Nicotine-Induced Norepinephrine Release in the Rat Amygdala and Hippocampus is Mediated through Brainstem Nicotinic Cholinergic Receptors

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ABSTRACT

Previous studies have shown that nicotine stimulates norepinephrine (NE) release in the rat hypothalamic paraventricular nucleus, which in turn activates the hypothalamo-pituitary-adrenal axis. In the present study, nicotine induced NE release in the amygdala (AMYG) and the hippocampus (HP) of the same rat in vivo. Nicotine (0.065–0.135 mg/kg i.v. at a rate of 0.09 mg/kg/60 sec) dose-dependently increased NE release at both sites with similar potencies. To determine whether the site of action of nicotine is in the brainstem, which contains the noradrenergic cell bodies projecting to AMYG and HP, nicotinic cholinergic receptor (NACHR) antagonists were injected into the cerebral aqueduct before i.v. nicotine. Use of the following antagonists enabled partial characterization of the NACHRs mediating NE secretion: mecamylamine (Mec), dihydro-β-erythroidine (DHβE), methyllycaconitine (MLA) and α-bungarotoxin (α-BTX). Mec inhibited 80% of NE release in AMYG and 87% in HP (IC₅₀ = 6 nmol for both regions). DHβE blocked 62% of NE release in AMYG (IC₅₀ = 8 nmol) and 63% in HP (IC₅₀ = 16 nmol). Similar to DHβE, MLA inhibited 60% of NE release in AMYG and 66% in HP (IC₅₀ = 5 nmol for both regions). In contrast, α-BTX had no effect on NE release in either region. These results indicate that brainstem NACHRs accessible from the fourth ventricle mediate nicotine-stimulated NE secretion in AMYG and HP. Taken together with prior investigations showing the brainstem expression of mRNAs encoding NACHR subtypes and the selectivity of antagonists for NACHR subtypes, the present studies suggest that brainstem alpha-3 subunit may be involved.

Nicotine is a psychoactive component in cigarette smoke that affects many physiological functions of the CNS. By stimulating brainstem noradrenergic and peptidergic pathways, nicotine activates the HPA axis, which leads to the secretion of stress-responsive hormones (Sharp and Beyer, 1986; Matta et al., 1990; Valentine, et al., 1996; Fu et al., 1997). Memory-enhancing effects of nicotine also have been reported in both human and animal studies (Warburton, 1990; McGehee and Role, 1996). Indeed, a loss of NACHRs was found in Alzheimer’s disease (Schroder et al., 1991), and the administration of intravenous nicotine to these patients appeared to improve memory transiently (Newhouse et al., 1988, 1990).

The central noradrenergic system is involved in stress-related responses and memory function (Bremner et al., 1996). Most CNS noradrenergic cell bodies are located in the LC, although a large group also are found in the NTS-A2 and the ventromedullary A1 region (Holets, 1990; Aston-Jones et al., 1995). These central noradrenergic neurons innervate brain regions such as the AMYG, HP, hypothalamus and frontal cortex, which also are anatomical substrates for stress responses and/or memory function (Holets, 1990; Bremner et al., 1996). Systemic nicotine stimulates NE release in vivo in the rat hypothalamic PVN (Sharp et al., 1993; Matta et al., 1995; Fu et al., 1997), HP (Brazell et al., 1991; Mitchell, 1993) and the cerebral cortex (Summers and Giacobini, 1995).

The amygdaloid complex is one of the limbic system structures which facilitate HPA axis responses (Feldman and Weidenfeld, 1996) and govern a wide array of autonomic functions. In addition, AMYG plays an essential role in emotional memory (Cahill et al., 1996; LeDoux, 1992; Izquierdo and Medina, 1995; Maren, 1996), in working memory performance (Ohno et al., 1993) and in the regulation of the storage of memory by other brain regions (McGaugh et al., 1990; Galvez et al., 1996). Evidence indicates that the release of NE

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is involved in both AMYG-mediated HPA responses (Feldman and Weidenfeld, 1996) and memory function (Intorini-Collison et al., 1996; McGaugh et al., 1988; Cahill and McGaugh, 1996). However, no studies have examined the effect of nicotine on NE release in the AMYG.

The HP is another limbic region known for its role in memory development (Lee et al., 1993) and HPA responses (Feldman et al., 1995). Direct connections between the HP and AMYG may be important in the limbic memory system (Saunders et al., 1988; Izquierdo and Medina, 1995). In addition, studies have shown that NE is involved in hippocampal functions that enhance memories of inhibitory avoidance and spatial habituation (Izquierdo et al., 1992; Izquierdo and Medina, 1995). It has been reported that systemically administered nicotine stimulates NE release in the HP and is sensitive to antagonist blockade (Mitchell, 1993). In that study, only Mec, an NAchR antagonist with limited specificity (Olney et al., 1978; Clarke et al., 1994), was tested. Thus, the subtype(s) of NAchRs mediating systemic nicotine-induced NE release in the HP is not known.

Neuronal NAchRs are pentameric receptors consisting of alpha (agonist binding) and beta subunits. To date, eight different alpha subunits (alpha 2 to 9) as well as four beta subunits (beta 2 to 5) have been identified (Lukas, 1995; McGeehe and Role, 1995; Vidal, 1996). Distinct receptor subtypes, consisting of various combinations of alpha and beta subunits, have been shown to co-exist in many brain regions, including the AMYG and HP (Wada et al., 1989; Flores et al., 1992; Rubboli et al., 1994). Several nicotinic antagonists have been described which are suitable for in vivo pharmacological investigations of these subtypes. Mec is, perhaps, the most commonly used antagonist. It is an ion channel blocker (Varanda et al., 1985) and is most effective at alpha-3 beta-4 receptors (Cachelin and Rust, 1995; Alkondon and Albuquerque, 1993). DHβE, a competitive antagonist, is most effective at alpha-4 beta-2 receptors (Luetje et al., 1990; Alkondon and Albuquerque, 1993). DHβE inhibits nicotine-elicted excitatory amino acid release in spinal cord (Khan et al., 1996) and reduces the number of infusions of self-administered nicotine (Corrigall et al., 1994). MLA, a toxin isolated from Delphinium sp., is another competitive NAchR blocker. It potently blocks α-BTX-sensitive alpha-7-containing NAchR at nanomolar concentrations (Alkondon et al., 1992). In contrast, micromolar concentrations of MLA are required to inhibit the response to α-BTX-insensitive (non-alpha-7) receptors (Alkondon and Albuquerque, 1993). Therefore, multiple antagonists can be used to obtain pharmacological evidence for the subtype(s) of NAchRs(s) that is involved in nicotine-stimulated NE release in specific regions of the brain.

In the present studies, NE release in AMYG and HP was detected concurrently in the same rat by in vivo microdialysis. Initial experiments were performed to establish dose-response relationships for NE release in both the AMYG and HP in response to i.v. infusions of nicotine. Then, experiments were performed to determine whether nicotine acts through receptors located in the brainstem, which harbors the noradrenergic cell bodies that project to the AMYG and HP. The NAchR subtypes mediating NE secretion in the AMYG and HP were characterized pharmacologically by determining the relative efficacies and potencies of the following NAchR antagonists: Mec, DHβE, MLA and α-BTX.
data were analyzed only from rats with blue in the fourth ventricle and none in the surrounding tissue.

**HPLC-electrochemical analysis.** Dialysis samples (16 μl) were immediately injected by a CMA 200 refrigerated autosampler onto a 150 × 3 mm ODS C18 column (ESA Inc., Chelmsford, MA) perfused by BAS 200A HPLC pumps at 0.5 ml/min with a mobile phase consisting of 80 mM sodium dihydrogen phosphate monohydrate, 2.0 mM 1-octanesulfonic acid sodium salt, 100 μl/μl triethylamine, 5 mM EDTA and 10% acetonitrile, pH 3.0. Samples were analyzed by an ESA Coulochem II 5200A electrochemical detector with an ESA 5041 high-sensitivity microbore analytical cell and an ESA 5020 guard cell (ESA). Electrochemical detection was performed at 220 mV and 1.0 nA with the guard cell at 350 mV. The limit of detection for NE was 0.5 pg.

**Experimental protocols.** A preliminary experiment was performed to determine the stability of both the basal NE levels and the responses to nicotine with repeated testing of each rat with a single probe. For each day's experiment, three consecutive preinfusion (basal) microdialysis samples were each collected for 20 min, and then nicotine was infused i.v. at 0.135 mg/kg for 90 sec, whereas dialysates were collected continuously at 20-min intervals for 40 min. This procedure was repeated on d3 and d5 in the same cohort of rats. The results showed that basal levels of NE in AMYG were reduced significantly on d3 and d5 compared with d1: 4.4 ± 0.5 pg/16 μl (mean ± S.E.M.) on d1, 2.5 ± 0.4 pg/16 μl on d3 (P < 0.05 compared with d1) and 2.3 ± 0.4 pg/16 μl on d5 (P < 0.01 compared with d1). However, no significant difference was observed between d3 and d5. Similar results were found in HP: 6.6 ± 0.5 pg/16 μl on d1, 4.2 ± 0.7 pg/16 μl on d3 (P < 0.05 compared with d1) and 3.6 ± 0.4 pg/16 μl on d5 (P < 0.01 compared with d1). In response to nicotine, the peak NE levels of NE were 7.8 ± 0.9 pg/16 μl on d1, 5.9 ± 0.6 pg/16 μl on d3 and 4.8 ± 0.5 pg/16 μl on d5. In HP, they were 13.8 ± 1.3 pg/16 μl on d1, 9.0 ± 1.1 pg/16 μl on d3 and 7.9 ± 0.8 pg/16 μl on d5. NE levels on d3 and d5 were lower than those detected on d1 in both regions (P < 0.05 compared with d1 for both brain regions). These measurements indicate that basal NE levels and NE responses to nicotine were stable between d3 and d5 in each region. Therefore, in all subsequent experiments, on d1 a probe was inserted for 10 min and removed thereafter without further microdialysis (sham microdialysis). On d3 and d5, probes were reinserted and rats received randomized treatments.

The second experiment was conducted to determine the dose-response relationship for nicotine-induced NE secretion in AMYG and HP. Rats randomly received infusions of saline or one of four doses of nicotine (each delivered at a constant rate of 0.09 mg/kg per 60 sec): 0.045 mg/kg for 30 sec, 0.065 mg/kg for 44 sec, 0.09 mg/kg for 60 sec or 0.135 mg/kg for 90 sec (Valentine et al., 1996). This dosing regimen was used to avoid aversive behavioral responses that might induce NE release, independently of nicotine. Because the behavioral responses to nicotine 0.135 mg/kg in some rats were not tested because they elicited agitated behavioral responses in many rats at doses larger than 1.25 nmol (Y. Fu, S. G. Matta and J. D. Valentine, unpublished observations).

To evaluate whether the effects of NAcHR antagonists administered into the cerebral aqueduct are localized to the brainstem, two experiments were performed. The first experiment was designed to determine whether a large fraction of the intra-aqueductal dose of an antagonist gaining access to the systemic circulation would effectively inhibit NAcHRs at an unspecified site(s). This was assessed by injecting i.v. the IC50 dose of Mec (6 nmol/0.1 ml for 60 sec), DHβE (15 nmol/0.1 ml for 60 sec) or CSP 15 min before a nicotine infusion (0.09 mg/kg i.v.). In the second experiment, the hypothetical delivery of NAcHR antagonists via the CSF circulation to presynaptic NAcHR in rostral brain sites was evaluated by administering Mec or DHβE directly into the AMYG and HP through a microdialysis probe. After three 20-min basal samples were collected, perfusates (1 μl/min) containing Mec (80 nmol/20 μl), DHβE (200 nmol/20 μl) or CSP (20 μl) were switched into the inflow catheter (using a CMA 110 liquid switch), and the microdialysis probe was perfused for 20 min. Thereafter, the antagonist solution was replaced by KRB, and 0.09 mg/kg nicotine was infused i.v. Because Mec and DHβE have molecular weights similar to NE, the amount of antagonist that diffused from the probe was estimated from experiments in which the in vitro diffusion of NE had been measured. Therefore, the dose of Mec (80 nmol) or DHβE (200 nmol) perfused through the probe was calculated based on 7.5%, the average in vitro recovery of NE by HP probes, and on the experimentally determined IC50 value of each antagonist.

**Data analysis and statistics.** Chromatographic data were collected and analyzed with the PowerChrom system (AD Instrument, Castle Hill, NSW, Australia) and expressed either as picograms per 16-μl sample or as a percentage of pre-infusion basal NE levels. Basal values were defined in each rat as the average NE levels of the three samples before administration of nicotine, antagonists or vehicle. Data were analyzed by one-way analysis of variance with StatView. Results were considered significant at P < 0.05. The number shown in parentheses (n) in the text and graphs is the number of rats within a specific treatment group.

**Results.**

Representative histological specimens illustrating the placement of concentric dialysis probes in AMYG and HP are shown in figure 1. Each probe was placed to maximally dialyze as much of the specific region as possible, without affecting surrounding structures. Figure 2 shows the HPLC chromatograms obtained from a NE standard (panel A) and representative dialysate samples (panels B–E). The NE peaks are symmetrical, and the retention time of peaks detected in dialysate samples are identical with synthetic NE. Figure 3, A and B, demonstrates the time course for NE release in the AMYG and HP in response to nicotine infusions during the active (dark) phase of the light cycle. Nicotine stimulated NE release in these two brain regions in a dose-dependent manner. NE concentrations were maximal within the first 20 min after the end of the nicotine infusions and returned to base-line levels immediately thereafter. The maximal responses to nicotine were approximately 2-fold greater than basal NE levels in both brain regions. The potency of nicotine was similar in both regions with approximate ED50 values (within the dosage range tested) of 0.073 mg/kg for the AMYG and 0.079 mg/kg for the HP. The specificity of these NE responses to nicotine is underscored by the release of serotonin only at doses greater than 0.09 mg/kg.
This dose-dependent release of different neurotransmitters by nicotine indicates specificity, rather than a nonselective secretagogue effect similar to KCl stimulation.

The results presented in figure 4 and table 1 demonstrate that systemic nicotine activated brainstem site(s), leading to NE secretion in the AMYG and HP. Figure 4A shows that nicotine-induced NE release in AMYG was inhibited by Mec, DHβE and MLA, whereas, by themselves, the antagonists did not affect basal NE levels (data not shown). Doses of Mec equal to or greater than 4 nmol, injected into the cerebral aqueduct, resulted in a dose-dependent blockade of NE release in response to 0.09 mg/kg nicotine. The IC$_{50}$ for Mec blockade of NE release was 6 nmol with 80% maximal inhibition. DHβE at a dose of 16.8 nmol or greater significantly inhibited NE secretion with an IC$_{50}$ of 8 nmol and maximal

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**Fig. 1.** Photomicrographs of probe placement in the AMYG (A, B) and HP (C). Rats were cardiac perfused with 4% paraformaldehyde in 0.05 M phosphate-buffered saline. Brains were removed, cryosectioned at 20 μm and stained with cresyl violet. Panels A and B show both sides of a coronal section in the same rat: panel A is the intact left AMYG and panel B shows the tissue track of the probe identified by the arrows in the right AMYG. Panel C shows the bilateral HP, the track of the probe is on the left and identified by arrows. Magnification bar, 400 μm; ot, optic tract.

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**Fig. 2.** Chromatograms of NE in standard solution and dialysate samples. The synthetic NE peak in the standard (10 pg) was symmetrical and had a retention time of 3.45 min (A). Panels B and D show representative basal levels of NE present in dialysates collected simultaneously from the AMYG and HP, respectively, of one rat. Panels C and E illustrate the increase in the level of NE in dialysates from the AMYG (C) and HP (E) after i.v. infusion of 0.09 mg/kg nicotine in the same rat.
inhibition of 62%. MLA at doses of 5.4 nmol or greater dose-dependently blocked NE release; its IC\textsubscript{50} was 5 nmol and NE secretion was maximally inhibited by 60%. Lower doses of MLA (0.4–1.8 nmol) had no effect on nicotine-induced NE release in the AMYG.

Figure 4B and table 1 show that nicotine-induced NE release in the HP also was blocked by Mec, DH\textsubscript{b}E and MLA, with potencies and efficacies similar to those observed for the AMYG. The IC\textsubscript{50} values were 6 nmol for Mec, 15 nmol for DH\textsubscript{b}E and 5 nmol for MLA. Similar to the inhibition seen in the AMYG, Mec also was more efficacious at inhibiting NE secretion in the HP (maximal inhibition, 87%) than DH\textsubscript{b}E and MLA.

In contrast, α-BTX had no effect on NE release in either of these regions (fig. 5; P = .621 for AMYG and .473 for HP, compared with CSF/nicotine). Higher doses of α-BTX could not be evaluated because they frequently produced considerable agitation, as indicated by gasping, running and jumping.

The anatomical specificity of NACHR antagonists for the brainstem, after their administration into the cerebral aqueduct, was evaluated in two ways. First, the potential diffusion of a large fraction of the delivered dose of an antagonist into the systemic circulation was assessed by injecting the IC\textsubscript{50} dose of Mec or DH\textsubscript{b}E (from table 1) into the jugular vein before infusing 0.09 mg/kg nicotine. The data presented in table 2 show that nicotine-induced NE secretion in both the AMYG and HP was unaffected. Second, the hypothetical delivery of NACHR antagonists via the CSF circulation to presynaptic NACHR in rostral brain sites was evaluated by administering Mec or DH\textsubscript{b}E directly into the AMYG and HP through a microdialysis probe. Again, no inhibition of nicotine-induced NE secretion was observed (table 2). Therefore, it appears that brainstem NACHRs were targeted by the antagonists administered into the cerebral aqueduct.

**Discussion**

NE neuronal cell bodies are localized exclusively within brainstem regions (designated “A” by convention) and project rostrally throughout the brain. Approximately 90%
of the NE neurons are located in the LC (A6 region, Holets, 1990; Aston-Jones et al., 1995), with most of the remaining neurons found in the NTS-A2 region of the dorsal medulla or in the ventromedullary A1 region (Holets, 1990). The AMYG receives input from the LC in rostral brainstem (Fallon et al., 1978; Jones and Yang, 1985; Petrov et al., 1993) and both the NTS and A1 in caudal brainstem (Kalina et al., 1985; Zardetto-Smith and Gray, 1990; Petrov et al., 1993; Roder and Ciriello, 1993), whereas the HP receives NE input primarily from LC (Aston-Jones et al., 1995). Brainstem NAchRs have been identified in studies demonstrating in situ hybridization for subunit mRNAs (Wada et al., 1989; Marks et al., 1992) or high-affinity binding of radiolabeled agonists (3H-nicotine and 125I-α-BTX; Clarke et al., 1985; Maley and Seybold, 1993). Although the precise cellular localization of NAchRs to NE somata or other neurons has not been reported, the brainstem appears to be the primary site of action of nicotine that leads to the activation of NE neurons.

Nicotinic antagonists have been used to show that the brainstem mediates the effects of systemic nicotine on NE secretion. Antagonists administered intraparenchymally (into LC, Mitchell 1993) or via the aqueduct (fig. 4) and fourth ventricle (Fu et al., 1997) effectively block nicotine-stimulated NE secretion in rostral targets (HP, AMYG and PVN, respectively). Diffusion of the antagonists from the CSF to the blood at sufficient concentration to act at unspecified sites in the periphery is an unlikely possibility. This possibility was eliminated by experiments in which the IC50 concentrations of Mec or DHβE were administered i.v. (table 2); nicotine-stimulated NE was unaffected. It also is possible that antagonists delivered into the aqueduct and/or fourth ventricle could gain access to regions of the brain other than the brainstem and block the effect of systemic nicotine. Such a site could be the HP itself, which is immediately accessible from the ventricular system. The HP contains multiple NAchRs (Wada et al., 1989, Flores et al., 1992; Hill et al., 1993), some of which appear to be presynaptic, because synaptosomal preparations released NE in response to nicotine (Clarke and Reuben, 1995; Vizi et al., 1995). However, when Mec was dialyzed directly into the HP, NE secretion in response to s.c. nicotine was unaffected (Mitchell, 1993). In the present investigations, when Mec or DHβE were dialyzed into the HP at concentrations calculated to approximate brainstem IC50 doses, NE secretion elicited by i.v. nicotine was unchanged (table 2). These findings do not preclude, however, an action of nicotine directly on Mec- or DHβE-

Fig. 4. Dose-dependent blockade of nicotine-induced NE release in the AMYG and HP after microinjection of antagonists into the cerebral aqueduct. Peak NE levels were measured in the samples collected 20 min after infusion of nicotine (0.09 mg/kg i.v.), and were expressed as percent of pre-infusion basal levels. The basal NE value (mean ± S.E.M.) in CSF/nicotine group for AMYG (A) was 1.8 ± 0.4 pg/16 μl in experiments performed with Mec and DHβE, and was 2.2 ± 0.5 pg/16 μl in those experiments performed with MLA. In the HP (B), the basal NE value was 4.3 ± 0.9 pg/16 μl for experiments with Mec and DHβE, and 4.8 ± 0.8 pg/16 μl in the MLA studies. Similar doses of Mec, DHβE and MLA inhibited nicotine-induced NE release in the AMYG and HP. * P < .05, ** P < .01, compared with microinjection of CSF (”0 nmol”) into the cerebral aqueduct followed by nicotine (0.09 mg/kg i.v.; n = 5 rats/treatment).

TABLE 1
Nicotinic antagonists injected into cerebral aqueduct block nicotine-stimulated NE release in AMYG and HP: approximate IC50 values and efficacies

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<th>Antagonists</th>
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<tr>
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<td>Approximate IC50</td>
<td>Maximal Inhibition</td>
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<td>Mec</td>
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<tr>
<td>DHβE</td>
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<td>MLA</td>
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<td>60</td>
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Fig. 5. Lack of antagonism by α-BTX injected into the cerebral aqueduct on nicotine-stimulated NE release in the AMYG and HP. No significant difference occurred between rats receiving a microinjection of CSF (CSF/nicotine) and those receiving 1.25 nmol of α-BTX 15 min before an i.v. infusion of 0.09 mg/kg nicotine (n = 4/group).
insensitive NAchRs located presynaptically on NE terminals within the HP itself (or AMYG).

The present study demonstrates that systemic nicotine stimulated NE release in both the AMYG and HP with similar potency and efficacy. This similarity could be explained by the common origin of the NE input to these structures. In contrast, NE secretion in the PVN is sensitive to a dose of nicotine (i.e., 0.045 mg/kg i.v.) that was ineffective in the AMYG or HP (Fu et al., 1997). This difference may be because the NTS provides 80% of the NE afferents to the PVN, whereas only 10% arise from the LC or the A1 (Sawchenko and Swanson, 1982; Cunningham and Sawchenko, 1988). Indeed, the LC and A1 have been shown to be less sensitive to systemic nicotine than the NTS in studies demonstrating dose-dependent stimulation of cFos expression (Matta et al., 1993, Valentine et al., 1996). The presence of NAchRs of differing affinity may account for this regional sensitivity to nicotine.

Two approaches have been used to demonstrate the presence of multiple NAchRs in the brainstem. In situ hybridization histochemistry has been used to localize mRNA transcripts for different rat alpha and beta subunits (Wada et al., 1989; Marks et al., 1992), and receptor autoradiography in rats and cats has shown high-affinity binding of both 3H-nicotine and 125I-alpha-BTX (Clarke et al., 1985; Maley and Seybold, 1993). alpha-BTX binding has been found in both the LC and NTS (Clarke et al., 1985; Maley and Seybold, 1993), which suggests the presence of alpha-7 subunits. This is based on evidence showing that most alpha-BTX-binding NAchRs contain alpha-7 subunits in the mouse brain (Stitzel et al., 1996). Although alpha-4 beta-2 is the dominant configuration of mammalian brain NAchRs with high affinity for 3H-nicotine (Flores et al., 1992), alpha-4 transcripts have not been found in the LC and are only expressed at very low levels in the NTS (Wada et al., 1989). The presence of specific NAchR subunits in the A1 region has not been reported. In contrast, the mRNAs for alpha-2, alpha-3, beta-2 and beta-4 have been localized in both the rat LC and NTS (Wada, et al., 1989). Therefore, it appears that multiple NAchR subtypes exist in both the LC and NTS.

In vitro oocyte preparations expressing specific combinations of NAchR subunits have been used to define the relative efficacy and potency of the currently available nicotinic antagonists (Luetje and Patrick, 1991; Drasdo et al., 1992; Cachelin and Rust, 1995; Harvey and Luejte, 1996). Based on these observations, the current study compared the relative efficacies of several nicotinic antagonists at blocking NE in response to systemic nicotine to define pharmacologically the receptor subtypes involved. For two reasons, alpha-7 subunits do not appear to be involved in the stimulation of NE secretion by brainstem NAchRs. 1) Injection of alpha-BTX, which is highly potent and selective for alpha-7 subunits, did not block the effect of nicotine in either the AMYG or HP. 2) Based on in vitro hippocampal studies, MLA is approximately 1000- to 10,000-fold more potent at blocking alpha-7-mediated currents than DHbetaE or Mec (Allkondon and Albuequerue, 1993; Briggs and McKenna, 1996). However, as shown in table 1, the IC50 value for blockade by MLA was not substantially less than the other two antagonists in either the AMYG or HP. This indicates that NE secretion in the AMYG and HP is not mediated by alpha-7-containing NAchRs in the brainstem.

Two lines of evidence obtained from published reports and the present investigations suggest that alpha-4 beta-2, the dominant high-affinity nicotine binding site in the CNS, is not likely to mediate nicotine-induced NE secretion. 1) Levels of alpha-4 mRNA are very low in the NTS and absent in the LC (Wada et al., 1989). 2) At alpha-4 beta-2 NAchRs, DHbetaE is reported to be much more potent than Mec, and its efficacy is similar to MLA (Luetje et al., 1990; Allkondon and Albuequerue, 1993). Table 1 shows, however, that the IC50 values for blockade of NE secretion by DHbetaE was not different from Mec in the AMYG and actually was 2- to 3-fold greater than Mec in HP, providing little evidence for stimulation of NE secretion by brainstem alpha-4 beta-2 receptors. However, these findings must be interpreted cautiously in view of possible differences in the diffusion of these antagonists in vivo.

In contrast, involvement of alpha-3 beta-2 receptors is suggested by the similar potency that all three antagonists have for NE secretion in AMYG and HP. Oocyte transfection studies indicate that only alpha-3 beta-2 subunits demonstrate relatively similar sensitivity to Mec (IC50 = 2.9 mU; Cachelin and Rust, 1995) and DHbetaE (IC50 = 0.41 mU; Harvey and Luejte, 1996). The only report of MLA with transfected alpha-3 beta-2 receptors showed an IC50 of 80 nM, a value approximately 2 orders of magnitude greater than its potency at alpha-7-containing NAchRs (Drasdo et al., 1992). Moreover, MLA appears to be much more potent at alpha-3 beta-2 than at alpha-3 beta-4 (0% inhibition of apparent alpha-3 beta-4 receptors with 100 nM MLA in hippocampal cultures; Allkondon and Albuequerue, 1993). Based on these differences in the potencies of the three antagonists used in the present investigations, our observations favor the involvement of alpha-3 beta-2 in nicotine-induced NE release in the AMYG and HP.

An additional contribution from alpha-3 beta-4 subunits is suggested by the greater efficacy of Mec (80-87% blockade; table 1), which has been shown to be more effective than DHbetaE at alpha-3 beta-4 receptors (Allkondon and Albuequerue, 1993). Other studies with either Mec or DHbetaE support these observations, in that Mec was more potent at alpha-3 beta-4 (IC50 = 0.19 mU, Cachelin and Rust, 1995) than DHbetaE (IC50 = 23.1 mU; Harvey and Luejte, 1996). The involvement of both alpha-3 beta-2 and alpha-3 beta-4 NAchRs also is supported by in situ hybridization studies demonstrating

### Table 2

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<th>Delivered via</th>
<th>AMYG</th>
<th>HP</th>
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<tr>
<td></td>
<td>CSF</td>
<td>Mec</td>
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<tr>
<td>i.v.</td>
<td>172.7 ± 8.8</td>
<td>163.8 ± 12.5</td>
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<tr>
<td>probe</td>
<td>170.2 ± 12.0</td>
<td>164.0 ± 8.8</td>
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that alpha-3 is the major nicotinic agonist-binding subunit found in both the LC and NTS, whereas moderate levels of both beta-2 and beta-4 subunit mRNAs are present (Wada et al., 1989). Therefore, a heterogeneous population of brainstem NAcRAs, which primarily comprises alpha-3 beta-2 and alpha-3 beta-4 subtypes, may mediate nicotine-stimulated NE release in the AMYG and HP.


