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Effects of galantamine, a nicotinic allosteric potentiating ligand, on nicotine-induced catecholamine release in hippocampus and nucleus accumbens of rats

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Abbreviations: acetylcholinesterase (AChE); allosteric potentiating ligand (APL); Alzheimer's disease (AD); dopamine (DA); nicotinic cholinergic receptors (nAChRs). norepinephrine (NE),

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Abstract

Galantamine, a drug for treatment of Alzheimer's disease, is a novel cholinergic agent with a dual mode of action that inhibits acetylcholinesterase and allosterically modulates nicotinic cholinergic receptors (nAChRs). Nicotine stimulates catecholamine secretion, inducing hippocampal norepinephrine (NE) release, and improves memory consolidation. Thus, the effect of galantamine on nicotine-induced hippocampal NE secretion was investigated. This was compared with the effect of galantamine on nicotine-induced DA release within the nucleus accumbens of the same rat. Nicotine (0.025-0.09 mg/kg; i.v.) dose-dependently increased NE and DA levels in microdialysates from the hippocampus and nucleus accumbens, respectively, of freely moving rats. Pretreatment with galantamine (3.0 mg/kg, s.c.) 3 h prior to nicotine either potentiated NE responses to doses of nicotine that were ineffective alone (0.025-0.045 mg/kg) or significantly enhanced (0.065 mg/kg) NE responses, whereas galantamine was ineffective when administered 2 or 4 h before nicotine. In contrast to its effects on NE, galantamine did not alter accumbal DA responses to any dose of nicotine. These selective effects of galantamine on nicotine-stimulated NE secretion may reflect differences in local neural circuits that utilize nAChRs to modulate hippocampal NE versus accumbal DA release.

Galantamine acts as an allosteric potentiating ligand (APL) at nicotinic cholinergic receptors (nAChRs), and also displays properties of a weak, reversible acetylcholinesterase (AChE) inhibitor (Thomsen et al., 1991; Santos et al., 2002). It is currently approved for the treatment of Alzheimer's disease, a common dementing disorder strongly associated with reduced expression of nAChRs in frontal and temporal cortex and hippocampus (Guan et al., 2000). Galantamine acts by binding at a site on the α subunit of nAChRs, which is conserved across species, and is close to the acetylcholine-binding site (Samochocki et al., 2003). Based on conservation of the sequence of the APL binding region present on known α subunits, it has been postulated that galantamine may bind to most, if not all, nAChRs (Maelicke and Albuquerque, 2000). This allosteric action of galantamine occurs only within a specific concentration range. At lower concentrations (0.02 to 2.0 μM), galantamine has been shown to enhance acetylcholine-driven depolarization of human embryonic kidney-293 cells transfected with human α4β2 nAChRs. In contrast, 10 μM galantamine inhibited this response to acetylcholine, apparently by direct blockade of the ion channel (Samochocki et al., 2000).

Nicotine, the major psychoactive compound in cigarette smoke, affects myriad physiological CNS functions by activating nAChRs. For example, in both human and animal studies, nicotine has been reported to enhance memory (Gray et al., 1996; McGehee and Role, 1996). The hippocampus, involved in memory formation (Lee et al., 1993), is abundantly innervated by brainstem noradrenergic projections (Aston-Jones et al., 1995) that have been shown to enhance memory in experimental paradigms of inhibitory avoidance and spatial habituation (Izquierdo and Medina, 1995). The systemic

administration of nicotine stimulates norepinephrine (NE) release *in vivo* in multiple brain regions including hippocampus (Mitchell, 1993; Fu et al., 1998). Therefore, the memory-enhancing effects of nicotine may be related, in part, to the stimulation of noradrenergic neurotransmission. Moreover, the direct allosteric actions of galantamine may amplify the effects of nicotine on hippocampal noradrenergic neurotransmission.

These studies were designed primarily to determine whether galantamine affects the hippocampal NE response to the systemic administration of nicotine. Since DA is also released by nicotine in multiple brain regions including the nucleus accumbens (Nisell et al., 1994) and the modulatory effects of galantamine on this interaction have not been described, studies of accumbal DA were a secondary objective of these investigations. The nucleus accumbens was selected because the release of DA in this region by systemically self-administered nicotine appears to be pivotal to the reinforcing properties of nicotine, and to drugs of abuse in general (Di Chiara, 2000). The effects of galantamine on both hippocampal NE and accumbal DA release were investigated by using a model in which microdialysis was concurrently performed through two dialysis probes placed into an alert, freely moving rat. In order to determine whether the inhibition of AChE was involved in the effects of galantamine, rats were pretreated with donepezil instead of galantamine. A dose of donepezil was administered that results in AChE inhibition comparable to that induced by galantamine. These experiments show that galantamine enhanced nicotine-induced hippocampal NE release, but not accumbal DA secretion, independent of any effects on AChE.

Materials and Methods

Materials.

(-)-Nicotine hydrogen tartrate salt (Sigma-Aldrich; St.Louis, MO) was diluted with saline, pH adjusted to 7.2 with 0.1 N NaOH (all doses based on free base). Galantamine hydrobromide was provided by Janssen Pharmaceutica (Belgium). Donepezil hydrochloride was obtained from Pfizer (NY). Norepinephrine hydrochloride, dopamine hydrochloride, and nomifensine maleate were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dihydrogen phosphate monohydrate, EDTA, 1-decanesulfonic acid, acetonitrile, methanol and phosphoric acid (Fisher Scientific, Fair Lawn, NJ) were used to prepare the mobile phase. The alert-rat microdialysis systems and CMA 110 liquid switches were obtained from CMA/Microdialysis (Chelmsford, MA). For constructing dialysis probes, cellulose fiber tubing was obtained from Spectrum (Laguna Hills, CA), and silica tubing (outer diameter, 148 µm; inner diameter, 73 µm; TSP 075150) was purchased from Polymicron Technologies Inc. (Phoenix, AZ). Cheminert automated injectors and a digital valve sequence programmer for on-line microdialysis were purchased from Valco Instruments (Houston, TX).

Animals

Adult male Holtzman rats (250-300 g, HSD, Madison, WI) were given access to standard rat chow and water *ad libitum.* They were housed individually on a 12-h light cycle (lights on at 9:00 AM, off at 9:00 PM) for 14 days before the microdialysis experiments. After housing for 7 days, they were anesthetized with xylazine-ketamine (5:35 mg/kg body weight im; Sigma, St. Louis, MO), and chronic guide cannulae (20 gauge) were

stereotaxically implanted into both the hippocampus and nucleus accumbens, according to the atlas coordinates of Paxinos and Watson (1986). The coordinates for hippocampus were AP, -3.0 mm, DV, 2.6 mm, and ML, 1.4 mm, from bregma with a flat skull. For nucleus accumbens, the coordinates were AP, 1.4 mm, DV, 0.5 mm, and ML, 6.0 mm. These exact coordinates have been used to localize microdialysis probes placed into the hippocampus and nucleus accumbens in previous studies from our laboratory, which included photomicrographic evidence of microdialysis probe placement (Fu et al., 1998; 2000). Five days later, rats received jugular cannulae under xylazine-ketamine and were allowed to recover for another 2 days. All procedures were conducted in accordance with National Institutes of Health Guidelines Concerning the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center.

In Vivo Microdialysis.

The microdialysis method has been described previously (Fu et al., 1998; 2000). Briefly, a 2-mm concentric probe (molecular mass cutoff, 13,000 Da; outer diameter, 235 µm) was constructed in our laboratory. The recovery rate of individual probes was determined by *in vitro* dialysis for 60 min at 22°C in a solution of 200 pg NE/16 µl or 200 pg DA/16µl respectively. The probes were perfused at 1 µl/min with standard perfusate (see below), and three 15-min samples were obtained; the average recovery rate was 7.1% ± 1.0 and 6.7% ± 0.8 for NE and DA respectively (n=10). On the day of microdialysis, rats were moved into the laboratory and placed in CMA 120 awake animal system with plastic bowl CMA/microdialysis (Acton, MA). The probe was perfused at 1 µl/min with a

solution of Krebs-Ringer Buffer (KRB: 147 mM NaCl, 2.7 mM KCl, 0.9 mM $MgCl_2$ and 1.2mM $CaCl_2$ using 0.2-µm filter sterilized and degassed) containing 5 µM nomifensine (NE uptake blocker; Lin et al., 1997). Nomifensine was used in order to facilitate reliable detection of basal NE levels.

Two hours after insertion of the probes through the guide cannulae into dorsal hippocampus (CA1) and the shell of nucleus accumbens, three consecutive samples were collected to measure basal NE and DA levels before drug administration. Using an on-line microdialysis system, samples were collected through a 1.5 m length silica tube, which ran from the probes directly to the gearbox of the two Cheminert automated injectors (one for hippocampal NE and another for accumbal DA from the same rat). Samples from each brain region were collected for 15 minutes before injection onto the column. Sequential injections were controlled by a digital valve controller. At the end of each experiment, the positions of probes were verified by histological examination, as demonstrated in our previous studies (Fu et al., 1998; 2000). Only data obtained from animals with probes identified in the correct location within the hippocampus and nucleus accumbens were included in the data set.

High Pressure Liquid Chromatography-Electrochemical Analysis.

Samples (15 µl) were injected immediately onto 150 x 2 mm ODS C18 columns (ESA, Chlemsford, MA) perfused by ESA model 582 solvent delivery modules at a rate of 0.25ml/min (ESA, Chelmsford, MA) with a mobile phase containing 50mM sodium dihydrogen phosphate monohydrate, 0.7 mM EDTA, 2mM 1-decanesulfonic acid, 11% methanol and 11% acetonitrile, pH 6.0. Samples were analyzed by an ESA Coulochem II

5200A electrochemical detector with an ESA 5041 high-sensitivity microbore analytical cell. Electrochemical detection was performed at 220 mV and 1.0 nA. The limit of detection for NE and DA was 100 fg per injection. Previous reports from our laboratory have shown representative chromatograms (Fu et al., 1998, 2000).

Experimental Protocols

The first set of experiments were performed to characterize the dose-dependent response of NE and DA to i.v. nicotine. Each animal received one dose of nicotine (0.025, 0.045, 0.065, 0.09 mg/kg free base, all doses delivered at a rate of 0.09mg/kg/60s i.v.) or saline. The second set of experiments evaluated the effect of pretreatment with galantamine on nicotine-induced NE and DA release. The dose of galantamine (3.0 mg/kg, s.c.) was chosen based on a preliminary study that measured rat brain galantamine concentrations over time, utilizing five doses from 0.05 – 5.0 mg/kg b.wt., s.c. (Dr. Christopher Grantham, Janssen Pharmaceutica Inc.; Beerse, Belgium; unpublished data). This study indicated that 0.1-1.0 μM concentrations of brain galantamine would be expected 2-3h after a subcutaneous injection of galantamine 3.0 mg/kg. Therefore, nicotine (0.045 or 0.065 mg/kg) was initially delivered 2, 3 or 4 h after galantamine. Since galantamine augmented nicotine-induced NE release only at the 3h time interval, additional concentrations of nicotine were evaluated at this time point. The third set of experiments determined whether the enhancement of NE release by galantamine was due to inhibition of AChE. Donepezil, a more potent *in vitro* AChE inhibitor than galantamine (IC_{50} values of 15-24 nM versus 2.8-3.9 μM, respectively; Thomsen 1991), has been shown to be 4-fold more potent than galantamine in the inhibition of rat brain AChE following *in vivo*

administration (IC_{50} values of 0.77 mg/kg for donepezil and 2.99 mg/kg for galantamine; Barnes 2000). Importantly, donepezil is devoid of APL activity (Samochocki et al., 2000). Therefore, a dose of donepezil (1.0 mg/kg, s.c.) that would result in comparable, if not greater, inhibition of AChE than galantamine (3.0 mg/kg), was administered 3h prior to nicotine.

Data Analysis and Statistics.

Chromatographic data were collected and analyzed with the PowerChrom system (AD Instruments, Castle Hill, New South Wales, Australia). Data (mean ± S.E.) were expressed as a percentage of basal levels of NE or DA. Basal values were defined as the mean level detected in the three samples obtained prior to nicotine administration. Peak levels were measured in the samples collected 15 min after i.v. nicotine. All data were analyzed by one-way analysis of variance, followed with *post hoc* analyses with Fisher's PLSD where appropriate (SPSS 7.5; Prentice Hall).

Results

The dose-dependency of nicotine-induced hippocampal NE and accumbal DA release is shown in figure 1. Panel A demonstrates that 0.025 or 0.045 mg/kg nicotine had not effect on NE release, as compared to their respective baseline values (pre-nicotine) and to the saline control group. Higher doses of nicotine significantly elevated NE levels (+60 min) in comparison to controls ($P<0.05$ or 0.01 for nicotine 0.065 and 0.09 mg/kg, respectively), and peak NE responses to 0.09 mg/kg nicotine were significantly greater than to 0.065 mg/kg ($P<0.05$). In panel B, DA levels were not stimulated by nicotine 0.025 mg/kg, whereas higher doses elevated DA levels above saline control values ($P<0.05$ for nicotine 0.045 and 0.065 mg/kg; $P<0.01$ for nicotine 0.09 mg/kg). Peak DA responses to 0.09 mg/kg nicotine were significantly greater than to lower doses ($P<0.05$). Furthermore, 0.09 mg/kg nicotine stimulated a prolonged DA response. Thus, similar dose-response relationships were found for NE and DA, although 0.045 mg/kg nicotine only stimulated DA. In addition, DA responses were, in general, longer lasting.

Figure 2 illustrates the effects of galantamine on nicotine-induced hippocampal NE release. Baseline NE levels (+15-45 min; see figure legend for actual values), measured in samples obtained 2.5-3 h after a single injection of galantamine (3.0 mg/kg), were not different from those in the control group pretreated with saline. Galantamine, administered 3 h prior to nicotine, significantly augmented peak NE responses (+60 min) to all but the highest dose of nicotine (0.09 mg/kg; panel B). Indeed, galantamine potentiated NE responses to 0.025 and 0.045 mg/kg nicotine (panel A), doses that were ineffective in the absence of galantamine.

Figure 3 shows peak hippocampal NE and accumbal DA responses as a function of different galantamine pretreatment time intervals and nicotine doses. Panel A demonstrates that pretreatment with galantamine at 3 h, but not 2 or 4 h, consistently and significantly augmented NE responses to nicotine doses in the range of 0.025-0.065 mg/kg nicotine. Considering the two doses of nicotine that were not sufficient to stimulate NE release, the magnitude of potentiation by galantamine was greater for the NE response to 0.045 mg/kg nicotine than to 0.025mg/kg. Galantamine also enhanced the response to nicotine 0.065 mg/kg, but not to 0.09 mg/kg (see fig. 2). Figure 4 shows the effects of galantamine on the dose-response relationship for nicotine-stimulated hippocampal NE release. In general, galantamine sensitized the NE response to nicotine without affecting the amount of NE released by a maximally stimulative dose of the drug (i.e., 0.09 mg/kg nicotine; a dose that would induce a maximal increase in hippocampal NE levels without affecting behavior). Higher doses of nicotine (e.g. 0.135 mg/kg), which stimulate NE release to a greater extent, were not tested because their effects on NE are potentially confounded by system-wide effects such as brief tremors, gasping and postural imbalance (Fu et al., 1998).

In contrast to the augmenting effect of galantamine on NE, panel B of figure 3 shows that galantamine pretreatment failed to alter peak DA responses to nicotine at any dose. Thus, although both NE and DA responses to nicotine were measured in samples obtained concurrently from the same rat, 2 or 3 h after galantamine, only hippocampal NE levels were augmented. In view of these findings, rats receiving galantamine 4h prior to

nicotine 0.045 mg/kg or 2h prior to nicotine 0.065 mg/kg did not receive guide cannulae in the nucleus accumbens.

Since galantamine is also a relatively weak AChE inhibitor, donepezil was substituted for galantamine to evaluate whether the effects of galantamine depend on cholinesterase inhibition (Thomsen, 1991). Figure 5 illustrates the results of experiments designed to evaluate the effect of pretreatment with donepezil on NE and DA responses to nicotine 0.065 mg/kg. Pretreatment (3 h) with donepezil had no effect on baseline hippocampal NE or accumbal DA levels. As expected, nicotine significantly elevated both NE and DA levels, but donepezil did not modify these responses. The efficacy of donepezil at inhibiting rat brain acetylcholinesterase (AChE) has been compared to galantamine at different time points after subcutaneous administration. At 3 h after injection, donepezil 1.5 and 5.0 mg/kg inhibited 27% and 33%, respectively, of brain AChE activity, whereas galantamine inhibited only 1% and 9%, respectively (personal communication from Hugo Geerts, Ph.D., In Silico Biosciences, Philadelphia, PA).

Discussion

In the present study, nicotine dose-dependently stimulated hippocampal NE release and galantamine enhanced this when administered 3h prior to nicotine. Galantamine potentiated responses to two doses of nicotine that were themselves ineffective (0.025-0.045 mg/kg), and amplified the response to a dose (0.065 mg/kg) sufficient to stimulate hippocampal NE release. The NE response to 0.09 mg/kg nicotine was not augmented by galantamine. Overall, galantamine (fig. 4) reduced the EC_{50} dose of nicotine without changing the maximum response. Furthermore, galantamine pretreatment intervals of 2 or 4 h were ineffective. Studies *in vitro* have reported that at concentrations of 0.1-1.0 µM galantamine functions as a nicotinic APL (Samochocki et al., 2003). In rats, pharmacokinetic studies indicate that subcutaneous administration of 3 mg/kg galantamine yielded peak brain tissue levels (>1 µM) in less than 1 h, and tissue concentrations between 0.1-1.0 µM would be expected within 2 – 3 h (unpublished data, Christopher Grantham, Ph.D., Janssen Inc). Thus, galantamine modulates nicotine-stimulated hippocampal NE release within the concentration range and time required for its APL activity *in vitro*. The failure of donepezil to substitute for galantamine indicates that the inhibition of acetylcholinesterase is not a substantive factor in the modulation of nicotine-stimulated NE release by galantamine. These findings, taken together with the effects of galantamine on the dose-response relationship between nicotine and hippocampal NE, are consistent with the known nicotinic APL effects of galantamine.

Potentiation by galantamine occurred after a pretreatment interval of three but not two hours. This may be explained by the known properties of galantamine acting as an open

channel blocker at brain concentrations >1 µM and an allosteric potentiator at lesser concentrations. At concentrations near 1 µM (present 2 hours after galantamine 3.0 mg/kg) both properties would be evident, resulting in a balance between the two and no detectable effect at the system level. At the receptor level, one would expect that some nAChRs are potentiated by galantamine bound only to APL sites while others are inhibited by drug bound to both APL sites and the channel blocking domain of the nAChR. These properties would yield an inverted U-shaped dose-response relationship, as observed previously (Samochocki et al., 2000).

nAChRs are positively modulated by physostigmine-like compounds that bind near the acetylcholine binding site on the α subunit of nAChRs (Okonjo et al., 1991). These compounds, collectively known as nicotinic APLs, enhance responsiveness to acetylcholine and are insensitive to blockade by nicotinic antagonists. Galantamine has been shown to be one of these allosteric ligands (Santos et al., 2002). In addition to the enhancing effect of galantamine on nicotine-induced hippocampal NE release, electrophysiological studies using patch-clamp technique have previously shown that galantamine facilitated glutamatergic or GABAergic neurotransmission through tonically active presynaptic nAChRs in rat hippocampal and human cortical slices (Santos et al., 2002). In those experiments, the nicotinic APL activity of galantamine was implicated in the facilitation of both tonically active and acetylcholine-driven neurotransmission due to the enhanced release of glutamate and GABA.

Whole-cell patch clamp studies of transfected human embryonic kidney-293 cells have shown galantamine-dependent enhancement of human α3β4, α4β2, and α6β4 nAChR activity induced by acetylcholine, indicating the breadth of the APL effect (Samochochi et al., 2003). Concentrations of 0.1-1.0 μM galantamine potentiated agonist-induced responses to a similar degree in all nAChRs, resulting in a shift to the left and increased slope of the concentration-response relationships; EC_{50} values were reduced approximately 50%. In contrast, no studies have directly determined the effects of galantamine on α6β3β2-containing nAChRs, the subunits potentially involved in the cholinergic regulation of noradrenergic neurons projecting from the locus coeruleus to the hippocampus (Léna et al., 1999). In other studies, utilizing microinjections directly into the locus coeruleus, both conotoxins M2 and AuIB blocked nicotine-induced hippocampal NE release, without evidence of additivity (Fu et al., 1999). Those observations provide additional evidence for the expression of nAChRs containing α6, β2 and non-β2 subunits by neurons in the ceruleo-hippocampal pathway. Therefore, the efficacy of galantamine in enhancing the effects of nicotine on this pathway indicates that it is likely to act as an APL on α6-containing nAChRs.

nAChRs are known to enhance NE release in multiple brain regions (Mitchell 1993; Fu et al., 1998) including the hippocampus where noradrenergic neurotransmission is involved in cognitive function (Friedman et al., 1999). Brainstem noradrenergic input to hippocampus is involved in several memory paradigms. For example, direct hippocampal infusion of NE enhanced long-term potentiation and strengthened the memory of inhibitory avoidance and spatial habituation (Izquierdo and Medina, 1995). Furthermore,

NE enhanced long-term potentiation in the CA1 region of hippocampal slice preparations through α1-adrenergic receptors (Izumi and Zorumski, 1999). On the other hand, intra-hippocampal timolol, a β-adrenergic receptor antagonist, induced retrograde amnesia and reduced performance in step-down inhibitory avoidance (Bevilaqua et al., 1997). In addition, lesions of the locus coeruleus, which attenuate cortical and hippocampal noradrenergic innervation produce cognitive deficits, including a reduction in sustained attention (Carli et al., 1983) and impaired learning (Anlezark et al., 1973).

There is also evidence of cholinergic-adrenergic interactions that enhance cognitive function. For example, idazoxan, an α2 adrenoceptor antagonist, potentiates the enhanced passive avoidance learning induced by AChE inhibitors (Camacho et al., 1996). This likely reflects elevated synaptic NE levels by a reduced presynaptic inhibition of NE release. Furthermore, combined (but not separate) administration of a β-adrenergic receptor antagonist and a muscarinic antagonist impaired learning on inhibitory avoidance performance and spatial learning in the Morris water maze (Decker et al., 1990). Finally, deprenyl, a monoamine oxidase inhibitor, substantially decreased the water maze escape latency in rats treated with tacrine, an AChE inhibitor (Dringenberg et al., 2000).

Treatments that augment cholinergic and adrenergic neurotransmission may improve the clinical status of patients with Alzheimer's disease (AD). Loss of neurons in the locus coeruleus appears to be a characteristic of AD that correlates with the severity of the dementia (Bondareff et al., 1981), and has not been reported in dementia caused by

multiple infarcts (Mann et al., 1982). Deprenyl has been reported to briefly improve cognition in a clinical AD trial (Wilcock et al., 2002). Indeed, the combination of L-deprenyl and physostigmine, an AChE inhibitor, has been shown to be more beneficial in patients with AD than physostigmine alone (Schneider et al., 1993). Clinical studies also have shown that galantamine had broad and sustained therapeutic effects in patients with mild to moderate AD, improving cognition, behavior and the ability to perform daily activities (Corey-Bloom, 2003). Although its mechanism of action has not been clarified, the inhibition of AChE may play a modest role in the efficacy of galantamine. This is suggested by the fact that galantamine is a weak AChE inhibitor compared to donepezil, yet treatment with galantamine yields comparable benefit. In two studies, galantamine produced a significant improvement in cognition as measured by the Alzheimer's Disease Assessment Scale (Wilcock et al., 2000; Raskind et al., 2000); and in a separate study, donepezil resulted in a similar degree of clinical improvement (Rogers et al., 1998). Based on the experiments reported herein, the APL effect of galantamine specifically on endogenous nicotinic cholinergic regulation of hippocampal NE secretion may be one mode whereby galantamine ameliorates some of the cognitive deficits in AD.

In contrast to the effect on hippocampal NE, galantamine did not alter the response of nucleus accumbens DA to nicotine at any dose or time interval. This may reflect two factors. *In vivo* microdialysis can only resolve neurochemical effects that occur on the time-scale of minutes. Thus, galantamine may have very brief effects on dopamine secretion, possibly at the DA terminal, (John Dani, Ph.D., unpublished observation) that may not yield sufficient DA for synaptic spill-over into the extracellular space, a

requirement for detection by microdialysis. Secondly, galantamine may interact with multiple nAChRs containing a variety of subunits expressed by neurochemically distinct neurons that regulate the activity of DA neurons intrinsic to the ventral tegmental area (some of these DA neurons project to nucleus accumbens). The relative impact of these phenotypically distinct neurons (i.e., glutamatergic and GABAergic) on the activity of DA neurons in response to nicotine (Schilstrom et al., 1998; Fu et al., 2000; Mansvelder et al., 2002) may be modified by galantamine, which may retard the desensitization of nAChRs (Maelicke et al., 2001). Under these circumstances, there may be no detectable net effect of galantamine, especially when the effect of nicotine is integrated across 15 min sampling intervals.

This study provides the first *in vivo* evidence that galantamine augments adrenergic neurotransmission within the hippocampus. Using *in vivo* microdialysis, the secretion of hippocampal NE, but not accumbal DA, was enhanced by galantamine. Thus, we postulate that galantamine may up-regulate noradrenergic neurotransmission in AD patients by augmenting the stimulative effects of endogenous nicotinic cholinergic circuits. This is supported by the fact that galantamine potentiated hippocampal NE secretion to sub-threshold doses of nicotine (i.e., 0.025 to 0.045 mg/kg). Since the loss of central noradrenergic function has been implicated in the progress of AD and noradrenergic agents may be beneficial in treatment, augmenting NE secretion may be one of multiple mechanisms underlying the therapeutic benefit of galantamine.

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Figure 1. Time course and dose-dependency of nicotine-stimulated hippocampal NE and accumbal DA release in the same animals. NE or DA levels are expressed as % of baseline levels obtained prior to nicotine (see Materials and Methods). Each animal received one i.v. infusion of nicotine (0.025 to 0.09 mg/kg). At doses of 0.025 or 0.045 mg/kg, nicotine alone had no effect on NE levels (panel A), whereas nicotine 0.065 and 0.09 mg/kg elevated NE levels (comparisons were made between peak responses at 60 min) [$F_{(3, 22)} = 16.61$; $P<0.001$]. Nicotine 0.025 mg/kg had no effect on peak DA levels (panel B), but peak DA responses to nicotine 0.045 mg/kg or higher were significantly greater than in saline controls [$F_{(3, 22)} = 13.96$; $P<0.01$]. * or **, $P<0.05$ or 0.01 compared to saline control group; #, $P<0.05$, comparison of nicotine 0.09 mg/kg to other effective doses; n=6-9 rats per treatment.

Figure 2. The effects of galantamine on nicotine-induced NE release in the hippocampus. Three hours prior to an infusion of nicotine (+45 min), rats received galantamine 3.0 mg/kg s.c. Pretreatment with galantamine potentiated NE responses (+60 min) to doses of nicotine that were ineffective alone (0.025 to 0.045 mg/kg; $P<0.05$ and 0.01, respectively, for comparison of galantamine/nicotine vs. saline/nicotine; panel A) and enhanced the NE response to nicotine 0.065 ($P<0.05$; panel B), but not to 0.09 mg/kg [$F_{(6,624)} = 12.64$, $P<0.001$]. In rats pretreated with saline or galantamine and then infused with 0.045 mg/kg nicotine or saline, the following baseline NE levels (mean ± SEM) were determined from the three samples obtained prior to administering nicotine or saline: 0.39 ±0.12 (saline/saline); 0.36 ± 0.10 pg/15 µl (galantamine/saline), 0.53 ± 0.11 pg/15 µl (galantamine/nicotine), 0.45 ± 0.15 pg/15 µl (saline/nicotine) [$F_{(2,16)} = 1.431$, $P=0.45$].

The following coefficients of variation for mean baseline NE values were calculated for all groups pretreated with either saline or galantamine: 0.15 or 0.19, respectively. Taken together, these absolute baseline NE values and the overall coefficient of variation for all groups pretreated with saline vs. galantamine demonstrate that baseline hippocampal NE levels, measured in samples obtained 2.5-3 h after the administration of galantamine, were not altered by the drug. * or ** are $P<0.05$ or 0.01, respectively, for comparisons between saline/nicotine and saline/saline; # or ## are $P<0.05$ or 0.01, respectively, for comparisons between galantamine/nicotine and saline/nicotine; n=6-9 rats per group.

Figure 3. Effect of galantamine pretreatment interval on peak hippocampal NE and accumbal DA responses to nicotine. Pretreatment with galantamine enhanced peak hippocampal NE (panel A), but not accumbal DA responses (panel B) to nicotine in the same animals. However, only the 3 h pretreatment with galantamine resulted in peak nicotine-stimulated NE levels that were higher than those in the galantamine/saline and saline/nicotine groups [$F_{(2,12)}=5.51$, $P<0.05$ for nicotine 0.025 mg/kg; $F_{(4,20)}=5.47$, $P<0.01$ for nicotine 0.045 mg/kg; $F_{(3,20)}=14.56$, $P<0.01$ for nicotine 0.065 mg/kg]. Panel B shows that peak nicotine-stimulated DA levels were not affected by galantamine pretreatment at any time interval. In rats given 0.045 or 0.065 mg/kg nicotine, peak DA levels in the galantamine/nicotine and saline/nicotine groups were higher than those in galantamine/saline rats [$F_{(3.17)}=8.28$, $P<0.01$ for nicotine 0.045 mg/kg; $F_{(2,16)}=5.35$, $P<0.05$ for nicotine 0.065 mg/kg]. In addition, baseline NAcc DA levels, measured in the 3 samples obtained before infusing nicotine, were not altered by galantamine [0.53 ± 0.11 pg/15 µl (galantamine/nicotine) versus 0.45 ± 0.15 pg/15 µl (saline/nicotine), $P>0.05$]. *

or ** are $P<0.05$ or 0.01, respectively, for comparisons of saline/nicotine and galantamine/nicotine vs. galantamine/saline; # or ## are $P<0.05$ or 0.01, respectively, for comparisons between galantamine/nicotine and saline/nicotine; n=6-9 rats per group

Figure 4. The effect of galantamine on the dose-response relationship between peak NE levels and nicotine. Galantamine sensitized the hippocampal NE response to nicotine without affecting the amount of NE released by a maximally stimulative dose of the drug (e.g., 0.09 mg/kg nicotine; a dose that induces a maximal increase in hippocampal NE levels without affecting behavior). In so doing, galantamine potentiated NE responses to doses of nicotine that were ineffective alone (0.025 to 0.045 mg/kg) and enhanced the NE response to nicotine 0.065. * or **, $P<0.05$ or 0.01 for comparisons between groups pretreated with saline versus galantamine.

Figure 5. The effect by donepezil on hippocampal NE and accumbal DA responses to nicotine. Pretreatment with donepezil, at a dose that results in comparable AChE inhibition to that induced by galantamine, had no effect on nicotine-induced NE secretion (panel A). Nicotine 0.065 mg/kg was used in order to achieve significant hippocampal NE release. There was no difference in peak NE levels found in the donepezil/nicotine or saline/nicotine groups; both treatments elevated NE levels in comparison to the donepezil/saline group [$F_{(2, 12)} = 9.96$; $P<0.01$]. Similar results were found with accumbal DA, in that donepezil did not modify DA responses to nicotine [$F_{(2, 12)} = 8.85$; $P<0.01$] (panel B). Baseline NE levels (mean ± SEM) were unaffected by donepezil pretreatment [0.33 ± 0.09 pg/15 µl (donepezil/saline) vs. 0.28 ± 0.09 pg/15 µl (donepezil/nicotine) vs.

0.18 ± 0.03 pg/15 µl (saline/nicotine); $F_{(2,12)}$= 1.542, P=0.261]. Combining the individual values of the donepezil/saline and donepezil/nicotine groups and then comparing these to the saline/nicotine group failed to show a difference in baseline NE levels (t=1.77, P=0.10). Baseline DA levels (mean ± SEM) were also unchanged by donepezil [0.22 ± 0.09 pg/15 µl (donepezil/saline) vs. 0.26 ± 0.09 pg/15 µl (donepezil/nicotine) vs. 0.19 ± 0.13 pg/15 µl (saline/nicotine); $F_{(2,14)}$= 1.031, P=0.823]. * or **, P<0.05 or 0.01, respectively, compared to donepezil/saline (n=4 rats per group).

Figure 1

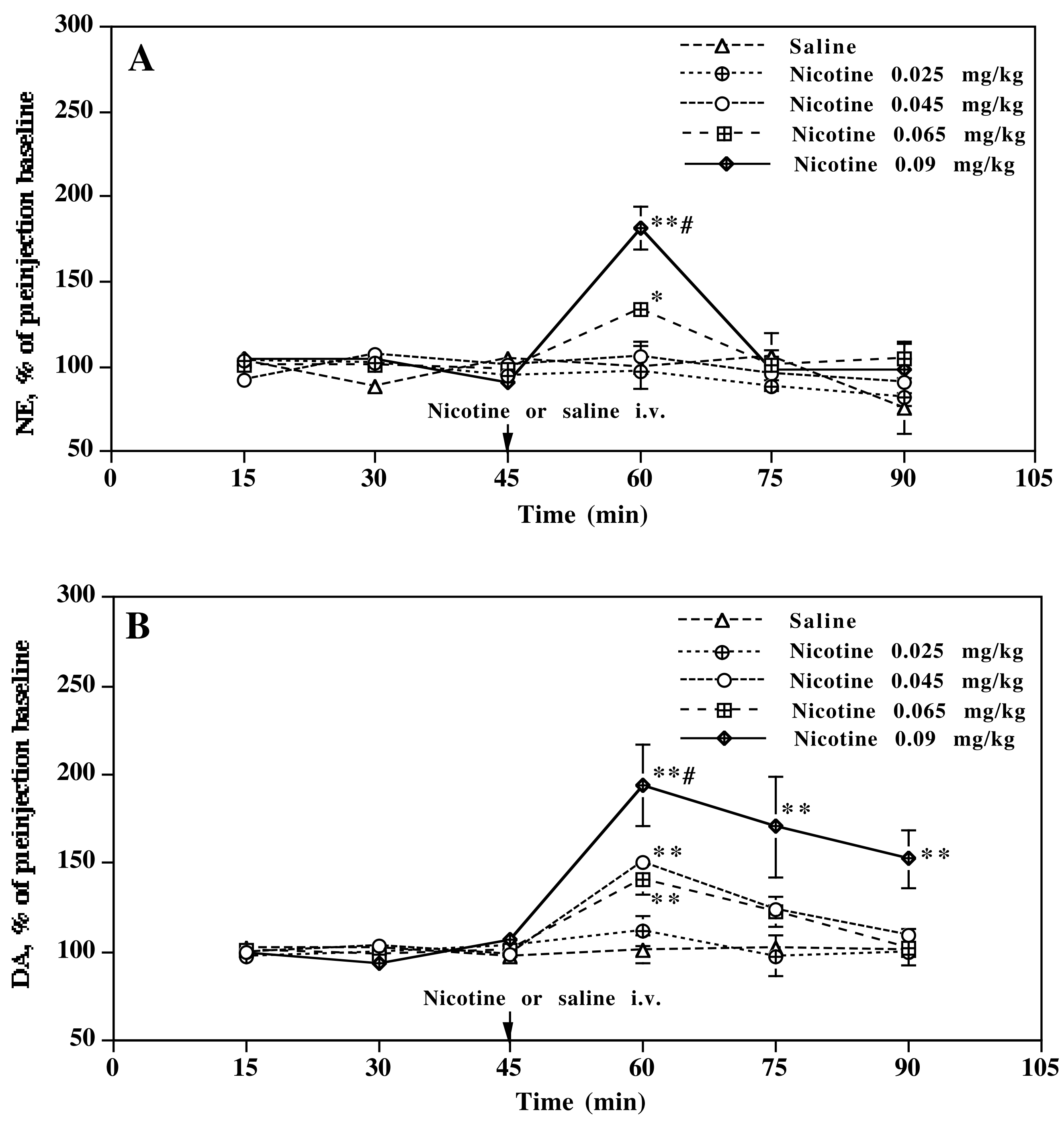


Figure 2

A

Saline/Saline
Galantamine/Saline
Saline/Nicotine 0.025
Galantamine/Nicotine 0.025
Saline/Nicotine 0.045
Galantamine/Nicotine 0.045

**##
*#

NE, % of preinjection baseline
230
200
170
140
110
80
50

Nicotine or saline i.v.

0 15 30 45 60 75 90 105
Time (min)

B

Saline/Saline
Galantamine/Saline
Saline/Nicotine 0.065
Galantamine/Nicotine 0.065
Saline/Nicotine 0.09
Galantamine/Nicotine 0.09

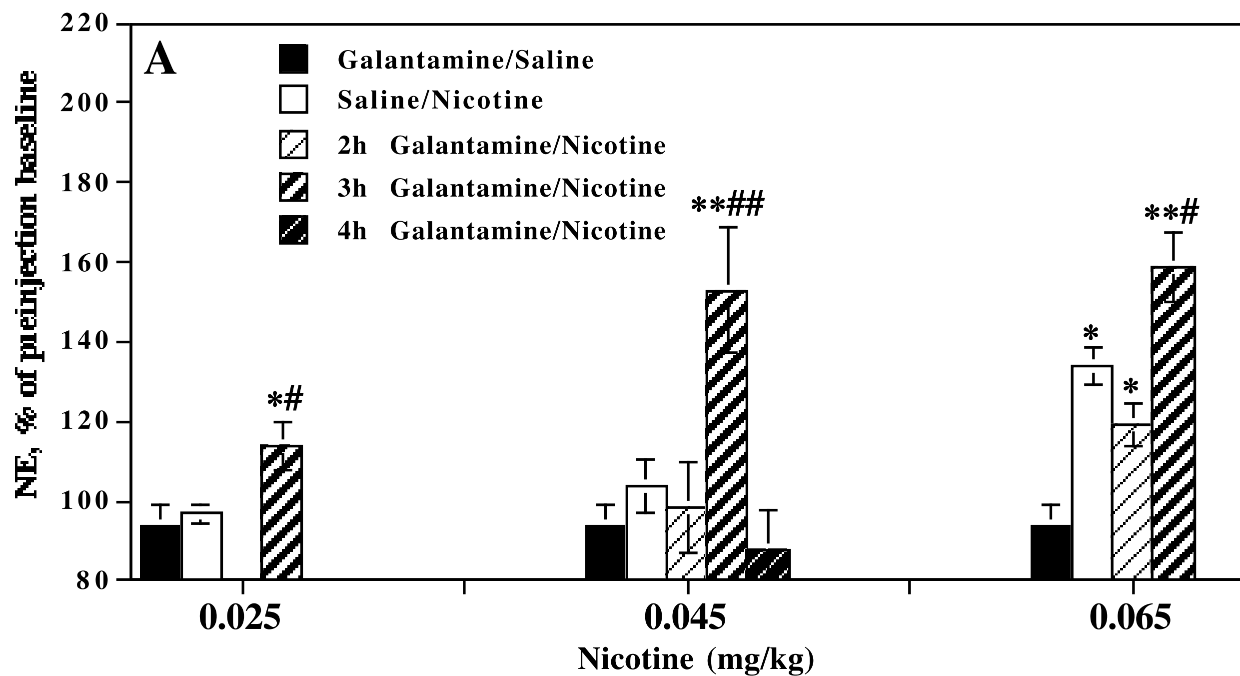
**
**
**#
*

NE, % of preinjection baseline
290
250
210
170
130
90
50

Nicotine or saline i.v.

0 15 30 45 60 75 90 105
Time (min)

Figure 3



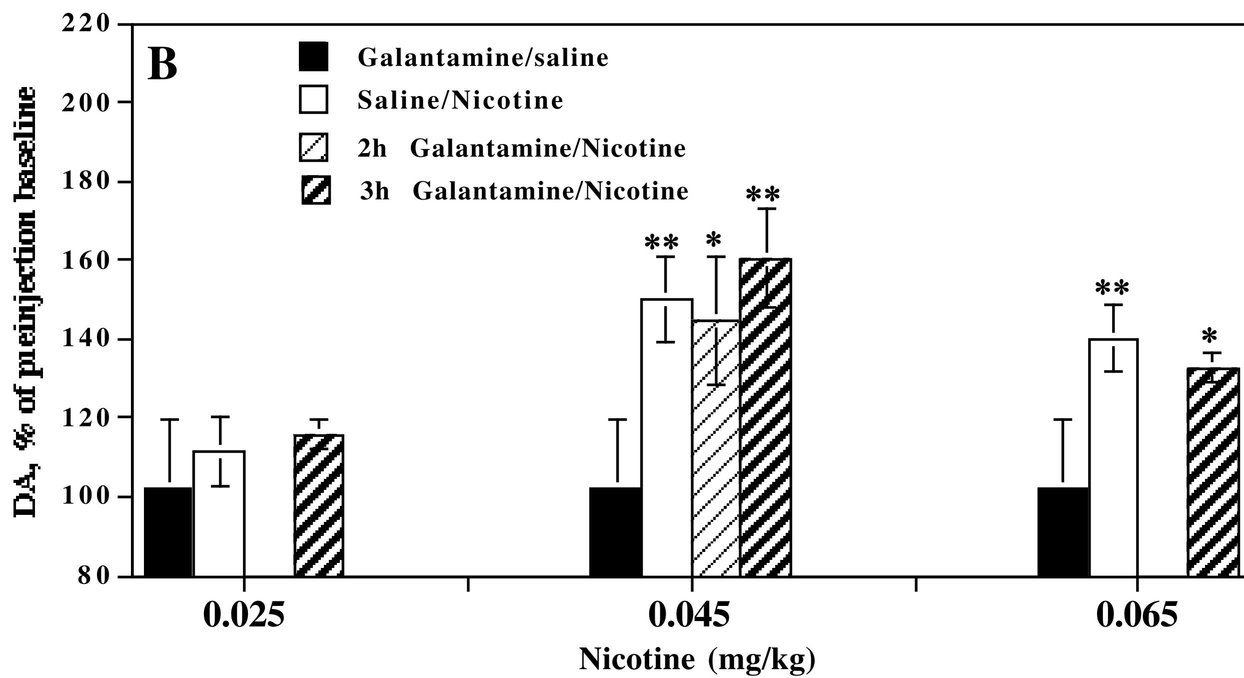


Figure 4

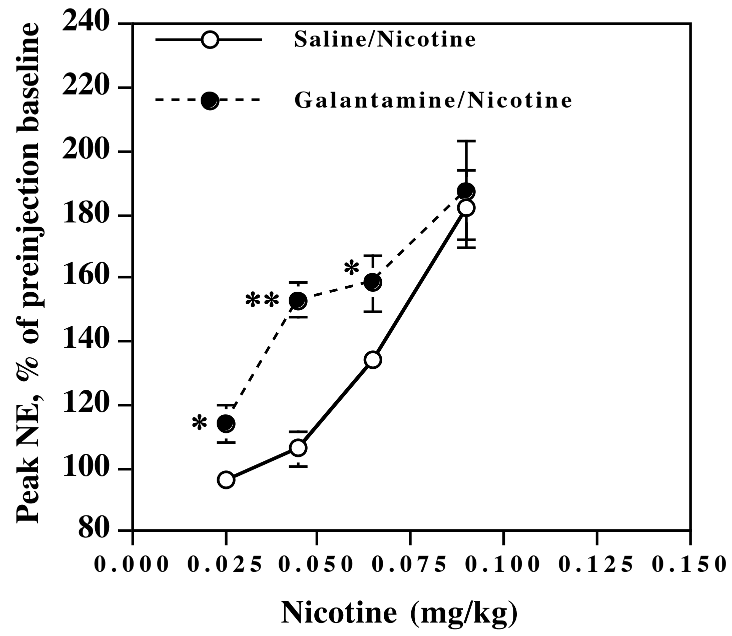


Figure 5