Differential effects of an NMDA and a non-NMDA receptor antagonist on medullary lateral tegmental field neurons

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The discharges of sympathetic nerves that project to the heart and vasculature of skeletal muscle and viscera contain a prominent cardiac-related rhythm (1–3, 18, 23–25, 35). In the cat, this rhythm arises from entrainment of low-frequency (≤6-Hz) oscillations in sympathetic nerve discharge (SND) to the arterial pulse (AP) by pulse-synchronous baroreceptor nerve activity (17, 24). Neurons in the rostral ventrolateral medulla (RVLM) with cardiac-related activity project directly to spinal preganglionic sympathetic neurons, and chemical inactivation of the RVLM reduces SND and blood pressure to levels seen after cervical spinal cord transection (for reviews see Refs. 5, 15, and 20). Thus the RVLM is critical for the maintenance of basal SND and the normotensive state. Nevertheless, data from single-neuron recording experiments and microinjection of excitatory amino acid (EAA) receptor antagonists support the view that neurons in the cat medullary lateral tegmental field (LTF) are also involved in the generation and baroreceptor reflex control of SND (1–3, 6, 18, 24).

This region of the dorsolateral medullary reticular formation (including portions of the nucleus reticularis parvocellularis and nucleus reticularis ventralis) contains at least two types of neurons with naturally occurring discharges that are correlated to the cardiac-related rhythm in SND (2, 3, 18). Putative LTF sympatheexcitatory neurons decrease their firing rate in parallel to SND during baroreceptor reflex activation, and their axons project to the vicinity of RVLM-spinal sympathoexcitatory neurons (2, 18). In contrast, the firing rate of putative LTF sympathoinhibitory neurons increases during the inhibition of SND produced by baroreceptor reflex activation, and their axons project to the vicinity of caudal medullary raphe-spinal sympathoinhibitory neurons (3, 18).

The effects produced by bilateral microinjection of N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists into the LTF provide direct support for the view that this region plays a critical role in the control of SND (6, 24). Specifically, power in SND (as determined with spectral analysis) and mean arterial pressure (MAP) were significantly reduced by microinjection of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo-[f]quinoxaline-7-sulfonamide (NBQX, a non-NMDA receptor antagonist) in baroreceptor-denervated cats (6). Microinjection of NBQX also reduced power in SND of cats with intact carotid sinus, aortic depressor, and vagus nerves; however, baroreceptor reflex control of SND was preserved (24). In contrast, although there was not a significant change in total power in SND, the cardiac-related rhythm and the sympathoinhibitory re-

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response to activation of the baroreceptor reflex were significantly reduced or eliminated by microinjection of D-(-)-2-amino-5-phosphono-pentanoic acid (D-AP5, an NMDA receptor antagonist) into the same region of the LTF (24).

On the basis of the results obtained with microinjection of EAA receptor antagonists into the LTF, the present study was designed to test the following predictions. First, microiontophoresis of NBQX onto individual LTF sympatoexcitatory neurons would decrease their mean firing rate without disrupting their relationship to the cardiac-related rhythm in SND. Second, microiontophoresis of D-AP5 onto these neurons would eliminate their cardiac-related activity without altering their overall basal firing rate. We used two indexes to quantify the degree of cardiac-related activity of LTF neurons: the ratio of peak to background counts in AP-triggered histograms of their activity and the coherence value relating LTF neuronal activity to the AP at the frequency of the heartbeat. The data support the hypothesis that the basal activity of LTF neurons with sympathetic nerve-related activity is dependent, at least in part, on activation of non-NMDA receptors, and activation of NMDA receptors is important for the entrainment of their activity to the cardiac cycle by pulse-synchronous baroreceptor nerve activity.

METHODS

General Procedures

The protocols used in this study on 11 cats were approved by the All-University Committee on Animal Use and Care of Michigan State University. Cats were anesthetized with an intraperitoneal injection of a mixture of sodium diallylbarbiturate (60 mg/kg), urethane (240 mg/kg), and monothelyurea (240 mg/kg). The right brachial artery and left and right femoral veins were cannulated to measure arterial pressure and to administer drugs, respectively. Cats were paralyzed (gallamine triethiodide, 4 mg/kg iv, initial dose), pneumothoracotomized, and artificially ventilated with room air. End-tidal CO₂ was monitored (Traverse Medical Monitors Capnometer model 2200), and rectal temperature was kept near 38°C with a heat lamp. Before neuromuscular blockade, the adequacy of anesthesia was indicated by the absence of a palpebral reflex. When cats were paralyzed, an adequate level of anesthesia was indicated by the inability of noxious stimuli (pinch, heat, or surgery) to increase blood pressure and desynchronize the frontal-parietal electroencephalogram (1).

An intravenous infusion of norepinephrine bitartrate (1–3 µg/min) in dextran (6% in saline) was used to set MAP at a level (~120 mmHg) adequate to produce a prominent cardiac-related rhythm in SND (24, 35). When necessary, the rate of infusion was adjusted during the experiment to keep MAP constant. To study the effect of baroreceptor reflex activation on LTF neurons, the abdominal aorta was obstructed for 5–10 s by rapid inflation of the balloon-tipped end of a Fogarty embolectomy catheter that was inserted via the femoral artery. This procedure led to a pressor response above the level of obstruction that was accompanied by inhibition of SND. The firing rates of LTF neurons were compared before and during two or more episodes of aortic obstruction. If the firing rate of an LTF neuron was decreased in parallel to SND, it was classified as a sympathoexcitatory neuron. If the firing rate of an LTF neuron was increased during the inhibition of SND, it was classified as a sympathoinhibitory neuron. SND and medullary neuronal activity are unaffected by aortic obstruction after baroreceptor denervation produced by bilateral section of the carotid sinus, aortic depressor, and vagus nerves (6).

Neural Recordings and Microiontophoresis

The methods used to make monophasic recordings of left inferior cardiac postganglionic SND have been described elsewhere (2, 3, 18). The preamplifier band pass was 1–1,000 Hz. The synchronized discharges of sympathetic fibers appear as slow waves (i.e., envelopes of spikes) when this band pass is used (18).

The dorsal surface of the brain stem was exposed by removing portions of the occipital and parietal bones and cerebellum. The obex and midline were used as landmarks for placement of the recording microelectrode into the region of the LTF where bilateral microinjections of NBQX or D-AP5 reduced basal levels of SND or the cardiac-related rhythm in SND, respectively (6, 24). Recording sites were in tracks on the left side of the medulla 2–4.5 mm rostral to the obex, 2.8–3 mm lateral to the midline, and at depths of 2.2–4.7 mm from the dorsal surface. This region contains putative sympa-thoexcitatory and sympathoinhibitory neurons (2, 3, 18).

The action potentials of LTF neurons were recorded extracellularly with one barrel of a five-barrel glass micropipette (6- to 10-µm composite tip diameter) filled with 2 M NaCl. The recording barrel had a tip impedance of 4–6 MΩ. Capacity-coupled preamplification with a band pass of 0.1–3 kHz was used. The reference electrode was a clip placed on crushed muscle overlaying the skull. The position of the recording electrode in the LTF was controlled with a stepping hydraulic microdrive (David Kopf Instruments).

The other four barrels of the micropipette were filled with 2 M NaCl (for automatic current balancing), 0.5 M l-glutamate, 2 mM NBQX, and 6 mM D-AP5. All solutions were adjusted to pH 8 (litmus paper test). A Neuro Phore BH-2 system (Medical Systems) was used for microiontophoresis of these drugs onto single LTF neurons. All drugs were ejected as anions with currents ranging from −10 to −40 nA. Cation retaining currents (20–30 nA) were applied between ejection periods to prevent leakage of the drugs. In a preliminary series of experiments, these concentrations and ejection currents for NBQX and D-AP5 were found to antagonize the excitatory effects of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (10 mM) and 100 mM NMDA, respectively, in a random sample of medullary neurons (n = 6). Concentrations of NBQX and D-AP5 as well as ejection currents used in the present study were similar to those used by others (11, 21, 30, 34).

For each of the LTF neurons included in this study, we verified that the recordings were made from the soma-dendritic region by producing an increase in their firing rate during microiontophoresis of l-glutamate. The duration of neuronal action potentials was ≥1.5 ms, and in some cases there was an inflection on the rising phase of the spike. These properties also indicate that recordings were made from cell bodies, rather than axons (22).

Data Analysis

Before all analyses on a Dell Optiplex GX110 computer, SND was low-pass filtered at 100 Hz; the Butterworth analog filter (AP circuit, model 260-5) has unity gain and a roll-off rate of 24 dB/octave. The action potentials of individual LTF neurons were isolated by using window discrimination (FHC
amplitude analyzer) and represented by 5-ms square-wave pulses (PX-934 pulse stretcher, CWE). Data were acquired (5-ms sampling interval) with software and an analog-to-digital converter board from RC Electronics (Santa Barbara, CA). Time-domain analyses used RC Electronics software. For frequency-domain analysis, a modified version (19) of the software of Cohen et al. (13) and Kocsis et al. (23) was used.

Spike-triggered averaging. Standardized square-wave pulses representing the action potentials of single LTF neurons were used as reference signals to construct normalized averages of SND. A series of randomly generated pulses with the same number and mean frequency as the neuronal spike train was used to construct a “dummy” average from the same data sample of SND. The discharges of a neuron considered to be correlated to SND if the amplitude (peak-to-trough voltage on the y-axis) of the slow wave surrounding time 0 on the x-axis in the normalized spike-triggered average was at least three times that of the largest deflection in the dummy average. Time 0 denotes LTF neuronal spike occurrence. Spike-triggered averages before and during microiontophoresis of NBQX or D-AP5 are plotted on the same voltage scale.

AP-triggered analysis. A point on the systolic phase of the AP was used as a reference to construct averages of the AP and SND and as a histogram of LTF neuronal activity. A neuron was classified as having cardiac-related activity if the histogram had a peak at the same phase of each of the APs in the average. The ratio of peak to background counts (number of spikes/bin) in the histogram was used as a measure of the degree of cardiac-related activity. The ratio was calculated by using the averages of the maximum and minimum numbers of phase-locked counts in the histogram over three or more cardiac cycles.

Interspike interval and ratemeter histograms. The mean firing rate of individual LTF neurons was calculated as the reciprocal of the mean interspike interval (ISI) measured with a 5-ms bin resolution. ISI histograms are displayed using ≥25-ms bin widths. A ratemeter histogram (bin resolution of 1–3 s) was used to provide a continuous record of LTF neuronal firing rate (spikes/s) in control and during microiontophoresis of EAA receptor antagonists.

Frequency-domain analyses. Fast Fourier transform was performed on 32 5-s windows to construct autospectra of SND, AP, and LTF single neuronal activity. Coherence functions relating pairs of these signals were also computed. When data blocks of <160 s were analyzed, data windows were overlapped to obtain 32 windows (e.g., 50% overlap for 80-s data blocks). Digital low-pass filtering (cutoff at 250 Hz) of the standardized pulses representing the action potentials of single neurons was performed by convolving the trains with a sinc function having parameters so that the autospectrum reflected the ISIs, rather than the shape of the pulses (12). The autospectrum of a signal shows how much power (in V²) is present at each frequency. Autospectra before and during microiontophoresis of NBQX or D-AP5 are plotted on the same power scale. The coherence function (normalized cross spectrum) is a measure of the strength of linear correlation of two signals at each frequency. The squared coherence value (referred to as coherence value) is 1 in the case of a perfect linear relationship and 0 if two signals are unrelated. A coherence value ≥0.1 was considered to reflect a statistically significant relationship when 32 windows were averaged (7). Spectral analysis was done over a frequency band of 0–100 Hz with a resolution of 0.2 Hz/bin. Only frequencies ≥20 Hz are shown in this report, since ≥90% of the total power in SND was within this band (23).

Experimental Protocol

Once we determined that the naturally occurring discharges of an LTF neuron were correlated to SND (on the basis of spike-triggered averaging), L-glutamate was applied for 3–5 s to ensure that the recording was in the vicinity of the soma-dendritic region of the neuron as indicated by an increase in firing rate. Data were then collected for the same length of time (80–160 s) before and during microiontophoresis of NBQX or D-AP5 onto the neuron. The neuron was then allowed to recover from the effects of the EAA receptor antagonist. We were successful in repeating the protocol using the second drug for 13 neurons. Once the effects of the EAA receptor antagonists were assessed, changes in neuronal firing rate produced by baroreceptor reflex activation were determined.

Statistical Analysis

Values are means ± SE. Paired t-tests were used to compare the following parameters before and during microiontophoresis of NBQX or D-AP5: mean firing rates of LTF neurons, the AP-LTF coherence value at the frequency of the
heartbeat, the ratio of peak to background counts in the
AP-triggered histograms of LTF neurons, and the interval
between neuronal spike occurrence and the peak of the infe-
rior cardiac sympathetic nerve slow wave measured from the
spike-triggered average. AP-LTF and LTF-SND coherence
values were essentially the same for individual neurons; thus
only the former value was used to assess the effects of EAA
receptor antagonists. Coherence values were subjected to z
transformation before statistical analysis. Microiontophore-
sis of NBQX and D-AP5 onto LTF neurons did not affect MAP
or SND; thus any changes in the parameters measured
cannot be attributed to changes in the level of baroreceptor
nerve activity. A paired t-test was also used to compare firing
rates of LTF neurons before and during baroreceptor reflex
activation (brief partial aortic obstruction). \( P \leq 0.05 \) indi-
cated statistical significance.

**Histology**

The brain stem was removed and fixed in 10% buffered
formalin. Frontal sections (30 μm thick) were cut and stained
with cresyl violet. LTF neuronal recording sites were esti-
imated with reference to the tracks made with the recording
microelectrode and the stereotaxic planes of Berman (8).

**RESULTS**

**LTF Neurons With Activity Correlated to the
Cardiac-Related Rhythm in SND**

We recorded from 23 LTF neurons with naturally
occurring discharges that were correlated to the car-
diac-related rhythm in inferior cardiac SND of 11 cats.
Figure 1 shows the recording sites of these neurons in
the nucleus reticularis ventralis and nucleus reticu-
laris parvocellularis 2–4.5 mm rostral to the obex.
These neurons were intermixed with a larger group of
neurons (\( n = 128 \)) with naturally occurring discharges
that were not correlated to SND.

As illustrated in Figs. 2 and 3, three methods were
used to identify LTF neurons with activity correlated
to the cardiac-related rhythm in SND: spike-triggered
averaging, AP-triggered analysis, and coherence anal-
ysis. The spike-triggered average in Fig. 2A, top, shows
SND for 500 ms before and after LTF neuronal spike
occurrence at \( t = 0 \). The slow waves in the average
were regularly spaced at \(~260\)-ms intervals (corre-
ponding to the period of the cardiac cycle), and their

![Fig. 2. Time-domain analysis showing effects of 1,2,3,4-tetrahydro-6-nitro-
2,3-dioxobenzof[1]quinoxaline-7-sulfon-
amide (NBQX) on an LTF neuron with
cardiac-related activity. A and C:
spike-triggered (top) and dummy (bot-
tom) averages of SND before and dur-
ing microiontophoresis of NBQX, re-
spectively. Number of reference spikes
was 850 and 321 in A and C, respec-
tively. Vertical calibration is 20 μV. B
and D: arterial pulse (AP)-triggered
analyses based on 600 trials. Vertical
calibration for SND is 70 μV. Bin
width is 5 ms for averages 10 ms for
AP-triggered histograms here and in
subsequent figures.](http://ajpregu.physiology.org/)
amplitudes (peak-to-trough voltage) greatly exceeded those of the deflections in the corresponding dummy average of SND (Fig. 2A, bottom). The interval between LTF neuronal spike occurrence and the first peak to the right of time 0 in the average of SND was 130 ms. In agreement with the results of spike-triggered averaging, AP-triggered analysis in Fig. 2B showed that this LTF neuron was most apt to fire at the beginning of the rising phase of the cardiac-related slow wave in inferior cardiac SND; the ratio of peak to background counts (number of spikes/bin) in the AP-triggered histogram of LTF neuronal activity was 2.2:1.

Spectral analysis confirmed the interrelations of LTF neuronal activity, SND, and the AP in this case. The sharp peak at 3.8 Hz in the autospectra of SND (Fig. 3A, middle) was at the frequency of the heartbeat (see autospectrum of AP in Fig. 3A, top). The autospectra of LTF neuronal activity (Fig. 3A, bottom) also contained a small peak at this frequency, and the AP-LTF and LTF-SND coherence values at the frequency of the heartbeat were 0.26 and 0.25, respectively (Fig. 3B, middle and bottom). These values were significantly different from zero coherence (7).

We monitored the changes in firing rate of 14 of the 23 LTF neurons with cardiac-related activity during a brief (5- to 10-s) episode of partial aortic obstruction that increased MAP from 117 ± 3 to 182 ± 4 mmHg. Nine of these neurons were classified as putative sympathoexcitatory neurons, because their firing rates were significantly decreased from 3.49 ± 0.83 to 0.27 ± 0.11 spikes/s during the inhibition of SND produced by aortic obstruction. Figure 4 shows a case of complete inhibition of LTF neuronal activity and SND during activation of the baroreceptor reflex. Two of the LTF neurons were classified as putative sympathoinhibitory neurons, because their firing rates were increased (from 2.0 to 8.6 and from 2.9 to 12.2 spikes/s) during the rise in MAP that inhibited SND. The firing rate of the other two LTF neurons did not show a consistent change in firing rate during two or more episodes of aortic obstruction; thus these neurons could not be...
Effects of Microiontophoresis of NBQX on LTF Neuronal Activity

We determined the effects of microiontophoresis of NBQX on 20 LTF neurons with cardiac-related activity. Figures 2, 3, and 5 show data from one of these neurons. As shown by the ratemeter histogram in Fig. 5A, the firing rate of this neuron was markedly reduced by NBQX. The histograms in Fig. 5, B and C, show the distribution of ISIs before and during microiontophoresis of NBQX, respectively. During microiontophoresis of NBQX, there was a substantial decrease in the number of <250-ms ISIs and an increase in the number of >1,000-ms ISIs; the mean firing rate of this LTF neuron was decreased from 5.3 to 2.0 spikes/s (mean ISIs of 188 and 491 ms, respectively).

Despite the reduction in mean firing rate, the discharges of this LTF neuron remained correlated to the cardiac-related rhythm in SND during microiontophoresis of NBQX. This can be discerned from the spike-triggered average (Fig. 2C, top), the AP-triggered histogram of LTF neuronal activity (Fig. 2D), and the results of frequency domain analysis (Fig. 3, C and D). The ratio of peak to background counts in the AP-triggered histogram was 3:1, and the AP-LTF coher-

Fig. 4. Effect of baroreceptor reflex activation (aortic obstruction) on firing rate of an LTF neuron with activity correlated to the cardiac-related rhythm in SND. Oscillographic traces (top to bottom): brachial arterial pressure (BP; mmHg), SND, standardized pulses representing the action potentials of the neuron, and time marker (1 s/division). Vertical calibration for SND is 30 μV.

Fig. 5. Changes in firing rate of 2 LTF neurons during microiontophoresis of NBQX or d(-)-2-amino-5-phosphonopentanoic acid (d-AP5). Data in A–C are from the same experiment as in Figs. 2 and 3; data in D–F are from the same experiment as in Figs. 7 and 8. Ratemeter histograms show firing rate before and during microiontophoresis of NBQX (A) or d-AP5 (D). Bin width is 3 s in A and 1 s in D. Interspike interval histograms before (B and E) and during microiontophoresis of NBQX (C) or d-AP5 (F); bin width is 25 ms.
ence value at the frequency of the heartbeat (0.22) was similar to the control value.

Figure 6, A–C, summarizes the effects of microiontophoresis of NBQX on 20 LTF neurons with activity correlated to the cardiac-related rhythm in SND. The mean firing rate of these neurons was significantly reduced to 51 $\pm$ 8% of control ($P < 0.0001$) during microiontophoresis of NBQX (Fig. 6A). The activity of five of these neurons was virtually eliminated (firing rate reduced to $\leq 0.1$ spikes/s). Only 2 of the 20 neurons tested did not show a decrease in firing rate of $\geq 20%$. As shown in Fig. 6B, the ratio of peak to background counts in the AP-triggered histogram of LTF neuronal activity was not significantly changed (118 $\pm$ 15% of control) for the 15 LTF neurons that remained active during microiontophoresis of NBQX. As shown in Fig. 6C, the AP-LTF coherence value at the frequency of the heartbeat was also not significantly changed from control (101 $\pm$ 20% of control). This comparison is based on data from seven neurons that satisfied two criteria: AP-LTF coherence $\geq 0.1$ before microiontophoresis of NBQX and mean firing rate $\geq 0.1$ spikes/s during microiontophoresis of this drug. Only 10 of the 20 LTF neurons met the first criterion. Factors leading to the absence of significant coherence between the discharges of an individual neuron and AP or SND under conditions when a relationship is revealed by time-domain analyses are discussed elsewhere (4). Although not shown, the interval between LTF neuronal spike occurrence and the peak of the inferior cardiac sympathetic nerve slow wave surrounding time 0 was not significantly changed by NBQX (123 $\pm$ 13 vs. 113 $\pm$ 19 ms).

We tested the effects of baroreceptor reflex activation on 12 of the LTF neurons with mean firing rates that were reduced by NBQX; 8 were classified as sympathoexcitatory and 2 as sympathoinhibitory. The firing rate of the other two LTF neurons did not show a consistent change in firing rate during several episodes of aortic obstruction.

Effects of Microiontophoresis of D-AP5 on LTF Neuronal Activity

We determined the effects of microiontophoresis of D-AP5 on 16 LTF neurons with activity correlated to the cardiac-related rhythm in SND. Figures 7 and 8 show data from one of these neurons. The cardiac-
related activity of this LTF neuron was virtually eliminated by D-AP5. This is evident by comparing the AP-triggered histograms of LTF neuronal activity in Fig. 7, B and D, and the AP-LTF coherence functions in Fig. 8, B and D. Comparison of the spike-triggered averages in Fig. 7, A and C, also shows weakening of the relationship between LTF neuronal activity and SND during microiontophoresis of D-AP5. As shown by the raterometer histogram in Fig. 5D and ISI histograms in Fig. 5, E and F, the mean firing rate of this LTF neuron was not changed by microiontophoresis of D-AP5. The mean firing rate of this neuron was 2.8 and 2.7 spikes/s before and during microiontophoresis of D-AP5, respectively (i.e., mean ISIs of 354 and 368 ms).

On a group basis, microiontophoresis of D-AP5 did not produce a significant change (151 ± 47% of control, n = 16) in the mean firing rates of LTF neurons with activity correlated to the cardiac-related rhythm in SND (Fig. 6D). However, the effect of this drug on neuronal firing rate varied from neuron to neuron. The mean firing rate of individual neurons was unchanged (from 4.1 ± 1.5 to 3.8 ± 1.4 spikes/s, n = 5), significantly increased (from 3.7 ± 1.8 to 7.7 ± 2.1 spikes/s, n = 5, P = 0.03), or significantly decreased (from 2.0 ± 0.2 to 1.0 ± 0.1 spikes/s, n = 6, P = 0.001) by D-AP5. The raterometer histogram in Fig. 9A and ISI histograms in Fig. 9B are for an LTF neuron with a mean firing rate that was increased from 3.8 to 8.9 spikes/s (mean ISIs of 262 and 112 ms, respectively) by D-AP5. The ISI histograms in Fig. 10, A and B, are for an LTF neuron with a firing rate that was decreased during microiontophoresis of D-AP5. In this case, the mean ISI was increased from 382 ms (Fig. 10A) to 797 ms (Fig. 10B), corresponding to a decrease in mean firing rate from 2.6 to 1.3 spikes/s. Independent of the direction of change in neuronal firing rate, microiontophoresis of D-AP5 weakened (Fig. 9C) or eliminated (Fig. 10D) the cardiac-related activity of these LTF neurons.

Figure 6, E and F, summarizes the effects produced by microiontophoresis of D-AP5 on the cardiac-related activity of 16 LTF neurons. The ratio of peak to background counts in the AP-triggered histograms was
significantly reduced by D-AP5 to 57 ± 9% of control ($P = 0.02$; Fig. 6E). Also, the AP-LTF coherence value at the frequency of the heartbeat was significantly decreased to 25 ± 10% of control ($P = 0.001$; Fig. 6F); it fell to a value not significantly different from zero in 8 of the 10 cases in which this type of analysis was possible. Although not shown, the interval between LTF neuronal spike occurrence and the peak of the cardiac-related slow wave in the spike-triggered average of inferior cardiac nerve activity was not significantly changed during microiontophoresis of D-AP5. The intervals were 120 ± 15 and 131 ± 21 ms ($n = 10$) before and during microiontophoresis of D-AP5, respectively.

We tested the effects of baroreceptor reflex activation on the firing rate of 12 of the LTF neurons in which cardiac-related activity was reduced or eliminated by D-AP5. Eight of these neurons were classified as sympathoexcitatory neurons. This includes neurons in which mean firing rate was increased ($n = 3$), decreased ($n = 3$), or unchanged ($n = 2$) during microiontophoresis of D-AP5. The other LTF neurons were classified as sympathoinhibitory ($n = 2$) or did not show a consistent change in firing rate during baroreceptor reflex activation ($n = 2$).

Comparison of Effects of NBQX and D-AP5 on Individual LTF Neurons

Thirteen of the LTF neurons with activity correlated to the cardiac-related rhythm in SND were studied during microiontophoresis of NBQX and D-AP5 (applied separately). Figure 10 compares the effects of microiontophoresis of D-AP5 and NBQX onto one of these neurons. Two important points are illustrated. First, as shown by the similarity in the two control (predrug) distributions of ISIs in Fig. 10, A and E, and AP-triggered histograms of LTF neuronal activity in Fig. 10, C and G, these neurons recovered from the effects of microiontophoresis of the first drug (D-AP5 in this case) before the second drug was applied. Second, even in cases when both drugs caused similar changes in mean firing rate, the actions of these two drugs on the cardiac-related activity of LTF neurons were strikingly different. In the example shown, the mean ISI increased from 382 to 797 ms with D-AP5 (Fig. 10, A and B) and from 395 to 836 ms with NBQX (Fig. 10, E and F). Cardiac-related activity of this LTF neuron was virtually eliminated by microiontophoresis of D-AP5 (Fig. 10D) but persisted during microiontophoresis of NBQX.
Although not shown, the AP-LTF coherence value at the frequency of the heartbeat was decreased from 0.12 to a value not significantly different from zero with d-AP5 and was increased from 0.11 to 0.20 with NBQX.

**DISCUSSION**

This is the first study to evaluate the effects of microiontophoresis of an NMDA and a non-NMDA receptor antagonist on individual LTF neurons in which naturally occurring discharges were correlated to the cardiac-related rhythm in SND. We found that microiontophoresis of the non-NMDA receptor antagonist NBQX significantly reduced their mean firing rates but did not disrupt the relationship between their residual discharges and the cardiac-related rhythm in SND. In contrast, microiontophoresis of the NMDA receptor antagonist d-AP5 markedly reduced their cardiac-related activity but had variable effects on their mean firing rate. These results provide corroborating evidence for the hypothesis that non-NMDA and NMDA receptors on medullary LTF neurons are involved in the generation and baroreflex control of SND, respectively (6, 24).
The NBQX-induced decrease in mean firing rate of LTF neurons without loss of their cardiac-related activity can explain the effects on SND produced by microinjection of this drug bilaterally into the LTF of baroreceptor-innervated and -denervated cats (6, 24). Specifically, blockade of non-NMDA receptors in the LTF significantly reduced total power in SND in both groups of cats. However, the baroreceptor reflex remained functional as evidenced by the persistence of the inhibition of residual SND during aortic obstruction and high coherence of SND to the AP at the frequency of the heartbeat. These data led us to propose that central drive to sympathetic nerves depends, in part, on activation of non-NMDA receptors in the LTF. We (6, 24) proposed that NBQX exerted its effects on SND by decreasing the mean firing rate of LTF sympatheexcitatory neurons that innervate RVLM-spinal sympatheexcitatory neurons (2). Indeed, 8 of 12 LTF neurons in which firing rate was decreased by NBQX were classified as sympatheexcitatory on the basis of their response to baroreceptor reflex activation. Despite the significant decrease in mean firing rate of LTF neurons during microiontophoresis of NBQX, their activity remained cardiac related as indicated by the absence of significant changes in the ratio of peak to background counts in the AP-triggered histogram and the AP-LTF coherence value at the frequency of the heartbeat.

The cardiac-related rhythm in SND results from entrainment of low-frequency (<6 Hz) slow waves to the AP by pulse-synchronous baroreceptor nerve activity (17, 24). In an earlier study (24), we showed that bilateral microinjection of d-AP5 into the LTF significantly reduced cardiac-related power without changing total power in SND. We interpreted this to signify that the NMDA receptor antagonist disrupted baroreceptor reflex-induced entrainment of low-frequency bursts of SND to the AP. Microinjection of d-AP5 into the LTF also prevented baroreceptor reflex-induced inhibition of SND. In baroreceptor-denervated cats, microinjection of d-AP5 into the LTF did not affect SND (6), further implying that this drug affected SND by selectively suppressing baroreceptor reflex control of LTF neurons. On this basis, we predicted that microiontophoresis of d-AP5 onto individual LTF neurons would disrupt their cardiac-related activity. Indeed, in the present study we showed that there was a significant decrease in the ratio of peak to background counts in the AP-triggered histogram of LTF neuronal activity and in the AP-LTF coherence value at the frequency of
the heartbeat. The majority of these LTF neurons were classified as sympathoexcitatory on the basis of their response to aortic obstruction. Thus microiontophoresis of D-AP5 onto LTF neurons appeared to interrupt baroreceptor reflex-induced entrainment of their activity to the AP. Because D-AP5 is an antagonist of EAA receptors, these data imply that excitatory neurotransmission is involved in the entrainment process. However, the precise mechanism for entrainment of LTF neuronal activity to the AP is unknown.

On the basis of the lack of a change in total power in SND with bilateral microinjection of D-AP5 into the LTF (24), we predicted that microiontophoresis of this drug onto individual LTF neurons would not significantly affect their mean firing rates. Indeed, on a group basis, there was not a significant change in the mean firing rate of LTF neurons in which cardiac-related activity was decreased by D-AP5. However, the effects of D-AP5 on the mean firing rate of LTF neurons was quite variable. Individual LTF neurons responded with no change, a decrease, or an increase in their basal firing rate during microiontophoresis of D-AP5. Neurons classified as sympathoexcitatory were included in all three response types.

D-AP5 can disrupt rhythmic activity of mitral cells in the olfactory bulb without significantly affecting their basal firing rates (28), a situation analogous to that in 5 of 16 LTF neurons in the present study. In the case of these five LTF neurons, D-AP5 prevented entrainment of their discharges to the cardiac cycle without affecting the actual number of action potentials being generated. In contrast, other investigators (30, 34) reported that application of D-AP5 decreased the mean firing rate of neurons in the nucleus of the tractus solitarius, including some with cardiac-related activity. A reduction in firing rate, which also occurred in about one-third of the LTF neurons studied here, is not unexpected on application of a drug that blocks EAA receptors. If it is assumed that the effects of D-AP5 were mediated directly on the neuron under study (see below), these data imply that NMDA receptors on at least some LTF neurons are involved in setting their basal firing rate.

More difficult to understand is how microiontophoresis of an EAA receptor antagonist could induce an increase in mean firing rate of some LTF sympathoexcitatory neurons. There are at least two possible explanations. One possibility is that D-AP5 blocked NMDA receptors on adjacent inhibitory interneurons, leading to disinhibition of the neuron under study. Blockade of these same NMDA receptors might have accounted for the loss of cardiac-related activity of LTF sympathoexcitatory neurons, if it is assumed that these inhibitory interneurons also had pulse-synchronous activity. Indeed, the LTF contains sympathoexcitatory neurons that could function in this capacity (3). Two of 14 LTF neurons tested in the present study were classified as sympathoexcitatory on the basis of their increased firing rate during aortic obstruction, and microiontophoresis of D-AP5 onto both of these neurons virtually eliminated their cardiac-related activity. The low incidence of such neurons in the present study is not surprising, because in an earlier study in which we recorded from 88 LTF neurons with activity correlated to SND, only 16 neurons (18%) were classified as sympathoinhibitory (18).

A second way to explain how D-AP5 increased LTF neuronal firing rate is to assume that this drug acted on a presynaptic receptor. Presynaptic glutamate receptors have been identified; although many of these are metabotropic receptors (26), there is also evidence for a role of presynaptic ionotropic glutamate receptors in modulating neurotransmission (10, 27, 31). Activation of NMDA receptors on nerve terminals has been shown to decrease the release of an excitatory neurotransmitter (27) and enhance the release of an inhibitory neurotransmitter (10). In either case, the activity of the postsynaptic neuron would be decreased. Thus blockade of such presynaptic receptors on axon terminals antecedent to LTF neurons with cardiac-related activity would increase their firing rate.

Because some neurons were studied during microiontophoresis of NBQX and D-AP5 (applied separately), differences in response characteristics of the two EAA receptor antagonists cannot be attributed to sampling different pools of neurons. However, because some of the effects of D-AP5 and NBQX could have been due to actions of these drugs on a presynaptic terminal or on the somata of an antecedent LTF interneuron, we cannot be certain that an individual LTF neuron contains NMDA and non-NMDA receptors. The fact that NMDA and non-NMDA receptors in the LTF serve different functions is not surprising. It is generally accepted that activation of non-NMDA receptors mediates the fast component of glutamate signaling, whereas activation of NMDA receptors mediates a slower-developing and longer-lasting component of glutamate signaling (14). Activation of NMDA receptors has frequently been associated with induction of rhythmic activity, including locomotion, swallowing, respiration, and gamma and theta electroencephalogram activity (9, 16, 21, 29, 32, 33).

Some LTF neurons with cardiac-related activity became quiescent when NBQX was applied with low ejection currents (10 nA), whereas other neurons remained active even when ejection currents of 40 nA were applied. This difference in responsiveness could be seen for two neurons in the same experiment; thus it was not likely due to a problem with the drug or micropipette. Does the difference in responsiveness indicate that LTF neurons are not equally dependent on activation of non-NMDA receptors for producing their action potentials? Although this may be the case, it is also possible that the micropipette was not always in the optimal position to have access to all the non-NMDA receptors of a given neuron. We did not attempt to use ejection currents >40 nA for NBQX or D-AP5, inasmuch as Zhang and Mifflin (34) reported that higher ejection currents were often accompanied by nonspecific effects.

The firing rates of the two LTF sympathoinhibitory neurons identified in the present study were decreased
(although one by <20%) during microiontophoresis of NBQX. If this is a common response for such neurons, the overall effect would be to increase SND. However, because the LTF appears to contain more sympathoexcitatory than sympathoinhibitory neurons (18), the NBQX-induced decrease in firing rate of the former group of neurons would likely override the effects of this drug on the LTF sympathoinhibitory neurons. This would account for the significant decrease in power in SND when this drug is microinjected bilaterally into the LTF (6, 24).

In summary, NMDA and non-NMDA receptors appear to have different roles in regulating the activity of LTF sympathoexcitatory neurons. Activation of NMDA receptors is important in synchronizing the activity of these neurons to the AP, whereas activation of non-NMDA receptors is critical in setting their basal level of activity. The results of the present study with microiontophoresis of d-AP5 and NBQX on LTF neurons can explain the changes in SND produced by microinjection of these EAA receptor antagonists in the same region (6, 24).

Perspectives

During the 1980s our laboratory completed a series of studies (including Refs. 1–3, 18) that led us to propose that the medullary LTF contains sympathoexcitatory and sympathoinhibitory neurons that provide driving inputs to their counterparts in the RVLM and caudal medullary raphe. First, we showed that this region contained neurons with naturally occurring discharges that were correlated to the cardiac-related rhythm in SND of baroreceptor- innervated cats and irregular, low-frequency oscillations in SND of baroreceptor-denervated cats. Second, LTF sympathoexcitatory and sympathoinhibitory neurons fired earlier during sympathetic nerve slow waves than their counterparts in the RVLM and raphe. Third, we used the technique of antidromic activation to show that, rather than projecting directly to the spinal cord, the axons of these LTF neurons projected to the vicinity of the RVLM and caudal medullary raphe neurons that innervated the thoracolumbar intermediolateral autonomic nucleus. Lacking in these studies was direct proof that the discharges of these neurons actually influenced SND. More recently, we found that blockade of non-NMDA and NMDA receptor neurotransmission in the LTF decreased power in SND and interfered with baroreflex control of SND, respectively (6, 24). The present study provides an important link between these results and our earlier single-neuron recording studies by showing that the effects of microinjection of EAA receptor antagonists into the LTF can be explained by an action (direct or indirect) of these drugs on LTF neurons with activity correlated to SND. This should kindle renewed interest in the role of the LTF in control of SND and MAP, a concept that actually dates back to the 1930s (for review see Ref. 5).

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REFERENCES

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