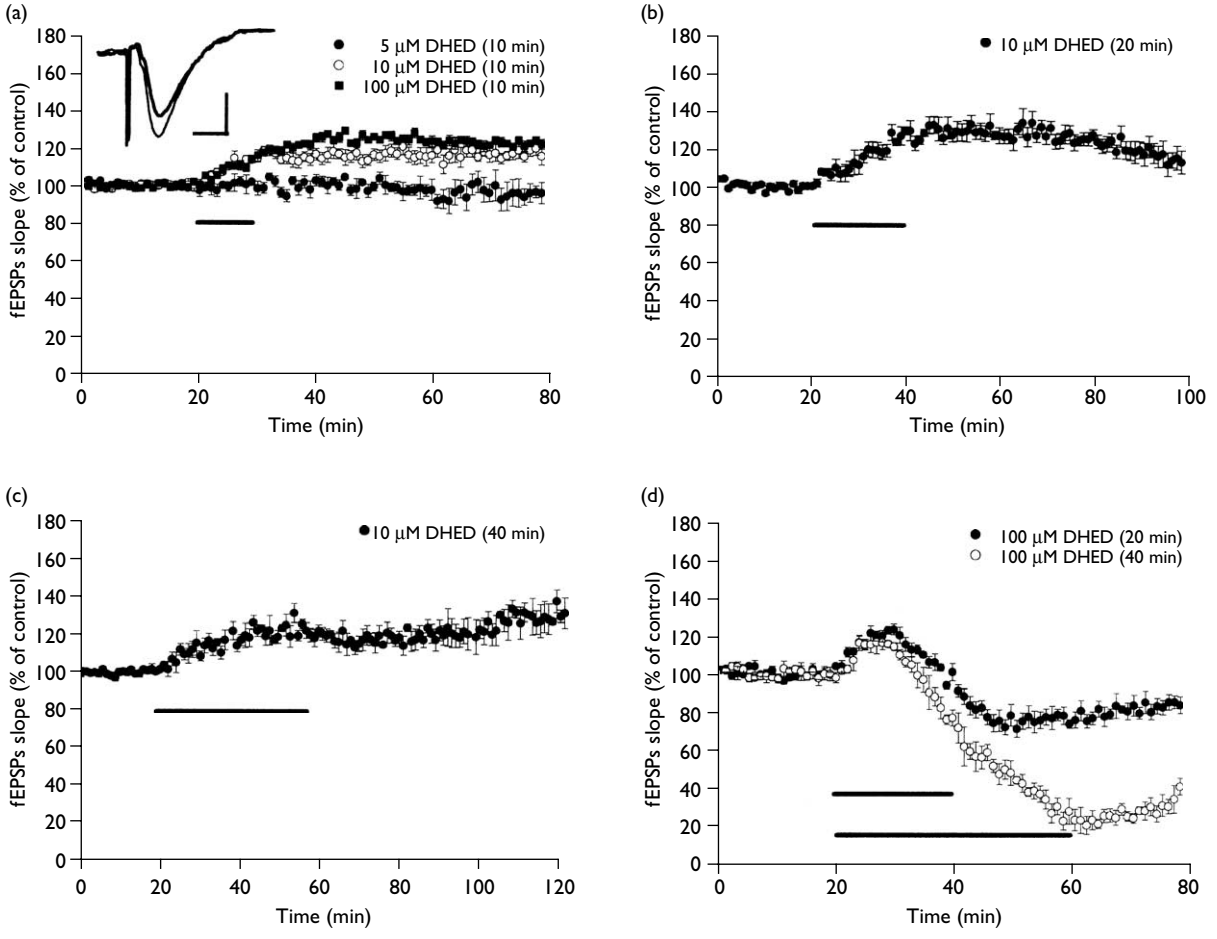

Fig. 1. Effects of DHED on synaptic transmission. (**a**) Bath application of DHED for 10 min induced long-lasting facilitation of fEPSPs, in a dose-dependent manner. Closed circles: 5 μM, $n = 10$; open circles: 10 μM, $n = 10$; closed squares: 100 μM, $n = 24$. fEPSPs before (solid trace) and 50 min (dotted trace) after 100 μM DHED treatment are shown superimposed at the top. The thick horizontal bar indicates the DHED treatment time. Calibration: 10 ms and 1 mV. (**b**,**c**) Application of 10 μM DHED for 20 ($n = 17$) or 40 min ($n = 18$) induced long-lasting facilitation in fEPSPs. (**d**) Effects of a high dose (100 μM) of DHED for 20 or 40 min. fEPSPs suppression was followed by a transient facilitation when DHED was applied for 20 (open circles, $n = 6$) or 40 min (open squares, $n = 7$).

tion. When 100 μM of DHED were treated for 20 and 40 min, fEPSPs were briefly increased to 123.7 ± 2.9% and to 116.8 ± 3.8% of control, and then gradually reduced to 88.4 ± 7.4% of control ($n = 20$) and 30.6 ± 7.1% of control ($n = 16$), respectively (Fig. 1d). The suppression of fEPSPs gradually recovered in 150–200 min after washing out DHED (data not shown).

Blockade of long-lasting facilitation by D-AP5 and atropine: The activation of NMDA receptors has been reported to be necessary for tetanus-induced long-term potentiation [11]. We, therefore, determined whether the facilitatory effect of DHED is also mediated by NMDA receptors. In the presence of 50 μM D-AP5, 10 min treatment with 100 μM DHED failed to induce long-lasting facilitation (Fig. 2a). In addition, D-AP5 prevented the transient facilitation of fEPSPs slope after 20 min treatment with 100 μM DHED, and D-AP5 also attenuated the suppressive effect of DHED (Fig. 2b). When treated 20 min after washing out DHED, D-AP5 failed to cause any change in synaptic response (Fig. 2c), indicating that NMDA receptors are required only for the induction of DHED-induced facilitation, not for its maintenance. Meanwhile, the non-NMDA receptor antagonist, CNQX (used at 100 μM), significantly reduced fEPSPs, suggesting that synaptic responses facilitated by DHED are mediated by AMPA/kainate receptors (Fig. 2d).

Next, we investigated whether the activation of muscarinic ACh receptors is required for DHED-induced facilitation. Atropine (10 μM) blocked the long-lasting facilitation induced by 10 μM DHED (Fig. 3a), and also prevented the transient facilitation of fEPSPs and its subsequent suppression by prolonged treatment with 100 μM DHED (Fig. 3b).

Finally, we tried to identify which subtypes of muscarinic receptors are involved in the altered responses induced by 100 μM DHED. Treatment with the M1 receptor antagonist, pirenzepine (1 μM), blocked the transient facilitation caused by 40 min treatment with 100 μM DHED, but did not attenuate the later suppression of fEPSPs (77.6 ± 6.5% of control, $n = 7$; Fig. 4a). Only a transient facilitation occurred in the presence of the M2 receptor antagonist methoctramine (1 μM), without the suppressive effect of a high concentration of DHED (Fig. 4b). When both the M1 and M2

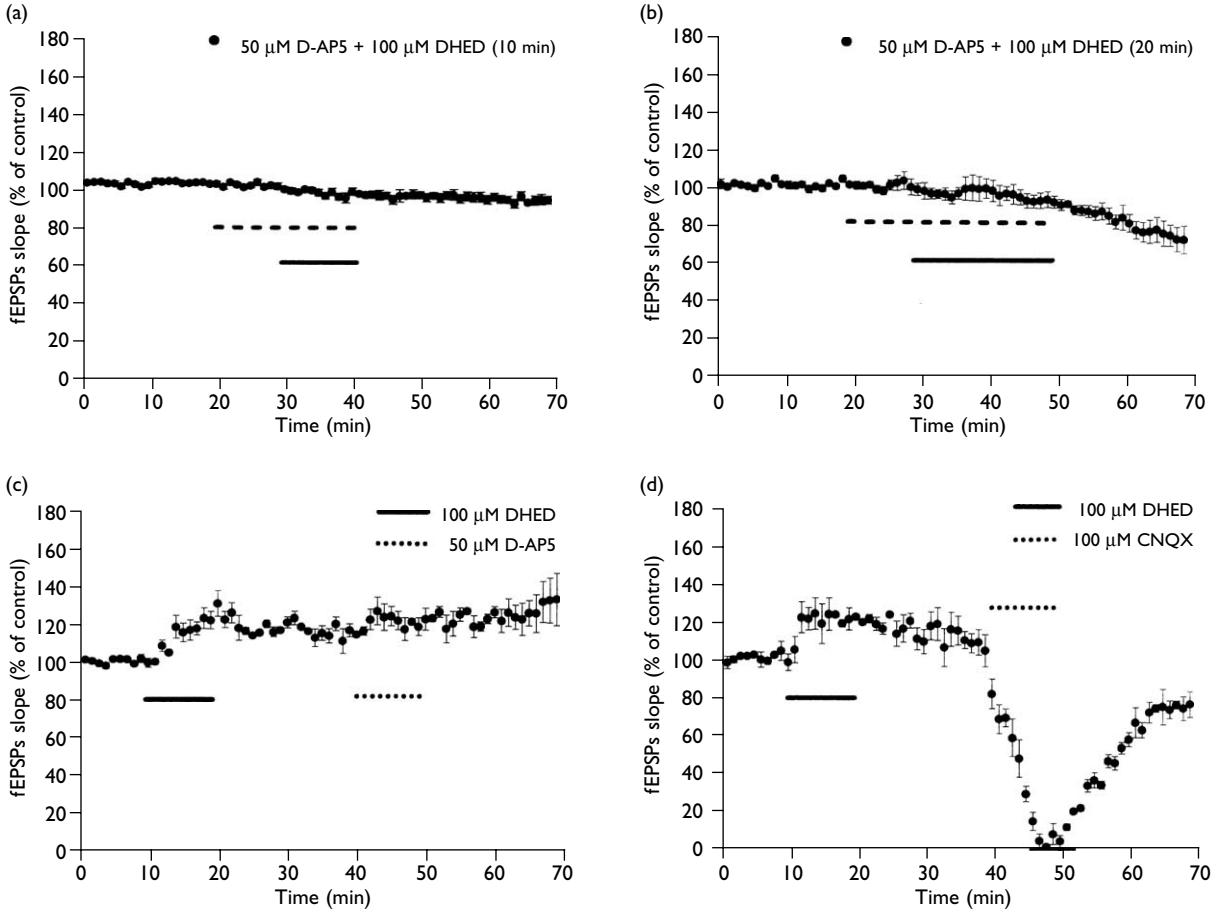

Fig. 2. Effects of glutamate receptor antagonists on DHED-induced long-lasting facilitation. (**a**) A 10 min treatment with 100 μM DHED failed to induce long-lasting facilitation in the presence of D-AP5 (50 μM; $n = 6$). Solid bar, DHED; Dotted bar, D-AP5. (**b**) When treated with 100 μM DHED for 20 or 40 min ($n = 9$ and 7, respectively), early transient facilitation was blocked by D-AP5. (**c**) D-AP5 failed to alter fEPSPs when treated 20 min after washing out the DHED ($n = 7$). (**d**) 100 μM CNQX abolished the fEPSPs enhanced by DHED ($n = 9$).

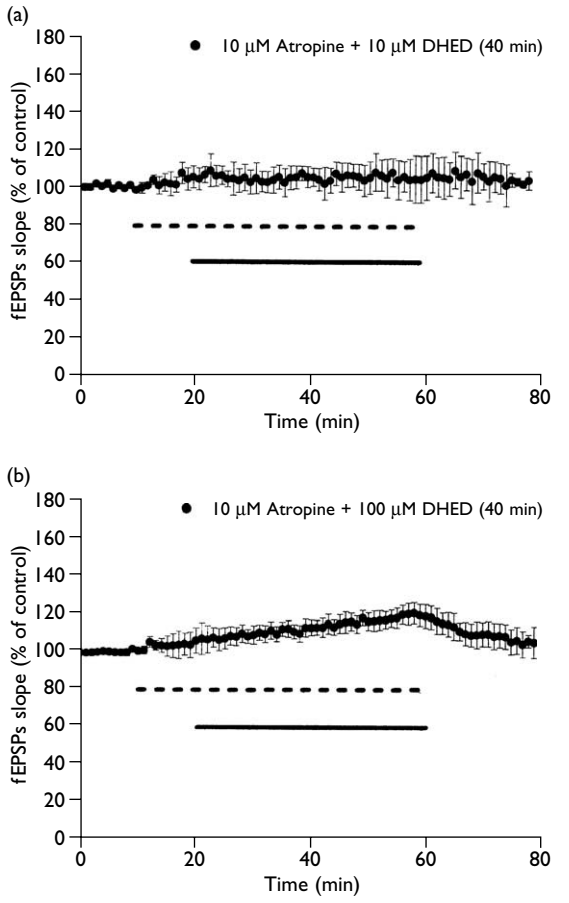

Fig. 3. Effects of atropine on DHED-induced long-lasting facilitation. (**a**) Long-lasting facilitation by 40 min treatment with 10 μM DHED was inhibited by atropine (10 μM; $n = 10$). Solid bar, DHED; Dotted bar, atropine. (**b**) The transient facilitation and subsequent suppression caused by treating for 40 min with 100 μM DHED was also prevented by atropine ($n = 10$).

receptor antagonists were applied, moderate suppression of fEPSPs by DHED was observed without any facilitation (Fig. 4c).

DISCUSSION

DHED has been known to have anti-acetylcholinesterase activity and anti-amnesic action [3,4]. In the present study, we found that the bath application of DHED produced long-lasting facilitation of synaptic responses in the CA1 region of rat hippocampal slices. This facilitation was blocked either by atropine, the muscarinic receptor antagonist, or by D-AP5, an NMDA receptor antagonist, which suggests that DHED-induced long-lasting facilitation involves both the muscarinic and NMDA receptors. When used at a higher concentration for a prolonged period (> 20 min), DHED was also observed to suppress synaptic response, and this effect was found to be completely blocked by atropine.

Several studies have reported effects of AChE inhibitors on synaptic transmission and tetanus-induced long-term potentiation (LTP). Physostigmine [12], HP 029 [13] and (−)huperzine [14] have been shown to modulate

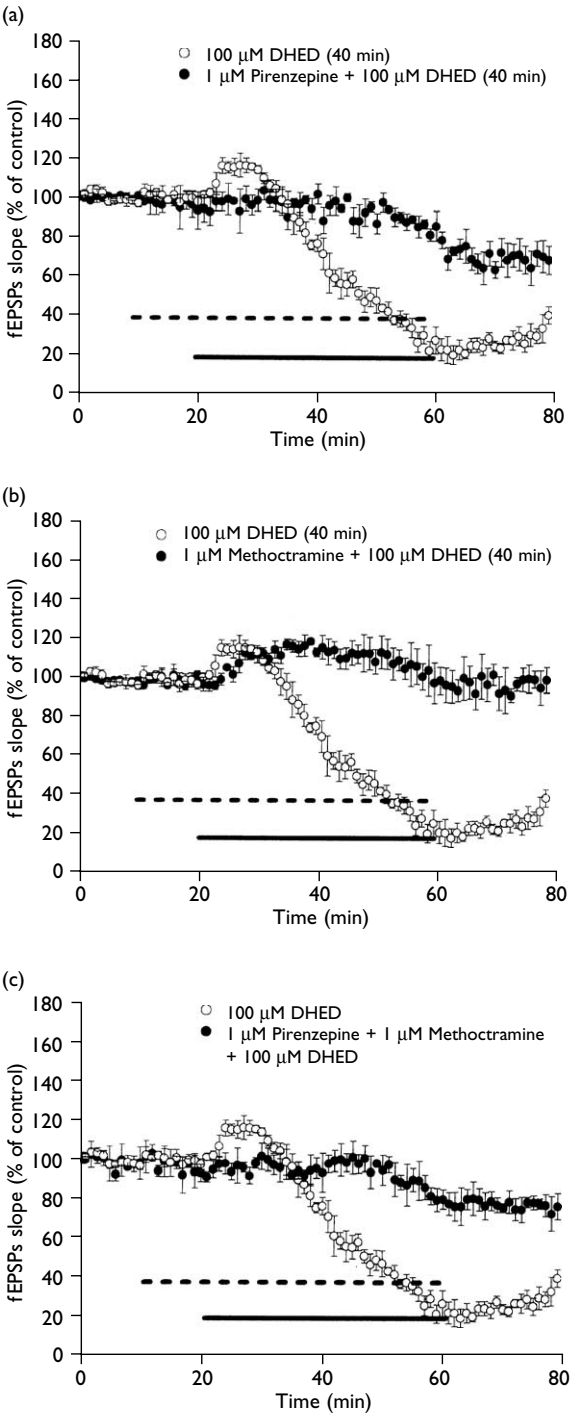

Fig. 4. Effects of M1 and M2 receptor antagonists on the altered synaptic responses caused by 100 μM DHED. (**a**) Early transient facilitation of fEPSPs was completely blocked in the presence of the M1 receptor antagonist, pirenzepine (1 μM; closed circles, $n = 6$). Solid bar, DHED; Dotted bar, pirenzepine. (**b**) fEPSPs were increased, without subsequent suppression, by DHED treatment in the presence of the M2 receptor antagonist, methoctramine (1 μM; closed circles, $n = 6$). Solid bar, DHED; Dotted bar, methoctramine. (**c**) Co-treatment with pirenzepine (1 μM) and methoctramine (1 μM) inhibited early facilitation and substantially prevented the subsequent suppression. (closed circles, $n = 6$). Open circles indicate the effect of 40 min treatment with 100 μM DHED alone.

tetanus-induced LTP in the rat hippocampus. These effects are believed to be important mechanisms for the amelioration of learning and cognitive impairments caused by AChE inhibitors. For instance, amyloid-β peptides are known to decrease endogenous ACh release evoked either by high concentrations of extracellular K^+ ions or by veratridine [15], and have also been shown to suppress the induction of tetanus-induced LTP in the CA1 region of rat hippocampal slices [16]. Interestingly, the AChE inhibitor (−)huperzine has been reported to block the suppressive action of amyloid-β peptide on LTP, suggesting that AChE inhibitor compensate for the pathological action of amyloid proteins [14].

Of the AChE inhibitors that have been studied, NIK-247 was first shown to induce the long-lasting facilitation of evoked synaptic activity in the CA1 pyramidal cell layer, and this facilitation was found to be blocked by atropine [17]. A recent study showed that AChE inhibitors, such as physostigmine and neostigmine increase the evoked discharges of neostriatal projection neurons, an effect blocked by pirenzepine [18], which is consistent with the findings of the present study (Fig. 4).

The extent of involvement of NMDA receptors in cholinergic agonist-induced potentiation remains controversial. In one study, carbachol selectively induced long-lasting facilitation of postsynaptic responses of AMPA receptors [19]. On the other hand, the topical application of acetylcholine produced long-lasting facilitation of EPSP, evoked by the stimulation of Schaffer collaterals afferents or by postsynaptic NMDA responses induced by ionophoretically applied NMDA [20,21]. Our results strongly suggest the involvement of NMDA receptors in the induction of long-lasting facilitation by DHED, an acetylcholinesterase inhibitor, because DHED failed to induce long-lasting facilitation in the presence of D-AP5 (Fig. 2c). The interaction between muscarinic and NMDA receptors is further supported by the finding that neostigmine enhances NMDA receptor-mediated current induced by the brief application of NMDA in projection cell of neostriatal brain slice, which is blocked by pirenzepine, but not by methoctramine [22]. Therefore, taken the present result that D-AP5 did not reduce facilitated synaptic responses when applied after a washout of DHED into consideration, the activation of NMDA receptors appears to be critical for the induction of DHED-induced facilitation, but not for its maintenance.

CONCLUSIONS

Our findings show that DHED, an acetylcholinesterase inhibitor, alters synaptic transmission in the CA1 region of rat hippocampal slices. DHED was found to induce long-lasting facilitation of synaptic transmission in a dose-dependent manner, which appears to require muscarinic and NMDA receptors activation. Therefore, we propose that DHED-induced facilitation may mechanistically underlie the antiamnesic effect of DHED in scopolamine-treated animals.

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