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EVIDENCE FOR CHOLINERGIC REGULATION OF BASAL NOREPINEPHRINE RELEASE IN THE RAT OLFACTORY BULB

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Abstract—The effects of locally infused cholinergic agonists on extracellular levels of norepinephrine in the olfactory bulb of anesthetized rats were determined using *in vivo* microdialysis coupled with high-performance liquid chromatography and electrochemical detection. Using chronically implanted microdialysis probes, the basal norepinephrine level in the olfactory bulb was 0.55 pg/10 μl dialysate. Local infusion of K^+ (30 mM) or the norepinephrine re-uptake inhibitor desipramine (1 μM) through the dialysis probe significantly increased basal norepinephrine levels. Focal activation of noradrenergic locus coeruleus neurons, the sole source of norepinephrine innervation of the olfactory bulb, increased norepinephrine levels by 247% of control. Local infusion of the acetylcholinesterase inhibitor soman (0.4 mM) into the olfactory bulb increased basal norepinephrine levels by 134% of control, suggesting that endogenously released acetylcholine modulates norepinephrine release. Intrabulbar infusion of acetylcholine (40 mM) or nicotine (40 mM) increased norepinephrine levels (317% and 178% of control, respectively), while infusion of the muscarinic receptor agonist pilocarpine (40 mM) reduced norepinephrine levels (54% of control).

These results demonstrate that basal norepinephrine release in the olfactory bulb is potently modulated by stimulation of local cholinergic receptors. Nicotinic receptors stimulate, and muscarinic receptors inhibit, norepinephrine release from locus coeruleus terminals. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: acetylcholine, locus coeruleus, olfactory bulb, muscarinic receptors, nicotinic receptors, *in vivo* microdialysis.

Convulsions elicited by systemic administration of cholinomimetic agents such as the irreversible acetylcholinesterase (AChE) inhibitor soman or muscarinic receptor agonists lead to massive depletion of the neurotransmitter norepinephrine (NE).[12,13,18] Soman-induced convulsions, for example, cause a rapid, 50% reduction of tissue NE content in the rat olfactory bulb and forebrain as early as 1 h following soman administration.[13] Based on the global reduction of NE levels, it is likely that cholinomimetic agents modify NE levels, at least in part, via actions on the noradrenergic system originating in the nucleus locus coeruleus (LC), which innervates most of the forebrain and is the sole source of NE input to the cerebral cortex, hippocampus and olfactory bulb.[4,32]

There are at least two potential mechanisms by which cholinergic agents could modify NE release from the LC system: (i) direct excitation of LC somata, leading to increased, impulse-driven terminal NE release; and (ii) local, presynaptic effects on LC terminals that could modulate NE release independently of impulse activity in LC somata. Previous studies in our laboratory and by others have shown that direct application of muscarinic or nicotinic receptor agonists, as well as AChE inhibitors, into the LC potently activate LC neurons.[3,9,10,12,14,15] It is not known, however, if cholinergic agents locally modulate NE release from axon terminals in the CNS *in vivo*. In the peripheral nervous system, nicotinic receptors stimulate, and muscarinic receptors inhibit, NE release.[48]

The goal of the present study, therefore, was to determine if activation of cholinergic receptors in LC terminal regions modulates basal NE release as measured by *in vivo* microdialysis coupled with high-performance liquid chromatography. The effects of cholinergic receptor stimulation on NE levels were investigated in the granule cell layer of the rat main olfactory bulb. The olfactory bulb was selected because: (i) the granule cell layer of the olfactory bulb receives a dense noradrenergic innervation that arises solely from LC neurons;[32,41,42] (ii) the granule cell layer is also targeted by cholinergic inputs (from the horizontal limb of the diagonal band), and this layer contains both muscarinic and nicotinic receptors;[22,24,25,29,49] (iii) previous studies demonstrate that activation of cholinergic projections to the olfactory bulb presynaptically modulates other centrifugal inputs to granule cells;[36] and (iv) cholinomimetic seizures rapidly deplete NE levels in the olfactory bulb.[12,13]

EXPERIMENTAL PROCEDURES

Materials

Soman (pinacolyl methylphosphonofluoridate) was supplied by the U.S. Army Institute for Chemical Defense (Aberdeen Proving Ground, Maryland). Acetylcholine (ACh) hydrochloride, pilocarpine hydrochloride, desipramine hydrochloride, (−)-nicotine, monochloroacetic acid, octyl sodium sulfate, NE bitartrate and ascorbate oxidase (EC 1.10.3.3) were obtained from Sigma Chemical Co. (St Louis, MO).

Microdialysis probe implantation

Experimental procedures were conducted so as to minimize animal suffering and the number of animals used. The following procedures

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ACSF, artificial cerebrospinal fluid; EDTA, ethylenediaminetetra-acetate; LC, locus coeruleus; NE, norepinephrine.

were approved by the animal welfare committee at the University of Cincinnati. Adult male Sprague–Dawley rats (280–350 g; Harlan Laboratories, Indianapolis, IN) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and concentric microdialysis CMA/12 probes (Carnegie Medicine, 2 mm active tip, 0.5 mm o.d.) were stereotaxically implanted in the granule cell layer of the left olfactory bulb (7.5 mm rostral to bregma, 0.5 mm lateral to the midline, 3.5 mm ventral to the brain surface). The olfactory bulb was studied in these experiments because: (i) the granule cell layer receives a rich noradrenergic innervation originating solely from the LC,[32,41,42] and the granule cell layer contains several classes of noradrenergic receptors;[22,25,42] and (ii) the granule cell layer of the bulb is targeted by cholinergic inputs (from the horizontal limb of the diagonal band) and this layer contains both muscarinic and nicotinic receptors.[22,24,25,28,29,49] The probe was surrounded with a plastic cap and secured to skull screws with dental acrylic. All microdialysis sampling experiments, including LC activation, were conducted one to two days later in methoxyflurane-anesthetized rats, as described below (see "Neurophysiological techniques"). The location of implanted probes was verified histologically (see below) at the end of the experiment.

Microdialysis and chromatographic methods

The microdialysis perfusate was an osmotically balanced artificial cerebrospinal fluid (ACSF; composition: 147 mM NaCl, 2.71 mM KCl, 1.22 mM $CaCl_2$, pH 7.4). The filtered and degassed ACSF, or ACSF containing dissolved pharmacological agents or elevated K^+, was placed in 2.5-ml microsyringes and continuously perfused through the probe at a flow rate of 2 μl/min using a microinfusion pump (CMA/100) equipped with an electronic syringe selector (CMA/111). The syringe selector allows delivery of perfusate from one of several syringes to the probe, while the others are voided to waste without the introduction of air bubbles; switching between syringes containing control ACSF did not alter baseline NE levels. Corrections were made to account for dead volume in tubing and in the dialysis probe. Dialysate samples (20 μl) were collected at 10-min intervals using a microfraction collector (CMA/140). Relative recovery of probes was calibrated before and after implantation by comparing the peak heights of NE in brain dialysate samples to those in a standard (20 pg/10 μl of ACSF, pH 7.4), as described previously.[37] The mean relative recovery of the dialysis probes in this study was 11.8 ± 0.5% (n=27).

In each experiment, NE efflux was monitored for a minimum of 90 min during continuous perfusion of normal (control) ACSF through the probe to establish stable, baseline NE levels; the last six 10-min samples were collected prior to experimental manipulations. Using the syringe selector (CMA/111), filtered ACSF containing high K^+ or a pharmacological agent (desipramine, soman, ACh, pilocarpine, nicotine) was then infused for 10 min in the olfactory bulb via the implanted probe. After local K^+ or drug infusion, control ACSF was allowed to perfuse for 1 h. In LC activation experiments (see below), only control ACSF was perfused through the probe before, during and after LC activation.

NE levels were determined using a Bioanalytical Systems (West Lafayette, IN) Model 200 Liquid Chromatograph, equipped with an LC-4B electrochemical detector (Bioanalytical Systems). The glassy carbon working electrode was held at 0.65 V against an Ag/AgCl reference electrode and the sensitivity of detection was kept at 0.2 nA (full scale). Chromatographic separations were effected with a 100 mm×3.2 mm Biophase ODS 3-μm (C18) column (Bioanalytical Systems) in a 14-min run. The mobile phase consisted of 63 mM monochloroacetic acid, 1.3 mM octyl sodium sulfate and 0.2 mM Na_2EDTA in the aqueous phase (pH 2.9), and 3% acetonitrile at a flow rate of 1.0 ml/min. The column and mobile phase were held at 40 and 35°C, respectively. Under these chromatographic conditions, injected brain dialysate samples (10 μl) have a large solvent front which interferes with the early eluting peaks. The main cause for this large solvent front is ascorbic acid. As reported previously,[31] this problem was mitigated by addition of 0.5 μl of ascorbic acid oxidase (activity: 250 units/0.5 ml of water) to each collected dialysate sample (20 μl). Each sample was vortexed and 10 μl of each treated sample was immediately injected into the high-performance liquid chromatograph. The NE peak in microdialysis samples was positively confirmed with spiking methods. NE levels are expressed as pg/10 μl sample corrected for probe recovery. Evoked NE levels are expressed as a percentage of the average baseline NE concentration.

Neurophysiological techniques

One to two days after microdialysis probe implantation, rats were intubated with a tracheal cannula under methoxyflurane (metofane) anesthesia (0.3–1.0% in moist air). A second ascending cannula was inserted into the distal (nasal) part of the trachea and connected to a respirator to produce moist airflow through the nasal cavity. Animals were mounted in a stereotaxic instrument with the incisor bar lowered to angle the skull 12° from the flat skull position. Core body temperature was maintained at 36–37°C with a feedback-controlled heating pad. A transcortical electroencephalographic electrode was implanted in the frontal cortex to maintain a constant plane of anesthesia at which a hindlimb pinch elicited a brief desynchronization of the electroencephalogram, as described previously.[23]

A hole was drilled in the skull above the left LC (4.0 mm posterior to lambda, 1.2 mm lateral to the midline), ipsilateral to the probe implanted in the left olfactory bulb. A composite recording/microinjection electrode was used to record extracellularly from single LC neurons during microinjection of ACh into the LC. The electrode consisted of a conventional, dye-filled glass recording micropipette (4–6 μm tip diameter, 5–10 MΩ impedance) glued to a calibrated injection micropipette (60–80 μm tip diameter), as described previously.[23] The tip of the recording pipette extended 100–200 μm beyond the tip of the injection pipette. Recordings from physiologically identified LC neurons were amplified and displayed continuously using conventional electrophysiological methods, as described previously.[23] The electrode was positioned so that the injection pipette tip was in the center of the LC. This was accomplished by carefully monitoring LC activity at several locations and selecting a site where LC activity could be recorded for at least 500 μm in the dorsal–ventral axis. Four 90-nl intracoerulear infusions of ACh (0.2 M in ACSF, pH 7.4) were made at 2.5-min intervals to activate LC neurons over a period of 10 min, corresponding to the interval for a single microdialysis sample. The conventional microelectrode continuously recorded an LC cell to determine the degree and the time-course of ACh-induced changes in LC discharge activity. At the end of the experiment, recording sites were marked by iontophoretic ejection of dye, and rats were deeply anesthetized and perfused with 10% formalin. Brains were removed, sectioned and stained with Cresyl Violet. All microdialysis and recording sites were verified histologically. The probe in every experiment was implanted in the olfactory bulb granule cell layer. Histological examination also confirmed that the LC injection/recording pipette tips were consistently placed in the center of the LC.

Statistical analysis

Statistical differences between mean baseline NE levels before and after experimental manipulations were assessed using one-way repeated measures ANOVA coupled with Duncan's multiple test. Similar tests were used to assess changes in LC neuronal discharge rate. The level of significance was established as $P<0.05$.

RESULTS

Basal and locus coeruleus-evoked norepinephrine levels in the olfactory bulb

The basal level of NE in the olfactory bulb of anesthetized rats recovered by *in vivo* microdialysis was 0.55 ± 0.11 pg/10 μl dialysate (n=21). In the first set of experiments, we determined if extracellular NE levels were modified by stimuli known to increase transmitter release. First, NE levels were measured before, during and after local depolarization by infusing a high K^+ ACSF solution through the microdialysis probe. As shown in Fig. 1A, infusion of ACSF (pH 7.4) containing 30 mM K^+ for 10 min increased NE levels to 166 ± 24% of baseline (n=6; $P<0.001$). NE levels decreased to 139 ± 20% of baseline ($P<0.05$) by 20 min, and returned to baseline levels by 30 min after K^+ infusion. NE levels were also increased by local infusion of the selective NE uptake inhibitor, desipramine. Infusion of ACSF containing desipramine (1 μM) significantly increased NE levels within 20 min

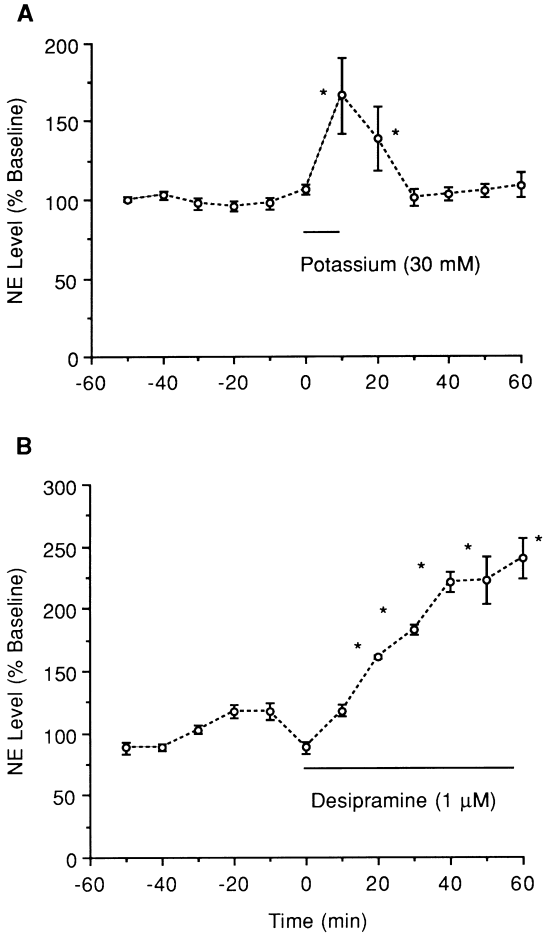

Fig. 1. Local depolarization and re-uptake inhibition increase NE levels in the olfactory bulb. (A) Brief infusion (10 min, shown at horizontal bar) of ACSF containing 30 mM K^+ through the microdialysis probe increased NE release to a maximum of 166% of baseline ($n=6$). (B) Addition of the selective re-uptake inhibitor desipramine (1 μM) to the ACSF for 1 h (shown at horizontal bar) gradually increased NE levels to a maximum of 238% of baseline ($n=5$). Results are expressed as mean ± S.E.M. *$P<0.05$ as compared to the mean of the six samples preceding infusion. In this and subsequent figures, the timing of the infusion is indicated by the horizontal bar.

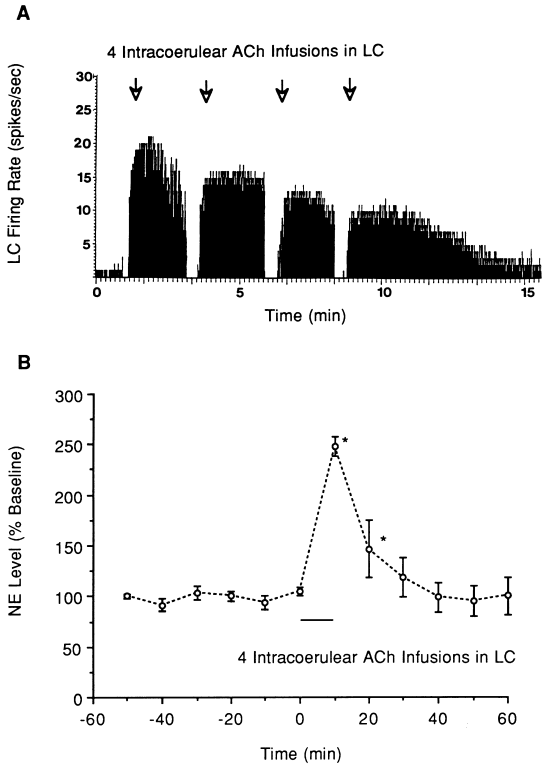

Fig. 2. Selective, confirmed activation of LC neurons increases NE levels in the olfactory bulb. (A) Ratemeter record showing the spontaneous discharge (spikes/s) of an LC neuron before and after four microinjections of 90 nl of 0.2 M ACh into the LC (at arrows). Four microinjections of ACh were made at 2.5-min intervals to produce an increase in LC discharge throughout a 10-min microdialysis sample collection interval. Intracoerulear injections of ACh increased the mean spontaneous discharge of the LC from an average rate of 1 spike/s to as high as 20 spikes/s; spontaneous activity of this cell could not be recorded during the injections, producing the gaps under each of the four arrows. (B) Activation of LC neurons rapidly increased NE levels in the olfactory bulb to 247% of baseline ($n=6$) in the first 10-min sample. Results are expressed as mean ± S.E.M. *$P<0.05$ as compared to the mean of the six samples preceding infusion.

(Fig. 1B); continuous infusion of desipramine for 1 h increased NE levels to 238 ± 16% of baseline ($n=5$; $P<0.001$).

Previous anatomical studies demonstrate that the sole source of noradrenergic inputs to the olfactory bulb is the LC.[32,41,42] This suggests that steady-state levels of NE recovered by microdialysis in the olfactory bulb represent NE tonically released LC terminals. Therefore, we next measured if focal activation of LC neurons increases NE levels. As shown in Fig. 2A, small microinjections of ACh (90 nl) into the LC produced a robust increase in LC discharge rate, beginning 5–7 s after injection. The baseline spontaneous discharge rates of LC neurons ranged from 1.3 to 3.5 spikes/s (mean: 1.9 ± 0.3 spikes/s, $n=7$). The discharge rates after ACh microinjections ranged from 7 to 22 spikes/s, with a mean of 14.0 ± 1.3 spikes/s ($n=6$; $P<0.001$). This corresponds to a 7.4-fold mean increase in LC neuronal discharge during the 10-min period of activation. LC activation increased olfactory bulb NE levels to 247 ± 9% of baseline ($n=6$; $P<0.001$) during the 10-min activation period (Fig. 2B). NE levels remained elevated for the next 10-min sample, and then decreased to baseline levels thereafter.

Effect of locally applied cholinergic agents on basal norepinephrine release

There is evidence in the autonomic nervous system and in brain slice preparations that presynaptic cholinergic receptors located on NE terminals modulate NE release.[48] In order to investigate possible local cholinergic control of terminal NE release in the olfactory bulb, basal NE levels were measured before, during and after a 10-min local infusion of cholinergic agents via the microdialysis probe. As shown in Fig. 3A, infusion of 40 mM ACh chloride in ACSF increased NE levels to 193 ± 42% of baseline ($n=4$; $P<0.05$) in the first 10-min sample and to a maximum level of 317 ± 30% of baseline ($P<0.001$) in the second 10-min sample. NE was still elevated to 297 ± 60% of baseline in the third 10-min sample ($P<0.001$), and by 1 h it returned to 137 ± 18% of baseline level ($P<0.001$).

As shown in Fig. 3C, local infusion (10 min) of nicotine (40 mM in ACSF) significantly increased olfactory bulb NE levels to a maximum of 178 ± 9% of baseline ($n=4$; $P<0.001$) by 20 min. This increase was maintained at

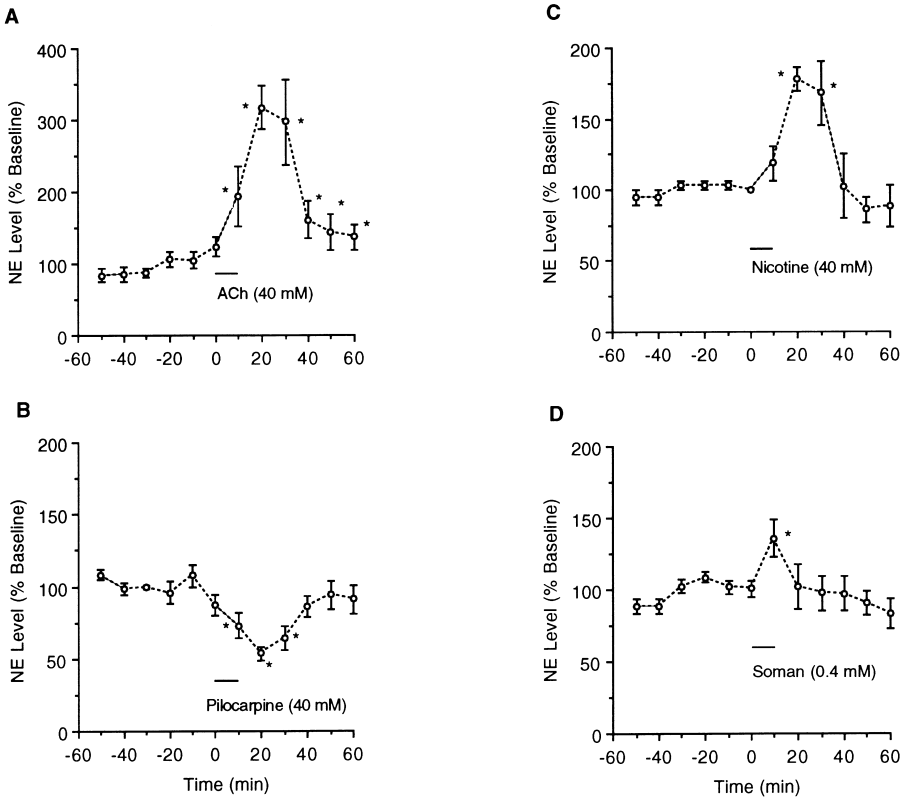

Fig. 3. Effects of local infusion of cholinergic agonists on NE levels in the olfactory bulb. (A) Infusion of ACh (40 mM) through the dialysis probe increased NE levels to a maximum of 317% of baseline ($n=4$). (B) Infusion of the muscarinic receptor agonist pilocarpine (40 mM) decreased NE levels to 50% of baseline ($n=6$). (C) Infusion of nicotine (40 mM) increased NE levels to 178% of baseline ($n=4$). (D) Infusion of the AChE inhibitor soman (0.4 mM) increased NE levels to 136% of baseline ($n=7$). Results are expressed as mean ± S.E.M. *$P<0.05$ as compared to the mean of the six samples preceding infusion.

30 min (168 ± 23; $P<0.001$) and then rapidly returned to basal levels. By contrast, local infusion of a selective muscarinic receptor agonist, pilocarpine chloride in ACSF (40 mM), significantly reduced NE levels, falling to a maximum of 54 ± 5% of baseline ($n=6$; $P<0.001$) in the second 10-min sample (Fig. 3B). NE levels were also significantly depressed in the third sample (64 ± 8% of baseline level; $P<0.001$), but then returned to near control levels afterwards.

In the final experiment, the AChE inhibitor soman was locally infused to determine if accumulation of endogenously released ACh modulated NE levels. Inhibition of AChE results in a rapid accumulation of ACh in the brain, and thereby activates both muscarinic and nicotinic receptors. As shown in Fig. 3D, a 10-min infusion of soman in ACSF (74 μg/ml, 0.4 mM) increased the NE level to 136 ± 13% of baseline ($n=7$; $P<0.001$) in the first 10-min sample.

DISCUSSION

The major finding of this study is that ACh regulates extracellular NE levels in the olfactory bulb via actions on nicotinic and muscarinic receptors. Nicotinic and muscarinic receptors in the olfactory bulb appear to exert opposing actions, as local perfusion of the nicotinic receptor agonist nicotine increased, and the muscarinic receptor agonist pilocarpine decreased, NE levels. Local infusion of ACh or the AChE inhibitor soman increased NE levels, suggesting that the action of increased ACh on nicotinic receptors predominates over the inhibitory influence of muscarinic receptor activation.

Technical considerations

Previous studies in cortical slices suggest that ACh alters NE release in a dose-dependent manner. At higher concentrations, ACh increases NE release via activation of nicotinic receptors, whereas, at lower concentration, it inhibits NE release via activation of muscarinic receptors.[38,40,47] Dose–response curves for the cholinergic agents tested, as well as cholinergic receptor antagonist studies, are needed to confirm the specificity of the present results. It is noteworthy, however, that nicotine, at concentrations comparable to those used here, increased NE release in brain slices; this effect was blocked by specific nicotine receptor antagonists.[47] Since ACh and pilocarpine were used in the form of chloride salts in the present study, it is also possible that the additional chloride anion introduced into the perfusate may have altered NE levels. This seems unlikely, however, as the ACh- and pilocarpine-induced NE changes were in different directions and of different magnitudes, despite the fact that they were used at the same concentration (40 mM).

While the present findings provide evidence that presynaptic cholinergic receptors modulate basal NE release in the olfactory bulb, we cannot exclude the possibility that NE release was influenced by postsynaptic actions of the cholinergic agents (e.g., altered transmitter release from neurons or glia in the granule cell layer, or increased extracellular K^+ levels and local depolarization resulting from increased neuronal activity). The GABAergic granule cell is the major neuronal element in the granule cell layer. Application of GABA has been reported to increase potassium-evoked

release of NE in rat olfactory bulb slices.[19] This raises the possibility that cholinergic agonists may have altered NE levels in the present experiments by increasing or decreasing GABA release from granule cells. It is difficult, however, to predict how cholinergic receptor stimulation would influence granule cells and GABA release. Previous studies on the influence of cholinergic inputs to these cells are discrepant. Cholinergic inputs have been suggested to either inhibit[11,35] or excite granule cells.[26,27] Furthermore, the pathway mediating these responses (mono- vs polysynaptic) and the involvement of muscarinic and nicotinic receptors is not known. Finally, it is unclear whether any changes in GABA release from granule cell dendrites in the external plexiform layer would influence NE release from LC terminals in the granule cell layer. As discussed below, cholinergic inputs have been found to presynaptically inhibit excitatory, presumably glutamatergic, centrifugal inputs to granule cells.[36] It is conceivable, therefore, that infusion of cholinergic agents may have presynaptically modified transmitter release from other inputs to granule cells which, in turn, altered NE release from LC terminals.

Basal and norepinephrine-evoked extracellular norepinephrine levels

The mean basal extracellular NE level in the rat olfactory bulb in anesthetized rats, corrected for relative probe recovery, was 0.55 pg/10 μl dialysate. This value is similar to estimates of extracellular NE levels in the hippocampus (~1.1 pg/20 μl dialysate) using comparable microdialysis probes in unanesthetized rats.[1] As tissue damage caused by probe implantation is known to disrupt synaptic release of neurotransmitter,[44] an important question is if the extracellular NE levels in the present study could be elevated by stimuli known to evoke synaptic release. Each of the three manipulations used in the present study (local depolarization by K^+, local inhibition of NE uptake by desipramine and direct activation of the LC) robustly increased NE levels, suggesting a synaptic site of origin of NE. The increase in NE levels produced by ACSF containing 30 mM K^+ (166% of baseline) is comparable to that in the hippocampus (196%) using an identical concentration of K^+ in the dialysate.[2] Similarly, the increase in NE levels following local infusion of desipramine (238% of baseline) is very similar to that in the medial prefrontal cortex (201% of baseline) after local infusion of an identical concentration (1 μM) of the uptake inhibitor.[21] Taken together, these findings indicate that the methods used in the present study accurately measured physiologically released NE.

The present results demonstrated that focal activation of LC neurons robustly increased NE levels in the olfactory bulb. Several previous studies reported increases in brain NE levels following stimulation of LC neurons or ascending LC axons,[5,6,30,43] but the relationship between the degree of LC neuronal activity and NE release was not examined. It was reported recently, however, that a two-fold increase in LC firing rate elicited by intra-LC infusion of corticotropin-releasing factor produced a 1.5-fold increase in extracellular NE levels in the prefrontal cortex.[7] The present results show that an approximately seven-fold increase in LC firing rate produces a 2.5-fold (i.e. 247% of baseline) increase in extracellular NE levels in the olfactory bulb. It should be noted that LC neurons distant from the injection site were probably not activated to the same degree. Taken together with the findings of Curtis *et al*.,[7] these results suggest that NE release increases in a relatively linear manner with tonic increases in LC discharge rate, at least within the range of firing rates tested in anesthetized animals.

Effect of cholinergic agents on basal extracellular norepinephrine release in vivo

Previous neurochemical studies demonstrate that NE release is regulated by several classes of presynaptic receptors on noradrenergic nerve terminals. Activation of presynaptic α_2 receptors inhibits basal or evoked release of NE.[2,6,16,17,30,48] NE release is increased by activation of presynaptic glutamate receptors.[16,17,20,34,45] In the autonomic nervous system, presynaptic muscarinic receptors inhibit, and presynaptic nicotinic receptors stimulate, NE release.[48] Presynaptic cholinergic regulation of NE release at central synapses is less clear. Stimulation of presynaptic nicotinic receptors in the CNS generally increases [^{3}H]NE release in brain slice preparations.[38,40,47] Activation of presynaptic muscarinic receptors has been reported to increase, decrease or have no effect on release of [^{3}H]NE from brain slices (also see below).[8,20,33,39,40,46] However, the influence of cholinergic receptors on synaptic release of NE from LC terminals *in vivo* has not been investigated previously.

The present *in vivo* microdialysis studies showed that local inhibition of AChE in the olfactory bulb with soman, a manipulation that should lead to increased ACh levels, significantly increased the extracellular levels of NE. This finding suggests that endogenously released ACh in the olfactory bulb tonically modulates NE release. It is interesting to note that ACh has been reported to presynaptically regulate other centrifugal inputs to the olfactory bulb. Activation of the horizontal limb of the diagonal band, the source of cholinergic innervation of the olfactory bulb,[42,49] presynaptically inhibits inputs to granule cells from the anterior olfactory nucleus.[36]

The effects of AChE inhibition on NE release were mimicked by local infusion of ACh. Thus, a build up of endogenously released ACh, or focal exogenous application of the transmitter, increased extracellular levels of NE. Infusions of nicotinic and muscarinic receptor agonists into the olfactory bulb suggest that the two cholinergic receptor subtypes exert opposing actions on NE release: nicotine increased, and the muscarinic receptor agonist pilocarpine decreased, extracellular levels of NE. Since ACh and soman increased NE levels, the present findings suggest that the stimulatory action of nicotinic receptors predominates over the inhibitory effect of muscarinic receptor activation, at least in the olfactory bulb.

Systemic administration of convulsive doses of soman, an AChE inhibitor, and pilocarpine rapidly deplete NE levels in the forebrain and olfactory bulb.[12,13,18] We hypothesized[13] that sustained cholinergic receptor activation of LC neurons caused by these agents[3,9,10,12,14,15] produces sustained release of NE, leading rapidly to depletion of NE in LC terminals. The present finding that soman, locally infused into the olfactory bulb, increased NE levels suggests that NE depletion produced by systemic administration of soman is also due to presynaptic, cholinergic receptor stimulation of NE release from LC terminals. Local infusion of pilocarpine, however, decreased basal release of NE in the present study. Since systemic administration of pilocarpine also depletes forebrain

NE,[12] this suggests that increased LC discharge caused by pilocarpine is able to override its presynaptic, muscarinic receptor-mediated inhibition of NE release from LC terminals. Additional studies are needed, however, to determine if local stimulation of cholinergic receptors modifies terminal NE release during activation of cell bodies in a manner similar to the presently observed effects of local cholinergic receptor stimulation on basal release of NE.

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REFERENCES

1. Abercrombie E. D. and Finlay J. M. (1991) Monitoring extracellular norepinephrine in brain using *in vivo* microdialysis and HPLC-EC. In *Techniques in the Behavioral and Neural Sciences*, Vol. 7: *Microdialysis in the Neurosciences* (eds Robinson T. E. and Justice J. B.), pp. 253–274. Elsevier, Amsterdam.
2. Abercrombie E. D., Kelley R. and Zigmond M. J. (1988) Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. *Neuroscience* **27,** 897–904.
3. Adams L. M. and Foote S. L. (1988) Effects of locally infused pharmacological agents on spontaneous and sensory-evoked activity of locus coeruleus neurons. *Brain Res. Bull.* **21,** 395–400.
4. Aston-Jones G., Shipley M. T. and Grzanna R. (1995) The locus coeruleus. In *The Rat Nervous System*, 2nd edn (ed. Paxinos G.), pp. 183–213. Academic, New York.
5. Bickford-Wimer P., Pang K., Rose G. M. and Gerhardt G. A. (1991) Electrically-evoked release of norepinephrine in the rat cerebellum: an *in vivo* electrochemical and electrophysiological study. *Brain Res.* **558,** 305–311.
6. Brun P., Suaud-Chagny M. F., Gonon F. and Buda M. (1993) *In vivo* noradrenaline release evoked in the anteroventral thalamic nucleus by locus coeruleus activation: an electrochemical study. *Neuroscience* **52,** 961–972.
7. Curtis A. L., Lechner S. M., Pavcovich L. A. and Valentino R. J. (1997) Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing-factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalograhic activity. *J. Pharmac. exp. Ther.* **281,** 163–172.
8. Diamant S., Lev-Ari I., Uzielli I. and Atlas D. (1988) Muscarinic agonists evoke neurotransmitter release: possible roles for phosphatidyl inositol biphosphate breakdown products. *J. Neurochem.* **51,** 795–802.
9. Egan T. M. and North R. A. (1985) Acetylcholine acts on m_2-muscarinic receptors to excite rat locus coeruleus neurones. *Br. J. Pharmac.* **85,** 733–735.
10. Egan T. M. and North R. A. (1986) Actions of acetylcholine and nicotine on rat locus coeruleus neurons *in vitro*. *Neuroscience* **19,** 565–571.
11. Elaagouby A., Ravel N. and Gervais R. (1991) Cholinergic modulation of excitability in the rat olfactory bulb: effect of local application of cholinergic agents on evoked field potentials. *Neuroscience* **45,** 653–662.
12. El-Etri M. M., Ennis M., Jiang M. and Shipley M. T. (1993) Pilocarpine-induced convulsions in rats: evidence for muscarinic receptor mediated activation of locus coeruleus and norepinephrine depletion in cholinolytic seizure development. *Expl Neurol.* **121,** 24–39.
13. El-Etri M. M., Nickell W. T., Ennis M., Skau K. A. and Shipley M. T. (1992) Brain norepinephrine reductions in soman intoxicated rats: association with convulsions and AChE inhibition, time-course, and relation to other monoamines. *Expl Neurol.* **118,** 153–163.
14. Engberg G. and Svensson T. H. (1980) Pharmacological analysis of a cholinergic receptor mediated regulation of brain norepinephrine neurons. *J. neural Transm.* **49,** 137–150.
15. Ennis M. and Shipley M. T. (1992) Tonic activation of locus coeruleus neurons by systemic or intracoerulear microinjection of an irreversible acetylcholinesterase inhibitor: increased discharge rate and induction of c-fos. *Expl Neurol.* **118,** 153–163.
16. Fink K. and Gothert M. (1993) Modulation of *N*-methyl-D-aspartate (NMDA)-stimulated noradrenaline release in rat brain cortex by presynaptic α_2-adrenoreceptors. *Naunyn-Schmiedeberg's Arch. Pharmac.* **348,** 372–378.
17. Forray M. I., Andres M. E., Bustos G. and Gysling K. (1995) Regulation of endogenous noradrenaline release from the bed nucleus of the stria terminalis. *Biochem. Pharmac.* **49,** 687–692.
18. Fosbraey P., Wetherell J. R. and French M. C. (1990) Neurotransmitter changes in guinea-pig brain regions following soman intoxication. *J. Neurochem.* **54,** 72–79.
19. Gervais R. (1987) Local GABAergic modulation of noradrenaline release in the rat olfactory bulb measured on superfused slices. *Brain Res.* **400,** 151–154.
20. Gonzales R. A., Roper L. C. and Westbrook S. L. (1993) Cholinergic modulation of *N*-methyl-D-aspartate-evoked [^{3}H]norepinephrine release from rat cortical slices. *J. Pharmac. exp. Ther.* **264,** 282–288.
21. Gresch P. J., Sved A. F., Zigmond M. J. and Finlay J. M. (1995) Local influence of endogenous norepinephrine on extracellular dopamine in rat medial prefrontal cortex. *J. Neurochem.* **65,** 111–116.
22. Hunt S. P. and Schmidt J. (1978) Some observations on the binding patterns of α-bungarotoxin in the central nervous system of the rat. *Brain Res.* **157,** 213–232.
23. Jiang M. R., Griff E. R., Ennis M., Zimmer L. A. and Shipley M. T. (1996) Activation of locus coeruleus enhances the responses of olfactory bulb mitral cells to weak olfactory nerve input. *J. Neurosci.* **16,** 6319–6329.
24. Kasa P., Hlavati I., Dobo E., Wolff A., Joo F. and Wolff J. R. (1995) Synaptic and non-synaptic cholinergic innervation of the various types of neurons in the main olfactory bulb of the adult rat: immunocytochemistry of choline acetyltransferase. *Neuroscience* **67,** 667–677.
25. Kobayashi R. M., Palkovits M., Hruska R. E., Rothschild R. and Yamamura H. I. (1978) Regional distribution of muscarinic cholinergic receptors in rat brain. *Brain Res.* **154,** 13–23.
26. Kunze W. A. A., Shafton A. D., Kemm R. E. and McKenzie J. S. (1991) Effect of stimulating the nucleus of the horizontal limb of the diagonal band on single unit activity in the olfactory bulb. *Neuroscience* **40,** 21–27.
27. Kunze W. A. A., Shafton A. D., Kemm R. E. and McKenzie J. S. (1992) Intracellular responses of olfactory bulb granule cells to stimulating the horizontal diagonal band nucleus. *Neuroscience* **48,** 363–369.
28. Le Jeune H., Aubert I., Jourdan F. and Quiron R. (1996) Developmental profiles of various cholinergic markers in the rat main olfactory bulb using quantitative autoradiography. *J. comp. Neurol.* **373,** 433–450.
29. Le Jeune H. and Jourdan F. (1991) Postnatal development of cholinergic markers in the rat olfactory bulb: a histochemical and immunocytochemical study. *J. comp. Neurol.* **314,** 383–395.
30. L'Heureux R., Dennis T., Curet O. and Scatton B. (1986) Measurement of endogenous noradrenaline release in the rat cerebral cortex *in vivo* by transcortical dialysis: effects of drugs affecting noradrenergic transmission. *J. Neurochem.* **46,** 1794–1801.
31. McKay L., Bradberry C. and Oke A. (1984) Ascorbic acid oxidase speeds up analysis from catecholamines, indoleamines and their metabolites in brain tissue using high-performance liquid chromatography with electrochemical detection. *J. Chromat.* **311,** 167–169.
32. McLean J. H., Shipley M. T., Nickell W. T., Aston-Jones G. and Reyher C. K. H. (1989) Chemoanatomical organization of the noradrenergic input from locus coeruleus to the olfactory bulb of the adult rat. *J. comp. Neurol.* **285,** 339–349.
33. Milusheva E., Baranyi M., Zelles T., Mike A. and Vizi E. S. (1994) Release of acetylcholine and noradrenaline from the cholinergic and adrenergic afferents in rat hippocampal CA1, CA3, and dentate gyrus regions. *Eur. J. Neurosci.* **6,** 187–192.

34. Neslon M. F., Zaczek R. and Coyle J. T. (1980) Effects of sustained seizures produced by intrahippocampal injection of kainic acid on noradrenergic neurons: evidence for local control of norepinephrine release. *J. Pharmac. exp. Ther.* **214,** 694–702.
35. Nickell W. T. and Shipley M. T. (1988) Neurophysiology of magnocellular forebrain inputs to the olfactory bulb in the rat: frequency potentiation of field potentials and inhibition of output neurons. *J. Neurosci.* **8,** 4492–4502.
36. Nickell W. T. and Shipley M. T. (1993) Evidence for presynaptic inhibition of the olfactory commissural pathway by cholinergic agonists and stimulation of the nucleus of the diagonal band. *J. Neurosci.* **13,** 650–659.
37. Nisenbaum L. K., Zigmond M. J., Sved A. F. and Abercrombie E. D. (1991) Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *J. Neurosci.* **11,** 1478–1484.
38. Sacaan A. I., Dunlop J. L. and Lloyd G. K. (1995) Pharmacological characterization of neuronal acetylcholine gated ion channel receptor-mediated hippocampal norepinephrine and striatal dopamine release from rat brain slices. *J. Pharmac. exp. Ther.* **274,** 224–230.
39. Schwartz L. and Atlas D. (1989) Synergy between membrane depolarization and muscarinic receptor activation leads to potentiation of neurotransmitter release (II). *Brain Res.* **503,** 62–67.
40. Sershen H., Balla A., Lajtha A. and Vizi E. S. (1977) Characterization of nicotinic receptors involved in the release of noradrenaline from the hippocampus. *Neuroscience* **77,** 121–130.
41. Shipley M. T., Halloran F. J. and de la Torre J. (1985) Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. *Brain Res.* **329,** 294–299.
42. Shipley M. T., McLean J. H., Zimmer L. A. and Ennis M. (1996) The olfactory system. In *Handbook of Chemical Neuroanatomy*, Vol. 12: *Integrated Systems of the CNS*, Part III (eds Björklund A., Hökfelt T. and Swanson L. W.), pp. 467–571. Elsevier, Amsterdam.
43. Smagin G. N., Swiergiel A. H. and Dunn A. J. (1995) Corticotropin-releasing factor administered into locus coeruleus, but not the parabrachial nucleus, stimulates norepinephrine release in the prefrontal cortex. *Brain Res. Bull.* **36,** 71–76.
44. Ungerstedt U. (1991) Introduction to intracerebral microdialysis. In *Techniques in the Behavioral and Neural Sciences*, Vol. 7: *Microdialysis in the Neurosciences* (eds Robinson T. E. and Justice J. B.), pp. 3–22. Elsevier, Amsterdam.
45. Wang J. K. T., Andrews H. and Thukral V. (1992) Presynaptic glutamate receptors regulate noradrenaline release from isolated nerve terminals. *J. Neurochem.* **58,** 204–211.
46. Westfall T. C. (1974) Effect of muscarinic agonists on the release of ^{3}H-norepinephrine and ^{3}H-dopamine by potassium and electrical stimulation from rat brain slices. *Life Sci.* **14,** 1641–1652.
47. Westfall T. C. (1974) Effect of nicotine and other drugs on the release of ^{3}H-norepinphrine and ^{3}H-dopamine from rat brain slices. *Neuropharmacology* **13,** 693–700.
48. Westfall T. C. (1980) Neuroeffector mechanisms. *A. Rev. Neurosci.* **42,** 383–397.
49. Zaborszky L., Carlsen J., Brashear H. R. and Heimer L. (1986) Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. *J. comp. Neurol.* **243,** 488–509.

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