Cholinergic Drugs for Alzheimer’s Disease Enhance In Vitro Dopamine Release

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Abstract

Alzheimer’s disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have non-cognitive symptoms, such as anxiety, depression, apathy, and psychosis that impair daily living. The most commonly prescribed treatments for Alzheimer’s disease are acetylcholinesterase inhibitors, such as donepezil (Aricept®) and galantamine (Reminyl®). Enhanced cholinergic functions caused by these compounds are thought to underlie improvements in learning, memory, and attention. The non-cognitive aspects of dementia, however, are usually linked to serotonin and dopamine rather than acetylcholine because those neurotransmitter systems most directly influence mood, emotional balance, and psychosis. Fast cyclic voltammetry applied to mouse striatal brain slices was used to measure the real-time release of DA arising from spontaneous activity or from single electrical stimulations. At concentrations that include their prescribed dosage ranges, donepezil (1 - 1000 nM) and galantamine (50 - 1000 nM) increase action potential dependent dopamine release. Consistent with previous literature, the data support slightly different modes of action for donepezil and galantamine. The ability of these commonly prescribed drugs to alter catecholamine release may underlie their influence over non-cognitive symptoms of dementia. Furthermore, these results suggest that acting via nicotinic receptors, these drugs may serve presently untapped therapeutic roles by altering dopamine release in other disorders involving dopaminergic systems.
Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that affects more than 15 million people worldwide, and it is on the increase as the elderly population proportionately rises (Palmer, 2002). Cognitive dysfunctions, particularly in learning and memory, are hallmarks of the disease. AD progresses to affect limbic structures, subcortical nuclei, and cortical regions, and in that way influences multiple neurotransmitter systems. The most well appreciated neuronal loss is in the cholinergic system (Perry, 1986; Fibiger, 1991). The decline of cortical cholinergic activity as measured in post mortem brains correlates with the severity of AD symptoms and with the intellectual deterioration observed in life (Coyle et al., 1983; Nordberg, 1999). As the disease worsens, cholinergic neurons are progressively lost and the number of nicotinic acetylcholine receptors (nAChRs) declines in the hippocampus and cortex (Paterson and Nordberg, 2000; Perry et al., 2000). Although loss of muscarinic acetylcholine (ACh) receptors is less widespread, a decline of M₄ muscarinic receptors has been reported in the hippocampus of AD patients (Mulugeta et al. 2003). For those reasons, mild to moderate AD is most commonly treated with acetylcholinesterase (AChE) inhibitors, such as donepezil (Aricept®) and galantamine (Reminyl®). Enhancement of the cholinergic system is thought to ameliorate mainly attentional processes and, thereby, improve cognitive abilities (Sarter and Bruno, 1997; Palmer, 2002).

Non-cognitive behavioral and neuropsychiatric symptoms often accompany AD and other forms of dementia (Assal and Cummings, 2002). Lyketsos et al. (2001) reported that 60% of AD patients in their study experienced problems ranging from depression and anxiety to hallucinations and delusions. The non-cognitive aspects of dementia usually arise from the dysfunction of the serotonergic and dopaminergic systems rather than the cholinergic systems.
(Assal and Cummings, 2002; Erkinjuntti, 2002). The dopaminergic systems are further implicated because parkinsonian indications are present in greater than 30% of AD patients (Tyrrell et al., 1990; Joyce et al., 1998), and in dementia with Lewy bodies dopaminergic neurons are lost, leading to a 40-70% decline in striatal dopamine (DA) (Walker et al., 2002).

There is evidence that AChE inhibitors used to treat the cognitive dysfunction of AD also positively affect non-cognitive deficits (Blesa, 2000; Feldman et al., 2001). Because the influence over behavioral problems is unlikely to arise directly from cholinergic mechanisms (Palmer, 2002), those findings prompted us to investigate whether donepezil and galantamine influence dopaminergic events. Either spontaneous or stimulus-evoked DA release was monitored by fast cyclic voltammetry using carbon-fiber microelectrodes placed into mouse striatal brain slices. We found that in the concentration range prescribed for patients, donepezil and galantamine boost DA release. The data support previous results indicating that galantamine is a weaker AChE inhibitor than donepezil and that galantamine potentiates nAChRs (Maelicke et al., 2000, 2001; Samochocki et al., 2000). The ability of the two compounds to enhance DA release at therapeutic concentrations suggests their potential for treatment of other disorders involving dopaminergic systems.

Materials and Methods

Wild-type C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine) were used at 3 to 6 months of age. Mice were housed and handled in accord with Baylor College of Medicine’s animal care committee. Under deep anesthesia (a combination of ketamine, xylazine, acepromazine), mice were decapitated, and the brains were rapidly dissected out. Horizontal striatal slices 400 µm thick were cut using a vibratome (as in Zhou et al., 2001). Slices were kept in a holding chamber
of the following (in mM): 125 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 25 NaHCO₃, 1.25 NaH₂PO₄ and 10 glucose at equilibrium with a mixture of 95% O₂ and 5% CO₂ at room temperature. After 1 hour a slice was transferred into a 1.0-ml recording chamber that was continuously perfused at 2.0 ml/min (30 ± 0.5 °C) with the same solution as the holding chamber.

Fast-scan cyclic voltammetry was performed using homemade carbon-fiber microelectrodes (10 µm diameter and about 50 µm exposed length; P55s, Amoco Polymers, Greenville, South Carolina) that were placed in the dorsal striatum and followed published procedures (Zhou et al., 2001). The electrode potential was linearly scanned (12 ms duration, 10 Hz) from 0 mV to -400 mV to 1,000 mV to -400 mV to 0 mV against a Ag/AgCl reference electrode at a rate of 300 mV/ms. An Axopatch 200B amplifier, a Digidata 1320 interface and a pClamp 8 system (Axon Instruments, Foster City, California) were used to acquire and analyze data. The voltammograms were sampled at 50 kHz, and the background current was subtracted digitally. The peak oxidation currents for DA in each voltammogram (at about 600 mV) were converted into concentration based on a post-experiment calibration against fresh solutions of 0.5 to 5 µM dopamine.

A bipolar tungsten stimulating electrode with a resistance of 0.5 MΩ was used to evoke DA release. The two poles of the stimulating electrode were placed on the surface of the slice about 100 µm apart. The carbon-fiber recording electrode was placed 100 to 150 µm away from the poles of the stimulating electrode. Single stimuli of 1 to 4 V in amplitude and 1 ms in duration were delivered via a stimulus isolator (WECO, Millbrae, CA) controlled by a Master–8 pulse generator (A.M.P. Instruments, Israel) every 2 or 2.5 min at 50 to 60% of the maximal response. In a set of control experiments, the stimulating electrode was also placed in the nigrostriatal bundle 1 to 1.5 mm away from the recording carbon-fiber electrode to determine the
difference between intra-striatum and extra-striatum stimulation. This placement ensured that the incoming DA fibers could be stimulated without stimulating other intrinsic striatal neurons (e.g., cholinergic interneurons) that are near to the carbon-fiber recording electrode. The quantitative effect of the drugs was the same for the two placements of the stimulating electrode, and only for that reason, the results were combined for the final statistical calculations.

After a stable control recording for $\geq 60$ min, the slices were exposed to a single concentration of galantamine, donepezil, or ambenonium for 54 to 60 min followed by a washout period of $\sim 100$ min. A range of concentrations was tested in different batches of brain slices. The control recordings for the last 30 min before the slices were exposed to drug were used for computing a baseline DA release for normalization. Drugs were washed into the slice for twenty minutes to achieve equilibration before averaging to obtain the drug-induced change in DA release. In the experiments using mixtures of drugs, ambenonium (1 nM) or galantamine (200 nM) was added into the holding chamber for 1 hour before the slices were transferred to the recording chamber, which also contained the same concentration of that drug. In all other cases, the drugs were bath applied, including experiments with dihydro-β-erythroidine (DHβE) and atropine. In all cases, the drugs were dissolved in the bath solution that was flowing into the chamber. All results are presented as mean $\pm$ S.E.M. Statistical comparison was made using one-way ANOVA with one repeated factor (drug conditions x time) or Kolmogorov-Smirnov test. Galantamine hydrobromide and ambenonium dichloride were purchased from Tocris (Ellisville, MO). Donepezil chloride was kindly provided by Dr. E.X. Albuquerque (University of Maryland, Baltimore, MD).

Results
Nicotinic Receptors and Acetylcholinesterase Regulate Dopamine Release

Fast-scan cyclic voltammetry was performed with carbon-fiber microelectrodes to monitor DA release in real time from mouse striatal slices. Bipolar stimulating electrodes were placed about 150 µm from the carbon-fiber microelectrode in the dorsal striatum. DA release was electrically evoked by a single-pulse stimulus at about 50-60% of the maximal response. Under those experimental conditions, the DA signal was stable for over 2 hours. It has been shown previously that endogenous spontaneous cholinergic activity modulates action potential dependent DA release in the striatum via nAChRs (Zhou et al., 2001). We confirmed that finding (Fig. 1A). Inhibition of nAChRs by bath application of 50 nM DHβE potently diminished DA release evoked by a single stimulus, but inhibition of muscarinic ACh receptors by 0.5 or 1.0 µM atropine had little affect (Fig. 1B). These results indicate that, in the striatum, the nicotinic cholinergic system is more involved in presynaptic regulation of transmitter release whereas the muscarinic cholinergic system may directly modulate the activity of striatal neurons (Calabresi et al. 2000; Zhou et al. 2003).

It also was shown previously that strong inhibition of acetylcholinesterase (AChE) excessively prolongs the presence of ACh, leading to desensitization of nAChRs, and consequently decreases DA release (Zhou et al., 2001). We have additionally found here that much weaker inhibition of AChE enhances DA release. At low concentrations, bath application of the AChE inhibitor, ambenonium (Hodge et al., 1992), increased evoked DA release (Fig. 2A, B). The maximum increase was 12 ± 1% in 5 nM ambenonium (n = 5, p < 0.001). The effect of ambenonium was reversed upon prolonged wash. Mild AChE inhibition only slightly increases extracellular ACh (Vinson et al., 1997), which likely enhanced nAChR activity and, in turn,
increased DA release (Fig. 2). A more complete AChE inhibition by 20 nM or higher concentrations of ambenonium decreased DA release (Fig. 2C).

**Galantamine and Donepezil dose-dependently Influence Dopamine Release**

Because cholinergic mechanisms strongly influence DA release in the striatum, we reasoned that galantamine and donepezil would inhibit AChE and alter DA release. Low concentrations of galantamine progressively enhanced evoked DA release up to a maximum increase of 24 ± 4% (n = 5, p < 0.001) in 400 nM galantamine (Fig. 3A,B). After reaching that maximum, higher concentrations of galantamine began inhibiting DA release (Fig. 3C). For example, DA release was inhibited by 48 ± 2% (n = 3, p < 0.001) in 10 µM galantamine and by 91 ± 2% (n = 3, p < 0.001) in 100 µM galantamine (Fig. 3C).

Because the electrical stimulation applied in the striatum to evoke DA release excites all the nearby fibers of different neurochemical identities, interactions among multiple neurotransmitter systems could have been created. Two different controls were conducted to avoid this stimulus-induced association. First, the stimulating electrode was moved out of the striatum and into the nigrostriatal bundle, 1 to 1.5 mm away from the tip of the carbon-fiber recording electrode. With this arrangement, 400 nM galantamine produced the same percent enhancement of DA release (23 ± 6%, n = 4; p > 0.05). Second, we monitored spontaneous DA release without any electrical stimulation (Zhou et al., 2001). Spontaneous action potential dependent DA release was monitored in the absence and presence of galantamine. As seen with electrical stimulation, low concentrations of galantamine (0.4 and 0.8 µM) enhanced spontaneous DA release (Fig. 3D). When we added galantamine, the amplitude of the DA-release events were larger, causing some events that were below our level of detection in the control to
reach the level of detection in galantamine. That process caused an “apparent” increase in the frequency of spontaneous DA-release events: the frequency of detectable DA-release events increased by 22% (from 1.1 ± 0.1 event/min to 1.4 ± 0.1 event/min). This apparent frequency increase confounds the statistical analysis of the amplitude distribution because the detection problem leads to a disproportional increase in small DA-release events in galantamine. To avoid this problem, we compared the largest 10% of the spontaneous DA-release events because these larger events are not subject to this detection confound. If galantamine increased the amplitude of DA-release events, then there should be larger events in a complete distribution in galantamine than in control. After the distribution was collected, we averaged the largest 10% of the events in control and compared them to the largest 10% of events in galantamine. We found a significant increase in the amplitude: 0.17 ± 0.01 µM in control and 0.23 ± 0.01 µM in galantamine (p < 0.05, n = 4). That result was consistent with the increase found with electrically evoked DA release.

We next examined the effects of donepezil on evoked striatal DA release. Low concentrations of donepezil applied to the bath enhanced evoked DA release up to a maximum of 20 ± 3% in 100 nM donepezil (n = 5, p < 0.001) (Fig. 4A,B). After reaching that maximum, higher concentrations of donepezil began inhibiting DA release. For example (Fig. 4C), 10 µM donepezil inhibited DA release by 66 ± 3% (n = 3, p < 0.001).

**Galantamine also Enhances Dopamine Release via a Second Mode of Action**

Both galantamine and donepezil are AChE inhibitors, but published evidence indicates that galantamine is a weaker AChE inhibitor that also has a second mode of action via increasing nAChR currents (Maelicke et al., 2000, 2001; Samochocki et al., 2000; Woodruff-Pak et al., 2002). To test whether these potential mechanistic differences could influence how these two
drugs enhance DA release, we did the following experiment. The brain slices were bathed in 1 nM ambenonium to cause a mild background inhibition of AChE. In separate experiments, 1 nM ambenonium caused a 10 ± 1% (n = 6, p < 0.001) increase in DA release (see Fig. 2). On this background of mild AChE inhibition, different concentrations of galantamine or donepezil were bath applied to produce dose-response relationships. The difference in the results with galantamine and donepezil is exemplified by comparing the effects at half the concentration that gave the maximum enhancement of DA release. Galantamine gave a maximum enhancement of DA release of 24% at 400 nM. Therefore, it was applied to the ambenonium background at 200 nM, giving a further increase in DA release of 23 ± 2% (n = 9, p < 0.001 compared to baseline) (Fig. 5A). Donepezil gave a maximum enhancement of DA release of 20% at 100 nM, and therefore it was applied at 50 nM. Unlike galantamine, adding donepezil (50 nM) onto a background of mild AChE inhibition caused a decrease in DA release of 14 ± 5% (n = 7, p < 0.001 compared to baseline) (Fig. 5B). Although ambenonium and donepezil are structurally quite different and may have different additional actions (see Hodge et al., 1992; Santos et al., 2002), they are both considered AChE inhibitors. The decrease in DA release caused by adding two rather strong AChE inhibitors is expected to arise from the dose-response curves, which show a switch from enhancement to inhibition of DA release as the concentration of AChE inhibitor increases (see Figs. 2C and 4C).

The dose-response relationships show that with mild AChE inhibition galantamine enhanced DA release over a wider range (Fig. 5C). After adding the 10% enhancement caused by 1 nM ambenonium, galantamine with extra mild AChE inhibition produced a larger maximum enhancement of DA release (10% + 23% = 33%) than with pure galantamine (24%, p < 0.05). Thus, ambenonium and galantamine together produced a greater enhancement of DA release than either drug alone. These results are consistent with the published results indicating
that galantamine is a mild AChE inhibitor (IC50 ~ 800 nM) (Woodruff-Pak et al., 2002) that also acts to enhance nAChR currents (Maelicke et al., 2000, 2001; Samochocki et al., 2000). On the other hand, mild inhibition of AChE mainly shifts the dose-response relationship for donepezil to the left without giving greater enhancement of DA release (Fig. 5D). Again, this result is consistent with donepezil being a stronger AChE inhibitor that does not greatly enhance nAChR activity as a separate mode of action.

The enhancement of DA release seen in Figure 5 with galantamine and ambenonium was the same when the order of application was reversed (Fig. 6). When 1 nM ambenonium was added onto a background of 200 nM galantamine, a 14 ± 4% (n = 5, p < 0.001 compared to baseline) increase in DA release was observed (Fig. 6). After adding the roughly 17% enhancement caused by 200 nM galantamine in the background to the additional 14% enhancement caused by ambenonium, the total enhancement is 31%, which is comparable to the 33% total enhancement seen when the applied drugs are reversed.

Discussion

Dopaminergic fibers originating in the midbrain and cholinergic fibers arising from local interneurons form an intertwined meshwork in the striatum that is the densest in the mammalian brain (Björklund and Lindvall, 1984; Woolf, 1991; Zhou et al., 2001, 2002). These DA and ACh fibers are associated with the densest expression of AChE (Butcher and Woolf, 1984; Zhou et al., 2001). The striatal cholinergic interneurons fire tonically at about 5 Hz (Aosaki et al., 1995; Bennett and Wilson, 1999), providing a pulsatile ACh signal that is rapidly terminated by AChE. This situation optimizes ongoing nAChR activity by avoiding desensitization. Histochemical studies showed that nAChRs are present on DA nerve terminals (Hill et al., 1993; Jones et al.,
2001), and functional studies revealed that the activity of presynaptic \( \beta_2^* \) nAChRs regulates action potential dependent striatal DA release (Marshall et al., 1997; Johnson et al., 2000; Grady et al., 2001; Zhou et al., 2001). This potent nicotinic mechanism controls DA release in the striatum, and can be modulated by acetylcholinesterase inhibitors that are used to treat Alzheimer’s disease. At low concentrations, donepezil and galantamine boost DA release evoked by a single-pulse stimulus by a maximum of 20% and 24%, respectively.

In the concentration range where DA release is enhanced, donepezil is mainly a pure AChE inhibitor (see Samochocki et al., 2000; Woodruff-Pak et al., 2002; Dajas-Bailador et al., 2003). It maximally enhanced DA release at 100 nM; but as donepezil’s concentration increased further, evoked DA release decreased, as was seen with the pure AChR inhibitor, ambenonium. These results are best explained by the overly strong AChE inhibition at high concentrations. Under that condition, ACh released from tonically firing cholinergic interneurons (Bennett and Wilson, 1999) is present at high concentrations for longer times, causing nAChR desensitization. As was shown when nAChRs were inhibited by DH\( \beta \)E (Fig. 1), desensitization of nAChRs likewise causes a decrease in DA release evoked by widely separated single stimuli (Zhou et al., 2001). It is interesting to note that although ambenonium and donepezil are both considered to act as AChE inhibitors they give different maximum levels of enhancement, 12% and 20% respectively. This difference may arise from mechanistic differences and secondary influences with these compounds, which are structurally quite different (see Hodge et al., 1992; Santos et al., 2002).

Although the effective constants for inhibition of AChE (IC50s) by galantamine or donepezil are difficult to estimate in vivo, rough estimates have been made, and there is agreement that galantamine is a weaker AChE inhibitor than donepezil (see Barnes et al., 2000; Woodruff-Pak et al., 2002). Separate from its action on AChE, galantamine (but not donepezil)
influences nAChRs currents by a putative allosteric mechanism (Samochocki et al., 2000). This effect has been shown in tissue culture preparations and in heterologous expression systems where AChE is not present (Maelicke et al., 2000, 2001; Samochocki et al., 2000). Galantamine was shown to increase nAChR currents by about 50% at concentrations between 0.1 to 1 µM (Maelicke et al., 2001). At higher concentrations, galantamine decreases nAChRs currents by a putative allosteric inhibition. Therefore, the enhancement of DA release we observed below 1 µM galantamine likely arose from mild AChE inhibition coupled to enhanced nAChR activity. The results with mixtures of galantamine and ambenonium support that conclusion.

When there was mild AChE inhibition caused by ambenonium, at half their most effective doses donepezil decreased and galantamine increased DA release. The combination of two strong AChE inhibitors (ambenonium and donepezil) likely overly extended the presence of ACh, leading to nAChR desensitization and decreased DA release, as was seen with either of these drugs at higher concentrations. The literature and results with ambenonium plus galantamine are consistent with the following explanation: a low concentration of a strong AChE inhibitor (ambenonium) with a weak AChE inhibitor (galantamine) did not overly inhibit AChE, and galantamine also enhanced the intrinsic activity of nAChRs. Those processes working together increased DA release. In fact, the total enhancement of DA release with this combination of ambenonium and galantamine was greater than the maximum enhancement seen with either of these drugs alone.

**Biological Significance and Implications of Anti-AChE Therapy**

The biological significance of these data arises because there is enhanced DA release at therapeutically relevant concentrations. Based on the pharmacokinetics, extrapolated plasma concentrations, and approximate IC50s, the brain concentrations can be estimated at 10 - 60 nM.
for donepezil and 100 - 600 nM for galantamine (see Bores et al., 1996; Barnes et al., 2000; Ogura et al., 2000; Mannens et al., 2002; Santos et al., 2002; Woodruff-Pak et al., 2002). In those concentration ranges, both drugs enhance DA release, and galantamine has its maximum effect well within that range. Santos et al. (2002) recently concluded that galantamine (but not donepezil) enhances glutamate transmission by allosterically enhancing nAChRs. Because presynaptic nAChR activity enhances the release of many neurotransmitters (see McGehee and Role, 1996; Role and Berg, 1996; Albuquerque et al., 1997; Wonnacott, 1997; Dani, 2001), the potentiating effect of galantamine on nAChRs suggests it also may influence the release of other neurotransmitters. That influence over the release of DA may contribute to the benefit of these drugs for non-cognitive symptoms (Blesa 2000; Assal and Cummings, 2002; Erkinjuntti, 2002; Lilienfeld, 2002).

The results also suggest that “cholinergic” drugs may be valuable in other disease cases. A range of neuropsychiatric symptoms, including anxiety, depression, apathy, and psychosis, are influenced by dopaminergic systems (Assal and Cummings, 2002; Erkinjuntti, 2002). Furthermore, parkinsonian symptoms commonly accompany AD (Tyrrell et al., 1990; Joyce et al., 1998; Werber and Rabey, 2001), and Parkinson’s disease is often linked with depression or dementia. There also is profound loss of DA neurons in dementia with Lewy bodies (Walker et al., 2002; see Galvin et al., 2001). A nicotinic deficit is further implicated because there is a reduced number of striatal nAChRs in AD, Parkinson’s disease, and dementia with Lewy bodies (Court et al., 2000). Therefore, improvements may be gained by enhancing nAChRs. The present results suggest that the tested drugs may offer benefits for dementia, parkinsonian symptoms, and specific neuropsychiatric dysfunctions of the dopaminergic systems.
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References


Alzheimer's disease using a dopaminergic presynaptic ligand. *J Neurol Neurosurg Psychiatry* **73**: 134-140.


Footnote

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Figure Legends

Figure 1. Nicotinic but not muscarinic ACh receptors strongly regulated evoked DA release in the striatum. (A) The DA responses were evoked under control conditions, during application of 50 nM dihydro-β-erythroidine (DHβE), and after recovery following a prolonged wash. Consistent with previous findings (Zhou et al., 2001), DA release was greatly reduced by DHβE, which is a specific β2* nAChR antagonist. (B) The DA responses were evoked under control conditions, during 1 µM atropine application, and after recovery following a prolonged wash. The two voltammograms on the right were obtained at the DA peak of the control traces.

Figure 2. Ambenonium dose dependently influences evoked DA release in the striatum. (A) Examples depicting electrically evoked DA release in the striatum under control conditions, during 1 nM ambenonium application, and after recovery from ambenonium. The voltammogram on the right was obtained at the DA peak of the control trace. (B) Average of the data in 1 nM ambenonium, which enhanced evoked DA release by 10 ± 1% (n = 6, p < 0.001). The data were normalized to the baseline obtained before adding ambenonium. (C) The evoked DA release as a function of bath applied ambenonium concentration (n = 3 to 6). The open circles at 0.1 and 1.0 µM ambenonium plot values taken from Zhou et al. (2001) for completeness. In this and other figures, the data points often obscure the S.E. bars, and the data points are connected for display purposes only.

Figure 3. Galantamine dose-dependently influences DA release in the striatum. (A) Examples of evoked DA release recorded under control conditions, in the presence of 0.4 µM galantamine,
and after recovery from galantamine. The voltammogram on the right was from the peak of the evoked DA release in 0.4 µM galantamine. (B) Average of the normalized amplitudes of evoked DA release versus time, showing that 0.4 µM galantamine reversibly increased DA release (n = 5). (C) The dose dependence of galantamine’s effect on evoked DA release (n = 3 to 9). (D) In the absence of electrical stimulation, low concentrations of galantamine also enhance spontaneous DA release in the striatum (n = 4). The left trace is a segment of recording under control conditions, and the right trace is a segment from the same recording after adding 0.4 µM galantamine to the bath. The voltammogram on the right was obtained from the peak of the spontaneous event indicated by the arrow.

**Figure 4.** Donepezil dose-dependently influences DA release in the striatum. (A) Examples of evoked DA release recorded under control conditions, in the presence of 100 nM donepezil, and after recovery from donepezil. The voltammogram on the right was from the peak of the control DA release. (B) Average of the normalized amplitudes of evoked DA release versus time, showing that 100 nM donepezil reversibly increased DA release (n = 5). (C) The dose dependence of donepezil’s effects on evoked DA release (n = 3 to 5).

**Figure 5.** With constant mild inhibition of AChE by 1 nM ambenonium, galantamine and donepezil had dose dependencies that showed different influences over evoked DA release. Ambenonium was present in the brain slice holding chamber to ensure equilibrium was achieved, and it was present during the entire experiment. (A) Average of the normalized amplitudes of evoked DA release versus time, showing that in the presence of 1 nM ambenonium, 200 nM galantamine increased evoked DA release (n = 9). (B) Average of the normalized amplitudes of evoked DA release versus time, showing that in the presence of 1 nM ambenonium, 50 nM...
donepezil decreased evoked DA release (n = 7). (C) In the presence of 1 nM ambenonium, galantamine enhanced evoked DA release over a broader concentration range. The 10% increase in DA release caused by 1 nM ambenonium was added to the dose dependence. The dose dependence in the absence of ambenonium is shown for comparison (dash curve, from Fig. 3C). (D) In the presence of 1 nM ambenonium, the dose-dependence for donepezil is mainly shifted to the left. The 10% increase in DA release caused by 1 nM ambenonium was added to the dose dependence. The dose dependence in the absence of ambenonium is shown for comparison (dash curve, from Fig. 4C).

**Figure 6.** In the background presence of 200 nM galantamine, 1 nM ambenonium increased evoked DA release. (A) Examples of evoked DA release recorded in 200 nM galantamine, in both 200 nM galantamine and 1 nM ambenonium, and after washing out ambenonium. Galantamine was present in the brain slice holding chamber to ensure equilibrium was achieved, and it was present during the entire experiment. The voltammogram on the right was from the peak of the middle trace. (B) Average of the normalized amplitudes of evoked DA release versus time, showing that in the presence of 200 nM galantamine, 1 nM ambenonium increased evoked DA release (n = 5).
Figure 1

Panel A: Control, 50 nM DHβE, Wash

Panel B: Control, 1 μM Atropine, Wash
Figure 2
Figure 3
Figure 4

A

Control

Donepezil

Wash

Voltammogram

1.0 μM

5 s

B

Donepezil

C

Relative DA Signal

Time (min)

Relative DA Signal

Donepezil (log M)
Figure 5
Figure 6