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RELATIONSHIP BETWEEN LOCUS COERULEUS DISCHARGE RATES AND RATES OF NOREPINEPHRINE RELEASE WITHIN NEOCORTEX AS ASSESSED BY *IN VIVO* MICRODIALYSIS

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Abstract—The relationship between discharge rates of locus coeruleus noradrenergic neurons and rates of norepinephrine release was examined in the anesthetized rat. Neuronal discharge rates of locus coeruleus neurons were altered and quantified using a combined recording-infusion probe. Peri-locus coeruleus infusions of either the cholinergic agonist, bethanechol, or the α_2-agonist, clonidine, were used to enhance or suppress neuronal discharge activity, respectively. Alterations in concentrations of extracellular norepinephrine within the prefrontal cortex were determined using *in vivo* microdialysis and high-pressure liquid chromatography with electrochemical detection. A linear relationship between locus coeruleus activity and norepinephrine dialysate concentration was observed between complete suppression of locus coeruleus discharge activity and approximately 300–400% of basal discharge levels (1.58 ± 0.29 Hz). Above these levels, increases in locus coeruleus discharge rates were not accompanied by similar increases in dialysate norepinephrine concentrations. In general, neither activation nor suppression of locus coeruleus neuronal discharge rates appeared to alter the relationship between discharge activity and norepinephrine efflux during subsequent epochs. The one exception to this was observed during recovery from relatively high-magnitude locus coeruleus activation. In two out of three cases in which locus coeruleus discharge rates were increased greater than 450%, a recovery of norepinephrine concentrations to basal levels occurred more quickly than the recovery of locus coeruleus neuronal discharge rates to basal levels.

Although limited, these latter observations suggest that dysregulation of norepinephrine release may occur following sustained activation of locus coeruleus at the highest rates examined, which may mimic those associated with intense arousal or stress.

Key words: norepinephrine, release, locus coeruleus, prefrontal cortex, microdialysis.

The locus coeruleus (LC) is the major source of brain norepinephrine (NE), providing the sole source of NE to neocortex and hippocampus. LC neurons display two primary modes of discharge activity: tonic and phasic. Tonic activity is characterized by sustained and highly regular discharge patterns, the firing rate of which is state dependent.[2,10] These and other observations have suggested a role of tonic activity of the LC-noradrenergic system in the modulation of behavioral state. Consistent with this hypothesis, increases in LC neuronal discharge rates elicit activation of forebrain EEG in the anesthetized rat,[5] whereas stimulation of noradrenergic β-receptors within the medial basal forebrain elicits waking in the unanesthetized, sleeping animal.[7]

In waking, phasic alterations in discharge rates are observed in response to salient sensory stimuli.[3,10] These responses, superimposed upon tonic activity, are comprised of a brief excitatory component followed by a more prolonged period of suppression of discharge activity. Phasic alterations in LC neuronal discharge rates have been posited to participate in processing sensory information and modulation of attention to sensory stimuli.[4,13]

Combined, these observations suggest LC neurons are important in the induction of a behavioral state appropriate for the collection of sensory stimuli. In waking, these neurons appear to modulate attentional and other cognitive processes involved in the processing of sensory information. Much of the current view of LC function in behavior stems from information regarding alterations in LC neuronal discharge rates across varying behavioral/cognitive tasks. In theories of LC function, it is generally assumed that rates of NE release are linearly related to LC neuronal discharge rates across the entire range of discharge rates displayed by these neurons. To date, this assumption has not been rigorously examined, although evidence from both *ex vivo* and *in vivo* studies indicates a positive relationship between LC discharge activity and NE release.[8,9,11]

We have described previously the use of a combined electrophysiological recording/infusion probe that permits the selective enhancement and suppression of LC tonic discharge activity using small infusions of pharmacological agents placed immediately adjacent to LC.[5,6] The use of this probe provides a number of advantages over previously available methodology, such as electrical stimulation. First, it employs a physiologically relevant, receptor-mediated alteration of LC activity. Second, it avoids placement of a relatively large infusion needle (or stimulating electrode) directly into LC, and thus minimizes damage to LC neurons. Third, it permits both suppression and enhancement of neuronal discharge activity. Finally, this probe permits assessment of the extent to which experimental manipulations alter discharge activity relative to basal activity levels.

The current studies combined use of this recording/infusion probe and *in vivo* microdialysis to examine the relationship between tonic LC discharge rates and rates of NE release within prefrontal cortex, in halothane-anesthetized rats. Alterations in prefrontal cortical extracellular NE concentrations

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Abbreviations: AECF, artificial extracellular fluid; EDTA, ethylenediaminetetra-acetate; EEG, electroencephalographic/electroencephalogram; HPLC–EC, high-pressure liquid chromatography with electrochemical detection; LC, locus coeruleus; NE, norepinephrine.

were assessed while LC discharge rates were systematically varied above and below spontaneous discharge rates. The range of LC discharge rates examined encompasses activity levels typically displayed by these neurons across a variety of behavioral states and environmental conditions.

Recent evidence suggests that the activity state of the terminal field may influence rates of NE release.[12] The ability of LC activation to alter the state of the forebrain [e.g., elicit activation of forebrain electroencephalographic activity (EEG); see above], is anesthesia dependent.[5] Therefore, in a subset of studies we compared alterations in rates of NE efflux following LC activation under conditions that either permitted or prevented activation of cortical EEG.

EXPERIMENTAL PROCEDURES

Animals and surgery

Male Sprague–Dawley rats weighing 290–320 g (Charles River, Wilmington, MA) were housed in groups of two or three and provided *ad libitum* access to food and water for approximately one week following arrival. On the day prior to testing, a microdialysis probe was lowered into the prefrontal cortex (A + 3.2; L 1.2; V − 5.0) over a 5–10 min period and an EEG recording electrode was implanted into the contralateral frontal cortex, under halothane anesthesia. The EEG electrode and dialysis probe were cemented into position using acrylic cement (Plastics One, Roanoke, VA). Care was taken to avoid even slight vibration of the dialysis probe, as previous observations indicated that small, rapid movements of the dialysis probe results in substantially lower NE dialysate concentrations. Following probe implantation, the animals were placed in a Plexiglas chamber and artificial extracellular fluid (AECF) was perfused through the dialysis probe at a flow rate of 0.5 μl/min overnight. All efforts were made to minimize animal suffering and to minimize the number of animals used.

Locus coeruleus recordings and infusions

On the day of testing, the animal was reanesthetized and placed in the stereotaxic apparatus with the incisor bar positioned at − 11.5 mm below the ear-bars. Infusion and recording procedures were those described previously.[5] In brief, the recording/infusion probe consisted of a stainless steel microelectrode glued parallel to a shorter piece of 26-gauge hypodermic tubing. A skull hole was drilled above the LC (− 4.0 A; 1.4 L, relative to lambda-midline intersection) and a recording/infusion probe was used to locate the LC. LC neurons were tentatively identified using previously described criteria.[16] In some cases, single-unit recordings were made for a 15–20 min period to assess discharge rate. Following this, the electrode was positioned for a multi-unit recording (often this required passing current through the electrode to decrease electrode impedance). Quantification of discharge activity was made using an amplifier/window discriminator (Fintronics, New Haven, CT). Once located, a 33-gauge infusion needle was inserted into the guide tube that had been loaded with drug and was connected to a 10 μl syringe via a length of PE20 tubing. The PE20 tubing was connected to the 33-gauge infusion needle via a 26-gauge sleeve attached to the needle. Each probe is constructed such that the ventral tip of the needle extends to the depth of the electrode tip with a lateral displacement of about 300–500 μm. This construction ensures that when the recording electrode is placed within LC, the infusion needle is positioned 200–400 μm outside of LC, avoiding needle-induced damage to LC.

One-hundred-and-fifty nanoliter peri-LC infusions of bethanechol (1–4 ng/nl) or clonidine (0.25–1.0 ng/nl), dissolved in AECF, were made over 90 s. To prevent leakage of clonidine into tissue, 150 nl of AECF was loaded into the infusion needle following loading clonidine. AECF and clonidine were separated by an approximately 100 nl air bubble. This also permitted determination of the effect of AECF infusions on LC neuronal discharge rates, prior to making drug infusions.

Under these experimental conditions LC neurons displayed activity rates of 1.58 ± 0.27 Hz ($n = 8$). In general, multi-unit recordings were utilized during the experiments. In two cases in which bethanechol infusions were made, single-unit activity was recorded. In these two cases, the magnitude and time-course of LC activation were similar to those obtained with multi-unit recordings.

Electroencephalographic recording

A bipolar surface-to-depth recording electrode was used to record cortical EEG in the hemisphere contralateral to the manipulated LC, as previously described.[5] EEG signals were amplified, filtered (0.1–50.0 Hz bandpass), and recorded on a polygraph and on magnetic tape. In previous studies, identical alterations in left and right cortical EEG activity patterns were observed with unilateral LC activation.[5] Thus, in terms of EEG activity patterns and temporal parameters of EEG responses, the contralateral hemisphere provides information comparable to that of the ipsilateral hemisphere. EEG electrodes were not implanted in the same hemisphere in which the microdialysis probe was implanted to minimize damage to that hemisphere.

Microdialysis and high-pressure liquid chromatographic analysis of norepinephrine concentrations

NE was measured in dialysate samples using high-pressure liquid chromatography (HPLC) with electrochemical detection (EC), as described previously.[15] In brief, AECF (147 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 2.5 mM KCl; pH 7.4) was delivered at a rate of 1.5 μl/min through PE20 tubing to a length of Spectra/Por hollow fiber (mol. wt cut-off 6000, o.d. 250 μm). The dialysis membrane was sealed and attached to the PE20 tubing with epoxy. The length of functional dialysis membrane in contact with tissue was 4 mm. A 6 inch length of fused silica (150 μm o.d., 75 μm i.d.) provided outflow.

At least three to four baseline samples were collected beginning approximately 30–60 min after the peri-LC infusion needle was lowered into the brainstem. Aliquots (6–20 μl) were injected on to an HPLC–EC system consisting of a Waters Model 510 pump set at 0.6 ml/min, a Velosep C18 100 × 3.2 mm cartridge, an ESA Model 5100A detector with two electrodes in series: − 0.025 V, +0.220 V. The mobile phase consisted of 60–80 mM sodium phosphate (pH 2.75), 100 μM EDTA, 1.16–1.46 mM sodium octyl-sulfate and 3–4% v/v methanol. The quantitation limit for NE (using a criterion of three times background noise) was approximately 0.3–0.5 pg of NE. The mean concentration per 20 μl sample was 2.1 ± 0.2 pg (range = 1.1–3.2 pg/20 μl). All data are expressed in pg/20 μl sample. In those cases where the sample volume was less than 20 μl (e.g., 5 min collection periods), values are corrected for expression as pg/20 μl. In developing the NE assay, the NE peak was initially identified based on the retention time of NE standards, the ability of NE added to the sample to increase the size of the NE peak, the ability of LC activation to increase peak size, and the ability of intraperitoneally (i.p.)-administered clonidine to reduce the NE peak below the levels of detection. Intermittently, during the course of the studies, clonidine (100 μg/kg) was administered i.p. at the end of the experiment to confirm that the NE peak could be reduced to below the limits of detection, verifying that: (i) this peak did contain NE; and (ii) this peak did not contain one or more contaminant.

Histological analyses and data selection

Placement of LC recording/infusion probes, dialysis probes and EEG electrodes was histologically verified in 50-μm-thick coronal sections stained with Neutral Red dye. Data from a given experiment were included only when histological analyses verified accurate placement of the LC recording/infusion and microdialysis probes, LC recordings were electrically adequate and stable, and NE concentrations were stable (± 10% of the mean) throughout the baseline portion of the experiment.

RESULTS

Effects of peri-locus coeruleus bethanechol-induced locus coeruleus activation on norepinephrine efflux

Figure 1 shows typical placement of dialysis probes within the prefrontal cortex and of the recording/infusion probe within LC. In general, minimal tissue damage was associated with placement of both dialysis and recording/infusion

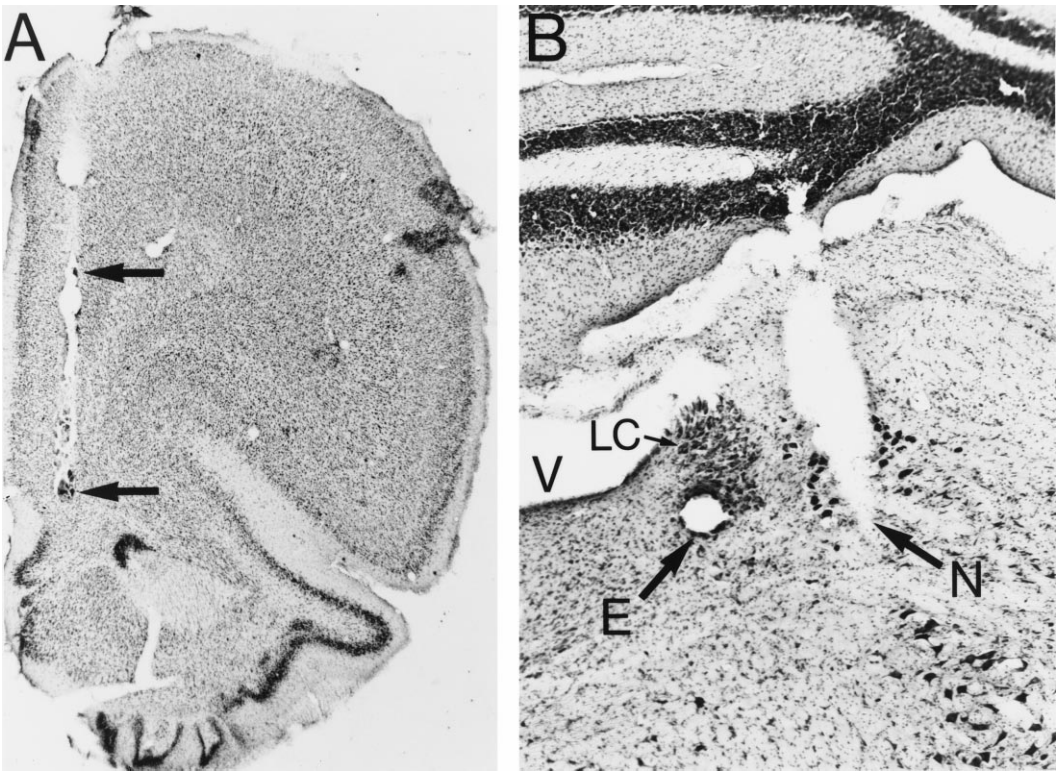

Fig. 1. Photomicrograph of LC recording, peri-LC infusion and prefrontal cortex dialysis probe implant sites. (A) Microdialysis probe implant site within prefrontal cortex in Neutral Red-stained 40 μm section. Arrows indicates track created by the dialysis probe. (B) LC recording/peri-LC infusions sites. The LC is the collection of small dark cells, the ventral border of which is even with the tip of the infusion needle. The recording electrode site (E) is marked by the small hole in the ventral portion of LC. The infusion site is the enlarged portion of the tract created by the infusion needle (N), lateral to LC. Me5, mesencephalic nucleus of the trigeminal nerve; (V) fourth ventricle.

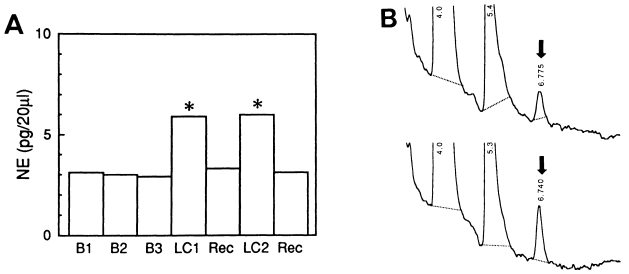

Fig. 2. Repeated LC stimulation elicits consistent enhancement of NE concentrations in cortical dialysis samples. (A) NE concentrations (pg/20 μl) prior to, during and following LC activation (asterisks indicate samples collected during LC activation). All samples were collected over 15 min. Peri-LC bethanechol infusions (1 ng/nl; 100 nl) were made immediately prior to samples LC1 and LC2. These infusions elevated LC discharge rates to a maximum of approximately 300% of baseline (approximately 10 min total duration of LC activation). LC activation was accompanied by an increase in NE concentrations from approximately 3.0 pg to approximately 6.0 pg/sample. (B) Chromatograms from same experiment. Shown are samples B3 and LC1. The peak corresponding to NE is indicated by the down arrow.

probes. In the first series of studies, 150 nl peri-LC infusions of the cholinergic agonist, bethanechol (1–4 ng/nl), were used to increase LC neuronal discharge rates to varying degrees above basal levels. Occasionally, following recovery of bethanechol-induced activation, LC discharge rates returned to a level that differed substantially from preinfusion levels. Because it was not possible to determine unambiguously whether this resulted from movement of tissue, neuronal damage or normal physiological processes, data from these cases were excluded from the analyses. In general, vehicle infusions ($n = 7$) did not result in alterations in LC neuronal activity or alterations in NE dialysate concentrations (data not shown). In one case, vehicle infusion elicited a stable and relatively small (e.g., approximately 35%) decrease in basal discharge rates, suggestive of a shift in tissue relative to the recording electrode, which was not accompanied by alterations in NE dialysate concentrations.

In all experiments, two bethanechol infusions were made, each separated by two or three 15 min recovery samples. In all cases in which stable baseline LC activity rates were observed through the recovery portion of both infusions, repeated peri-LC bethanechol infusions elicited similar effects on LC neuronal discharge rates and comparable increases in NE dialysate concentrations ($n = 8$; e.g., see Fig. 2). The bethanechol-induced increase in LC activity was characterized by a 3–5 min period during which maximal LC activity rates were observed, followed by a steady decline to pre-infusion levels over the next 5–10 min (Fig. 3). To better assess the relationship between LC activity and NE efflux, two 5 min dialysate samples were collected beginning at near-maximal LC activation followed by at least two 15 min recovery samples ($n = 17$). For these analyses, only data from one set of infusions per animal were included. The 5 min samples corresponded to maximal and intermediate levels of LC activation. Peri-LC bethanechol increased LC discharge rates to approximately 100–600% of basal levels, when averaged over the first 5 min stimulation epoch. As shown in Fig. 3, alterations in NE concentrations closely followed alterations in LC activity (data are corrected for expression as pg/20 μl sample).

The magnitude of the NE response, as measured by

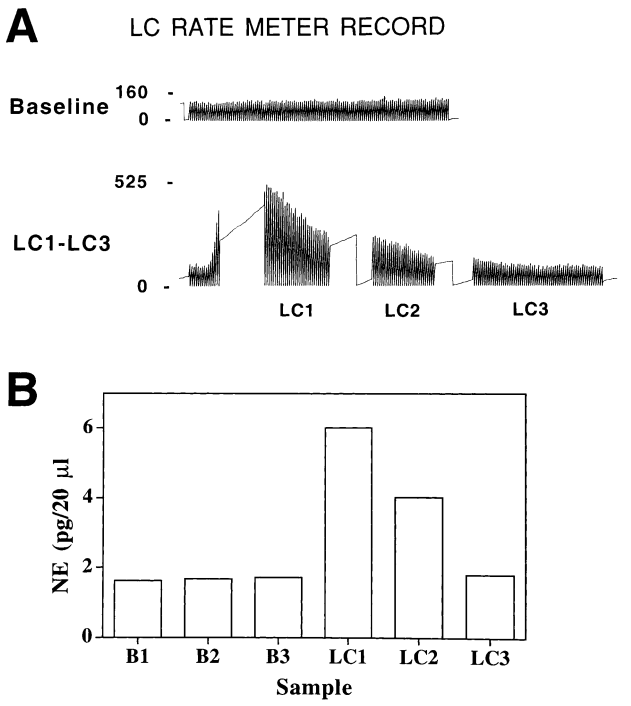

Fig. 3. Typical time-course of LC and NE responses following peri-LC bethanechol. (A) Rate-meter record of LC multi-unit activity immediately prior to (baseline) and following (LC1–LC3) peri-LC bethanechol. (B) NE concentrations of dialysate samples from same experiment shown in (A). When appropriate (5 and 10 min samples), NE concentrations are corrected for expression as pg/20 µl of sample. All baseline samples (B1–B3) were collected for 15 min. LC1 and LC2 were 5 min samples and LC3 was a 10 min sample. Immediately preceding LC1, a 150 nl bethanechol infusion was made that increased LC discharge levels an average of 370% during the first 5 min sample and 195% in the second 5 min sample, relative to basal levels. LC activation increased NE concentrations to 360% and 240% of baseline in LC1 and LC2, respectively.

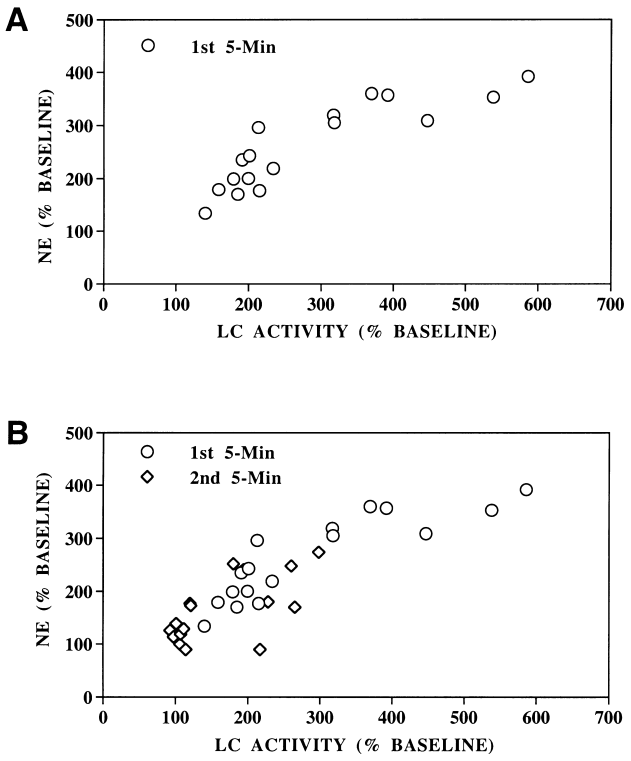

Fig. 4. Relationship between bethanechol-induced increases in LC activity and NE efflux. (A) Shown are NE dialysate concentrations vs LC activity for the first 5 min sample collected immediately following peri-LC bethanechol infusions. Both NE concentrations and LC activity are expressed as percentage of baseline levels. NE baseline levels were determined by averaging the three baseline dialysis samples that immediately preceded the infusion. LC baseline activity levels were determined by averaging trigger counts (action potentials)/10 s during the 15 min sample that immediately preceded the infusion. A relatively linear relationship between enhancement of LC discharge rates and increases in NE concentrations is observed up through approximately 300–400% of basal LC discharge rates. Increases in LC discharge rates beyond 300–400% basal levels do not elicit corresponding increases in NE efflux. (B) Data are presented from both the first 5 min (maximum activation) and the second 5 min (recovery) samples. In general, a comparable relationship is observed between NE concentrations and LC activity for the first 5 min and recovery samples. However, in two out of three cases in which the maximum LC activation was greater than 450% of baseline, NE concentrations approached pre-infusion levels more rapidly than LC activity rates.

percentage of pre-infusion levels, is largely linearly proportional to the magnitude of LC activation, up to approximately 300–400% of basal discharge levels (Fig. 4A). Beyond this level, increases in LC discharge rates do not appear to elicit comparable increases in NE concentrations (see below). During recovery, alterations in NE concentrations largely followed alterations in LC neuronal activity. Thus, in general, a similar relationship between NE concentrations and LC discharge rates was observed for both the first 5 min and second 5 min samples. An exception to this was observed in two out of three cases in which LC activation was greater than or equal to 450% of baseline during the first 5 min epoch of LC activation. In these cases, NE concentrations approached basal levels more rapidly than did LC activity during the second 5 min epoch (see Fig. 4B).

Effects of forebrain activity state, as measured by electroencephalographic activity, on rates of norepinephrine efflux

To assess the extent to which the activity state of the terminal field modulates rates of NE release, the above-described studies were conducted under two different experimental conditions. In the first ($n = 12$), the level of halothane anesthesia was adjusted such that neither tail-pinch, nor LC activation, elicited an activation of cortical EEG. In the second condition ($n = 5$), halothane was adjusted such that both tail-pinch and LC activation elicited a robust activation of cortical EEG.[5] Cortical EEG activation is characterized by a profound suppression of large-amplitude, slow-wave activity and the presence of low-amplitude, high-frequency activity (Fig. 5A). This activation of the EEG was maintained for at least 5 min, throughout the period of maximal LC activation corresponding to the first 5 min dialysis sample collected during LC activation. As shown in Fig. 5B, comparable increases in dialysate NE concentrations were observed whether LC activation was accompanied by EEG activation or not.

Effects of peri-locus coeruleus clonidine-induced locus coeruleus suppression on norepinephrine efflux

In a second series of studies, the effects of suppression of basal LC discharge rates on NE dialysate concentrations were examined. Suppression of LC discharge rates was accompanied by a decrease in NE concentrations in dialysate samples (15 min collection time). Maximal clonidine-induced suppression was often not obtained until 15–25 min (one to

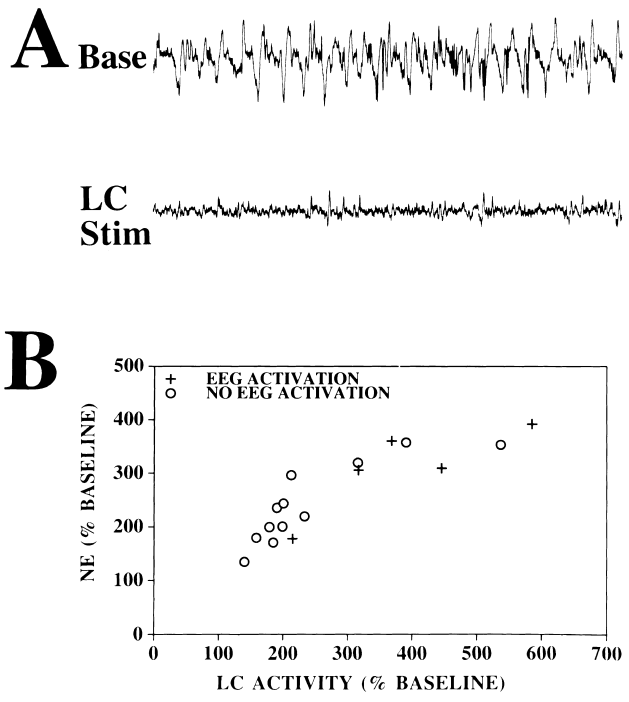

Fig. 5. Relationship between LC activity and NE efflux is not influenced by activity state of the terminal field, as measured by EEG. In all experiments, cortical EEG was recorded and baseline EEG activity was characterized by the stable presence of large-amplitude, slow-wave activity. Halothane level was adjusted to permit or prevent EEG activation following peri-LC bethanechol-induced enhancement of LC discharge rates. (A) Raw cortical EEG activity from one of the five cases in which peri-LC bethanechol induced robust EEG activation. Shown are 40 s segments of EEG immediately preceding (top trace; base) and following bethanechol-induced stimulation of LC (bottom trace; LC Stim). Robust EEG activation persisted for approximately 6 min. (B) Relationship between NE dialysate concentrations (expressed as a percentage of baseline) and LC activity (expressed as percentage of baseline) for the first 5 min epoch following peri-LC bethanechol infusions.

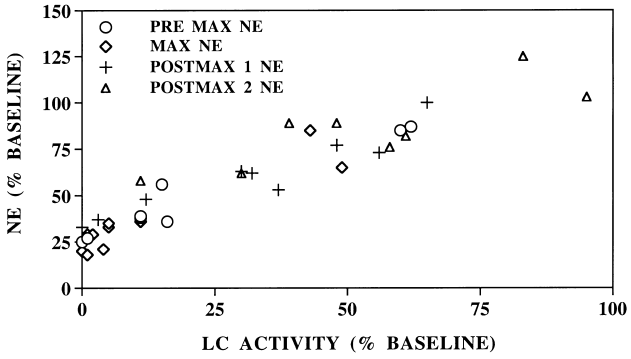

Fig. 6. Relationship between LC activity and NE efflux following clonidine-induced LC suppression. Maximal LC suppression was achieved within the first one to two samples following peri-LC clonidine. The magnitude of maximal suppression ranged from 50 to 0% of basal LC discharge rates. Shown are data from four 15 min epochs, determined relative to maximal suppression of LC discharge rates: pre-maximal LC suppression (PRE-MAX); maximal LC suppression (MAX); and the first two samples from LC recovery (POSTMAX 1 and POSTMAX 2). Not all experiments had pre-maximal suppression samples. Across this range of LC activity (0–100%), there appears to be a linear relationship between LC discharge activity and NE concentrations. At zero LC discharge activity, NE concentrations were approximately 25% of basal levels. For both pre-maximal and recovery samples, the relationship between NE concentrations and LC activity appears similar.

two samples) following the infusion. Thus, for many cases, pre-maximal, maximal and post-maximal (recovery) samples were collected. The relationship between decreases in LC neuronal discharge rates and decreases in NE dialysate concentrations appears linear (Fig. 6). It is possible that LC suppression for many minutes might alter the relationship between LC activity and NE efflux. However, in the current studies a similar relationship between LC activity and NE concentrations was observed whether samples were collected immediately prior to or following maximal suppression (Fig. 6).

Following complete unilateral suppression of LC neuronal activity, the size of the NE peak approached the limit of detection of the NE assay. However, in all cases, the NE peak in the chromatogram was distinct. This is in contrast to that observed following systemic administration of clonidine, when NE concentrations were typically below the level of detection (Fig. 7).

Combined data from peri-locus coeruleus bethanechol and clonidine studies

The combined data from both the peri-LC clonidine and peri-LC bethanechol studies are presented in Fig. 8. As can be seen, there appears to be a linear relationship between LC neuronal discharge activity and NE release, up to approximately 300–400% basal discharge rates. Beyond this value, it appears that NE efflux tends to asymptote. The conclusion that there is a stronger linear relationship between LC discharge rates and NE efflux is supported by results of linear regression analyses. A correlation coefficient of $r^2 = 0.931$ was obtained across the entire range of LC activity rates examined (0–600%). However, this appears to derive primarily from data collected below 300–400% LC activity levels (0–400%, $r^2 = 0.963$). Between 300–600% LC activity and NE efflux are not as strongly correlated ($r^2 = 0.452$). Further evidence that the relationship between LC activity and NE efflux may not be strictly linearly related across the entire range of LC activity levels examined is the apparent better description of this relationship by a second-order polynomial ($r^2 = 0.964$).

DISCUSSION

General observations

The current studies characterized the relationship between LC discharge rates and NE efflux within neocortex, assessed using *in vivo* microdialysis. In these studies, a linear relationship between LC activity and NE efflux was observed across LC discharge rates that ranged from complete suppression to approximately 300–400% of basal discharge levels (1.58 ± 0.29 Hz). Beyond 300–400% of basal levels, increases in LC discharge rates were not accompanied by comparable increases in NE dialysate concentrations.

Complete unilateral suppression of LC neuronal activity (peri-LC clonidine) results in a decrease of NE concentrations to approximately 25% of pre-suppression levels. Precise measurement of NE in these samples is difficult owing to the fact that these levels approach the limit of detection for our assay. However, in virtually all cases, there was a discernible NE peak present in the chromatogram. This is in contrast to that observed following i.p. clonidine, which resulted in suppression of NE levels below the limits of detection. Thus,

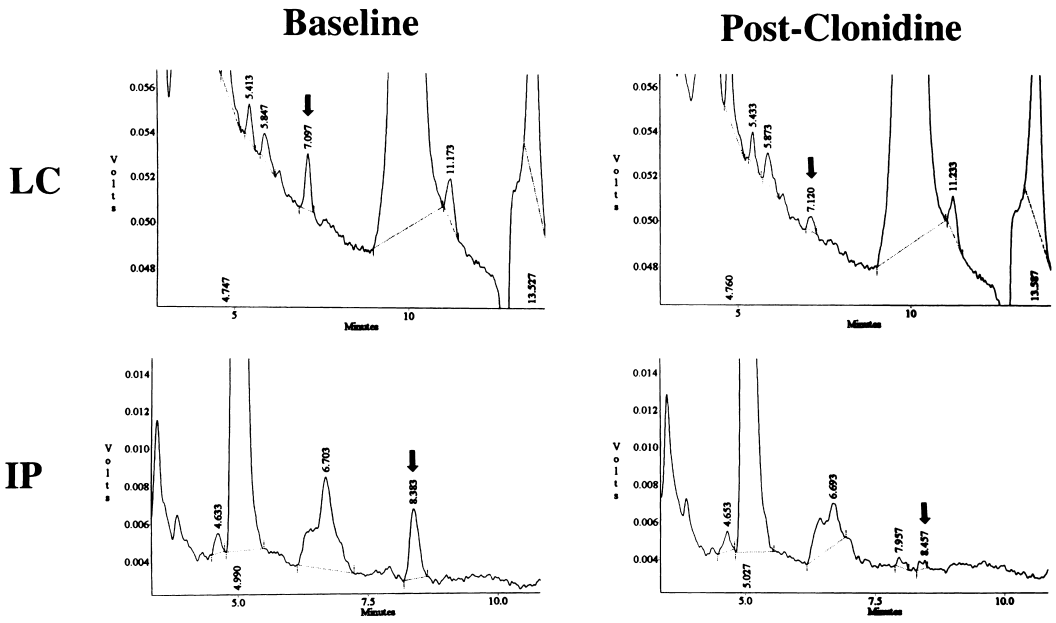

Fig. 7. Effects of peri-LC and i.p. clonidine on NE concentrations. Shown are chromatograms from baseline (pre-clonidine) and post-clonidine dialysate samples. The NE peak is indicated by the down arrow. Data from peri-LC and i.p. infusions are from separate cases. In these cases, both peri-LC and i.p. clonidine resulted in the complete cessation of LC neuronal discharge activity for the entire period of sample collection. Both treatments substantially decreased the size of the NE peak. Intraperitoneal clonidine consistently resulted in a decrease in the NE peak to below detection levels. In contrast, peri-LC clonidine infusions consistently decreased the NE peak to a size that, although substantially smaller than pre-infusion levels, was readily discernible.

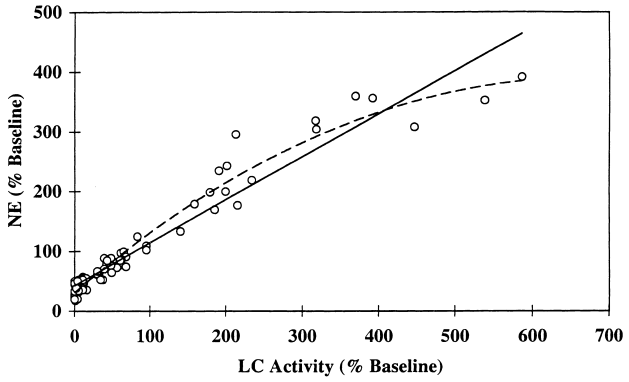

Fig. 8. Relationship between LC activity and NE efflux following both LC activation (first 5 min epoch only) and LC suppression. The figure combines data from both series of studies displayed in Figs 4A and 7. There appears to be a strong linear relationship between LC activity and NE efflux across the range of LC activity examined, as indicated by linear regression analysis (solid line; $r^2 = 0.931$). However, this appears to derive mostly from data collected below 300–400% basal LC discharge rates ($r^2 = 0.963$). Beyond 300–400%, NE levels appear to asymptote and NE levels and LC activity are not as strongly correlated (300–600%, $r^2 = 0.452$). Further evidence that the relationship between LC activity and NE levels is not strictly linear across the entire range of LC activity levels examined is the apparent better description of this relationship by a second-order polynomial (dotted line).

it appears that there is appreciable NE present in the ipsilateral cortex following complete suppression of LC neuronal discharge activity. Presumably, at least a portion of NE remaining within the ipsilateral cortex following complete unilateral suppression of LC discharge activity results from noradrenergic fibers originating from the contralateral LC.[17]

In general, neither activation nor suppression of LC discharge rates appeared to alter the relationship between LC activity and NE efflux during subsequent epochs. This indicates that sustained alterations (10 min for activation, 20–60 min for suppression) in LC discharge rates do not impact subsequent rates of NE release. The one exception to this was observed during recovery from relatively high-magnitude LC activation. In two out of three cases in which the average LC discharge rate exceeded 450% of preinfusion levels during the first 5 min sample, a dissociation between LC activity and NE efflux was observed during the recovery portion of the LC response (the second 5 min sample). In these cases, NE concentrations in the second 5 min sample approached pre-LC activation concentrations more rapidly than LC activity approached basal levels. This dissociation between infusion-induced alterations in NE concentrations and LC discharge activity during recovery from high LC activation could reflect physiological limitations of LC neurons to maintain NE release. Alternatively, this could reflect actions of rate-dependent autoregulatory mechanisms (e.g., re-uptake or autoreceptor). Finally, the variable nature of this observation may reflect non-physiological mechanisms, such as the extent of probe-induced tissue damage. Additional studies are required to provide an unambiguous answer to this question.

Methodological considerations

The current studies utilized *in vivo* microdialysis and multi-unit extracellular recordings to assess rates of NE release following peri-LC infusion-induced alterations in LC neuronal discharge activity. These methods permit assessment of alterations in NE efflux within an LC terminal field across the broad range of activity levels typically displayed by these neurons, both above and below basal levels. Further, pharmacologically induced alterations in LC neuronal discharge rates involve physiological, receptor-mediated mechanisms bypassed with electrical stimulation. The temporal resolution of microdialysis as well as the time-course of peri-LC-induced alterations in LC neuronal discharge rates are well suited to the study of rates of NE

release associated with fluctuations in LC tonic discharge activity typically observed in the unanesthetized animal.

In the present studies, assessment of the relationship between LC discharge rates and NE release was primarily dependent on expression of NE concentrations in terms of percentage of pre-infusion baseline. Absolute levels of dialysate NE concentrations are subject to influence from both physiological (such as probe placement location) and non-physiological factors (such as probe-related tissue damage, diffusion kinetics of NE from tissue to probe). These factors are not well understood or under strict experimental control. This makes interpretation of differences in absolute levels of NE between animals difficult. Similarly, the use of multi-unit recordings precludes determination of absolute LC activity rates. Multi-unit recordings were preferable for these studies because stable and prolonged single-unit recordings are extremely difficult to obtain under these experimental conditions. The use of multi-unit recordings necessitated expression of experimentally induced alterations in LC discharge rates as a percentage of pre-infusion rates.

Limited evidence suggests that regardless of whether NE and/or LC responses are expressed as absolute levels or as percentage of pre-infusion means, a similar relationship between LC discharge rates and NE release is obtained. First, consistent with previous observations,[5] in two cases in which single-unit LC recordings were obtained and bethanechol was used to enhance LC discharge rates, the magnitude of the increases in LC activity was similar to that obtained with multi-unit recordings. This suggests that multi-unit recordings describe accurately alterations in single-unit activity. In addition, within a given animal, the relationship between LC activity and absolute levels of NE concentration, across the range of LC activity levels examined in that animal, was similar to that observed when NE levels are plotted as a percentage of pre-infusion means (e.g., see Fig. 3). Finally, the results obtained with bethanechol-induced activation of LC neurons are entirely consistent with previous observations on the effects of LC electrical stimulation on various indices of NE release.[8,9,11]

Previous studies

Results obtained in the current study are consistent with those obtained in previous studies in which a positive relationship between LC discharge rates and NE efflux was observed. For example, Korf *et al.*[11] demonstrated frequency-dependent increases in tissue concentrations of the NE catabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), following electrical stimulation of LC. Similar rate-dependent increases in NE utilization have been observed utilizing *in vivo* measures. For example, rate-dependent increases in NE were observed in prefrontal cortical microdialysis samples following electrical stimulation of LC.[9] The use of a NE re-uptake blocker in these latter studies could have altered the normal relationship between LC discharge rates and NE release. However, electrical stimulation of the dorsal noradrenergic bundle or infusions of glutamate into LC also elicited frequency/concentration-dependent increases in oxidation current corresponding to NE using *in vivo* voltammetry.[8] *In vivo* voltammetry is not able currently to provide measures of baseline extracellular NE concentrations. As such, use of this method is restricted to the examination of NE efflux at LC activity rates above basal discharge levels.

Effects of activity state of the terminal field

It has been proposed that the activity state of the terminal field may exert a substantial influence on rates of NE efflux, via actions of excitatory thalamocortical afferents.[12] Under experimental conditions utilized in the current studies, LC activation elicits an anesthesia-dependent activation of the forebrain, as measured by EEG.[5] Given that EEG activation is typically associated with activation of thalamocortical afferents, we compared NE responses following LC activation under conditions that permit or prevent LC activation-induced EEG activation. Comparable NE responses were observed whether LC activation was accompanied by an activation of cortical EEG or not. This indicates that the activity state of the terminal field, as measured by EEG, is not a primary factor determining rates of NE efflux, under these experimental conditions.

Relevance to behavior

The range of LC activity rates examined in the current studies encompasses those rates typically displayed by LC neurons in the unanesthetized animal. These manipulations elicited discharge rates ranging from those observed in rapid-eye movement (REM) sleep, in which LC neurons are virtually silent, to those corresponding to alert, active waking.[2,3,10] Results obtained in these studies demonstrate that across this wide range of discharge activity, there is a linear relationship between LC neuronal discharge activity and NE efflux, that is maintained up to 300–400% of basal levels (corresponding to approximately 4.5–6.5 Hz).

Within waking, LC discharge rates of rat and monkey are positively correlated with behavioral and EEG indices of arousal, with tonic discharge rates up to 5 Hz typically observed.[10] These rates of discharge activity are often maintained for prolonged epochs. Thus, the sustained 300–400% increase in LC discharge rates elicited in the current studies approximates that typically observed in unanesthetized animals.

It is less clear whether LC activation greater than 400% of basal levels reflects physiologically relevant activity levels. In monkeys, complex sensory stimuli that increase behavioral and EEG indices of arousal elicit brief epochs of tonic discharge rates of approximately 7–15 Hz.[10] However, it is currently not known whether prolonged exposure to high arousal-inducing conditions, such as exposure to moderate-to-strong stressors, results in LC activity rates in this range over prolonged periods (minutes) in either rat or monkey.

Behavioral deficits have been observed following prolonged exposure to intense stressors that appear to involve actions of LC neurons and alterations in rates of NE release.[18] The current observations suggest that the ability to maintain high rates of NE release during sustained periods of elevated LC discharge activity may be limited. Future studies will need to address the degree to which rat and monkey LC neurons display prolonged epochs of discharge rates above 4–5 Hz under stressful, or other intense arousal-inducing conditions, and the extent to which NE efflux is maintained under these conditions. In the cat, LC neurons do not display activity rates greater than approximately 3 Hz in the presence of stressors.[1]

However, it should be noted that the cat may not be an appropriate model for either the primate or rat LC-noradrenergic system. For example, in addition to anatomical differences between cat and both rat and monkey LC,[14] LC discharge rates of rat and monkey are higher than those observed in cat during alert waking.[1] Further, cat LC neurons appear to be less responsive to arousal-enhancing environmental stimuli than those observed in either rat or monkey.[1,14]

LC neurons display two primary modes of neuronal discharge activity. Each mode probably serves distinct behavioral functions. First, LC neurons display a state-dependent, tonic mode of discharge activity.[10] Second, superimposed upon tonic discharge activity are phasic responses to salient sensory stimuli.[10] The relatively prolonged (minutes) pharmacological manipulation of LC neuronal discharge rates used in these studies closely mimics changes in tonic discharge rates. Given this time-scale of alterations in discharge rates, microdialysis is an appropriate method for assessing alterations in NE efflux associated with the pharmacological manipulations of LC discharge rates. Additional studies utilizing voltammetric methods are needed to assess rapid fluctuations in NE efflux associated with phasic LC discharge activity.

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