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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 noncognitive 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; Eisai Inc., Teaneck, NJ) and galantamine (Reminyl; Janssen Pharmaceutica Products, Titusville, NJ). Enhanced cholinergic functions caused by these compounds are believed to underlie improvements in learning, memory, and attention. The noncognitive 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-scan cyclic voltammetry applied to mouse striatal brain slices was used to measure the real-time release of dopamine 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 noncognitive 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.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects more than 15 million people worldwide, and it is on the rise as the elderly population proportionately increases (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, it 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 postmortem 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 M_4 muscarinic receptors has been reported in the hippocampus of patients with AD (Mulugeta et al., 2003). For those reasons, mild to moderate AD is most commonly treated with acetylcholinesterase (AChE) inhibitors, such as donepezil (Aricept; Eisai Inc., Teaneck, NJ) and galantamine (Reminyl; Janssen Pharmaceutica Products, Titusville, NJ). Enhancement of the cholinergic system is believed to ameliorate mainly attentional processes and thereby improve cognitive abilities (Sarter and Bruno, 1997; Palmer, 2002).

Noncognitive behavioral and neuropsychiatric symptoms often accompany AD and other forms of dementia (Assal and Cummings, 2002). Lyketsos et al. (2001) reported that 60% of patients with AD in their study experienced problems ranging from depression and anxiety to hallucinations and delusions. The noncognitive 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 more than 30% of patients with AD (Tyrrell et al., 1990; Joyce et al., 1998); in dementia with Lewy bodies, dopaminergic neurons are lost, leading to a 40 to 70% decline in striatal dopamine (DA) (Walker et al., 2002).

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ABBREVIATIONS: AD, Alzheimer's disease; nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; AChE, acetylcholinesterase; DA, dopamine; DHβE, dihydro-β-erythroidine.

There is evidence that AChE inhibitors used to treat the cognitive dysfunction of AD also positively affect noncognitive 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-scan 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 aged 3 to 6 months (Jackson Laboratory, Bar Harbor, ME) were used. Mice were housed and handled in accordance with the guidelines set forth by the animal care committee at Baylor College of Medicine. Under deep anesthesia (a combination of ketamine, xylazine, and acepromazine), mice were decapitated, and the brains were rapidly dissected out. Horizontal striatal slices 400 μm thick were cut using a vibratome (Zhou et al., 2001). Slices were kept in a holding chamber containing the following: 125 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 10 mM glucose at equilibrium with a mixture of 95% O_2 and 5% CO_2 at room temperature. After 1 h, a slice was transferred into a 1.0-ml recording chamber that was continuously perfused at 2.0 ml/min (30 $\pm$ 0.5°C) with the same solution as that contained in the holding chamber.

Fast-scan cyclic voltammetry was performed using homemade carbon-fiber microelectrodes (10 μm diameter and approximately 50 μm exposed length; P55s, Amoco Polymers, Greenville, SC) that were placed in the dorsal striatum, and previously published procedures were followed (Zhou et al., 2001). The electrode potential was linearly scanned (12 ms duration, 10 Hz) from 0 to -400 to 1000 to -400 to 0 mV against a silver/silver chloride reference electrode at a rate of 300 mV/ms. An Axopatch 200B amplifier, a Digidata 1320 interface, and a pClamp 8 system (Axon Instruments Inc., Union City, CA) 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 approximately 600 mV) were converted into concentration from a postexperiment 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 approximately 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, Jerusalem, 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 intrastriatum and extrastriatum 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 for that reason only, 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 20 min 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 h 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 means $\pm$ S.E.M. Statistical comparison was made using one-way analysis of variance with one repeated factor (drug conditions $\times$ time) or Kolmogorov-Smirnov test. Galantamine hydrobromide and ambenonium dichloride were purchased from Tocris Cookson Inc. (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 approximately 150 μm from the carbon-fiber microelectrode in the dorsal striatum. DA release was electrically evoked by a single-pulse stimulus at approximately 50 to 60% of the maximal response. Under those experimental conditions, the DA signal was stable for more than 2 h. 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 effect (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 AChE excessively prolongs the presence of ACh, leading to desensitization of nAChRs, and it 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. 2, A and B). The maximum increase was 12 $\pm$ 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 and Justice, 1997), which probably 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 to a maximum increase of 24 ± 4% ($n = 5$, $p < 0.001$) in 400 nM galantamine (Fig. 3, A and 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 of 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 percentage of 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 was larger, causing some events that were lower than 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 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 with the largest 10% of events in galantamine. We found a significant increase in the amplitude: 0.17 ± 0.01 μM in control and

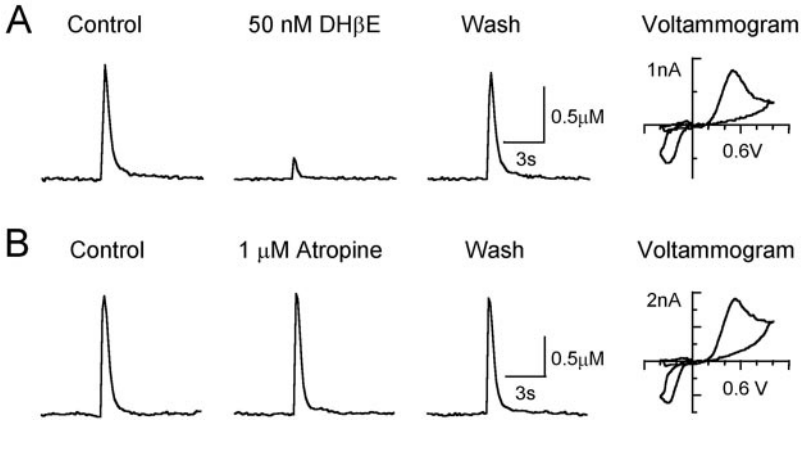

Fig. 1. Nicotinic but not muscarinic ACh receptors strongly regulated evoked DA release in the striatum. A, the DA responses were evoked under control conditions, during application of 50 nM DHβE, and after recovery following a prolonged wash. Consistent with previous findings (Zhou et al., 2001), DA 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 (right) were obtained at the DA peak of the control traces.

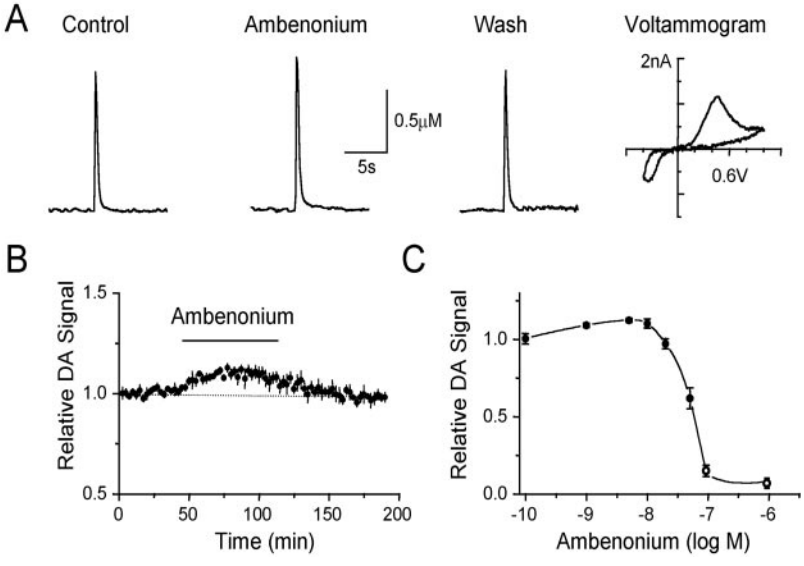

Fig. 2. Ambenonium dose-dependently influences evoked DA release in the striatum. A, examples depicting electrically evoked DA release in the striatum under control conditions, during 1 nM ambenonium application, and after recovery from ambenonium. The voltammogram (right) was obtained at the DA peak 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 ($n = 3$–6). ○ 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.

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 $20 \pm 3\%$ in 100 nM donepezil ($n = 5$, $p < 0.001$) (Fig. 4, A and B). After reaching that maximum, higher concentrations of donepezil began inhibiting DA release. For example (Fig. 4C), 10 μM donepezil inhibited DA release by $66 \pm 3\%$ ($n = 3$, $p < 0.001$).

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 performed 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 $10 \pm 1\%$ ($n = 6$, $p < 0.001$) increase in DA release (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

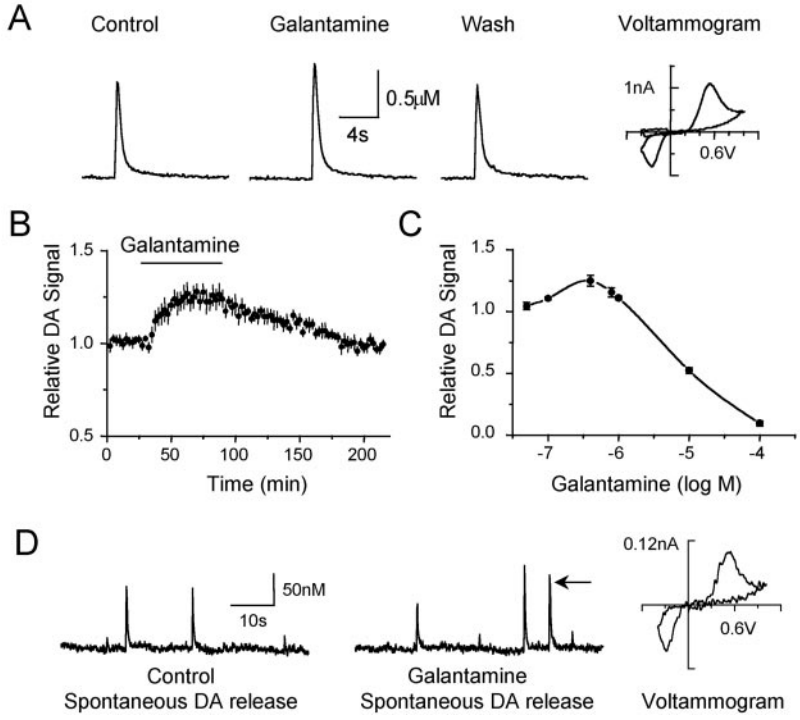

Fig. 3. Galantamine dose-dependently influences DA release in the striatum. A, examples of evoked DA release recorded under control conditions, in the presence of 0.4 μM galantamine, and after recovery from galantamine. The voltammogram (right) was from the peak of the evoked DA release in 0.4 μM galantamine. 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 ($n = 3$–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 (right) was obtained from the peak of the spontaneous event indicated by the arrow.

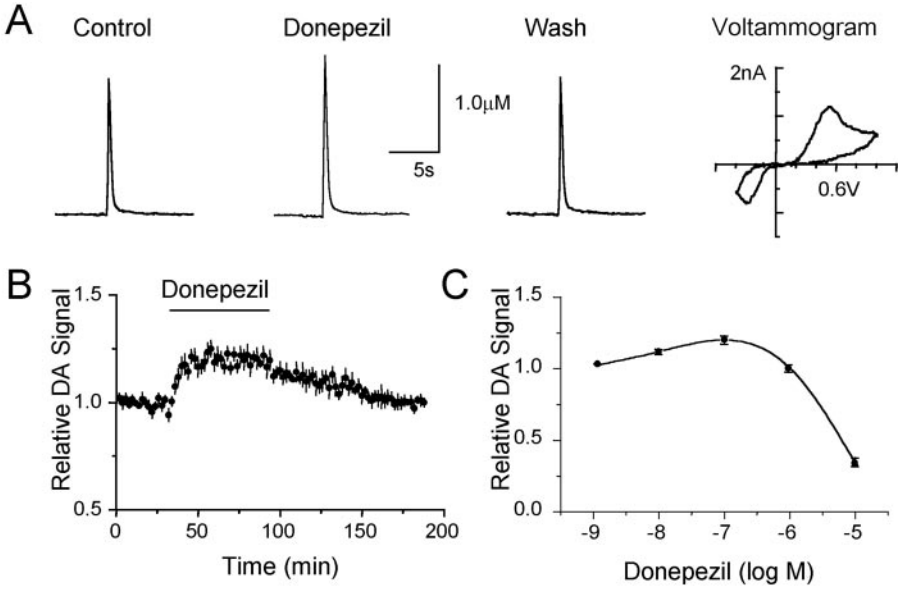

Fig. 4. Donepezil dose-dependently influences DA release in the striatum. A, examples of evoked DA release recorded under control conditions, in the presence of 100 nM donepezil, and after recovery from donepezil. The voltammogram (right) was from the peak of the control DA release. B, average of the 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 ($n = 3$–5).

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 with 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 with baseline) (Fig. 5B). Although ambenonium and donepezil are structurally quite different and may have different additional actions (Hodge et al., 1992; Santos et al., 2002), they are 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 (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 along with additional 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 did either drug alone. These results are consistent with the published results indicating that galantamine is a mild AChE inhibitor (IC_{50} of ~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, additional 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 Fig. 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 with 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 with 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 approximately 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;

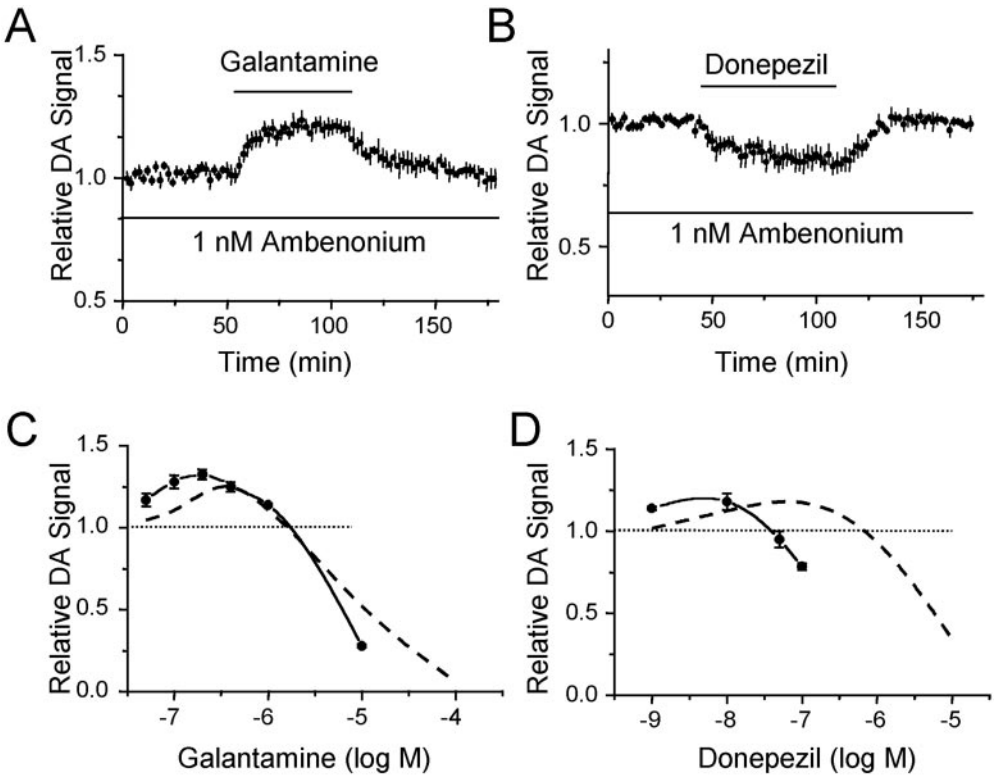

Fig. 5. With constant mild inhibition of AChE by 1 nM ambenonium, galantamine and donepezil had dose dependencies that showed different influences over evoked DA release. Ambenonium was present in the brain-slice holding chamber to ensure that equilibrium was achieved, and it was present during the entire 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 (broken 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 (broken curve, from Fig. 4C).

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 in which 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 the donepezil 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 DHβE (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 of 12 and 20%, respectively. This difference may arise from mechanistic differences and secondary influences with these compounds, which are structurally quite different (Hodge et al., 1992; Santos et al., 2002).

Although the effective constants for inhibition of AChE (IC_{50} values) 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 (Barnes et al., 2000; Woodruff-Pak et al., 2002). Separate from its action on AChE, galantamine (but not donepezil) influences nAChR currents by a putative allosteric mechanism (Samochocki et al., 2000). This effect has been shown in tissue culture preparations and in heterologous expression systems in which AChE is not present (Maelicke et al., 2000, 2001; Samochocki et al., 2000). Galantamine was shown to increase nAChR currents by approximately 50% at concentrations between 0.1 and 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 at concentrations lower than 1 μM galantamine probably arose from mild AChE inhibition coupled with enhanced nAChR activity. The results with mixtures of galantamine and ambenonium support that conclusion.

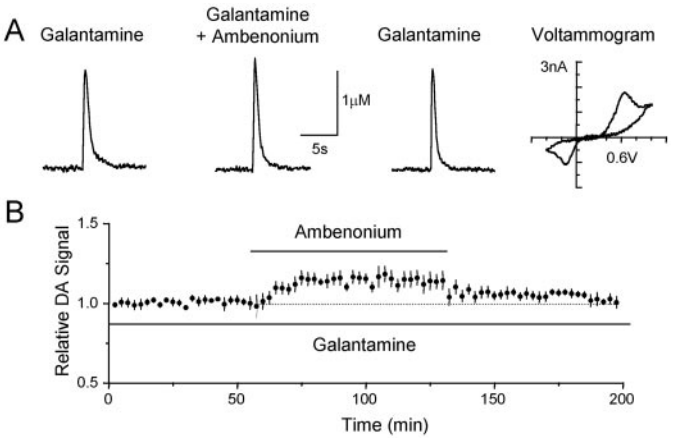

Fig. 6. In the background presence of 200 nM galantamine, 1 nM ambenonium increased evoked DA release. A, examples of evoked DA release recorded in 200 nM galantamine, in both 200 nM galantamine and 1 nM ambenonium, and after washing out ambenonium. Galantamine was present in the brain-slice holding chamber to ensure that equilibrium was achieved, and it was present during the entire experiment. The voltammogram (right) was from the peak of the middle trace. B, the 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 ($n = 5$).

When there was mild AChE inhibition caused by ambenonium, at half of their most effective doses, donepezil decreased and galantamine increased DA release. The combination of two strong AChE inhibitors (ambenonium and donepezil) probably 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. From the pharmacokinetics, extrapolated plasma concentrations, and approximate IC_{50} values, the brain concentrations can be estimated at 10 to 60 nM for donepezil and 100 to 600 nM for galantamine (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 (McGehee and Role, 1996; Role and Berg, 1996; Albuquerque et al., 1997; Wonnacott, 1997; Dani, 2001), the potentiating effect of galantamine on nAChRs suggests that 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 noncognitive 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; 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). There-

fore, 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

Albuquerque EX, Alkondon M, Pereira EF, Castro NG, Schrattenholz A, Barbosa CT, Bonfante-Cabarcas R, Aracava Y, Eisenberg HM, and Maelicke A (1997) Properties of neuronal nicotinic acetylcholine receptors: pharmacological characterization and modulation of synaptic function. *J Pharmacol Exp Ther* **280:**1117–1136.

Aosaki T, Kimura M, and Graybiel AM (1995) Temporal and spatial characteristics of tonically active neurons of the primate's striatum. *J Neurophysiol* **73:**1234–1252.

Assal F and Cummings JL (2002) Neuropsychiatric symptoms in the dementias. *Curr Opin Neurol* **15:**445–450.

Barnes CA, Meltzer J, Houston F, Orr G, McGann K, and Wenk GL (2000) Chronic treatment of old rats with donepezil or galantamine: effects on memory, hippocampal plasticity and nicotinic receptors. *Neuroscience* **99:**17–23.

Bennett BD and Wilson CJ (1999) Spontaneous activity of neostriatal cholinergic interneurons in vitro. *J Neurosci* **19:**5586–5596.

Björklund A and Lindvall O (1984) Dopamine-containing systems in the CNS, in *Classical Transmitters in the CNS, Part I* (Björklund A, Hökfelt T eds) pp 55–122, Elsevier, Amsterdam.

Blesa R (2000) Galantamine: therapeutic effects beyond cognition. *Dement Geriatr Cogn Disord* **11 Suppl 1:**28–34.

Bores GM, Huger FP, Petko W, Mutlib AE, Camacho F, Rush DK, Selk DE, Wolf V, Kosley RW Jr, Davis L, et al. (1996) Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. *J Pharmacol Exp Ther* **277:**728–738.

Butcher LL and Woolf NJ (1984) Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons, in *Classical Transmitters and Transmitter Receptors in the CNS* (Björklund A, Hökfelt T, and Kuhar MJ eds) pp 1–50, Elsevier, Amsterdam.

Calabresi P, Centonze D, Gubellini P, Pisani A, and Bernardi G (2000) Acetylcholine-mediated modulation of striatal function. *Trends Neurosci* **23:**120–126.

Court JA, Piggott MA, Lloyd S, Cookson N, Ballard CG, McKeith IG, Perry RH, and Perry EK (2000) Nicotine binding in human striatum: elevation in schizophrenia and reductions in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease and in relation to neuroleptic medication. *Neuroscience* **98:**79–87.

Coyle JT, Price DL, and DeLong MR (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science (Wash DC)* **219:**1184–1190.

Dajas-Bailador FA, Heimala K, and Wonnacott S (2003) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine is transduced into cellular responses in neurons: Ca^{2+} signals and neurotransmitter release. *Mol Pharmacol* **64:**1217–1226.

Dani JA (2001) Overview of nicotinic receptors and their roles in the central nervous system. *Biol Psychiatry* **49:**166–174.

Erkinjuntti T (2002) Treatment options: the latest evidence with galantamine (Reminyl). *J Neurol Sci* **203–204:**125–130.

Feldman H, Gauthier S, Hecker J, Vellas B, Subbiah P, and Whalen E (2001) A 24-week, randomized, double-blind study of donepezil in moderate to severe Alzheimer's disease. *Neurology* **57:**613–620.

Fibiger HC (1991) Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends Neurosci* **14:**220–223.

Galvin JE, Lee VM, and Trojanowski JQ (2001) Synucleinopathies: clinical and pathological implications. *Arch Neurol* **58:**186–190.

Grady SR, Meinerz NM, Cao J, Reynolds AM, Picciotto MR, Changeux JP, McIntosh JM, Marks MJ, and Collins AC (2001) Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum. *J Neurochem* **76:**258–268.

Hill JA Jr, Zoli M, Bourgeois JP, and Changeux JP (1993) Immunocytochemical localization of a neuronal nicotinic receptor: the β2-subunit. *J Neurosci* **13:**1551–1568.

Hodge AS, Humphrey DR, and Rosenberry TL (1992) Ambenonium is a rapidly reversible noncovalent inhibitor of acetylcholinesterase, with one of the highest known affinities. *Mol Pharmac*ol **41:**937–942.

Johnson JH, Zhao C, James JR, and Rosecrans JA (2000) Individual variability of dopamine release from nucleus accumbens induced by nicotine. *Brain Res Bull* **51:**249–253.

Jones IW, Bolam JP, and Wonnacott S (2001) Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *J Comp Neurol* **439:**235–247.

Joyce JN, Murray AM, Hurtig HI, Gottlieb GL, and Trojanowski J (1998) Loss of dopamine D2 receptors in Alzheimer's disease with parkinsonism but not Parkinson's or Alzheimer's disease. *Neuropsychopharmacology* **19:**472–480.

Lilienfeld S (2002) Galantamine—a novel cholinergic drug with a unique dual mode of action for the treatment of patients with Alzheimer's disease. *CNS Drug Rev* **8:**159–176.

Lyketsos CG, Sheppard JM, Steinberg M, Tschanz JA, Norton MC, Steffens DC, and Breitner JC (2001) Neuropsychiatric disturbance in Alzheimer's disease clusters into three groups: the Cache County study. *Int J Geriatr Psychiatry* **16:**1043–1053.

Maelicke A, Samochocki M, Jostock R, Fehrenbacher A, Ludwig J, Albuquerque EX, and Zerlin M (2001) Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. *Biol Psychiatry* **49:**279–288.

Maelicke A, Schrattenholz A, Samochocki M, Radina M, and Albuquerque EX (2000) Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease. *Behav Brain Res* **113:**199–206.

Mannens GS, Snel CA, Hendrickx J, Verhaeghe T, Le Jeune L, Bode W, van Beijsterveldt L, Lavrijsen K, Leempoels J, Van Osselaer N, et al. (2002) The metabolism and excretion of galantamine in rats, dogs and humans. *Drug Metab Dispos* **30:**553–563.

Marshall DL, Redfern PH, and Wonnacott S (1997) 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* **68:**1511–1519.

McGehee DS and Role LW (1996) Presynaptic ionotropic receptors. *Curr Opin Neurobiol* **6:**342–349.

Mulugeta E, Karlsson E, Islam A, Kalaria R, Mangat H, Winblad B, and Adem A (2003) Loss of muscarinic M4 receptors in hippocampus of Alzheimer patients. *Brain Res* **960:**259–262.

Nordberg A (1999) PET studies and cholinergic therapy in Alzheimer's disease. *Rev Neurol (Paris)* **155 Suppl 4:**S53–63.

Ogura H, Kosasa T, Kuriya Y, and Yamanishi Y (2000) Comparison of inhibitory activities of donepezil and other cholinesterase inhibitors on acetylcholinesterase and butyrylcholinesterase in vitro. *Methods Find Exp Clin Pharmacol* **22:**609–613.

Palmer AM (2002) Pharmacotherapy for Alzheimer's disease: progress and prospects. *Trends Pharmacol Sci* **23:**426–433.

Paterson D and Nordberg A (2000) Neuronal nicotinic receptors in the human brain. *Prog Neurobiol* **61:**75–111.

Perry E, Martin-Ruiz C, Lee M, Griffiths M, Johnson M, Piggott M, Haroutunian V, Buxbaum JD, Nasland J, Davis K, et al. (2000) Nicotinic receptor subtypes in human brain ageing, Alzheimer and Lewy body diseases. *Eur J Pharmacol* **393:**215–222.

Perry EK (1986) The cholinergic hypothesis—ten years on. *Br Med Bull* **42:**63–69.

Role LW and Berg DK (1996) Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* **16:**1077–1085.

Samochocki M, Zerlin M, Jostock R, Groot Kormelink PJ, Luyten WH, Albuquerque EX, and Maelicke A (2000) Galantamine is an allosterically potentiating ligand of the human alpha4/beta2 nAChR. *Acta Neurol Scand Suppl* **176:**68–73.

Santos MD, Alkondon M, Pereira EF, Aracava Y, Eisenberg HM, Maelicke A, and Albuquerque EX (2002) The nicotinic allosteric potentiating ligand galantamine facilitates synaptic transmission in the mammalian central nervous system. *Mol Pharmacol* **61:**1222–1234.

Sarter M and Bruno JP (1997) Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. *Behav Brain Res* **83:**7–14.

Tyrrell PJ, Sawle GV, Ibañez V, Bloomfield PM, Leenders KL, Frackowiak RS, and Rossor MN (1990) Clinical and positive emission tomographic studies in the extrapyramidal syndrome of dementia of the Alzheimer type. *Arch Neurol* **47:**1318–1323.

Vinson PN and Justice JB Jr (1997) Effect of neostigmine on concentration and extraction fraction of acetylcholine using quantitative microdialysis. *J Neurosci Methods* **73:**61–67.

Walker Z, Costa DC, Walker RW, Shaw K, Gacinovic S, Stevens T, Livingston G, Ince P, McKeith IG, and Katona CL (2002) Differentiation of dementia with Lewy bodies from Alzheimer's disease using a dopaminergic presynaptic ligand. *J Neurol Neurosurg Psychiatry* **73:**134–140.

Werber EA and Rabey JM (2001) The beneficial effect of cholinesterase inhibitors on patients suffering from Parkinson's disease and dementia. *J Neural Transm* **108:**1319–1325.

Wonnacott S (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci* **20:**92–98.

Woodruff-Pak DS, Lander C, and Geerts H (2002) Nicotinic cholinergic modulation: galantamine as a prototype. *CNS Drug Rev* **8:**405–426.

Woolf NJ (1991) Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* **37:**475–524.

Zhou FM, Liang Y, and Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nature Neurosci* **4:**1224–1229.

Zhou FM, Wilson CJ, and Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* **53:**590–605.

Zhou FM, Wilson CJ, and Dani JA (2003) Muscarinic and nicotinic cholinergic mechanisms in the mesostriatal dopamine systems. *Neuroscientist* **9:**23–36.

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