Published in final edited form as:

J Alzheimers Dis. 2009 June ; 17(2): 369–382. doi:10.3233/JAD-2009-1058.

Acute but not chronic Donepezil administration increases muscarinic receptor-mediated brain signaling involving arachidonic acid in unanesthetized rats

Mireille Basselin, Henry N. Nguyen, Lisa Chang, Jane M. Bell, and Stanley I. Rapoport

Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

Abstract

Donepezil, an acetylcholinesterase (AChE) inhibitor that is used to treat patients with Alzheimer's disease, is thought to act by increasing brain extracellular acetylcholine (ACh), and thus ACh binding to cholinergic receptors. Cholinergic muscarinic receptors may be coupled to cytosolic phospholipase A₂ (cPLA₂) activation and arachidonic acid (AA) release from synaptic membrane phospholipid, and this activation can be imaged in rodents as an AA incorporation coefficient k^* , using quantitative autoradiography. To examine acute and chronic effects of Donepezil on the AA signal, k^* for AA was measured with quantitative autoradiography in 81 brain regions of unanesthetized rats. Twenty min after a single oral dose (3.0 mg/kg) of Donepezil compared with saline, k^* was increased significantly in 37 brain regions, whereas k^* did not differ from control 7 h afterwards or following chronic (21 days) of Donepezil. Pretreatment with atropine prevented the 20-min increments in k^* following Donepezil. Donepezil also increased the brain ACh concentration and reduced brain AChE activity, but did not change cPLA₂ activity, regardless of administration regimen. These results show that Donepezil acutely increases the brain AA signal that is mediated by ACh acting at muscarinic receptors, but that this signal is rapidly desensitized despite continued elevated brain ACh concentration. In contrast, the AA signal in response to arecoline was not altered following Donepezil.

Keywords

Donepezil; muscarinic receptors; acetylcholine; arachidonic acid; phospholipase A₂; acetylcholinesterase; anticholinesterase; Alzheimer disease; imaging; brain; desensitization

Introduction

Alzheimer's disease is an age-associated neurodegenerative disorder that affects an estimated 5.1 million Americans [1]. The postmortem brain from Alzheimer's disease patients shows several indices of reduced cholinergic function, including deficits in the enzyme responsible for the synthesis of acetylcholine (ACh), choline acetyltransferase, reduced ACh release, and loss of cholinergic perikarya in the nucleus basalis of Meynert [2,3]. These observations, together with an accepted role of ACh in learning and memory, have suggested that cholinergic dysfunction contributes to cognitive and behavioral deficits in Alzheimer's disease [4]. Thus, drugs that potentiate central cholinergic function have been approved for treating Alzheimer's

disease, including the reversible acetylcholinesterase (AChE) inhibitor, Donepezil [(±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one hydrochloride] [5]. In some randomized clinical trials, patients on Donepezil compared with placebo performed better on memory, language, orientation and attention [6-8]. However, two trials found no benefit of Donepezil [9,10], and progression of brain atrophy did not differ in patients with mild cognitive impairment on or off Donepezil [11,12], suggesting only symptomatic relief.

Donepezil (2.5 mg/kg, p.o.), when given to rats, inhibited whole brain AChE activity and increased the synaptic ACh concentration in the hippocampus [13]. Chronic daily Donepezil (14 to 21 days) increased brain ACh [14,15] and nicotinic receptor levels [16,17], and enhanced memory on behavioral tests [18]. It did not affect baseline synaptic transmission after one week [16], expression of cholinergic muscarinic M₃ receptor genes, or muscarinic receptor binding at baseline or after activation by carbachol [15,19].

Postsynaptic muscarinic M_{1,3,5} receptors can be coupled *via* G_{q/11} proteins to Ca²⁺-dependent cytosolic phospholipase A₂ (cPLA₂), which when activated selectively releases the second messenger, arachidonic acid (AA, 20:4n-6), from synaptic membrane phospholipid [20-22]. The signaling process has been imaged using quantitative autoradiography in unanesthetized rodents administered the nonspecific muscarinic receptor agonist, arecoline, and could be prevented by pretreatment with muscarinic receptor antagonist, atropine. In those studies, radiolabeled AA was injected intravenously, and regional brain AA incorporation coefficients k* and incorporation rates J_{in} were measured using quantitative autoradiography [23-27]. These parameters represent brain AA metabolic consumption and are unaffected by changes in cerebral blood flow [28-31], and thus are ideal for flow-independent imaging of resting and activated brain AA metabolism. AA and its metabolites can influence many physiological processes, including membrane excitability, gene transcription, apoptosis, sleep, brain blood flow and behavior [32].

In this study, we tested the hypothesis that k* for AA would be elevated in rats given acute or chronic Donepezil, insofar as both regimens increase the brain extracellular ACh concentration (see above). The fatty acid method was used with quantitative autoradiography to image k* for AA in unanesthetized rats at 20 min or 7 h after receiving 3.0 mg/kg p.o. Donepezil, or after receiving 1.5 mg/kg p.o. Donepezil twice daily for 21 days. These doses and times are reported to inhibit AChE activity and to increase the ACh concentration in rat brain [13-15]. In the rat, the brain Donepezil concentration reaches a peak 30 min after a single oral dose, then declines rapidly [13,33]. Effects of 5.0 mg/kg arecoline and of atropine on k* for AA [23,25,27] also were measured, as were the cortical ACh concentration and AChE activity, and whole brain cPLA₂ activity.

Materials and Methods

Animals

Experiments were conducted following the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Two- or 3-month-old male Fischer CDF (F-344)/CrIBR rats (Charles River Laboratories, Wilmington, MA) were acclimated for 1 week in an animal facility in which temperature, humidity and dark-light cycle were regulated. The rats had *ad libitum* access to water and food (Rodent NIH-31 auto 18-4 diet, Zeigler Bros, Gardners, PA). The diet contained (as percent of total fatty acids) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α-linolenic, 0.02% AA, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid [34].

Drug administration

For the chronic study, rats received 1.5 mg/kg p.o. Donepezil twice daily (7:30 A.M. and 3:30 P.M.) for 21 days (Donepezil HCl, 99% pure, Ivy Fine Chemicals, Cherry Hill, NJ), as done previously [14]. For the 20-min and 7-h acute Donepezil studies, rats received a single dose of 3.0 mg/kg p.o.. A pilot study showed that a single dose of 1.5 mg/kg, p.o. Donepezil did not significantly decrease total brain cholinesterase (ChE) activity after 7 h (36.10 ± 12.67 versus 40.83 ± 12.36 nmol/min/mg protein, $n = 8$, $p = 0.4623$). Donepezil was dissolved in saline (0.9% NaCl). Control groups received the same volume of saline under parallel conditions. Arecoline hydrobromide, methylatropine bromide and atropine were purchased from Sigma-Aldrich (St Louis, MO). The competitive muscarinic receptor antagonist, methylatropine bromide (4 mg/kg, s.c.), which does not enter the brain, was given 17 min before arecoline to block peripheral autonomic effects of arecoline [35]. Atropine (5 mg/kg s.c.), which enters the brain but does not affect k^* [26], was given 17 min before administration of Donepezil.

Surgical procedures and tracer infusion

Three hours after receiving the single dose of Donepezil or 7 h after the last of the 21-day dose or after receiving saline, a rat was anesthetized with 2-3% halothane in O₂. Polyethylene catheters were inserted into the right femoral artery and vein, as described [25]. The rat was allowed to recover from anesthesia for 3 h in a sound-dampened temperature-controlled box, with its hindquarters loosely wrapped and taped to a wooden block. Arterial blood pressure and heart rate were measured with a blood pressure recorder (CyQ 103/302; Cybersense, Inc., Nicholasville, KY).

After the rat had recovered from anesthesia, it was given methylatropine, then arecoline or saline. Three min later, [1-¹⁴C]AA (170 µCi/kg; 53 mCi/mmol, > 99% pure, Moravek Biochemicals, Brea, CA) in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml of bovine serum albumin essentially fatty acid free, was infused into the femoral vein for 5 min at a rate of 400 µl/min using an infusion pump (Harvard Apparatus Model 22, Natick, MA) [25]. For the 20-min study, saline, Donepezil (3.0 mg/kg, p.o.) or atropine was administered 20 min before [1-¹⁴C]AA infusion. Twenty min after beginning tracer infusion, the rat was killed with Nembutal→ (80 mg/kg, i.v.). Its brain was removed within 30 s, frozen in 2-methylbutane maintained at -40°C with dry ice, and stored at -80°C.

Chemical analysis

Thirteen arterial blood samples were collected before, during and after [1-¹⁴C]AA infusion and were centrifuged immediately (30 s at 18,000 g) to determine radioactivity of unesterified AA in the plasma. Total lipids were extracted from 30 µl of plasma with 3 ml chloroform:methanol (2:1, by vol) and 1.5 ml 0.1 M KCl, using the Folch procedure [36]. Radioactivity was determined in 100 µl of the organic phase by liquid scintillation counting. As reported, following [1-¹⁴C]AA infusion, greater than 97% of plasma radioactivity is radiolabeled AA at 5 min, and brain phospholipids account for greater than 81% of brain lipid radioactivity over 2 h [37]. Concentrations of unlabeled unesterified fatty acids were determined in 100-150 µl of the arterial plasma. Total lipids were extracted [36] and were separated by thin layer chromatography on silica gel 60 plates using the solvent system heptane:diethylether:glacial acetic acid (60:40:3, by vol). Unesterified fatty acids were scraped from the plate and methylated with 1% H₂SO₄ in anhydrous methanol for 3 h at 70°C. Fatty acid methyl esters were then separated and quantified by gas chromatography using an internal standard heptadecanoic acid (17:0).

Quantitative autoradiography

Frozen brains were cut in 20- μ m-thick coronal sections in a cryostat at -20°C. The sections were exposed together with [14 C]methylmethacrylate standards to Ektascan C/RA film (Eastman Kodak Company, Health Imaging Group, Rochester, NY) for 5 weeks. Radioactivity (nCi/g of brain) in 81 anatomically identified regions [38] was measured bilaterally six times by quantitative densitometry using the public domain NIH Image program 1.62 (<http://rsb.info.nih.gov/nih-image/>). Regional AA incorporation coefficients k^* (ml/s/g brain) of AA were calculated as [28],

$$k^* = \frac{c_{\text{brain}}^* (20 \text{ min})}{\int_0^{20} c_{\text{plasma}}^* dt} \quad (\text{Eq. 1})$$

where c_{brain}^* (nCi/g brain) is brain radioactivity at 20 min after the onset of infusion as determined by densitometry, c_{plasma}^* (nCi/ml plasma) is the arterial plasma concentration of labeled unesterified AA as determined by scintillation counting, and t (min) is time after beginning [14 C]AA infusion. Integrals of plasma radioactivity (input function in denominator) were determined in each experiment by trapezoidal integration, and divided into c_{brain}^* to calculate k^* for each experiment.

The regional rate of incorporation of unesterified AA from plasma into brain phospholipids, J_{in} (fmol/s/g), was calculated as,

$$J_{\text{in}} = k^* c_{\text{plasma}} \quad (\text{Eq. 2})$$

where c_{plasma} (nmol/ml) is the plasma concentration of unlabeled unesterified AA.

Brain cPLA₂ activity

After being given Donepezil or saline, a rat was anesthetized with Nembutal→ (50 mg/kg, i.p.) and decapitated. The brain was removed within 30 s, frozen in 2-methylbutane maintained at -40°C with dry ice, and stored at -80°C. Half-brains were homogenized using a Tenbroeck tissue grinder on ice (Kontes Glass Co., Vineland, NJ) in 3 vol of cold buffer containing 10 mM HEPES, pH 7.5, 1 mM EDTA, 0.34 M sucrose and protease inhibitor cocktail tablet (Complete, Roche, Mannheim, Germany). The homogenates were centrifuged at 100,000 g for 1 h at 4°C (Beckman L8-M Ultracentrifuge, Fullerton, CA). The supernatants corresponding to the cytosolic fractions were assayed for cPLA₂ activity, using a cPLA₂ assay kit and secretory PLA₂ and Ca²⁺-independent PLA₂ inhibitors (Cayman, Ann Arbor, MI). The protein concentration of the cytosolic fraction was determined by the Bradford method [39].

Brain cholinesterase activity

Cerebral cortical samples weighing 65-90 mg were homogenized in 5 volumes of cold 0.1 M phosphate buffer (pH = 7.5) in a Dounce tissue grinder on ice (Kontes Glass Co., Vineland, NJ). Samples were centrifuged at 1,000 g for 10 min at 4°C, and 10 μ l of the supernatant was used for the assay with a Quantichrom™ AChE assay kit (BioAssay Systems, Hayward, CA) and acetylthiocholine chloride as substrate. Since acetylthiocholine chloride is a good substrate for both AChE and butyrylcholinesterase, a selective AChE inhibitor, BW284C51 (Sigma-Aldrich), was added to a final concentration of 0.01 mM.

Acetylcholine concentration

In separate experiments, a rat was anesthetized with Nembutal→ (45 mg/kg, i.p.) and immediately subjected to head-focused microwave irradiation (5.5 kW, 90% power, 3.2 s; Cober Electronics, Stamford, CT) to inactivate ChE prior to ACh analysis [40]. Cortical samples of about 300-450 mg were homogenized in 2.4 volumes of choline assay buffer provided with the choline/ACh quantification kit (BioVision, Mountain View, CA) in a Dounce tissue grinder on ice. Samples were centrifuged at 2,000 g for 15 min at 4°C, and 50 µl of the supernatant was used for the assay.

Statistical analyses

Physiological parameters before and after drug administration were compared in the same animal using paired *t*-tests (GraphPad Software, San Diego, CA, www.graphpad.com). Enzyme activities and ACh and plasma unesterified fatty acid concentrations were compared using an unpaired *t*-test. Regional values of k^* following 20-min Donepezil, atropine plus Donepezil, or saline were compared by a one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of k^* , J_{in} and the input function after 7-h or 21-day Donepezil, followed by saline or arecoline, were compared by a two-way ANOVA. Where interactions between Donepezil and arecoline were statistically significant, probabilities of main effects are not reported because they cannot be interpreted clearly [41]. Instead, a Bonferroni post hoc test with correction for three comparisons was performed to compare arecoline and saline responses between Donepezil- and saline-treated rats. Other comparisons were not considered relevant. Data are reported as mean $\pm$ SD, with statistical significance taken as $p \leq 0.05$.

Results

Physiology and arterial plasma radioactivity

There was no significant effect 20 min after acute (single-dose) Donepezil compared to saline on mean rectal temperature, arterial blood pressure, pH, pCO_2 or pO_2 , whereas heart rate was increased significantly by 11% ($p = 0.03$). The latter change could be blocked by atropine. Donepezil produced tremors and repetitive head movements at 20 min, as reported [42], and these behaviors could be prevented by atropine pretreatment. We did not quantify them.

Acute Donepezil at 7 h, or 21 days of daily Donepezil, did not significantly change mean body weight, arterial blood pressure, heart rate or rectal temperature compared with saline. Arecoline significantly increased mean heart rate in all treatment groups, a change that has been ascribed to its effect on brain ACh [43].

Neither Donepezil after 20 min or 7 h, nor 21 days of Donepezil treatment, nor acute arecoline, modified the time-course of arterial plasma [$1-^{14}C$]AA radioactivity, the input function for calculating k^* in Eq. 1 (data not shown).

Plasma concentrations of unesterified fatty acids

The mean arterial plasma concentration of each of 8 measured unesterified fatty acids, including AA, was not significantly affected by 21 days of Donepezil compared with saline (Table 1). Arecoline's effects were not measured, because no effects were found in a comparable prior study [24].

Regional brain AA incorporation coefficients, k^*

Figure 1 presents representative coronal autoradiographs from brains of rats at 20 min and 7 h after acute Donepezil or saline, or after 21 days of repeated administration. The effects of pretreatment with atropine at 20 min also are shown. k^* for AA was increased compared with

saline at 20 min but not at 7 h after a single dose or after 21 days of Donepezil, and atropine blocked the 20 min effects on k^* .

Mean values of k^* for AA, determined in each of 81 brain regions, were subjected to a one- or two-way ANOVA for each of the three treatment paradigms. Twenty min after the single Donepezil dose compared with saline, k^* was elevated significantly by 22-42% in 37 of 81 brain regions (Table 2). Affected were prefrontal cortex layers I and IV (22-23%), frontal (10) and (8) layers I and IV (26-44%), pyriform (33%), anterior cingulate (33%), motor layers I to VI (28-35%), somatosensory layers I to VI (29-37%), auditory layers IV and VI (38-41%) cortex, preoptic area (30%), caudate putamen (28-33%), medial septal nucleus (26%), medial and lateral habenular nuclei (34-41%), dorsal lateral and medial geniculate nuclei (22-42%), paratenial (26%), anteroventral (28%), anteromedial (30%) and paraventricular (38%) thalamus nuclei, lateral (38%) and anterior (39%) hypothalamus area and interpeduncular nucleus (30%). Atropine pretreatment prevented all significant elevations.

Seven h after the single Donepezil dose, none of the 81 regions had a statistically significant Donepezil $\times$ arecoline interaction with regard to k^* for AA, whereas Donepezil had a main effect in 9 regions (data not shown). Arecoline had a significant main effect on k^* in 49 regions, elevating k^* for AA by 12-69%. Affected were prefrontal layer IV, frontal, anterior cingulate, motor, somatosensory, auditory and visual cortex, olfactory tubercle, caudate putamen, habenular nuclei, dorsal lateral geniculate nucleus, thalamus (6 of 8 regions), hypothalamus (2 of 7 regions), mesencephalon (6 of 6 regions) and rhombencephalon (3 of 3 regions).

None of the 81 regions had a statistically significant Donepezil $\times$ arecoline interaction or a Donepezil main effect with regard to k^* after 21 days of daily Donepezil (Table 3). Arecoline had a significant main effect on k^* for AA in 32 regions, increasing k^* by 15-46%. Many of these regions were the same as at 7 h after single dose of Donepezil. k^* responses to arecoline did not differ significantly between animals treated chronically with Donepezil compared with saline.

Regional incorporation rates of unesterified plasma AA into brain

Because mean plasma unesterified AA concentrations did not differ significantly between animals given Donepezil or saline daily for 21 days (Table 1), baseline differences and percent changes in J_{in} for AA corresponded to the differences and percent changes in respective values of k^* (Table 3). In chronic saline-treated rats, baseline J_{in} ranged from 11.5 fmol/s/g in the internal capsule to 23.6 fmol/s/g in the inferior colliculus. In the 21-day Donepezil-treated rats, baseline J_{in} ranged from 11.3 fmol/s/g in white matter of the cerebellum to 24.6 fmol/s/g in the interpeduncular nucleus.

Cortical cholinesterase activity

Mean cortical ChE activity was decreased significantly by 50% ($n = 4$), 55% ($n = 4$) and 68% ($n = 8$) at 20 min and 7 h after the single Donepezil dose, and after 21 days of repeated Donepezil, respectively (Fig 2A). This activity largely represented AChE activity because it was reduced by 90% in homogenates from saline- or Donepezil-treated rats following incubation with the selective AChE inhibitor, BW284C51 (Fig. 2B), consistent with the literature [44].

Cortical acetylcholine concentration

Twenty min and 7 h after a single Donepezil dose and after 21 days of repeated Donepezil, compared to saline, the cortical ACh concentration was increased by 141% ($n = 4$), 214% ($n = 4$) and 259% ($n = 6$), respectively (Fig 2C).

Brain cPLA₂ activity

Whole brain cPLA₂ activity was not significantly changed at 7 h after a single dose of Donepezil (n = 4) or after 21 days of daily drug (n = 8) (Fig. 2D). Since a calcium chelator is used to determine cPLA₂ activity *in vitro* (see Methods), we did not measure cPLA₂ activity following arecoline because we could not reproduce increments in intracellular Ca²⁺ likely caused by the drug [20].

Discussion

At 20 min following a single (acute) dose of 3.0 mg/kg p.o. Donepezil compared with saline, k* for AA was increased significantly in 37 of 81 brain regions in unanesthetized rats; these increases could be prevented by pretreatment with atropine. There were no relevant significant effects on k* at 7 h after the single dose or after 21 days of twice daily 1.5 mg/kg p.o. Donepezil. Arecoline-induced elevations in k* or J_{in} in saline-treated rats were unaffected at 7 h after the single dose or after 21 days of daily Donepezil. Each of three Donepezil regimens significantly inhibited cortical AChE activity and elevated the cortical ACh concentration, without changing global brain cPLA₂ activity.

The increments in k* at 20 min after the single dose of Donepezil likely arose from muscarinic receptor activation by an elevated brain ACh concentration, as they could be blocked by atropine and occurred in anterior brain regions also stimulated by arecoline (arecoline effects were blocked by atropine) and having high densities of muscarinic receptors [23,26,27]. The lack of significant changes in k* at 7 h after the single dose and after chronic Donepezil, despite an elevated brain ACh concentration at these times, suggests desensitization of the cPLA₂-coupled muscarinic receptors and the AA response to increased synaptic ACh [20-22,45,46]. This desensitization likely was unrelated to altered muscarinic receptor number [15] and was distinct from the lack of desensitization of the signal to arecoline.

One possible explanation for desensitization of the Donepezil but not the arecoline effect on k* is that the tonically elevated synaptic concentration of ACh, a full agonist in neuronal cells [47], caused internalization of surface muscarinic receptors into a lipophilic environment [48]. Arecoline, a lipophilic muscarinic agonist like [³H]quinuclidinyl benzilate (QNB) [49], would have access to both non-internalized and internalized muscarinic receptors, each of which remained coupled to cPLA₂. Indeed, internalization of muscarinic receptors without a change in receptor affinity was reported with acute and chronic AChE inhibitors in rodents by using as a ligand [³H]N-methylscopolamine (NMS), which detects only cell surface receptors [48,50,51]. Internalization did not result in desensitization of the phosphoinositide-turnover response in neuronal cell lines in response to muscarinic agonists [46,47,49,52]. Internalization following Donepezil could be tested by measuring [³H]NMS binding.

The elevations in the brain ACh concentration and in AChE inhibition at 20 min and 7 h following 3 mg/kg p.o. Donepezil are consistent with prior observations [13]. Additionally, the data obtained 7 h after the 21-day Donepezil regimen agree with a report that AChE remained inhibited for about 12 h after a single dose of Donepezil [13]. The 60% AChE inhibition after 21 days is comparable to the percent inhibition in cerebrospinal fluid of Alzheimer's disease patients treated with Donepezil [53].

Donepezil 2.0 mg/kg i.p. was reported to increase rat brain levels of dopamine, serotonin and norepinephrine in rats and to potentiate N-methyl-D-aspartic acid (NMDA) systems *in vitro* [54,55]. Although these changes might activate cPLA₂-coupled neuroreceptors so as to increase k* for AA [56-58], since the k* responses to Donepezil at 20 min were blocked by atropine, they likely were mediated entirely by cholinergic muscarinic receptors. Nicotinic receptors, which are largely presynaptic, also can be activated by Donepezil [59], but in

separate studies nicotine inhibited neuronal PLA₂ [60] and transiently reduced k* for AA in unanesthetized rats [61].

Chronically administered AChE inhibitors did not desensitize muscarinic receptor function in a number of studies. Fourteen days of Donepezil (10 mg/kg twice daily) did not desensitize muscarinic receptor-coupled G proteins in rat brain or spinal cord [19]. Chronic tacrine, metrifonate or diisopropylfluorophosphate did not alter muscarinic receptor densities, or their phosphoinositide responses at baseline or after muscarinic agonist administration in rodents [62-64]. Prolonged exposure of human neuroblastoma cells to galantamine or rivastigmine also did not affect muscarinic receptor-evoked increases in intracellular Ca²⁺ [65]. On the other hand, a life-long genetic deletion of AChE [66] or galantamine administration to transgenic mice that overexpressed AChE [67] downregulated M₁ receptors and reduced responses to cholinergic drugs.

The absence of a significant change in brain cPLA₂ activity at 7 h after a single dose of Donepezil or after 21 days of daily administration is consistent with the lack of a significant Donepezil effect on k* for AA at these times. In contrast, elevated baseline values of k* for AA were accompanied by increased cPLA₂ activity in rats treated for 3 weeks with fluoxetine, which increases the synaptic serotonin concentration to activate 5-HT_{2A/2C} receptors coupled to cPLA₂ [68,69], and in rats chronically administered NMDA to activate NMDA receptors coupled to cPLA₂ [70,71].

Arecoline-induced increments in k* for AA in saline-pretreated rats were reported in frontal cortical regions having high densities of M_{1,3,5} receptors and high AChE activity [23,25,27, 72]. Arecoline may activate nicotinic as well as muscarinic receptors [73], but since its effects on k* were prevented by pre-treatment with atropine [26], a nicotine receptor contribution to arecoline-induced increments in k* is unlikely.

In some randomized trials, Alzheimer's disease patients on Donepezil compared with placebo performed better on memory, language, orientation and attention [6-8], but in others only a "small" benefit [74] or no effect [9,10] was noted. Furthermore, progression of brain atrophy, a marker of structural disease [75], was not slowed by Donepezil [11,12]. Thus, despite the hypothesis that Donepezil would ameliorate cognitive changes due to cholinergic deficits in Alzheimer's disease (see Introduction), it is not certain that the drug actually slows underlying disease progression. Our data suggest that a lack of effect of chronic Donepezil may be related to desensitization of ACh-induced muscarinic receptor-initiated signaling involving AA. Reduced functional G protein coupling of the M₁ muscarinic receptor subtype also has been suggested [76,77]. On the other hand, Donepezil may be neuroprotective in Alzheimer's disease patients independently of its effect on muscarinic transmission [78,79]. The results of this study put into question the mechanism of action of chronically administered Donepezil and other AChE inhibitors in patients with Alzheimer's disease.

Acknowledgments

This work was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging. None of the authors has a financial or other conflict of interest related to this work. We thank Mrs. Kathy Benjamin for proofreading this paper.

Abbreviations

AA, arachidonic acid; ACh, acetylcholine; AChE, acetylcholinesterase; ChE, cholinesterase; cPLA₂, cytosolic PLA₂; NMDA, N-methyl-D-aspartate.

References

- [1]. Benjamin B, Burns A. Donepezil for Alzheimer's disease. *Expert Rev Neurother* 2007;7:1243–1249. [PubMed: 17939763]
- [2]. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, DeLong MR. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* 1982;215:1237–1239. [PubMed: 7058341]
- [3]. Atack JR, Perry EK, Bonham JR, Perry RH, Tomlinson BE, Blessed G, Fairbairn A. Molecular forms of acetylcholinesterase in senile dementia of the Alzheimer type: Selective loss of the intermediate (10S) form. *Neurosci. Lett* 1983;40:199–204. [PubMed: 6633975]
- [4]. Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982;217:408–414. [PubMed: 7046051]
- [5]. Seltzer B. Donepezil: an update. *Expert Opin Pharmacother* 2007;8:1011–1023. [PubMed: 17472546]
- [6]. Black SE, Doody R, Li H, McRae T, Jambor KM, Xu Y, Sun Y, Perdomo CA, Richardson S. Donepezil preserves cognition and global function in patients with severe Alzheimer disease. *Neurology* 2007;69:459–469. [PubMed: 17664405]
- [7]. Wallin AK, Andreasen N, Eriksson S, Batsman S, Nasman B, Ekdahl A, Kilander L, Grut M, Ryden M, Wallin A, Jonsson M, Olofsson H, Londos E, Wattmo C, Jonhagen M, Eriksdotter, Minthon L. Donepezil in Alzheimer's disease: what to expect after 3 years of treatment in a routine clinical setting. *Dement Geriatr Cogn Disord* 2007;23:150–160. [PubMed: 17312368]
- [8]. Bullock R, Dengiz A. Cognitive performance in patients with Alzheimer's disease receiving cholinesterase inhibitors for up to 5 years. *Int J Clin Pract* 2005;59:817–822. [PubMed: 15963209]
- [9]. Courtney C, Farrell D, Gray R, Hills R, Lynch L, Sellwood E, Edwards S, Hardyman W, Raftery J, Crome P, Lendon C, Shaw H, Bentham P. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. *Lancet* 2004;363:2105–2115. [PubMed: 15220031]
- [10]. Howard RJ, Juszcak E, Ballard CG, Bentham P, Brown RG, Bullock R, Burns AS, Holmes C, Jacoby R, Johnson T, Knapp M, Lindesay J, O'Brien JT, Wilcock G, Katona C, Jones RW, DeCesare J, Rodger M. Donepezil for the treatment of agitation in Alzheimer's disease. *N Engl J Med* 2007;357:1382–1392. [PubMed: 17914039]
- [11]. Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. *PLoS Med* 2007;4:e338. [PubMed: 18044984]
- [12]. Jack CR Jr, Petersen RC, Grundman M, Jin S, Gamst A, Ward CP, Sencakova D, Doody RS, Thal LJ. Longitudinal MRI findings from the vitamin E and donepezil treatment study for MCI. *Neurobiol Aging* 2008;29:1285–1295. [PubMed: 17452062]
- [13]. Kosasa T, Kuriya Y, Matsui K, Yamanishi Y. Effect of donepezil hydrochloride (E2020) on basal concentration of extracellular acetylcholine in the hippocampus of rats. *Eur J Pharmacol* 1999;380:101–107. [PubMed: 10513568]
- [14]. Scali C, Casamenti F, Bellucci A, Costagli C, Schmidt B, Pepeu G. Effect of subchronic administration of metrifonate, rivastigmine and donepezil on brain acetylcholine in aged F344 rats. *J Neural Transm* 2002;109:1067–1080. [PubMed: 12111444]
- [15]. Haug KH, Bogen IL, Osmundsen H, Walaas I, Fonnum F. Effects on cholinergic markers in rat brain and blood after short and prolonged administration of donepezil. *Neurochem Res* 2005;30:1511–1520. [PubMed: 16362770]
- [16]. Barnes CA, Meltzer J, Houston F, Orr G, McGann K, Wenk GL. Chronic treatment of old rats with donepezil or galantamine: effects on memory, hippocampal plasticity and nicotinic receptors. *Neuroscience* 2000;99:17–23. [PubMed: 10924948]
- [17]. Reid RT, Sabbagh MN. Effects of donepezil treatment on rat nicotinic acetylcholine receptor levels in vivo and in vitro. *J Alzheimers Dis* 2003;5:429–436. [PubMed: 14757932]
- [18]. Cutuli D, Foti F, Mandolesi L, Bartolo PD, Gelfo F, Federico F, Petrosini L. Cognitive performance of healthy young rats following chronic donepezil administration. *Psychopharmacology (Berl)* 2008;197:661–673. [PubMed: 18309476]

- [19]. Clayton BA, Hayashida K, Childers SR, Xiao R, Eisenach JC. Oral donepezil reduces hypersensitivity after nerve injury by a spinal muscarinic receptor mechanism. *Anesthesiology* 2007;106:1019–1025. [PubMed: 17457135]
- [20]. Clark JD, Schievella AR, Nalefski EA, Lin LL. Cytosolic phospholipase A₂. *J Lipid Mediat Cell Signal* 1995;12:83–117. [PubMed: 8777586]
- [21]. Bayon Y, Hernandez M, Alonso A, Nunez L, Garcia-Sancho J, Leslie C, Crespo M Sanchez, Nieto ML. Cytosolic phospholipase A₂ is coupled to muscarinic receptors in the human astrocytoma cell line 1321NI: characterization of the transducing mechanism. *Biochem. J* 1997;323:281–287. [PubMed: 9173894]
- [22]. Felder CC, Dieter P, Kinsella J, Tamura K, Kanterman RY, Axelrod J. A transfected m₅ muscarinic acetylcholine receptor stimulates phospholipase A₂ by inducing both calcium influx and activation of protein kinase C. *J Pharmacol Exp Ther* 1990;255:1140–1147. [PubMed: 2124620]
- [23]. Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI. Chronic lithium administration potentiates brain arachidonic acid signaling at rest and during cholinergic activation in awake rats. *J Neurochem* 2003;85:1553–1562. [PubMed: 12787074]
- [24]. Basselin M, Villacreses NE, Langenbach R, Ma K, Bell JM, Rapoport SI. Resting and arecoline-stimulated brain metabolism and signaling involving arachidonic acid are altered in the cyclooxygenase-2 knockout mice. *J Neurochem* 2006;96:669–679. [PubMed: 16405503]
- [25]. Basselin M, Villacreses NE, Lee H-J, Bell JM, Rapoport SI. Flurbiprofen, a cyclooxygenase inhibitor, reduces the brain arachidonic acid signal in response to the cholinergic muscarinic, arecoline, in awake rats. *Neurochem Res* 2007;32:1857–1867. [PubMed: 17562170]
- [26]. DeGeorge JJ, Nariai T, Yamazaki S, Williams WM, Rapoport SI. Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. *J. Neurochem* 1991;56:352–355. [PubMed: 1824784]
- [27]. Nariai T, DeGeorge JJ, Lamour Y, Rapoport SI. In vivo brain incorporation of [1-¹⁴C]arachidonate in awake rats, with or without cholinergic stimulation, following unilateral lesioning of nucleus basalis magnocellularis. *Brain Res* 1991;559:1–9. [PubMed: 1723641]
- [28]. Robinson PJ, Noronha J, DeGeorge JJ, Freed LM, Nariai T, Rapoport SI. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: Review and critical analysis. *Brain Res. Brain Res. Rev* 1992;17:187–214. [PubMed: 1467810]
- [29]. Rapoport SI. In vivo approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism. *J Pediatr* 2003;143:S26–34. [PubMed: 14597911]
- [30]. Rapoport SI, Chang MC, Spector AA. Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J. Lipid Res* 2001;42:678–685. [PubMed: 11352974]
- [31]. Chang MC, Arai T, Freed LM, Wakabayashi S, Channing MA, Dunn BB, Der MG, Bell JM, Sasaki T, Herscovitch P, Eckelman WC, Rapoport SI. Brain incorporation of [1-¹¹C]-arachidonate in normocapnic and hypercapnic monkeys, measured with positron emission tomography. *Brain Res* 1997;755:74–83. [PubMed: 9163542]
- [32]. Bosetti F. Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models. *J Neurochem.* 2007
- [33]. Matsui K, Mishima M, Nagai Y, Yuzuriha T, Yoshimura T. Absorption, distribution, metabolism, and excretion of donepezil (Aricept) after a single oral administration to Rat. *Drug Metab Dispos* 1999;27:1406–1414. [PubMed: 10570021]
- [34]. DeMar JJ, Lee HJ, Ma K, Chang L, Bell JM, Rapoport SI, Bazinet RP. Brain elongation of linoleic acid is a negligible source of the arachidonate in brain phospholipids of adult rats. *Biochim Biophys Acta* 2006;1761:1050–1059. [PubMed: 16920015]
- [35]. Brezenoff HE, Xiao YF, Vargas H. A comparison of the central and peripheral antimuscarinic effects of atropine and methylatropine injected systemically and into the cerebral ventricles. *Life Sci* 1988;42:905–911. [PubMed: 3343890]
- [36]. Folch J, Lees M, Stanley GH Sloane. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem* 1957;226:497–509. [PubMed: 13428781]
- [37]. DeGeorge JJ, Noronha JG, Bell JM, Robinson P, Rapoport SI. Intravenous injection of [1-¹⁴C] arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J. Neurosci. Res* 1989;24:413–423. [PubMed: 2512392]

- [38]. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; New York: 1987.
- [39]. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254. [PubMed: 942051]
- [40]. Stavinoha WB, Weintraub ST, Modak AT. The use of microwave heating to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. *J Neurochem* 1973;20:361–371. [PubMed: 4698283]
- [41]. Motulsky, H. Prism 4 Statistics Guide-Statistical analyses for laboratory and clinical researchers. GraphPad Software Inc.; San Diego: 2003.
- [42]. Dronfield S, Egan K, Marsden CA, Green AR. Comparison of donepezil-, tacrine-, rivastigmine- and metrifonate-induced central and peripheral cholinergically mediated responses in the rat. *J Psychopharmacol* 2000;14:275–279. [PubMed: 11106308]
- [43]. Janowsky DS, Risch SC. Cholinomimetic and anticholinergic drugs used to investigate an acetylcholine hypothesis of affective disorders and stress. *Drug Dev Res* 1984;4:125–142.
- [44]. Kousba AA, Poet TS, Timchalk C. Characterization of the in vitro kinetic interaction of chlorpyrifos-oxon with rat salivary cholinesterase: a potential biomonitoring matrix. *Toxicology* 2003;188:219–232. [PubMed: 12767693]
- [45]. Lippa AS, Critchett DJ, Joseph JA. Desensitization of muscarinic acetylcholine receptors: possible relation to receptor heterogeneity and phosphoinositides. *Brain Res* 1986;366:98–105. [PubMed: 2870767]
- [46]. Maloteaux JM, Hermans E. Agonist-induced muscarinic cholinergic receptor internalization, recycling and degradation in cultured neuronal cells. Cellular mechanisms and role in desensitization. *Biochem Pharmacol* 1994;47:77–88. [PubMed: 8311846]
- [47]. Baumgold J, Cooperman BB, White TM. Relationship between desensitization and sequestration of muscarinic cholinergic receptors in two neuronal cell lines. *Neuropharmacology* 1989;28:1253–1261. [PubMed: 2556656]
- [48]. Fisher SK. Recognition of muscarinic cholinergic receptors in human SK-N-SH neuroblastoma cells by quaternary and tertiary ligands is dependent upon temperature, cell integrity, and the presence of agonists. *Mol Pharmacol* 1988;33:414–422. [PubMed: 3357485]
- [49]. Thompson AK, Fisher SK. Preferential coupling of cell surface muscarinic receptors to phosphoinositide hydrolysis in human neuroblastoma cells. *J Biol Chem* 1991;266:5004–5010. [PubMed: 1848233]
- [50]. Cioffi CL, el-Fakahany EE. Decreased binding of the muscarinic antagonist [3H]N-methylscopolamine in mouse brain following acute treatment with an organophosphate. *Eur J Pharmacol* 1986;132:147–154. [PubMed: 3816972]
- [51]. Tang J, Carr RL, Chambers JE. Changes in rat brain cholinesterase activity and muscarinic receptor density during and after repeated oral exposure to chlorpyrifos in early postnatal development. *Toxicol Sci* 1999;51:265–272. [PubMed: 10543028]
- [52]. Harden TK, Petch LA, Traynelis SF, Waldo GL. Agonist-induced alteration in the membrane form of muscarinic cholinergic receptors. *J Biol Chem* 1985;260:13060–13066. [PubMed: 4055732]
- [53]. Darreh-Shori T, Meurling L, Pettersson T, Hugosson K, Hellstrom-Lindahl E, Andreassen N, Minthon L, Nordberg A. Changes in the activity and protein levels of CSF acetylcholinesterases in relation to cognitive function of patients with mild Alzheimer's disease following chronic donepezil treatment. *J Neural Transm* 2006;113:1791–1801. [PubMed: 16868793]
- [54]. Hatip-Al-Khatib I, Iwasaki K, Yoshimitsu Y, Arai T, Egashira N, Mishima K, Ikeda T, Fujiwara M. Effect of oral administration of zanapezil (TAK-147) for 21 days on acetylcholine and monoamines levels in the ventral hippocampus of freely moving rats. *Br J Pharmacol* 2005;145:1035–1044. [PubMed: 15951830]
- [55]. Moriguchi S, Zhao X, Marszalec W, Yeh JZ, Narahashi T. Modulation of N-methyl-D-aspartate receptors by donepezil in rat cortical neurons. *J Pharmacol Exp Ther* 2005;315:125–135. [PubMed: 15951396]
- [56]. Felder CC, Kanterman RY, Ma AL, Axelrod J. Serotonin stimulates phospholipase A₂ and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc. Natl. Acad. Sci. USA* 1990;87:2187–2191. [PubMed: 2315313]

- [57]. Vial D, Piomelli D. Dopamine D₂ receptors potentiate arachidonate release via activation of cytosolic, arachidonic-specific phospholipase A₂. *J. Neurochem* 1995;64:2765–2772. [PubMed: 7760057]
- [58]. Weichel O, Hilgert M, Chatterjee SS, Lehr M, Klein J. Bilobalide, a constituent of Ginkgo biloba, inhibits NMDA-induced phospholipase A₂ activation and phospholipid breakdown in rat hippocampus. *Naunyn Schmiedebergs Arch Pharmacol* 1999;360:609–615. [PubMed: 10619176]
- [59]. Marshall DL, Redfern PH, Wonnacott S. Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: comparison of naive and chronic nicotine-treated rats. *J Neurochem* 1997;68:1511–1519. [PubMed: 9084421]
- [60]. Marin P, Hamon B, Glowinski J, Prémont J. Nicotine-induced inhibition of neuronal phospholipase A₂. *J Pharmacol Exp Ther* 1997;280:1277–1283. [PubMed: 9067314]
- [61]. Chang L, Rapoport SI, Nguyen HN, Greenstein D, Chen M, Basselin M. Acute nicotine reduces brain arachidonic acid signaling in unanesthetized rats. *J Cereb Blood Flow Metab* 2009;29:648–658. [PubMed: 19142197]
- [62]. Kiefer-Day JS, el-Fakahany EE. Muscarinic receptor function and acetylcholinesterase activity after chronic administration of tacrine to mice at therapeutic drug concentrations. *Pharmacology* 1992;44:71–80. [PubMed: 1315062]
- [63]. Abdallah EA, el-Fakahany EE. Lack of desensitization of muscarinic receptor-mediated second messenger signals in rat brain upon acute and chronic inhibition of acetylcholinesterase. *J Biochem Toxicol* 1991;6:261–268. [PubMed: 1663554]
- [64]. Hinz VC, Kolb J, Schmidt BH. Effects of subchronic administration of metrifonate on cholinergic neurotransmission in rats. *Neurochem Res* 1998;23:931–938. [PubMed: 9690734]
- [65]. Barik J, Dajas-Bailador F, Wonnacott S. Cellular responses to nicotinic receptor activation are decreased after prolonged exposure to galantamine in human neuroblastoma cells. *Br J Pharmacol* 2005;145:1084–1092. [PubMed: 15937519]
- [66]. Li B, Duysen EG, Volpicelli-Daley LA, Levey AI, Lockridge O. Regulation of muscarinic acetylcholine receptor function in acetylcholinesterase knockout mice. *Pharmacol Biochem Behav* 2003;74:977–986. [PubMed: 12667913]
- [67]. Svedberg MM, Bednar I, Nordberg A. Effect of subchronic galantamine treatment on neuronal nicotinic and muscarinic receptor subtypes in transgenic mice overexpressing human acetylcholinesterase. *Neuropharmacology* 2004;47:558–571. [PubMed: 15380373]
- [68]. Lee HJ, Rao JS, Ertley RN, Chang L, Rapoport SI, Bazinet RP. Chronic fluoxetine increases cytosolic phospholipase A(2) activity and arachidonic acid turnover in brain phospholipids of the unanesthetized rat. *Psychopharmacology (Berl)* 2007;190:103–115. [PubMed: 17093977]
- [69]. Qu Y, Chang L, Klaff J, Seemann R, Greenstein D, Rapoport SI. Chronic fluoxetine upregulates arachidonic acid incorporation into the brain of unanesthetized rats. *Eur Neuropsychopharmacol* 2006;16:561–571. [PubMed: 16517130]
- [70]. Lee HJ, Rao JS, Chang L, Rapoport SI, Bazinet RP. Chronic N-methyl-D-aspartate administration increases the turnover of arachidonic acid within brain phospholipids of the unanesthetized rat. *J Lipid Res* 2008;49:162–168. [PubMed: 17957090]
- [71]. Rao JS, Ertley RN, Rapoport SI, Bazinet RP, Lee HJ. Chronic NMDA administration to rats up-regulates frontal cortex cytosolic phospholipase A₂ and its transcription factor, activator protein-2. *J Neurochem* 2007;102:1918–1927. [PubMed: 17550430]
- [72]. Levey AI. Immunological localization of m1-m5 muscarinic acetylcholine receptors in peripheral tissues and brain. *Life Sci* 1993;52:441–448. [PubMed: 8441326]
- [73]. Borea PA, Varani K, Gessi S, Merighi S, Piaz A Dal, Gilli P, Gilli G. Receptor binding thermodynamics at the neuronal nicotinic receptor. *Curr Top Med Chem* 2004;4:361–368. [PubMed: 14754451]
- [74]. Greenberg SM, Tennis MK, Brown LB, Gomez-Isla T, Hayden DL, Schoenfeld DA, Walsh KL, Corwin C, Daffner KR, Friedman P, Meadows ME, Sperling RA, Growdon JH. Donepezil therapy in clinical practice: a randomized crossover study. *Arch Neurol* 2000;57:94–99. [PubMed: 10634454]
- [75]. Leber P. Observations and suggestions on antidementia drug development. *Alzheimer Dis Assoc Disord* 1996;10(Suppl 1):31–35. [PubMed: 8876787]

- [76]. Flynn DD, Weinstein DA, Mash DC. Loss of high-affinity agonist binding to M1 muscarinic receptors in Alzheimer's disease: implications for the failure of cholinergic replacement therapies. *Ann Neurol* 1991;29:256–262. [PubMed: 2042942]
- [77]. Tsang SW, Pomakian J, Marshall GA, Vinters HV, Cummings JL, Chen CP, Wong PT, Lai MK. Disrupted muscarinic M1 receptor signaling correlates with loss of protein kinase C activity and glutamatergic deficit in Alzheimer's disease. *Neurobiol Aging* 2007;28:1381–1387. [PubMed: 16828202]
- [78]. Akaike A. Preclinical evidence of neuroprotection by cholinesterase inhibitors. *Alzheimer Dis Assoc Disord* 2006;20:S8–11. [PubMed: 16772755]
- [79]. Jacobson SA, Sabbagh MN. Donepezil: potential neuroprotective and disease-modifying effects. *Expert Opin Drug Metab Toxicol* 2008;4:1363–1369. [PubMed: 18798705]

Figure 1.

Coronal autoradiographs of effects of Donepezil, atropine and arecoline on k^* for AA in rat brain. (A) Effect of atropine (5.0 mg/kg, s.c.) on 20-min Donepezil's stimulation of k^* for AA. (B) Effect of 7-h (3.0 mg/kg, p.o.) and 21-day Donepezil (1.5 mg/kg, twice daily, p.o.) and arecoline (5 mg/kg, i.p.) on k^* for AA. Values of k^* are color-coded. Abbreviations: Acg, anterior cingulate cortex; CPu, caudate putamen; Mot, motor cortex.

Figure 2.

Effects of Donepezil on cortical cholinesterase activity, cortical acetylcholine concentration and cPLA₂ activity in rat brain. Each bar represents mean $\pm$ SD. *** $p < 0.0001$ compared to saline (t-test). (A) Effects of 20-min, 7-h and 21-day Donepezil administration on cortical cholinesterase activity. (B) Effects of BW284C51 on cortical cholinesterase activity in vitro. (C) Effects of 20-min, 7-h and 21-day Donepezil administration on cortical acetylcholine concentration. (D) Effects of 7-h and 21-day Donepezil administration on cPLA₂ activity.

Table 1

Plasma concentrations of unesterified fatty acids after 21 days of Donepezil

Fatty Acid	Plasma unesterified fatty acid (nmol/ml)	
	Saline	Donepezil
Palmitic (16:0)	311.1 ± 100.7	265.6 ± 76.1
Palmitoleic (16:1n-7)	39.8 ± 16.1	31.3 ± 10.1
Stearic (18:0)	79.9 ± 19.3	70.4 ± 15.3
Oleic (18:1n-9)	229.7 ± 82.0	193.8 ± 51.3
Linoleic (18:2n-6)	310.9 ± 127.7	255.3 ± 82.5
α -Linolenic (18:3n-3)	21.7 ± 13.2	14.9 ± 4.9
Arachidonic (20:4n-6)	34.2 ± 7.5	33.9 ± 6.7
Docosahexaenoic (22:6n-3)	31.3 ± 10.3	27.6 ± 8.4

Values are mean ± SD (n = 12), measured from arterial plasma collected before [1-¹⁴C]AA infusion.

Table 2

Mean values of arachidonic acid incorporation coefficients k^* in rats, at baseline (in response to saline), in response to 20-min Donepezil and, following atropine + Donepezil

Brain Region	Saline	Donepezil	Atropine + Donepezil
Prefrontal cortex layer I	3.40 ± 0.42	4.20 ± 0.42 [*]	3.38 ± 0.60
Prefrontal cortex layer IV	3.61 ± 0.51	4.41 ± 0.50 [*]	3.68 ± 0.58
Primary olfactory cortex	3.63 ± 0.76	4.43 ± 0.52	3.63 ± 0.94
Frontal cortex (10)			
Layer I	3.44 ± 0.58	4.44 ± 0.39 [*]	3.57 ± 0.66
Layer IV	4.02 ± 0.80	5.06 ± 0.52 [*]	3.99 ± 0.75
Frontal cortex (8)			
Layer I	3.78 ± 0.75	5.01 ± 0.96 [*]	3.90 ± 0.70
Layer IV	4.07 ± 0.78	5.87 ± 1.17 [*]	4.49 ± 1.01
Pyriform cortex	2.60 ± 0.41	3.47 ± 0.43 [*]	2.85 ± 0.66
Anterior cingulate cortex	4.03 ± 0.99	5.36 ± 0.57 [*]	4.29 ± 0.88
Motor cortex			
Layer I	3.17 ± 0.41	4.27 ± 0.78 [*]	3.59 ± 0.64
Layer II — III	3.28 ± 0.46	4.25 ± 0.69 [*]	3.52 ± 0.70
Layer IV	3.83 ± 0.80	4.96 ± 0.50 [*]	4.03 ± 0.97
Layer V	2.98 ± 0.58	3.82 ± 0.32 [*]	3.10 ± 0.60
Layer VI	2.73 ± 0.44	3.65 ± 0.36 [*]	2.76 ± 0.40
Somatosensory cortex			
Layer I	3.46 ± 0.59	4.46 ± 0.65 [*]	3.54 ± 0.66
Layer II—III	3.47 ± 0.70	4.56 ± 0.44 [*]	3.70 ± 0.75
Layer IV	3.95 ± 0.85	5.41 ± 0.76 [*]	4.35 ± 0.96
Layer V	3.41 ± 0.45	4.54 ± 0.74 [*]	3.84 ± 0.71
Layer VI	3.35 ± 0.57	4.44 ± 0.87 [*]	3.65 ± 0.61
Auditory cortex			
Layer I	4.13 ± 1.55	4.66 ± 1.51 [*]	3.61 ± 0.48
Layer IV	4.16 ± 1.31	5.87 ± 1.03 [*]	4.46 ± 0.65
Layer VI	3.31 ± 0.66	4.56 ± 1.05 [*]	3.85 ± 0.54
Visual cortex			
Layer I	3.40 ± 0.99	3.65 ± 1.06	3.32 ± 0.61
Layer IV	4.04 ± 1.13	4.36 ± 1.18	3.69 ± 0.52
Layer VI	3.48 ± 1.00	4.09 ± 1.12	3.28 ± 0.57
Preoptic area (LPO/MPO)	2.62 ± 0.32	3.40 ± 0.70 [*]	2.96 ± 0.53
Suprachiasmatic nu	2.60 ± 0.55	3.46 ± 0.74	2.96 ± 0.56
Globus pallidus	2.55 ± 0.42	3.35 ± 0.76	2.89 ± 0.54
Bed nu stria terminalis	3.03 ± 0.36	3.66 ± 0.76	2.98 ± 0.77
Olfactory tubercle	3.71 ± 0.90	4.07 ± 0.76	3.57 ± 0.51
Diagonal band Dorsal	3.11 ± 0.57	3.81 ± 0.79	3.52 ± 0.70
Ventral	3.22 ± 0.49	3.97 ± 0.71	3.26 ± 0.87
Amygdala basolat/med	3.25 ± 0.49	3.96 ± 0.67	3.09 ± 0.49
Hippocampus			
CA1	2.86 ± 0.42	3.24 ± 0.52	2.60 ± 0.61
CA2	3.16 ± 0.58	3.30 ± 0.65	2.87 ± 0.39
CA3	2.96 ± 0.43	3.24 ± 0.65	2.75 ± 0.42
Dentate gyrus	3.02 ± 0.33	3.51 ± 0.79	3.04 ± 0.58
SLM	3.78 ± 1.10	4.17 ± 1.13	3.81 ± 1.06
Accumbens nucleus	3.77 ± 0.81	4.36 ± 0.81	3.74 ± 0.69
Caudate putamen			
Dorsal	3.52 ± 0.54	4.50 ± 0.47 [*]	3.56 ± 0.57
Ventral	3.50 ± 0.57	4.65 ± 0.39 [*]	3.63 ± 0.58
Lateral	3.59 ± 0.65	4.65 ± 0.43 [*]	3.57 ± 0.62
Medial	3.46 ± 0.55	4.57 ± 0.37 [*]	3.56 ± 0.63
Septal nucleus lateral	2.91 ± 0.35	3.60 ± 0.78	2.82 ± 0.64
medial	3.56 ± 0.79	4.49 ± 0.37 [*]	3.58 ± 0.70
Habenular nu lateral	4.33 ± 0.76	5.82 ± 0.41 [*]	5.04 ± 1.30
medial	4.64 ± 1.05	6.54 ± 0.66 ^{**}	5.02 ± 1.41
Dorsal lateral geniculate nu	3.67 ± 1.05	5.20 ± 1.19 [*]	4.10 ± 0.97
Medial Geniculate nu	3.84 ± 0.46	4.70 ± 0.57 [*]	4.30 ± 0.44
Thalamus			
Ventroposterior nu lat	4.12 ± 1.02	3.90 ± 1.13	3.37 ± 0.47
Ventroposterior nu med	3.72 ± 0.87	3.95 ± 1.08	3.29 ± 0.45
Paratenial nu	3.21 ± 0.44	4.05 ± 0.69 [*]	3.37 ± 0.52
Anteromedial nu	4.78 ± 1.15	6.12 ± 0.92 [*]	5.54 ± 0.58
Anteromedial nu	3.56 ± 0.57	4.63 ± 0.47 [*]	3.75 ± 0.73
Reticular nu	3.78 ± 0.68	4.55 ± 0.81	4.25 ± 0.84
Paraventricular nu	3.29 ± 0.35	4.53 ± 0.59 ^{**}	3.42 ± 0.60

Brain Region	Saline	Donepezil	Atropine + Donepezil
Parafascicular nu	3.86 ± 1.20	3.86 ± 1.32	3.62 ± 1.49
Subthalamic nucleus	3.82 ± 1.05	3.91 ± 1.22	4.35 ± 0.53
Hypothalamus			
Supraoptic nu	3.01 ± 0.60	3.56 ± 0.62	2.75 ± 0.40
Lateral	2.74 ± 0.40	3.78 ± 1.00*	3.02 ± 0.55
Anterior	2.79 ± 0.31	3.88 ± 0.87*	2.86 ± 0.42
Periventricular	2.30 ± 0.37	2.99 ± 0.88	2.26 ± 0.29
Arcuate	2.74 ± 0.32	3.35 ± 0.75	2.90 ± 0.51
Ventromedial	2.85 ± 0.22	3.44 ± 0.57	3.04 ± 0.52
Posterior	3.70 ± 0.82	3.73 ± 1.05	3.57 ± 0.63
Mammillary nucleus	3.37 ± 1.19	3.11 ± 0.81	2.84 ± 0.50
Interpeduncular nu	5.01 ± 1.23	6.52 ± 0.56*	5.62 ± 1.20
Substantia nigra	3.43 ± 1.06	3.37 ± 1.12	3.05 ± 0.39
Pretectal area	3.98 ± 1.31	4.40 ± 1.15	3.60 ± 0.66
Superior colliculus	3.73 ± 0.97	3.93 ± 1.02	3.36 ± 0.56
Deep layers	3.69 ± 0.85	4.47 ± 1.17	3.67 ± 0.35
Inferior colliculus	5.52 ± 1.50	6.18 ± 1.99	6.11 ± 1.45
Flocculus	4.01 ± 1.02	4.08 ± 1.00	4.42 ± 1.03
Cerebellar gray matter	3.70 ± 0.86	3.97 ± 1.12	3.60 ± 0.57
Molecular layer cerebellar gray matter			
	4.63 ± 1.08	5.46 ± 1.76	5.27 ± 1.15
White matter			
Corpus callosum	2.44 ± 0.43	2.21 ± 0.54	2.80 ± 0.42
Zona incerta	2.84 ± 0.47	3.04 ± 1.19	2.89 ± 0.45
Internal capsule	2.07 ± 0.52	2.63 ± 0.69	2.33 ± 0.42
Cerebellar white matter	2.10 ± 1.07	2.58 ± 0.62	2.46 ± 0.45
Non-blood-brain barrier regions			
Subfornical organ	4.09 ± 0.61	4.47 ± 0.74	4.05 ± 0.78
Median eminence	3.07 ± 0.46	3.26 ± 0.54	3.05 ± 0.52
Choroid plexus	16.89 ± 2.85	15.66 ± 3.18	17.32 ± 3.57

Abbreviations: nu, nucleus; lat, lateral; med, medial; SLM, stratum lacunosum-molecular of the hippocampus $k^* = (\text{ml/s/g}) \times 10^{-4}$

Donepezil administration: 3.0 mg/kg p.o. 20 min

Atropine plus Donepezil administration: 5.0 mg/kg s.c., 17 min following by Donepezil 3.0 mg/kg, p.o. 20 min

Each value is a mean ± S.D. (n = 6).

*
p < 0.05

**
p < 0.01; compared with the saline group (one-way ANOVA Dunnett tests).

Table 3

Effect of 21-day Donepezil or saline pretreatment on saline- or arecoline-induced K^* responses for AA in specific brain regions.

Brain region	Saline		Donepezil		Saline $\times$ Donepezil interaction <i>p</i> -value	Donepezil effect <i>p</i> -value	Arecoline effect <i>p</i> -value
	Saline (<i>n</i> = 13)	Arecoline (<i>n</i> = 11)	Saline (<i>n</i> = 10)	Arecoline (<i>n</i> = 10)			
Prefrontal cortex layer I	4.11 $\pm$ 1.69	5.06 $\pm$ 1.38	4.88 $\pm$ 1.63	5.18 $\pm$ 2.11	NS	NS	NS
Prefrontal cortex layer IV	4.93 $\pm$ 1.60	5.86 $\pm$ 1.62	5.48 $\pm$ 1.93	6.70 $\pm$ 1.86	NS	NS	0.0487
Primary olfactory cortex	4.79 $\pm$ 1.77	5.25 $\pm$ 1.44	5.46 $\pm$ 2.00	5.55 $\pm$ 1.90	NS	NS	NS
Frontal cortex (10)							
Layer I	4.58 $\pm$ 1.52	5.12 $\pm$ 1.34	5.21 $\pm$ 1.90	5.41 $\pm$ 1.85	NS	NS	NS
Layer IV	5.03 $\pm$ 1.66	6.36 $\pm$ 1.62	5.73 $\pm$ 2.06	7.23 $\pm$ 2.06	NS	NS	0.0153
Frontal cortex (8)							
Layer I	4.67 $\pm$ 1.52	5.65 $\pm$ 1.60	5.23 $\pm$ 1.80	5.85 $\pm$ 1.92	NS	NS	NS
Layer IV	5.61 $\pm$ 1.89	7.25 $\pm$ 1.95	6.09 $\pm$ 2.29	7.82 $\pm$ 2.15	NS	NS	0.0102
Pyriform cortex	3.94 $\pm$ 1.34	4.81 $\pm$ 1.17	3.81 $\pm$ 2.37	5.28 $\pm$ 1.83	NS	NS	0.0289
Anterior cingulate cortex	5.37 $\pm$ 1.83	7.21 $\pm$ 1.61	6.16 $\pm$ 2.20	7.99 $\pm$ 2.04	NS	NS	0.0030
Motor cortex							
Layer I	4.44 $\pm$ 1.56	5.53 $\pm$ 1.49	5.12 $\pm$ 1.78	5.74 $\pm$ 1.92	NS	NS	NS
Layer II - III	4.71 $\pm$ 1.50	6.23 $\pm$ 1.80	5.47 $\pm$ 2.00	6.87 $\pm$ 2.08	NS	NS	0.0121
Layer IV	5.49 $\pm$ 1.76	8.02 $\pm$ 2.27	6.13 $\pm$ 2.34	8.81 $\pm$ 2.30	NS	NS	0.0003
Layer V	4.92 $\pm$ 1.51	6.51 $\pm$ 1.71	5.60 $\pm$ 1.92	7.27 $\pm$ 2.01	NS	NS	0.0043
Layer VI	5.10 $\pm$ 1.54	6.60 $\pm$ 1.78	5.78 $\pm$ 2.06	7.57 $\pm$ 2.15	NS	NS	0.0060
Somatosensory cortex							
Layer I	4.67 $\pm$ 1.42	5.51 $\pm$ 1.54	5.44 $\pm$ 1.97	6.05 $\pm$ 2.09	NS	NS	NS
Layer II - III	4.94 $\pm$ 1.59	6.07 $\pm$ 1.64	5.66 $\pm$ 2.09	6.67 $\pm$ 1.09	NS	NS	0.0356
Layer IV	5.69 $\pm$ 1.87	7.39 $\pm$ 1.87	6.29 $\pm$ 2.27	8.19 $\pm$ 2.29	NS	NS	0.0061
Layer V	4.99 $\pm$ 1.58	6.12 $\pm$ 1.67	5.62 $\pm$ 2.02	7.55 $\pm$ 1.92	NS	NS	0.0156
Layer VI	5.11 $\pm$ 1.60	6.52 $\pm$ 1.80	5.81 $\pm$ 2.00	7.33 $\pm$ 2.05	NS	NS	0.0126
Auditory cortex							
Layer I	4.49 $\pm$ 1.45	5.18 $\pm$ 1.70	4.74 $\pm$ 1.06	5.88 $\pm$ 1.25	NS	NS	0.0371
Layer IV	5.13 $\pm$ 1.79	6.37 $\pm$ 2.01	5.44 $\pm$ 1.54	7.08 $\pm$ 1.77	NS	NS	0.0115
Layer VI	4.59 $\pm$ 1.47	5.95 $\pm$ 1.72	4.56 $\pm$ 1.15	5.41 $\pm$ 1.30	NS	NS	0.0152
Visual cortex							
Layer I	4.45 $\pm$ 1.32	5.19 $\pm$ 1.63	4.43 $\pm$ 0.73	5.78 $\pm$ 0.82	NS	NS	0.0100
Layer IV	5.05 $\pm$ 1.81	6.63 $\pm$ 2.01	5.18 $\pm$ 1.12	7.38 $\pm$ 1.40	NS	NS	0.0005
Layer VI	4.82 $\pm$ 1.63	5.97 $\pm$ 1.95	4.90 $\pm$ 0.96	6.82 $\pm$ 1.55	NS	NS	0.0027
Preoptic area (LPO/MPO)	4.46 $\pm$ 1.28	4.38 $\pm$ 1.91	5.15 $\pm$ 1.74	4.86 $\pm$ 1.84	NS	NS	NS
Suprachiasmatic nu	4.54 $\pm$ 1.28	4.23 $\pm$ 1.79	5.12 $\pm$ 1.60	4.89 $\pm$ 1.76	NS	NS	NS
Globus pallidus	4.18 $\pm$ 1.54	4.74 $\pm$ 1.24	4.97 $\pm$ 1.60	5.03 $\pm$ 1.86	NS	NS	NS
Bed nu stria terminalis	3.95 $\pm$ 1.49	4.29 $\pm$ 1.01	4.87 $\pm$ 1.44	4.74 $\pm$ 1.80	NS	NS	NS
Olfactory tubercle	4.79 $\pm$ 1.64	5.49 $\pm$ 1.33	4.49 $\pm$ 2.12	6.85 $\pm$ 1.92	NS	NS	0.0065
Diagonal band dorsal	5.04 $\pm$ 1.50	5.14 $\pm$ 2.32	5.73 $\pm$ 1.92	5.88 $\pm$ 2.08	NS	NS	NS
ventral	4.97 $\pm$ 1.62	5.45 $\pm$ 1.54	5.66 $\pm$ 1.96	5.87 $\pm$ 2.03	NS	NS	NS
Amygdala basolateral/medial	4.53 $\pm$ 1.35	4.95 $\pm$ 1.64	4.91 $\pm$ 1.52	5.54 $\pm$ 1.81	NS	NS	NS
Hippocampus							
CA1	4.12 $\pm$ 1.04	4.69 $\pm$ 1.56	4.05 $\pm$ 1.52	5.14 $\pm$ 1.69	NS	NS	NS
CA2	4.29 $\pm$ 1.19	4.88 $\pm$ 1.66	4.32 $\pm$ 1.58	5.27 $\pm$ 1.76	NS	NS	NS
CA3	4.26 $\pm$ 1.22	4.70 $\pm$ 1.58	4.18 $\pm$ 1.40	5.10 $\pm$ 1.69	NS	NS	NS
Dentate gyrus	4.53 $\pm$ 1.47	5.27 $\pm$ 1.78	4.66 $\pm$ 1.48	5.71 $\pm$ 1.93	NS	NS	NS
SLM	4.94 $\pm$ 1.73	6.03 $\pm$ 1.86	5.57 $\pm$ 1.75	6.51 $\pm$ 1.79	NS	NS	NS
Accumbens nucleus	4.93 $\pm$ 1.55	5.94 $\pm$ 1.67	5.52 $\pm$ 1.94	6.33 $\pm$ 2.02	NS	NS	NS
Caudate putamen							
dorsal	4.73 $\pm$ 1.61	5.16 $\pm$ 1.29	5.38 $\pm$ 1.84	5.90 $\pm$ 1.82	NS	NS	NS
ventral	4.89 $\pm$ 1.68	5.26 $\pm$ 1.53	5.41 $\pm$ 1.91	5.66 $\pm$ 1.97	NS	NS	NS
lateral	4.79 $\pm$ 1.64	5.56 $\pm$ 1.47	5.44 $\pm$ 1.91	6.18 $\pm$ 1.89	NS	NS	NS

Brain region	Saline			Donepezil		Saline × Donepezil interaction p-value	Donepezil effect p-value	Arecoline effect p-value
	Saline (n = 13)	Arecoline (n = 11)	Saline (n = 10)	Arecoline (n = 10)				
medial Septal nu lateral Septal nu medial Habenular nu lateral Habenular nu medial Lateral geniculate nu dorsal Medial geniculate nu Thalamus	4.86 ± 1.70	5.18 ± 1.37	5.42 ± 1.85	5.82 ± 2.01	NS	NS	NS	NS
	4.11 ± 1.37	4.43 ± 1.00	4.80 ± 1.49	4.79 ± 1.79	NS	NS	NS	NS
	5.06 ± 1.66	5.44 ± 1.51	5.53 ± 1.97	5.88 ± 1.86	NS	NS	NS	NS
	6.05 ± 2.23	7.48 ± 1.95	6.85 ± 1.87	8.38 ± 2.18	NS	NS	NS	0.0295
	5.38 ± 1.90	7.32 ± 1.90	6.24 ± 2.03	8.11 ± 2.03	NS	NS	NS	0.0311
	5.43 ± 1.94	6.29 ± 2.02	5.78 ± 1.75	6.74 ± 1.85	NS	NS	NS	NS
	5.28 ± 1.97	6.54 ± 2.04	5.66 ± 1.24	6.86 ± 1.96	NS	NS	NS	0.0340
	5.04 ± 1.85	5.99 ± 1.83	5.67 ± 1.74	6.54 ± 1.70	NS	NS	NS	NS
	5.08 ± 1.84	6.17 ± 1.90	5.77 ± 1.65	6.64 ± 1.64	NS	NS	NS	NS
	4.83 ± 1.44	5.09 ± 1.34	5.43 ± 1.90	5.62 ± 1.86	NS	NS	NS	NS
Paratentorial nu Anteroventral nu Anteromedial nu Reticular nu Paraventricular nu Parafascicular nu Subthalamic nu Hypothalamus	6.13 ± 2.25	7.92 ± 1.84	7.20 ± 2.67	8.99 ± 2.24	NS	NS	NS	0.0125
	5.11 ± 1.69	5.45 ± 1.58	5.66 ± 1.92	6.17 ± 1.85	NS	NS	NS	NS
	5.21 ± 1.67	5.87 ± 1.66	5.85 ± 2.17	6.81 ± 2.01	NS	NS	NS	NS
	4.80 ± 1.54	5.08 ± 1.27	5.49 ± 2.03	5.60 ± 1.80	NS	NS	NS	NS
	4.92 ± 1.19	6.20 ± 1.02	5.60 ± 1.58	6.46 ± 1.66	NS	NS	NS	0.0136
	5.08 ± 1.78	6.20 ± 1.96	5.93 ± 1.95	6.90 ± 1.67	NS	NS	NS	NS
	4.44 ± 1.39	4.77 ± 1.09	5.36 ± 1.90	5.59 ± 2.25	NS	NS	NS	NS
	4.10 ± 1.44	4.50 ± 1.04	4.97 ± 1.56	4.91 ± 1.78	NS	NS	NS	NS
	4.19 ± 1.29	4.43 ± 0.92	5.03 ± 1.62	4.99 ± 1.78	NS	NS	NS	NS
	4.28 ± 1.15	4.32 ± 0.90	5.07 ± 1.61	4.96 ± 1.67	NS	NS	NS	NS
Arcuate Ventromedial Posterior Mammillary nu Interpeduncular nu Substantia nigra Pretectal area Grey layer superior colliculus Superior colliculus Inferior colliculus Flocculus Cerebellar gray matter Molecular layer cerebellar gray White matter	4.40 ± 1.21	4.52 ± 1.44	5.03 ± 1.35	5.23 ± 1.64	NS	NS	NS	NS
	4.45 ± 1.31	4.53 ± 1.42	4.75 ± 1.42	5.18 ± 1.68	NS	NS	NS	NS
	4.68 ± 1.55	5.13 ± 1.72	4.96 ± 1.49	5.68 ± 1.84	NS	NS	NS	NS
	4.41 ± 1.23	4.73 ± 1.59	4.66 ± 1.15	5.32 ± 1.36	NS	NS	NS	NS
	6.65 ± 2.01	8.33 ± 2.17	7.27 ± 2.21	8.78 ± 1.34	NS	NS	NS	0.0110
	4.55 ± 1.42	4.85 ± 1.41	4.42 ± 1.20	5.54 ± 1.27	NS	NS	NS	NS
	5.13 ± 1.76	6.21 ± 1.81	5.45 ± 1.25	6.73 ± 1.55	NS	NS	NS	0.0214
	5.10 ± 1.84	6.85 ± 1.83	5.75 ± 1.77	6.17 ± 1.50	NS	NS	NS	0.0476
	5.30 ± 1.89	6.19 ± 1.85	5.50 ± 1.23	7.03 ± 1.63	NS	NS	NS	0.0234
	6.90 ± 2.76	8.72 ± 1.80	7.23 ± 1.48	9.71 ± 1.67	NS	NS	NS	0.0013
Cerebellar gray matter Flocculus Cerebellar white matter Non-blood-brain barrier regions Subfornical organ Median eminence	5.49 ± 1.83	6.49 ± 2.11	5.74 ± 1.47	7.07 ± 1.68	NS	NS	NS	0.0407
	4.98 ± 1.87	5.65 ± 1.75	4.98 ± 1.00	6.22 ± 1.61	NS	NS	NS	NS
	5.97 ± 2.42	7.63 ± 2.18	6.42 ± 1.64	8.53 ± 2.22	NS	NS	NS	0.0064
	3.56 ± 1.33	3.96 ± 0.74	4.34 ± 1.19	3.96 ± 0.74	NS	NS	NS	NS
	4.61 ± 1.53	5.14 ± 1.76	4.82 ± 1.38	5.52 ± 1.43	NS	NS	NS	NS
	3.37 ± 1.25	3.86 ± 0.70	4.27 ± 1.16	3.86 ± 0.70	NS	NS	NS	NS
	3.81 ± 0.78	3.81 ± 1.16	3.32 ± 1.19	4.41 ± 0.96	NS	NS	NS	NS
	4.34 ± 1.37	4.45 ± 1.04	4.94 ± 1.49	4.45 ± 1.04	NS	NS	NS	NS
	4.49 ± 1.28	4.64 ± 1.53	5.12 ± 1.35	5.22 ± 1.35	NS	NS	NS	NS

Abbreviations: nu, nucleus; SLM, stratum lacunosum-molecular of the hippocampus

Each k* value is a mean ± S.D.

NS: not significant.

k* = (ml/s/g) × 10⁻⁴.

Rats were pre-treated with saline or Donepezil (1.5 mg/kg, twice daily, p.o.) for 21 days and injected with arecoline (5.0 mg/kg, i.p.) or saline. Each bar represents the mean $\pm$ SD. Statistics were calculated by 2-way ANOVA.