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Effects of Local and Repeated Systemic Administration of (−)Nicotine on Extracellular Levels of Acetylcholine, Norepinephrine, Dopamine, and Serotonin in Rat Cortex

K. L. Summers[1] and E. Giacobini[1,2]

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Systemically administered (−)nicotine (0.2-1.2 mg/kg, s.c.) significantly increased the release of acetylcholine (ACh), norepinephrine (NE) and dopamine (DA) in rat cortex. The lowest dose of (−)nicotine examined (0.2 mg/kg, s.c.) also significantly elevated extracellular serotonin (5-HT) levels, and the maximal increases of extracellular ACh (122% at 90 min post injection) and DA levels (249% at 120 min post-injection) were observed following this dose. In contrast, the maximal increase of NE release (157% at 30 min post-injection) was observed following the highest dose of (−)nicotine injected (1.2 mg/kg, s.c.). This higher dose consistently produced generalized seizures. Repeating the (−)nicotine (0.58 mg/kg, s.c.) injection four hours after the first administration significantly elevated extracellular NE levels and also appeared to increase DA and ACh release. In addition, extracellular ACh and DA levels increased significantly in the dialysate after (−)nicotine was administered directly to the neocortex through the microdialysis probe membrane. Norepinephrine levels appeared to be elevated in the cortex following local administration as well.

KEY WORDS: Neurotransmitters; cortex; nicotine; microdialysis.

INTRODUCTION

Treatment strategies employing nicotinic agonists have been proposed for a variety of disorders including Parkinson's disease (PD), Alzheimer disease (AD), attention deficit disorder (ADD), ulcerative colitis (1), schizophrenia and Tourette's syndrome (2). Characterization of the responses evoked by nicotine administration may lead to a better understanding of the pharmacology of this drug and of the cholinergic aspects of these disorders.

Currently, much attention is being focused on the possible use of nicotinic agonists in the treatment of AD. Nicotine has been reported to alleviate memory deficits seen in aged rats (3) and monkeys (4) implicating the importance of nicotinic acetylcholine receptors (nAChR) in cognitive function. Loss of these receptors in aging humans has been related with an accelerated decay apparent in AD (5–7). Preliminary studies of nicotine administration to AD patients appear encouraging as improvements in cognitive tasks, particularly attentional ones, have been reported (8–11). The basis for this improvement would appear to be related to increased functional synaptic transmission of the cholinergic system. Working memory, or memory of information that changes from trial to trial and which may be analogous to recent memory in humans, has been suggested to involve the cholinergic neurons of the basal forebrain and their cortical projections (12). This system is severely impaired in AD with marked cell loss in the nucleus basalis, concurrent with loss of cholinergic synapse

[1] Department of Pharmacology #1222 Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, Illinois 62794-9230. Phone: 217/785-2184 Fax: 217/524-0145 Email: EGIACOBINI@WPSMTP.SIUMED.EDU

[2] To whom to address reprint requests.

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markers such as choline acetyltransferase (ChAT) (13). Impairment of other neurotransmitter systems is also seen in AD and interactions between the dopaminergic (14,15) and serotonergic (16) systems and nicotine with regard to working memory function have been observed.

Few investigations have addressed the effects of nicotine on cortical neurotransmitter release in vivo. Two groups have published microdialysis studies indicating that systematically administered (−)nicotine (0.50-0.58 mg/kg, s.c.) significantly increases cortical extracellular acetylcholine (ACh) (17,18). Little information is available regarding systemic effects of nicotine on other cortical neurotransmitters. Toth et al. (18) examined responses of nicotine administered in the cortex through the dialysis probe. Elevated levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were observed. We have preliminarily reported increased cortical extracellular levels of NE and DA following subcutaneous nicotine administration (17). Nicotine administered directly to the cortex also appears to increase extracellular glutamate levels (20).

In this study, we have determined the effects of subcutaneously administered (−)nicotine (0.2-1.2 mg/kg) on extracellular levels of ACh, DA, 5-HT and NE in the frontoparietal cortex of the rat under awake and freely-moving conditions. As the nAChR is known to desensitize (21), a second injection of (−)nicotine was administered four hours after the initial injection to determine the effects of consecutive repeated injections. (−)Nicotine was also administered directly into the cortex through the dialysis probe membrane to examine the role of cortical nAChR in the observed responses.

EXPERIMENTAL PROCEDURE

Mecamylamine hydrochloride and (−)nicotine free base were purchased from Sigma Chemical Co. (St. Louis, MO). Drugs were dissolved in saline and injected subcutaneously in a volume of 2.0 ml/kg or added to the dialysis probe perfusing solution. All concentrations (0.2 mg = 1.2 μmol/kg, 0.58 mg = 3.6 μmol/kg, 1.2 mg = 7.2 μmol/kg) are expressed as free base of the drug.

Surgery and Microdialysis. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 250-400 g were anesthetized with sodium pentobarbital (62.5 mg/kg, i.p.) and placed in a Kopf stereotaxic apparatus with the incisor bar set at 3.3 mm below the interaural line. AN69HF dialysis fibers (Hospal, Meyzieu, France) with an outer diameter of 340 μm were inserted transversely into the cortex [coordinates A +1.0, V −2.0, measured from bregma, according to the atlas of Paxinos and Watson (22)] through the trephined holes in each temporal bone. Dialysis was confined to the cortex by completely covering the fiber with epoxy (Boston, Milan, Italy) with the exception of the 8 mm portion within the cortical tissue. Both ends of the dialysis fiber were inserted into small steel tubes which were secured vertically to the skull by dental cement attached to bone screws. PE-10 tubing (internal diameter = .28 mm) was connected to the steel tubes and Ringer's solution (NaCl 147 mM; KCl 4.0 mM; $CaCl_2$ 2.3 mM) was passed through the probe. Following surgery, the rats were allowed to recover for at least 16 hours prior to experiments.

On the morning following surgery, the probes were perfused with Ringer's solution at 1.1 μl/min for at least 90 min before collection of dialysate. No cholinesterase inhibitors (ChEI) or other drugs were added to the perfusate to inhibit neurotransmitter degradation or reuptake. Fractions of the dialysate were collected every 30 min into 250 μl vials containing 2 μl acetic acid (1.0 N) to prevent degradation of the catecholamine. The vials were kept at 4°C in a refrigerated fraction collector (CA 200, Bioanalytical Systems, West Lafayette, IN) prior to analysis. Six baseline samples were collected, and control levels were defined as an average of these baseline vials. Corrections were made to account for dead volume. After the experiments, the brains were dissected and the location of the probe in the cortex verified by visual examination.

Analysis of Dialysate. The collected samples were divided into two aliquots to determine the neurotransmitter levels on two separate high performance liquid chromatography (HPLC) systems. For analysis of ACh, 15 μl of the dialysate was injected across an ACh-3 analytical column (150 × 3 mm; Environmental Sciences Associates, Inc. Bedford, MA) coupled to a post-column immobilized enzyme reactor (Bioanalytical Systems) containing acetylcholinesterase and choline oxidase. Consequently, electrochemically active hydrogen peroxide was detectable on a PEEK/platinum electrode. The columns were maintained at a constant temperature of 35°C. A Coulochem II electrochemical detector (Environmental Sciences Associates, Inc.) was employed with the electrode potential set at +300 mV. The mobile phase consisted of 100 mM NaH_2PO_4, 0.5 mM tetramethylammonium chloride, 150 μM EDT, 0.0005% Reagent MB (a microbicide, Environmental Sciences Associates, Inc.), and 2.0 mM 1-octanesulfonic acid sodium salt (pH = 8.0), and was passed through the system at a flow rate of 0.35 ml/min by an ESA-580 pump (23).

The amount of NE, DA, and 5-HT in the collected fractions was determined on a Coulochem II electrochemical detector (Environmental Sciences Associates, Inc.) equipped with dual porous graphite electrodes. The first potential was established at −40 mV, and the second was set at a potential of +320 mV. A guard cell set at a potential of +400 mV preceded an HR-80 analytical column (8 mm, 3 μm particles; Environmental Sciences Associates, Inc.). The mobile phase contained 75 mM NaH_2PO_4, 1.0 mM sodium dodecyl sulfate, 100 μM EDTA, 1.48 mM triethylamine, 13% methanol and 15% acetonitrile (pH = 5.6), and was passed through the system at a flow rate of 1.0 ml/min by a Shimadzu LC-10AD pump.

Peaks were displayed, integrated, and stored with Kontron Instruments Data System 450. Quantification of all substances was made by comparing peak areas of the samples to a standard curve.

Statistics. Paired *t*-tests were used to assess the differences between baseline (6 points) and post-injection time points within rats. $P < 0.05$ (*) and $P < 0.01$ (**) considered significant. Results are expressed as the mean ± SEM.

RESULTS

Basal ACh concentrations in the dialysate averaged 2.91 ± 0.40 nM (mean ± SEM; n = 19). As seen in Fig. 1, systemic administration of (−)nicotine (0.2 mg/kg, s.c.) significantly elevated extracellular ACh levels

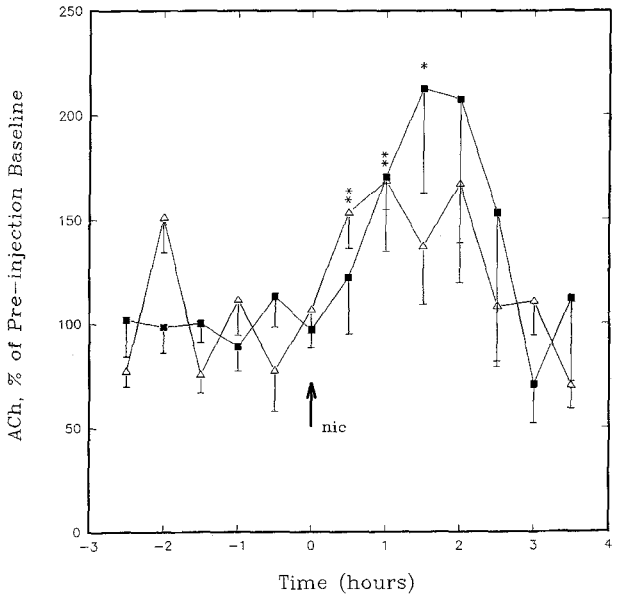

Fig. 1. Effects of a low systemic dose of (−)nicotine (0.2 mg/kg, s.c.; squares) and a higher dose of (−)nicotine (1.2 mg/kg, s.c.; triangles) on extracellular levels of ACh in rat cortex. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to injection = 100%); mean ± SEM; n = 7 for the low dose and n = 4 for the high dose. *P < 0.05 by paired Student *t*-test analysis.

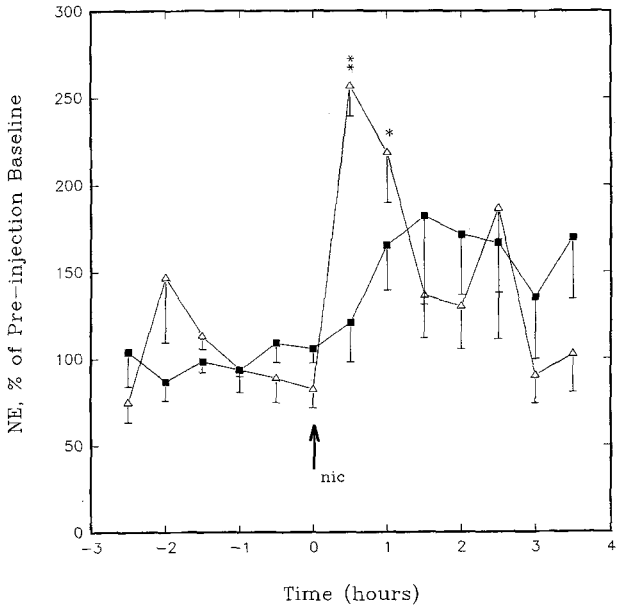

Fig. 2. Effects of a low systemic dose of (−)nicotine (0.2 mg/kg, s.c.; squares) and a higher dose of (−)nicotine (1.2 mg/kg, s.c.; triangles) on extracellular levels of NE in rat cortex. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to injection = 100%); mean ± SEM; n = 7 for the low dose and n = 4 for the high dose. *P < 0.05 by paired Student *t*-test analysis.

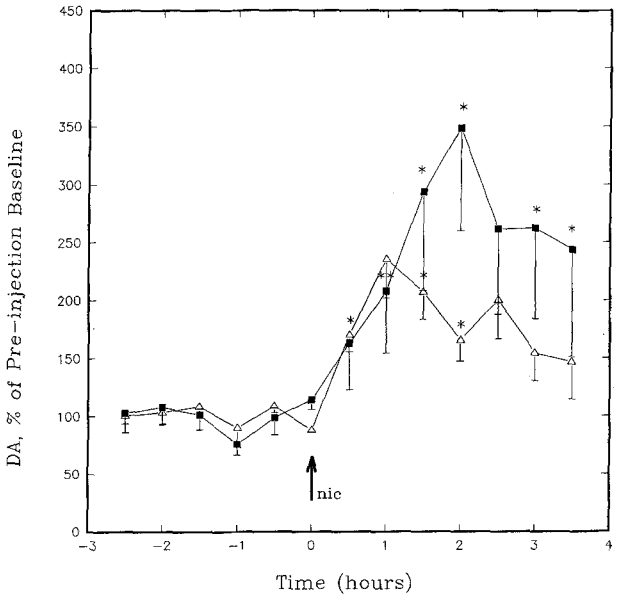

Fig. 3. Effects of a low systemic dose of (−)nicotine (0.2 mg/kg, s.c.; squares) and a higher dose of (−)nicotine (1.2 mg/kg, s.c.; triangles) on extracellular levels of DA in rat cortex. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to injection = 100%); mean ± SEM; n = 7 for the low dose and n = 4 for the high dose. *P < 0.05 by paired Student *t*-test analysis.

in frontoparietal cortex, reaching a maximum of 122% above basal levels 90 min after injection. A higher dose of (−)nicotine (1.2 mg/kg, s.c.) also significantly elevated ACh levels, but with a lower peak effect (69% at 60 min).

Basal NE concentrations in the dialysate averaged 1.55 ± 0.20 nM (mean ± SEM; n = 19). As shown in Fig. 2, systemic administration of (−)nicotine (0.2 mg/kg, s.c.) significantly increased NE levels in the dialysate 68% over basal values for at least 60 min post-injection. Increasing the amount of (−)nicotine administered also increased the release of NE, as 1.2 mg/kg, s.c. increased the levels by 157% within the first 30 min after injection.

Basal DA concentrations in the dialysate averaged 0.76 ± 0.13 nM (mean ± SEM; n = 19). (−)Nicotine (0.2 mg/kg, s.c.) significantly increased extracellular levels of DA, reaching a maximum of 249% at 2 hours post-injection. A higher dose of (−)nicotine (1.2 mg/kg, s.c.) also significantly elevated extracellular DA levels in rat frontoparietal cortex, but the maximal effect was achieved at 1 hour and was only 136% above basal values (Fig. 3).

Basal concentrations of 5-HT in the dialysate averaged 1.46 ± 0.22 nM (mean ± SEM; n = 19). No significant alterations of extracellular levels of this neu-

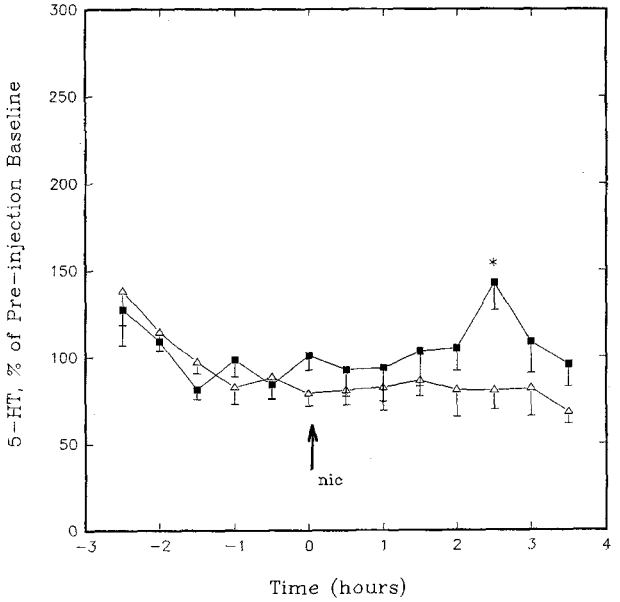

Fig. 4. Effects of a low systemic dose of (−)nicotine (0.2 mg/kg, s.c.; squares) and a higher dose of (−)nicotine (1.2 mg/kg, s.c.; triangles) on extracellular levels of 5-HT in rat cortex. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to injection = 100%); mean ± SEM; n = 7 for the low dose and n = 4 for the high dose. *P < 0.05 by paired Student *t*-test analysis.

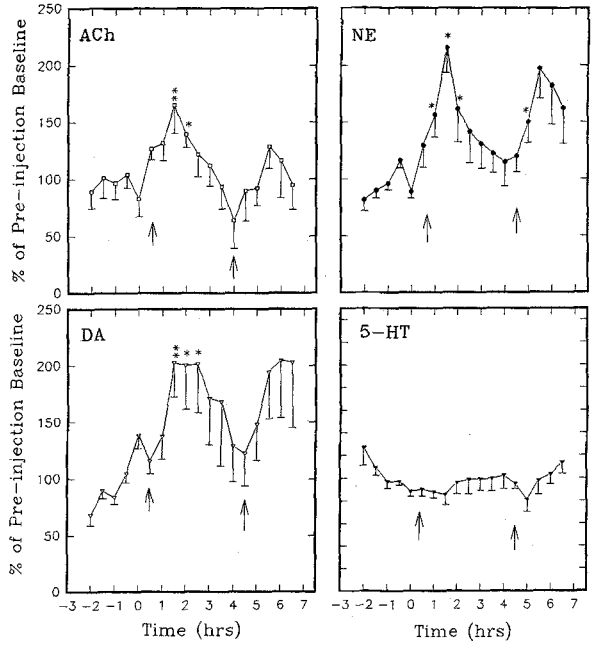

Fig. 5. Effects of (−)nicotine (0.58 mg/kg, s.c.) injection, followed by a repeated administration of the same dose four hours later, on extracellular levels of ACh (square), NE (circle), DA (empty triangles) and 5-HT (filled triangles) in rat cortex. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to first injection = 100%), mean ± SEM; n = 8. *P < 0.05 by paired Student *t*-test analysis.

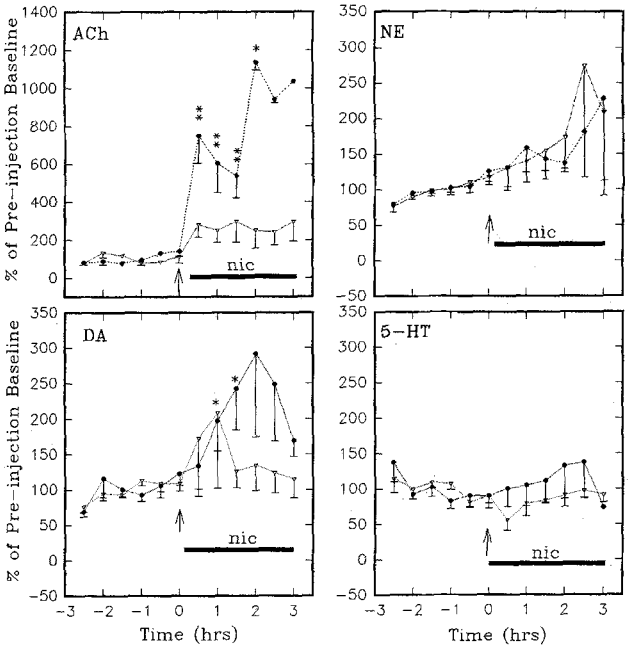

Fig. 6. Effects of (−)nicotine administered to the neocortex through the dialysis probe (250 μM; triangles and 2.5 mM; circles) on extracellular levels of ACh, NE, DA, and 5-HT. Data are expressed as a percentage of the pre-injection control levels (average of six samples prior to first injection = 100%), mean ± SEM; n = 5 for each group. *P < 0.05 by paired Student *t*-test analysis.

rotransmitter in the rat cortex were observed following a high dose of (−)nicotine (1.2 mg/kg, s.c.). However, a slight but significant increase in 5-HT levels was observed with a lower dose (0.2 mg/kg, s.c.). At 2.5 hours after the injection, extracellular levels of 5-HT were 36% above basal values (Fig. 4).

A first systemic injection of (−)nicotine (0.58 mg/kg, s.c.) significantly increased cortical extracellular levels of ACh to a maximum of 65% at 60 min, of NE to a maximum of 115% at 60 min, and of DA to a maximum of 103% at 60 min. No changes in extracellular levels of 5-HT were observed. In the same animals, a second injection of the same dose of [−]nicotine (0.58 mg/kg, s.c.) administered four hours after the first dose significantly increased NE levels by 50% within 30 min post-injection. No significant increases of extracellular levels of ACh, DA or 5-HT were seen after the second dose. However, as shown in Fig. 5, there was an obvious trend to an increase of both ACh and DA.

Upon the addition of (−)nicotine (2.5 mM) to the perfusing Ringer's solution, ACh and DA release in the rat cortex increased significantly to a maximum of 649% and 143% respectively (Fig. 6). A lower dose of (−)nicotine (250 μM) also appeared to increase the extracellular levels of these neurotransmitters, but the observed

effects did not reach statistical significance. Similarly, NE appeared to increase in response to both doses of (−)nicotine administered. No effects on 5-HT levels were visible.

DISCUSSION

Subcutaneous (−)nicotine administration (0.2 – 1.2 mg/kg, s.c.) significantly increased the cortical release of ACh, NE and DA in vivo. A delayed increase in extracellular levels of 5-HT was also observed. These effects appeared to be reproducible following a second (−)nicotine challenge with the exception of 5-HT. However, statistical significance was reached only with NE levels.

The elevation of cortical extracellular NE in response to systemic (−)nicotine appeared to be dose-dependent. Conversely, the increase in extracellular levels of ACh and DA did not appear to be dose-dependent. A previous study from this laboratory has shown that these cortical responses to systemic (−)nicotine administration (0.58 mg/kg, s.c.) are receptor-mediated, as mecamylamine antagonizes the effects. Basal release is also calcium dependent and tetrodotoxin (TTX) sensitive suggesting a relation to neuronal function. No effects of subcutaneous saline injection on cortical neurotransmitter levels were observed (17).

The ability of (−)nicotine to increase ACh and DA levels in the cortex after subcutaneous injection would appear to be maximal at the lowest dose investigated (0.2 mg/kg, s.c.), a dose within the range found to improve memory performance in rats (24). (−)Nicotine at this lower dose (0.2 mg/kg, s.c.) is thought to result in a plasma concentration that resembles that of smoking human subjects (25). This could be a clinically relevant dose as this level of nicotine is well tolerated by human subjects while appearing to have centrally mediated effects.

The highest dose of (−)nicotine (1.2 mg/kg, s.c.) employed in this study consistently produced seizures. No seizures were observed in any of the rats administered the lower (−)nicotine dosages. For clinical applications it would be ideal to find nicotinic agonists that improve cognitive function without this toxicity.

Our experiments were performed with no drugs designed to inhibit degradation or re-uptake of neurotransmitters added to the dialysate probe perfusing solution. Previous studies have indicated that artifactual responses may be elicited by local application through the probe of agents designed to increase collected levels of neurotransmitters (26). Most prior investigations of ACh release in the cortex of the intact animal employed a ChEI, such as physostigmine, to prevent degradation and thus increase ACh to detectable levels. Evidence indicates a distinct site on the nAChR that binds physostigmine (27,28), and it appears to interact with the receptor as an agonist capable of inducing desensitization and blockade (29,30). Many investigators employ biogenic amine uptake inhibitors to increase the amount of these neurotransmitters in order to reach detectable levels. It is also possible that artifactual responses could result from manipulating the normal levels of biogenic amines in the brain.

The possible sites of action of the nAChR responsible for the effects on release reported here may be multiple. Local application of (−)nicotine into the cortex significantly increased ACh and DA release and appeared to increase NE release as well. Upon systemic administration, the increased cortical levels of ACh, NE, and DA could be due to direct local action presynaptically in the cortex and by subcortical activation of nAChR present on cortically projecting neurons in the nucleus basalis magnocellularis/Meynert (nbm) (31), locus coeruleus (LC) (32,33) and ventral tegmental area (VTA) (34), respectively. The increase in cortical extracellular levels of 5-HT reported is likely due to direct effects of nicotine on nAChR located on cortically projecting cell bodies in the dorsal raphe nuclei (DRN) as nicotine binding sites have not been observed on serotonergic axons in the cortex (35), and no change in 5-HT levels was observed when (−)nicotine was applied directly to the cortical tissue.

Indirect effects, such as interactions with other neurotransmitter systems, are possible as well. Pyramidal cell bodies located in the cortex have been immunocytochemically labeled with antibodies to nAChR subunit proteins (6,34,36,37), and nAChR subunit transcripts are present in cortical neurons (38). A number of nAChR appear to be present on glutamatergic terminals arising from the thalamus (39,40), and nicotine has been reported to potentiate cortical glutamatergic excitatory post-synaptic potentials (EPSP) (41).

(−)Nicotine's effects on noradrenergic, dopaminergic, and cholinergic systems in this study may correlate with behavioral aspects of nicotine administration. Nicotine has been shown to improve vigilance in animals with cognitive deficits due to cholinergic forebrain degeneration. Effects on selective attention appear to be related to interactions with the ascending mesolimbic dopaminergic system (42). Resistance to extinction attributed to nicotine results from effects on the ascending noradrenergic system innervating the cortex through the dorsal noradrenergic bundle (43).

Nicotinic agonists have been proposed as a therapeutic strategy to provide symptomatic relief for patients

suffering from a variety of disorders including AD, PD, ADD, Tourette's syndrome, ulcerative colitis, anxiety, and even obesity. Discerning the neuropharmacological effects that result from systemic administration of (−)nicotine in awake, freely-moving animals may help to correlate improvement in symptoms with physiological responses. Selective nicotinic agonists that affect subtypes of this receptor, allowing fewer side effects while maintaining beneficial therapeutic results, may thus eventually be developed.

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