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Research Article

Memantine Does Not Influence AChE Inhibition in Rat Brain by Donepezil or Rivastigmine but Does with DFP and Metrifonate in In Vivo Studies

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT This in vivo study investigated whether the *N*-methyl-D-aspartate receptor antagonist, memantine (MEM), interacts with inhibition of acetylcholinesterase (AChE) by reversible (donepezil and rivastigmine) and irreversible (diisopropyl fluorophosphate (DFP) and metrifonate) AChE inhibitors (AChEIs) in rat brain regions (cortex and hippocampus), which are affected in humans with Alzheimer's disease. MEM (10 mg/kg, e.g., two to four times greater than the therapeutically relevant dose) was administered 15 min prior to donepezil (0.75 or 1.5 mg/kg), rivastigmine (0.35 or 0.7 mg/kg), metrifonate (55 or 110 mg/kg), or DFP (1.5 or 3.0 mg/kg). DFP was used as positive control. Rats were sacrificed at the time of maximal AChE inhibition (determined from time course studies; 15 min after donepezil, 30 min after rivastigmine or metrifonate, 60 min after DFP) to determine AChE activity in the brain region homogenates. Neither MEM nor AChEIs produced any behavioral effects at any time during the study, except metrifonate, which produced muscle tremors and fasciculations at 110 mg/kg. The present studies showed that i) MEM itself did not inhibit AChE in any brain area; ii) MEM did not interact with AChE inhibition induced by therapeutically used AChEIs (donepezil and rivastigmine) at either dose level; iii) MEM prevented AChE inhibition caused by DFP or metrifonate; and (iv) MEM prevented metrifonate-induced tremors and fasciculations. These findings indicate that MEM does not influence AChE inhibition by donepezil or rivastigmine, and therefore the possibility exists that either of the two antidementic drugs can be used concurrently with MEM. Drug Dev. Res. 64:71–81, 2005.

Key words: Memantine, donepezil, rivastigmine, acetylcholinesterase, brain

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease whose pathogenesis involves both cholinergic and noncholinergic mechanisms. The losses of cholinergic neurons occur primarily in the cortex and the hippocampus, the brain structures that play important roles in memory and cognitive function [Bartus et al., 1982; Coyle et al., 1983]. Loss of cholinergic neurons results in up to 90% reduction in the activity of choline acetyltransferase (ChAT), the enzyme needed for the synthesis of the neurotransmitter acetylcholine (ACh) [Murphy et al., 1998]. In advanced AD, ACh levels are reduced by as much as

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90%, especially in the cerebral cortex and the hippocampus. For this reason, various therapeutic strategies have been used to increase synaptic ACh to enhance cholinergic transmission in the brain for the successful treatment of AD symptoms. Such approaches included i) increasing the synthesis of ACh by treatment with ACh precursors, e.g., choline or lecithin; and ii) stimulation of muscarinic ACh receptors with specific agonists, such as bethanechol. These strategies were abandoned because of their poor therapeutic efficacy [Harbaugh et al., 1984; Little et al., 1985; Gauthier et al., 1986; Penn et al., 1988]. The objective for these kinds of therapies was to maintain a long-lasting, steady-state concentration of synaptic ACh with the absence of severe side effects. These conditions were not met with the above-mentioned cholinergic agents for the therapy for AD [Becker and Giacobini, 1988].

Acetylcholinesterase inhibitors (AChEIs) are commonly used to boost the levels of ACh, as an alternative to ACh agonist, to enhance the central cholinergic transmission and thereby improving cognitive function [Krall et al., 1999; Weinstock, 1999; Giacobini, 2000; Grutzendler and Morris, 2001]. The cholinesterases, and in particular butyrylcholinesterase (BuChE), have been associated with the pathogenesis and progression of AD [Guillozet et al., 1997; Darvesh et al., 2003]. Furthermore, it has been hypothesized that those cholinesterase inhibitors that inhibit both BuChE and acetylcholinesterase (AChE) stabilize disease progression better than those that inhibit only AChE in AD patients [Ballard, 2002; Giacobini et al., 2002]. The most widely studied AChEIs have been carbamates, such as physostigmine [Thal et al., 1986; Giacobini et al., 1988; Levy et al., 1994] and tacrine [Summers et al., 1986]. Physostigmine's very short duration of action, and tacrine's frequent dosing along with the need for monitoring liver enzymes, have made the use of these compounds obsolete. Other compounds with improved specificity and tolerability were developed, e.g., donepezil (Aricept, Pfizer-Eisai) in 1996 and rivastigmine (Exelon, Novartis) in 2000 [Scali et al., 2002]. However, these compounds are only approved for mild to moderate stages of AD, and confer only modest benefits in disease treatment. Also, these compounds are known to produce some adverse effects, which are related to cholinergic activity. Such adverse effects are experienced more with rivastigmine than with donepezil.

Degeneration of cholinergic nerve cells appears to be due to excessive activation of *N*-methyl-D-aspartate (NMDA) receptors [Cacabelos et al., 1999]. Memantine (MEM) is a specific, uncompetitive, voltage-dependent NMDA receptor antagonist with low- to moderate-affinity, strong voltage dependency, and rapid blocking/unblocking receptor kinetics [Danysz et al., 2000] and is effective in moderate to severe cases of dementia [Danysz et al., 2000; Reisberg et al., 2003; Wilcock, 2003]. Numerous data demonstrate that at higher doses, MEM induced attenuation of in vitro/in vivo AChE inhibition by organophosphates and carbamates [Gupta and Kadel, 1989, 1990, 1991; Gupta and Dettbarn, 1992; McLean et al., 1992; Gupta, 1994]. In an in vitro study, Wenk et al. [2000] found no interaction of MEM with AChE inhibitors, including donepezil, tacrine, and galantamine. Similar findings are observed with MEM and rivastigmine in in vitro and ex vivo studies conducted in rats [Enz and Gentsch, 2004]. Therefore, the present investigation was undertaken to clarify whether, in vivo, MEM influences AChE inhibition by donepezil, rivastigmine, metrifonate, and diisopropyl fluorophosphate (DFP).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley (Sasco) rats (each weighing approximately 150 g) were purchased from Charles River Labs (Wilmington, MA). They were housed five per cage (large size) in a room with controlled conditions: temperature $21 \pm 1^{\circ}C$, humidity $50 \pm 10\%$, and light–dark cycle 12:12 h. Every third day, rats were placed in clean cages. They had free access to pelleted food (Rodent Laboratory Chow 5001, Purina Mills, St. Louis, MO) and tap water. The animals were acclimatized to these conditions for 5–7 days before being used for the experiments. During pretreatment or treatment, rats were placed in individual small cages. The animal facility is approved by the Institutional Animal Care and Use Committee and all experiments were conducted in accordance with guidelines from the National Institutes of Health, with adequate measures taken to minimize any discomfort to the rats.

Chemicals

The following drugs/chemicals were provided by Merz Pharmaceuticals: memantine HCl (MEM), donepezil HCl, and rivastigmine tartrate. Diisopropyl fluorophosphate (DFP), metrifonate, acetylcholine iodide, and tetraisopropylpyrophosphoramide were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were American Chemical Society certified and purchased from Fisher Scientific (Fair Lawn, NJ).

Experimental Design

Selection of brain areas

Cerebral cortex and hippocampus are the two brain regions primarily affected by AD. Therefore, these brain areas were selected for the measurement of AChE activity.

Rationale for dose selection

Based on preliminary studies, for each AChEI, a dose was established (DFP, 1.5 mg/kg; metrifonate, 55 mg/kg; donepezil, 0.75 mg/kg; and rivastigmine, 0.35 mg/kg, ip) that produced about 50% AChE inhibition. This was based on literature demonstrating that the cortex and hippocampus are the two major brain areas most affected by AD, and 40–50% AChE inhibition by a drug is required to enhance the levels of ACh necessary in AD patients [Bartus et al., 1982; Coyle et al., 1983].

Time course of AChE inhibition

AChE activity was determined in cortex and hippocampus homogenates before (control), and 15 min, 30 min, 1 h, 2 h, and 24 h after administration of each drug (DFP, metrifonate, donepezil, and rivastigmine) to determine the time at which maximal AChE inhibition occurred.

Interaction study

Rats were pretreated with a single dose of memantine (MEM, 10 mg/kg, ip) 15 min prior to injection of an AChE inhibitor (DFP, 1.5 or 3 mg/kg; metrifonate, 55 or 110 mg/kg; donepezil, 0.75 or 1.5 mg/kg; and rivastigmine, 0.35 or 0.7 mg/kg, ip). Rats were sacrificed at the time of maximum AChE inhibition (i.e., donepezil, 15 min; rivastigmine or metrifonate, 30 min; and DFP, 1 h). Time of maximum AChE inhibition was determined from time course studies (see previous paragraph). Details of experimental protocol are provided in Table 1.

Assay of acetylcholinesterase activity (AChE, EC 3.1.1.7)

AChE activity was determined in brain region (cortex and hippocampus) homogenates according to the modified method of Hestrin [Gupta and Kadel, 1989; Gupta et al., 2000]. The enzyme activity was determined and calculated as micromole substrate (ACh iodide) hydrolyzed/g/h and was expressed in terms of percent remaining activity compared to controls (100%). Tissue homogenates were kept at crushed ice temperature, and enzyme assay was performed at room temperature.

TABLE 1. Schedule for prophylactic treatment with Memantine (MEM) against Acetylcholinesterase Inhibition (AChEI)

Group	Number of animals/treatment	Drug I	Drug 2
I	6	Saline	Saline
II	6	MEM	Saline
III	6	Saline	DFP, metrifonate, donepezil, rivastigmine
IV	6	MEM	DFP, metrifonate, donepezil, rivastigmine

Drugs and dosages: MEM (10 mg/kg), Diisopropyl fluorophosphate (DFP) (1.5 or 3 mg/kg), metrifonate (55 or 110 mg/kg), donepezil (0.75 or 1.5 mg/kg), and rivastigmine (0.35 or 0.7 mg/kg). MEM was administered 15 min before administration of an AChEI.
Route of administration: All drugs were administered ip.
Sacrifice time: Rats were sacrificed 15 min after donepezil, 30 min after rivastigmine or metrifonate, and 1 h after DFP administration.

Statistical analysis

AChE activity data are presented as means $\pm$ SEM (n=6), and were statistically analyzed for significance using analysis of variance coupled, when significant, with the Tukey-Kramer post-hoc test ($P < 0.05$).

RESULTS

Results of this study are presented to (1) establish a dose of each AChE inhibitor (DFP, metrifonate, donepezil, or rivastigmine) that causes about 50% AChE inhibition in the brain cortex and hippocampus from time course studies, and (2) determine whether MEM pretreatment influences AChE inhibition caused by AChEI at the time of maximum AChE inhibition. At the time of maximal AChE inhibition, the double dose of each AChEI produced a greater degree of AChE inhibition compared to the lower dose, and therefore it was of interest to determine whether MEM influences AChE differently with either dose.

The results revealed that a single dose of DFP (1.5 mg/kg, ip) caused approximately 50% AChE inhibition in both brain regions. In a detailed time course study, rats receiving DFP at this dose showed significant AChE inhibition as early as 15 min with progression to maximal inhibition at 1 h that remained at that level until 2 h (Fig. 1a). When determined after 24 h, enzyme activity was still significantly depressed. DFP with a 3 mg/kg dose induced significantly greater inhibition of AChE than that observed with the 1.5 mg/kg dose (Fig. 1b). At no time did the rats show any signs of adverse effects at either dose of DFP.

In interaction studies, MEM pretreatment with a single dose (10 mg/kg, ip) 15 min before DFP (1.5 or 3.0 mg/kg, ip) administration provided significant

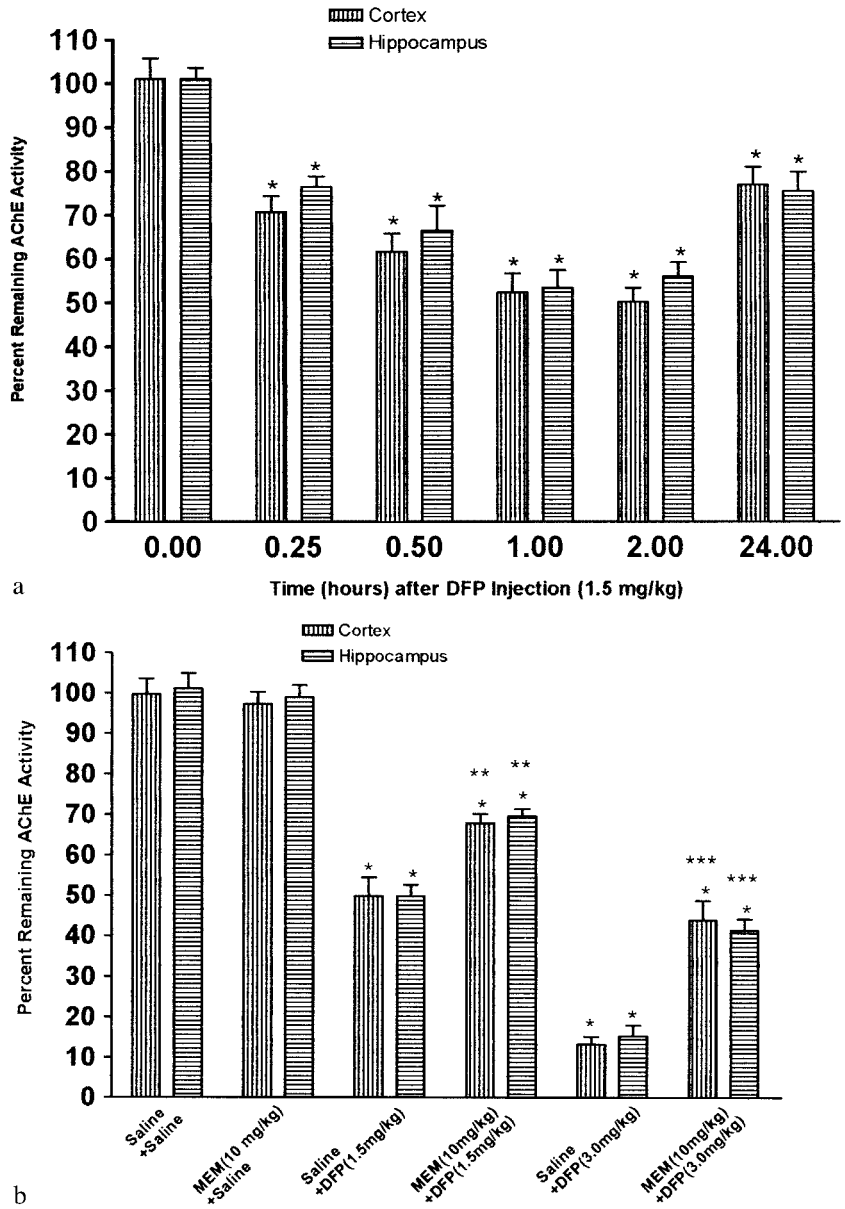
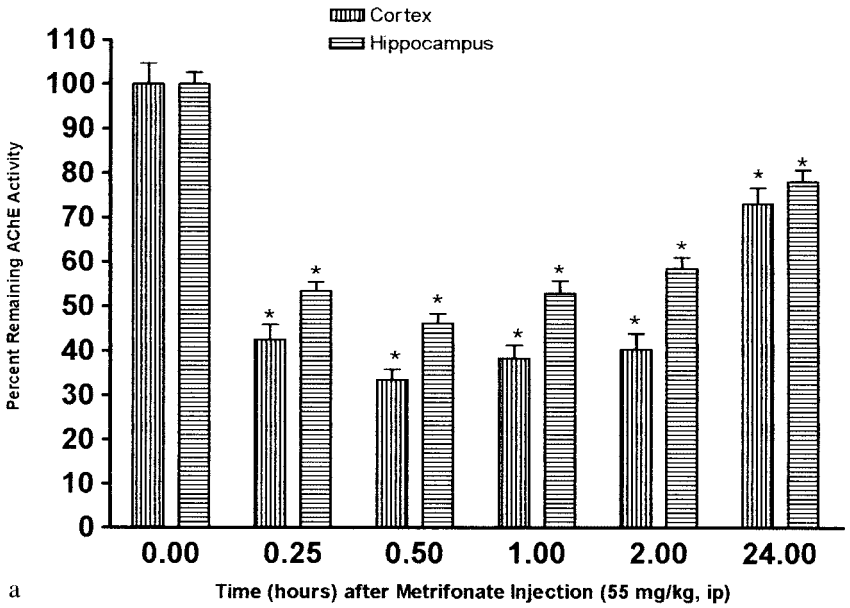


Fig. 1. **a:** Effect of diisopropyl fluorophosphate (DFP) (1.5 mg/kg, ip) on acetylcholinesterase (AChE) activity in brain regions (cortex and hippocampus) of rats at 0.25, 0.5, 1, 2, and 24 h after a single injection. AChE activity was determined as μmol/g/h and expressed as percent remaining activity compared to controls (100%). *Significant difference between controls and DFP-treated rats ($P<0.05$). **b:** Effect of memantine (MEM) (10 mg/kg, ip) and/or DFP (1.5 or 3.0 mg/kg) on AChE activity in brain regions of rats. Rats were sacrificed 1 h after the last drug administration. *Significant difference between controls (saline+saline) and any other treated group of rats ($P<0.05$). **Significant difference between saline+DFP (1.5 mg/kg) and MEM+DFP (1.5 mg/kg) ($P<0.05$). ***Significant difference between saline+DFP (3 mg/kg) and MEM+DFP (3 mg/kg) ($P<0.05$).

protection of AChE in both brain regions against DFP at either dose level (Fig. 1b). DFP was used as a positive control.

Data presented in Fig. 2a show that maximal AChE inhibition occurred in both brain regions after 0.5 h after a single dose of metrifonate (55 mg/kg). At this time, AChE inhibition in both brain regions was dose dependent, and rats receiving 110 mg/kg metrifonate displayed tremors and muscle fasciculations.

Data of the interaction study (Fig. 2b) revealed that MEM (10 mg/kg) pretreatment provided significant protection of AChE in both brain regions against metrifonate at either dose level (55 or 110 mg/kg).



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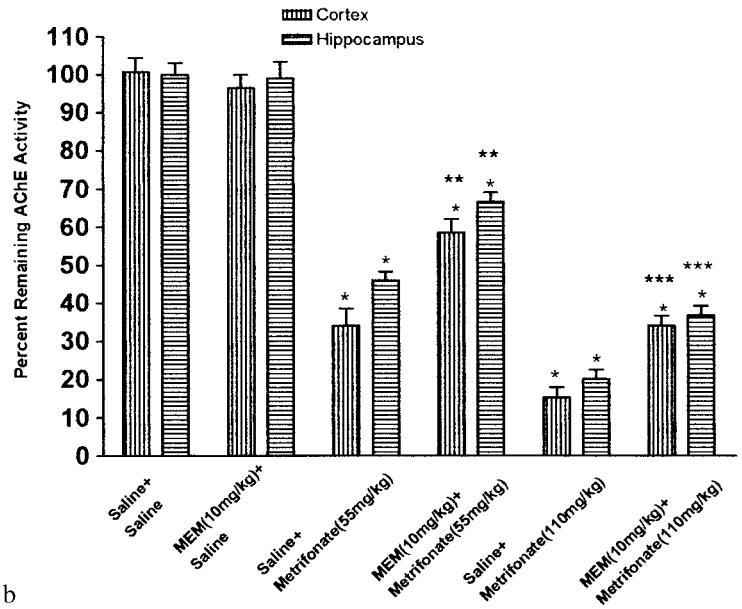

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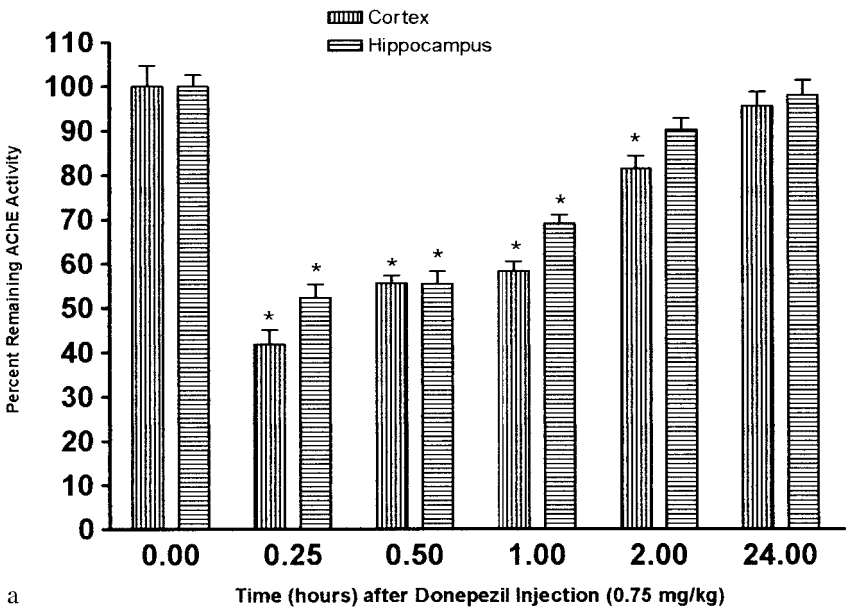
Fig. 2. **a:** Effect of metrifonate (55 mg/kg, ip) on acetylcholinesterase (AChE) activity in brain regions of rats at 0.25, 0.5, 1, 2, and 24 h after a single injection. AChE activity is expressed as percent remaining activity compared to controls (100%). *Significant difference between controls and metrifonate treated rats ($P<0.05$). **b:** Effect of memantine (MEM) (10 mg/kg) and/or metrifonate (55 or 110 mg/kg) on AChE activity in brain regions of rats. Rats were sacrificed 0.5 h after the last drug administration. *Significant difference between controls (saline+saline) and any other treated group of rats ($P<0.05$). **Significant difference between saline+metrifonate (55 mg/kg) and MEM (10 mg/kg)+metrifonate (55 mg/kg) ($P<0.05$). ***Significant difference between saline+metrifonate (110 mg/kg) and MEM+metrifonate (110 mg/kg) ($P<0.05$).

Data presented in Fig. 3a show that significant and maximal AChE inhibition (about 50%) occurred 15 min after a single dose of donepezil HCl (0.75 mg/kg). Within 2 h, AChE activity returned to normal range in the hippocampus but not in the cortex. At a higher dose (1.5 mg/kg), donepezil produced greater AChE inhibition than that seen with the 0.75 mg/kg dose (Fig. 3b). Data of interaction studies (Fig. 3b) revealed that MEM (10 mg/kg) did not interfere with the activity of AChE in any of the brain regions against either dose of donepezil (0.75 or 1.5 mg/kg).

The time course of AChE inhibition in brain regions of rats receiving a single dose of rivastigmine (0.35 mg/kg) is presented in Fig. 4a. Maximal inhibition



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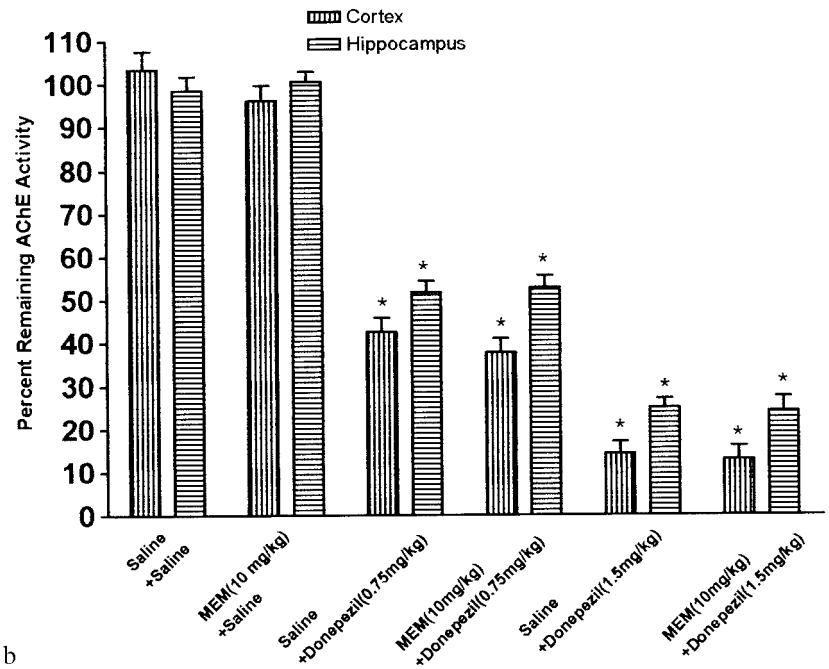

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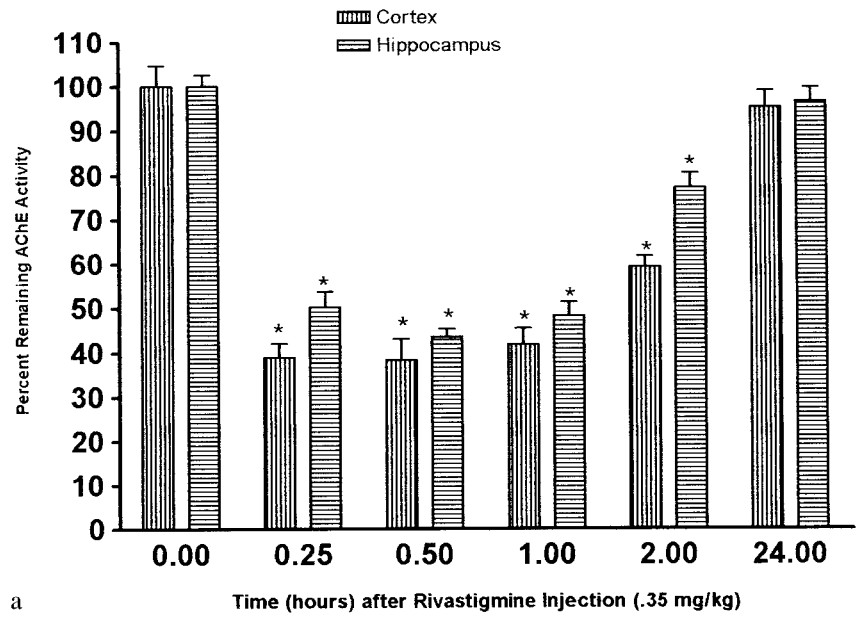
Fig. 3. **a:** Effect of donepezil (0.75 mg/kg, ip) on acetylcholinesterase (AChE) activity in brain regions of rats at 0.25, 0.5, 1, 2, and 24 h after a single injection. AChE activity is expressed as percent remaining activity compared to controls (100%). *Significant difference between controls and donepezil-treated rats ($P<0.05$). **b:** Effect of memantine (MEM) (10 mg/kg) and/or donepezil (0.75 or 1.5 mg/kg) on AChE activity in brain regions of rats. Rats were sacrificed 0.25 h after the last drug administration. *Significant difference between controls (saline+saline) and any other treated group of rats ($P<0.05$). **Significant difference between saline+donepezil (0.75 mg/kg) and MEM+donepezil (0.75 mg/kg) ($P<0.05$). ***Significant difference between saline+donepezil (1.5 mg/kg) and MEM+donepezil (1.5 mg/kg) ($P<0.05$).

of AChE occurred 0.5 h after rivastigmine administration. Inhibition of AChE was comparatively much greater in the cortex than in the hippocampus. AChE activity remained significantly inhibited until 2 h post-treatment. AChE inhibition by rivastigmine was dose dependent. Data of interaction studies revealed that MEM (10 mg/kg) did not interfere with AChE activity in any brain regions against rivastigmine at either dose level (0.35 or 0.7 mg/kg) (Fig. 4b). Rats treated with rivastigmine at either dose level did not show any behavioral signs of adverse effects.



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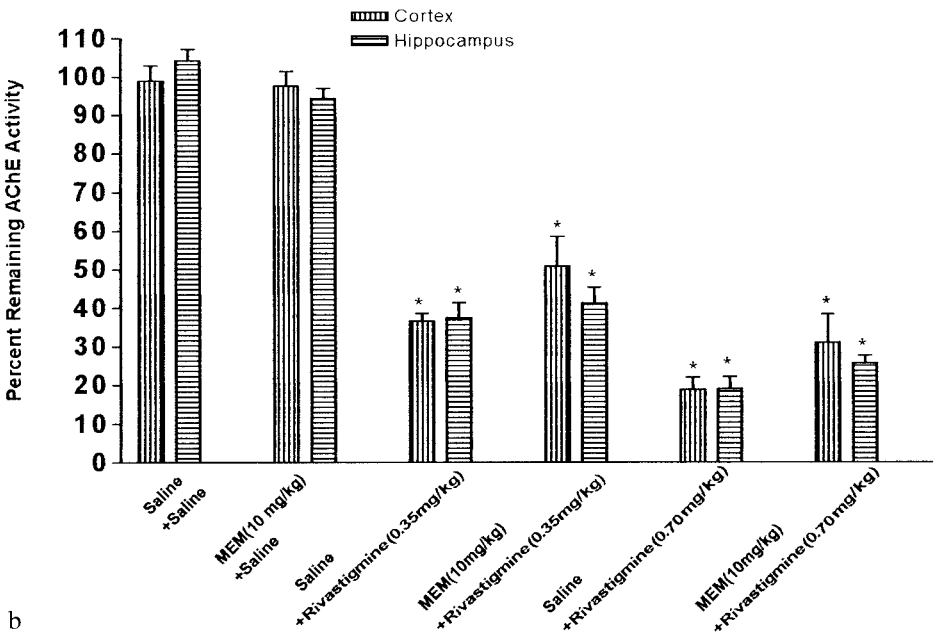

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Fig. 4. **a:** Effect of rivastigmine (0.35 mg/kg, ip) on acetylcholinesterase (AChE) activity in brain regions of rats at 0.25, 0.5, 1, 2, and 24 h after a single injection. AChE activity is expressed as percent remaining activity compared to controls (100%). *Significant difference between controls and rivastigmine treated rats ($P<0.05$). **b:** Effect of memantine (MEM) (10 mg/kg) and/or rivastigmine (0.35 or 0.7 mg/kg) on AChE activity in brain regions of rats. Rats were sacrificed 0.5 h after the last drug administration. *Significant difference between controls (saline+saline) and any other treated group of rats ($P<0.05$). **Significant difference between saline+rivastigmine (0.35 mg/kg) and MEM (10 mg/kg)+rivastigmine (0.35 mg/kg) ($P<0.05$). ***Significant difference between saline+rivastigmine (0.7 mg/kg) and MEM (10 mg/kg)+rivastigmine (0.7 mg/kg) ($P<0.05$).

DISCUSSION

In the aging population, AD is the most common cause of dementia, which leads to slowly progressive irreversible mental dysfunction. Although there are many hypotheses (cholinergic and noncholinergic) proposed, mounting evidence suggests that in the disease course, degeneration of cholinergic nuclei is localized in the basal forebrain [Ibach and Haen, 2004]. Impairment of the cholinergic system, which projects into large areas of the limbic system and the neocortex, is followed by disturbance of attentional processes and

cognitive decline. Therefore, AChEIs increase ACh availability and potentially enhance neuronal transmission, and consequently can relieve some symptoms of AD [Weinstock, 1999; Wynn and Cummings, 2004]. In addition to the cholinergic hypothesis, there is compelling evidence for a noncholinergic hypothesis related to an overstimulation of the NMDA receptor by glutamate, which is implicated in the pathogenesis of AD [Cacabelos et al., 1999].

Presently, donepezil and rivastigmine are commonly used drugs that can delay the progress of mental deterioration and reduce neuropsychiatric symptoms, and therefore represent a therapeutic approach for the treatment of mild to moderate AD [Taylor, 1998; Krall et al., 1999; Sramek et al., 2002; Grossberg and Desai, 2003; Ibach and Haen, 2004; Wynn and Cummings, 2004]. In October 2003, MEM was approved for moderate to severe cases of AD [Hecht and Hecht, 2004]. Among AChEIs, donepezil is the most widely prescribed drug in the treatment of dementia, even though it also has side effects [Rogers et al., 1998; Wengel et al., 1998; Wilkinson, 2001]. In clinical trials, a combined therapy of donepezil and MEM has demonstrated good tolerability and confirmed that MEM treatment is efficacious even in patients already treated with donepezil [Hartman and Möbius, 2003; Farlow et al., 2003; Tariot et al., 2004]. MEM, a specific, uncompetitive, voltage-dependent NMDA receptor antagonist with low- to moderate-affinity, strong voltage dependency, and rapid blocking/unblocking receptor kinetics [Danysz et al., 2000], has good tolerability and safety [Parsons et al., 1999; Orgogozo et al., 2002; Wilcock, 2003; Möbius, 2003; Reisberg et al., 2003]. MEM can also prevent neurotoxicity associated with excitatory amino acids without interfering with the physiological actions of glutamate required for memory and learning [Areosa-Sastre and Sherriff, 2003]. Gortelmeyer and Erbler [1992] noted that MEM provided benefits in a group of patients for whom other medications had not previously proven to be beneficial.

In in vitro interaction studies, Wenk et al. [2000] showed that MEM does not influence AChE inhibition in rat brain induced by donepezil, tacrine, or galantamine. These findings are further strengthened by the recent observations with rivastigmine in in vitro and in vivo studies [Enz and Gentsch, 2004]. However, numerous in vivo studies have demonstrated that at higher doses MEM attenuates AChE inhibition against several organophosphate (OP) nerve agents (soman, sarin, tabun, and VX), an OP prototype compound (DFP) and an insecticide (methyl parathion), and carbamate insecticides (carbofuran, aldicarb, oxamyl, and methomyl) [Gupta and Kadel, 1989, 1990, 1991; Gupta and Dettbarn, 1992; McLean et al., 1992; Gupta, 1994; Gupta and Goad, 2000]. Both in vitro and in vivo studies [McLean et al., 1992; Stojiljkovic et al., 2002; Antonijevic et al., 2002] further confirmed that MEM pretreatment protects AChE in rat and mice brain against soman-induced AChE inhibition. The doses of MEM used in these investigations were several folds higher than the therapeutic dose recommended in AD patients. At higher doses, MEM is also known to exert multiple pharmacological mechanisms, including (1) blockage of the nicotinic ACh–ion channel complex [Masuo et al., 1986], (2) prevention of neural hyperexcitability [McLean et al., 1992], (3) attenuation of high-frequency of repetitive activation of nerves [Wesemann and Eckenna, 1982; Wesemann et al., 1983], and (4) blockage of NMDA receptors [Parsons et al., 1999; Danysz et al., 2000]. It appears that more than one mechanism might be involved in protection by MEM of OP-poisoned animals.

Data of the present study (Figs. 1b and 2b) demonstrated that MEM pretreatment at the 10 mg/kg dose significantly attenuated AChE inhibition induced by DFP (1.5 or 3.0 mg/kg) or metrifonate (55 mg or 110 mg/kg) in both brain regions (cortex and hippocampus) of rats. DFP at either dose did not exert any behavioral signs, whereas metrifonate at higher dose (110 mg/kg) produced tremors and muscle fasciculations of moderate intensity, which were partially protected by MEM (10 mg/kg) pretreatment. Numerous in vivo studies have clearly established that MEM alone (18–72 mg/kg) does not produce AChE inhibition [Gupta and Kadel, 1990; Antonijevic et al., 2002; Stoljiljkovic et al., 2002], ruling out the possibility of interaction of MEM at the active center anionic site (the binding site for donepezil) or esteratic site (the binding site for rivastigmine) of AChE.

Data presented in Figure 3a show that a single injection of donepezil (0.75 mg/kg) caused approximately 50% AChE inhibition in the cortex and hippocampus of rats within 15 min. Donepezil at a 1.5 mg/kg dose induced AChE inhibition markedly greater than that caused by a low dose. It should be noted that donepezil is a reversible and mixed (competitive and noncompetitive) AChEI with a relative selectivity for AChE as compared to BuChE. Neither dose of donepezil exerted behavioral effects in rats. In contrast to the findings reported above for DFP and metrifonate, interaction of MEM with donepezil at AChE revealed that MEM pretreatment does not interfere with the activity of AChE against either dose of donepezil (0.75 or 1.5 mg/kg).

The present data show that a single injection of rivastigmine (0.35 mg/kg) caused 40–60% AChE inhibition in the cortex and hippocampus after 30 min of

drug administration. At this time, rats receiving double the dose of rivastigmine (0.7 mg/kg) showed significantly greater AChE inhibition compared to that seen with the low dose. Data of previous animal studies indicate that rivastigmine selectively increases the availability of ACh in the cortex and hippocampus [Scali et al., 2002]. In the present study, rivastigmine appears to be a stronger AChE inhibitor when compared to donepezil because the mechanism of action of rivastigmine differs from that of donepezil in the sense that donepezil is an AChE-selective inhibitor, whereas rivastigmine is a dual inhibitor of both AChE and BuChE [Ogura et al., 2000; Ballard, 2002]. Furthermore, unlike donepezil, rivastigmine is bound more tightly to the active center of AChE than a naturally occurring choline ester. The decarbamylation time for the rivastigmine–AChE complex is reported to be about 10 h [Ibach and Haen, 2004]. Therefore, it seems that MEM does not interact with the esteratic binding site or modulatory sites close to the binding site. It appears that like donepezil (a piperidine derivative), rivastigmine (a carbamate compound)-induced AChE inhibition was also not modulated by MEM (10 mg/kg) in either of the brain regions of rats receiving 0.35 or 0.7 mg/kg rivastigmine (Fig. 3b). Like physostigmine, rivastigmine is a noncompetitive pseudo-irreversible AChE inhibitor, which binds to the active center "esteratic binding site." Rivastigmine selectively inhibits monomeric AChE, especially in cortex and hippocampus, and thereby is thought to facilitate cholinergic neurotransmission by slowing the degradation of ACh released by functionally intact cholinergic neurons [Polinsky, 1998; Ibach and Haen, 2004].

It is noteworthy that MEM (10 mg/kg, ip) provided protection of a critical fraction of AChE activity against OPs (DFP or metrifonate) but not against donepezil or rivastigmine. This selectivity appears to be due to the fact that various AChEIs inactivate AChE via different mechanisms, e.g., phosphorylation by OPs, carbamylation by carbamates, and so on. In a recent report, Pope [1999] demonstrated that even not all OPs follow the same mechanism. Obviously, in the present study, MEM protected AChE from phosphorylation by DFP or metrifonate, but not from carbamylation by rivastigmine or donepezil. Our ongoing in vivo and in vitro studies will explore the underlying mechanisms.

From the findings presented here and elsewhere, it can be concluded that MEM neither inhibits AChE nor it influences AChE inhibition induced by reversible AChEIs (donepezil or rivastigmine) because MEM and donepezil/rivastigmine operate through different pharmacological mechanisms. MEM, however, provides protection of AChE against irreversible AChEIs (DFP and metrifonate).

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