Role of the medullary lateral tegmental field in reflex-mediated sympathoexcitation in cats

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Orer, Hakan S., Gerard L. Gebber, Shaun W. Phillips, and Susan M. Barman. Role of the medullary lateral tegmental field in reflex-mediated sympathoexcitation in cats. Am J Physiol Regul Integr Comp Physiol 286: R451–R464, 2004. First published November 6, 2003; 10.1152/ajpregu.00569.2003.—We tested the hypothesis that blockade of N-methyl-D-aspartate (NMDA) and non-NMDA receptors on medullary lateral tegmental field (LTF) neurons would reduce the sympathoexcitatory responses elicited by electrical stimulation of vagal, trigeminal, and sciatic afferents, posterior hypothalamus, and midbrain periaqueductual gray as well as by activation of arterial chemoreceptors with intravenous NaCN. Bilateral microinjection of a non-NMDA receptor antagonist into LTF of urethane-anesthetized cats significantly decreased vagal afferent-evoked excitatory responses in inferior cardiac and vertebral nerves to 29 ± 2 and 24 ± 6% of control (n = 7), respectively. Likewise, blockade of non-NMDA receptors significantly reduced chemoreceptor reflex-induced increases in inferior cardiac (from 210 ± 22 to 129 ± 13% of control; n = 4) and vertebral nerves (from 253 ± 41 to 154 ± 20% of control; n = 7) and mean arterial pressure (from 39 ± 7 to 21 ± 5 mmHg; n = 8). Microinjection of muscimol, but not an NMDA receptor antagonist, caused similar attenuation of these excitatory responses. Sympathoexcitatory responses to the other stimuli were not attenuated by microinjection of a non-NMDA receptor antagonist or muscimol into LTF. In fact, excitatory responses elicited by stimulation of trigeminal and in some cases sciatic, afferents were enhanced. These data reveal two new roles for the LTF in control of sympathetic nerve activity in cats. One, LTF neurons are involved in mediating sympathoexcitation elicited by activation of vagal afferents and arterial chemoreceptors, primarily via activation of non-NMDA receptors. Two, non-NMDA receptor-mediated activation of other LTF neurons tonically suppresses transmission in trigeminal-sympathetic and sciatic-sympathetic reflex pathways.

arterial chemoreceptor reflex; excitatory amino acid receptors; sciatic-sympathetic reflex; trigeminal-sympathetic reflex; vagal-sympathetic reflex

FIVE LINES OF EVIDENCE support the view that the medullary lateral tegmental field (LTF), including portions of nucleus reticularis parvocellularis and nucleus reticularis ventralis in the dorsolateral medullary reticular formation, contains neurons that control sympathetic nerve discharge (SND) and mean arterial pressure (MAP). First, chemical stimulation of this region leads to increases or decreases in SND and MAP in cats, rabbits, and rats (12, 15, 21, 30, 40). Second, several laboratories (3–5, 8, 19, 24, 45, 46) have shown that the naturally occurring discharges of LTF neurons are correlated to the rhythmic discharges in sympathetic nerves in cats. Putative LTF sympathoexcitatory neurons decrease their firing rate in parallel to SND during baroreceptor reflex activation, and their axons project to the vicinity of rostral ventrolateral medulla (RVLM)-spinal sympathoexcitatory neurons (3). In contrast, the firing rate of putative LTF sympathoinhibitory neurons increases during baroreceptor reflex activation, and their axons project to the vicinity of caudal medullary raphespinal sympathoexcitatory neurons (4). Importantly, the discharges of LTF neurons maintain their relationship to low-frequency (<6 Hz) oscillations in SND after baroreceptor denervation (19). These studies led to the proposal that LTF neurons are antecedent to RVLM and raphe neurons in sympathoexcitatory and sympathoinhibitory pathways, respectively.

Third, LTF neurons play a critical role in mediating baroreceptor reflex control of SND as evidenced by elimination of the cardiac-related rhythm in SND and reflex-mediated sympathoinhibition after microinjection of an N-methyl-D-aspartate (NMDA) receptor antagonist into the LTF (33). In addition, microiontophoresis of an NMDA receptor antagonist onto LTF sympathoexcitatory neurons essentially abolished synchronization of their discharges to the arterial pulse (8). Fourth, the results obtained with microinjection or microiontophoresis of a selective non-NMDA receptor antagonist support the view that LTF neurons play an important role in setting the basal level of SND. Specifically, blockade of non-NMDA receptors in the LTF decreased SND and MAP without interfering with the baroreceptor reflex (6, 33), and the microiontophoresis of a non-NMDA receptor antagonist onto LTF sympathoexcitatory neurons reduced their firing rate (8).

Fifth, LTF neurons respond to such procedures as activation of the Bezold-Jarisch reflex (45), hindlimb muscular contraction (24), arterial chemoreceptor activation (29), and electrical stimulation of vestibular afferents (50), hypothalamus (2, 49), and cardiac sympathetic afferents (23). In most of these reports (2, 23, 24, 49, 50), the proposal was made that LTF neurons were involved in mediating sympathetic nerve and cardiovascular responses to these stimuli. However, other than for the report on baroreceptor reflex control of SND (33), no studies have directly assessed the impact of blockade of synaptic transmission in the LTF on sympathetic nerve responses elicited by activation of other peripheral afferents or supramedullary sites.

The current study was designed to determine whether LTF neurons play a role in mediating sympathoexcitatory responses to electrical stimulation of vagal, trigeminal, and sciatic afferents, midbrain periaqueductal gray (PAG), and posterior hypothalamus as well as to activation of arterial chemoreceptors...
with intravenous NaCN in cats. We hypothesized that blockade of excitatory amino acid (EAA) receptors on LTF neurons would reduce the magnitude of the sympathoexcitatory responses elicited by one or more of these stimuli. Thus we tested the effects of bilateral microinjection of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxborenzo-quinoxla-line-7-sulphonamide (NBQX, a selective non-NMDA receptor antagonist) into the LTF on stimulus-induced excitatory responses in the inferior cardiac and vertebral sympathetic nerves. We then compared the effects of microinjection of NBQX with those produced by microinjection of a selective NMDA receptor antagonist, (–)-2-amino-5-phosphonopentanoic acid (d-AP5) and a γ-aminobutyric acid (GABA) receptor agonist, muscimol, which produces a generalized depression of neuronal activity (31). The results demonstrate that the LTF plays an important role in mediating sympathoexcitatory responses to stimulation of vagal afferents and arterial chemoreceptors primarily via activation of non-NMDA receptors.

METHODS

General Procedures

The protocols used in these studies on 52 adult cats (1.8–4.0 kg) were approved by the All-University Committee on Animal Use and Care of Michigan State University. Cats were initially anesthetized with 2.5% isoflurane mixed with 100% O₂. The right femoral artery and left and right femoral veins were cannulated to measure arterial pressure and to administer drugs, respectively. Urethane (1.2–1.8 g/kg iv, initial dose) was then administered, and isoflurane mixed with 100% O₂. The right femoral artery and left inferior cardiac and/or left vertebral postganglionic sympathetic nerves can be found in earlier reports (19, 20). The micropipette was positioned in tracks located 2 and 3 mm from the dorsal surface of the medulla. In seven cats, test data blocks were collected 10 min after microinjection and then at 15– to 30-min intervals for up to 90 min to allow for partial or full recovery. The data block collected 10 min after microinjection was used to quantify the effects of these drugs on sympathoexcitatory responses because our past work showed that the maximum changes were produced within 10 min after microinjection of NBQX or d-AP5 into the LTF after recovery from effects of microinjection of NBQX into the same area. In 13 cats, drugs were also injected bilaterally into the RVLM at depths of 5 and 6 mm from the dorsal surface in tracks located 4.5 and 5.5 mm rostral to the obex and 3.5 mm to the left and right of the midline.

The baroreceptor denervation

The carotid sinus, aortic depressor, and vagus nerves were sectioned bilaterally in 36 of the cats. Two observations verified the completeness of baroreceptor denervation. First, spectral analysis failed to show a cardiac-related rhythm in SND; i.e., there was no sharp peak in the autospectrum of SND at the frequency of the heart beat, and coherence values relating SND to the AP were <0.1 at this frequency. Second, SND was not reflexly inhibited during the pressor response produced by an injection of norepinephrine bitartrate (1–2 μg/kg iv).

Sympathetic Nerve Recordings

The methods used to make monophasic recordings from the cut central ends of the left inferior cardiac and/or left vertebral postganglionic sympathetic nerves can be found in earlier reports (19, 20). These nerves project to the heart and vasculature of the forelimb, respectively. The preamplifier band pass was usually set at 1–1,000 Hz. The synchronized discharges of sympathetic nerve fibers appear as slow waves (i.e., envelopes of spikes) when this band pass is used (19). In the experiments in which we activated the arterial chemoreceptor reflex and stimulated the PAG at high frequency, a band pass of 30–1,000 Hz was used to record SND.

Microinjections

The sites of microinjection into the brain stem as well as the doses of EAA receptor antagonists and muscimol were the same as those used by us in earlier studies (5, 6, 33). Figure 1 of Ref. 6 shows the location of injection sites in the LTF and RVLM. Drugs were microinjected through a glass micropipette (~40-μm tip diameter) that was glued (cyanoacrylate) to the needle of a 5-μL Hamilton syringe. The micropipette was filled with a 1.25 mM solution of NBQX disodium salt, a 3 mM solution of d-AP5, or a 10 mM solution of muscimol. Drugs were diluted in 0.9% saline, and the solution was adjusted to a pH of 6–8 (litmus paper test) to ensure their solubility. The syringe and micropipette were mounted on a microinjection unit (Kopf Instruments, model 5000). A 100-nl injection of NBQX (125 pmol), d-AP5 (300 pmol), or muscimol (1 nmol) was made slowly (~20 s) at each medullary site by turning the calibrated micrometer on the microinjection unit.

The dorsal surface of the brain stem was exposed by removing portions of the occipital bone and cerebellum. The midline, obex, and dorsal medullary surface were used as landmarks for placement of the micropipette. Microinjections were made at four sites in the LTF on each side of the medulla (total of 400 nl/side) over a period of ~6 min. Multiple injections were made because neurons with activity correlated to SND are distributed over several millimeters within the LTF (19). The micropipette was positioned in tracks located 2 and 3 mm rostral to the obex and 2.8 mm lateral to the midline; microinjections were made bilaterally at depths of 3 and 4.5 mm from the dorsal surface. In seven cats, test data blocks were collected 10 min after microinjection and then at 15– to 30-min intervals for up to 90 min to allow for partial or full recovery. The data block collected 10 min after microinjection was used to quantify the effects of these drugs on sympathoexcitatory responses because our past work showed that the maximum changes in basal SND produced by microinjection of these drugs had occurred at this time, and SND had reached a new steady-state level (5, 6, 33). The time courses of action of NBQX, d-AP5, and muscimol were similar to those reported in other studies in which cardiovascular or respiratory changes were monitored after microinjection of these drugs into the brain (5, 6, 12, 16, 33). NBQX, d-AP5, and muscimol were purchased from Sigma (St. Louis, MO). No attempt was made in the current study to test directly the selectivity of NBQX or d-AP5 on different classes of EAA receptors. However, the concentrations and doses of NBQX and d-AP5 used here were similar to or lower than those used in other studies in which their ability to antagonize selectively NMDA or non-NMDA receptors was demonstrated (12, 16, 28). As a control, in two cats, vehicle (saline adjusted to a pH of 6–8) was microinjected bilaterally into the LTF 30 min before microinjection of NBQX.

Electrical Stimulation

The cut central ends of the left cervical vagus nerve, the supraoral branch of the left trigeminal nerve, and a branch of the left sciatic
nerve were placed on bipolar stimulating electrodes. In cats in which the cervical vagus nerve was stimulated, the ipsilateral aortic depressor nerve was sectioned at its junction with the superior laryngeal nerve; the vagus nerve was also separated from the cervical sympathetic nerve. Thus the effects of stimulation could be attributed exclusively to activation of vagal afferent fibers. A concentric bipolar stainless steel electrode with 0.25-mm tip exposure separated by 0.75 mm (Rhodes, SNE-100) was positioned into supramedullary sites on the left side of the neuraxis. The PAG was stimulated at stereotaxic coordinates anterior (A) 1 to 3, lateral (L) 1.5 to 2, and horizontal (H) 0 to -2; the posterior hypothalamus was stimulated at stereotaxic coordinates A 7 to 9, L 1.5 to 2, and H 0 to -4. The responses to electrical stimulation of the PAG and hypothalamus may have resulted from activation of cell bodies, fibers of passage, or both. The electrodes were connected to constant-current stimulus isolation units (Grass Instrument, PSIU6). Short trains (8 ms) of three pulses (333 Hz; 1.5-ms duration) were applied once every 2 s to the peripheral nerves or central sites. The stimulus intensity ranged from 0.2 to 1.5 mA for the vagus nerve, PAG, and hypothalamus; the intensity was 1.5–3 mA for the trigeminal and sciatic nerves. In most cats, the responses to stimulation of two inputs were evaluated. Vaginal afferent stimulation was paired with the trigeminal (n = 8) or sciatic (n = 2) nerve; trigeminal afferent stimulation was also paired with PAG (n = 2), sciatic nerve (n = 1), or posterior hypothalamus (n = 1); and sciatic nerve stimulation was also paired with PAG (n = 6) or posterior hypothalamus (n = 6).

Arterial Chemoreceptor Activation

An intravenous injection of NaCN (100 μg/kg) was used to activate arterial chemoreceptors (26, 35) in 16 cats in which the vagus, aortic depressor, and carotid sinus nerves remained intact. At least two NaCN injections, separated by a minimum of 15 min, were made in each cat to demonstrate reproducibility of the sympathoexcitatory response before microinjection of a drug into the LTF. In 13 of these baroreceptor-intact cats, we also recorded a sympathoexcitatory response to high-frequency (25 Hz) PAG stimulation for a period (5–10 s) close to that of the duration of the increase in SND produced by arterial chemoreceptor activation.

Data Processing

Data were acquired using a Digidata1322A digitizer (Axon Instruments; Union City, CA) and were stored on a DAT Data Recorder (model RD-145T; TEAC America, Montebello, CA). Axoscope software was used to construct an on-line average (1-ms resolution) of the sympathetic nerve responses to 50 short trains of stimuli applied to supramedullary sites or peripheral afferents. The amplitude of the averaged sympathoexcitatory response was defined as the difference between the voltage readings at the onset and at the peak of the initial negative potential.

We also collected ~1-min data blocks (1-ms resolution) during activation of arterial chemoreceptors with intravenous NaCN and during high-frequency (25 Hz) PAG stimulation. SND was recorded with a 30- to 1,000-Hz band pass and then integrated off-line with a reset interval of 500 ms by using Datapac software from RUN Technologies (Mission Viejo, CA). To quantify the increase in SND produced by these stimuli, we compared the average amplitude of the integrated SND signal for a 5- to 10-s period before and after administration of NaCN (or before and during PAG stimulation). We also quantified the change in MAP produced by these stimuli.

Frequency-domain analysis was performed on 3-min data blocks (5-ms sampling interval). Details of the methods used to construct autospectra of SND can be found in other reports from this laboratory (5, 6, 20, 33). In the context of the current study, these data were used to confirm the results of past studies (5, 6, 33) in which we quantified the effects of microinjection of these drugs into the LTF on basal SND of baroreceptor-denervated and baroreceptor-innervated cats.

Statistical Analysis

Data are expressed as means ± SE. Student’s paired t-test was used to compare the following parameters before and after microinjection of drugs into the brain stem: 1) the amplitude of sympathoexcitatory responses elicited by short trains of electrical stimuli, 2) the response latencies, 3) the magnitude of the increases in SND and MAP produced by activation of arterial chemoreceptors and high-frequency PAG stimulation, and 4) MAP and total power in SND. An unpaired t-test was used to compare the effects of different drugs on the amplitude of the evoked responses elicited by a particular stimulus and to compare the effects of microinjection of a drug into the LTF on the amplitude of the evoked responses in the inferior cardiac and vertebral nerves. P ≤ 0.05 indicated statistical significance.

RESULTS

Effects of Microinjection of EAA Receptor Antagonists and Muscimol on Basal SND

Bilateral microinjection of NBQX into the LTF significantly (n = 30; P < 0.0001) reduced total power in the autospectrum of inferior cardiac SND to 67 ± 6% of control and reduced MAP from 120 ± 4 to 106 ± 5 mmHg. Microinjection of muscimol significantly (n = 22; P < 0.0001) reduced total power in inferior cardiac SND to 59 ± 7% of control and MAP from 124 ± 4 to 104 ± 6 mmHg. In contrast, microinjection of t-AP5 into the LTF (n = 12) did not significantly change total power in inferior cardiac SND (88 ± 11% of control) or MAP (113 ± 6 vs. 114 ± 9 mmHg). The effects of microinjection of these drugs into the LTF on total power in vertebral SND were not significantly different from those for inferior cardiac SND. These results are similar to those obtained in earlier studies from our laboratory (5, 6, 33).

Characteristics of Sympathetic Nerve Responses Elicited by Electrical Stimulation

The computer-averaged responses to 50 8-ms trains of three pulses applied once every 2 s to vagal, trigeminal, and sciatic nerve afferents as well as to the PAG and posterior hypothalamus had at least one feature in common. As shown by the representative examples in Figs. 1 and 3–5, the evoked responses were triphasic: an initial period of excitation (upward negative deflection), followed by a period of reduced activity (downward positive potential), and then a “rebound” excitation. This study deals primarily with the effects of microinjection of drugs into the brain stem on the initial excitatory response. The term “excitatory response” in this report refers to this initial phase of the evoked response. Table 1 shows the average onset latency and time to peak of the excitatory responses in the inferior cardiac and vertebral nerves elicited by electrical stimulation of vagal, trigeminal, and sciatic nerve afferents, PAG, and posterior hypothalamus. Note that the onset latency and time to peak response were longer for the vertebral nerve than the inferior cardiac nerve. Except where noted, microinjection of NBQX, t-AP5, and muscimol did not alter the onset latency or time to peak of the excitatory response. Short trains (8 ms) of stimuli separated by 2 s to the peripheral afferents and supramedullary sites did not induce changes in arterial pressure.

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Effects of EAA Receptor Antagonists and Muscimol Microinjections into the LTF on Sympathoexcitatory Responses Elicited by Electrical Stimulation of Peripheral Afferents and Supramedullary Sites

Vagal-sympathetic reflex. Figure 1A shows data from one of the seven cats in which the response in the inferior cardiac nerve to short trains of three pulses applied to vagal afferents was compared before (trace 1) and 10 min after bilateral microinjection of NBQX into the LTF (trace 2). The excitatory response was reduced to 38% of control after the injection; however, the positive potential after the excitation was essentially unchanged. Microinjection of NBQX did not affect the positive potential of the inferior cardiac or vertebral nerve response to vagal nerve stimulation in four cats. In the other three cats, the excitatory response and positive potential were reduced in parallel. In the example shown in Fig. 1A, the response to vagus nerve stimulation fully recovered within 1 h of microinjecting NBQX (trace 3).

As summarized in Fig. 2A, bilateral microinjection of NBQX into the LTF significantly (P < 0.0001; n = 7) reduced the vagal stimulus-induced excitatory responses in the inferior cardiac and vertebral nerves. The effects of NBQX were reversible; the excitatory response in these nerves recovered to 92±13 and 99±21% of control, respectively, in the five cats that were studied for up to 1 h after microinjecting NBQX. In the four cats in which there was residual excitation after microinjection of NBQX into the LTF, the onset latency of the inferior cardiac nerve response was significantly (P = 0.0274) increased.

Fig. 1. Attenuation of vagal-sympathetic reflex by bilateral microinjection of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo-[f]quinoxaline-7-sulfonamide (NBQX) or d-AP5 into the medullary lateral tegmental field (LTF). A: evoked responses to stimulation of the cut central end of the left cervical vagus nerve before (1), 10 min (2) and 1 h after microinjection of NBQX (5), and 10 min after microinjection of muscimol (4). B: evoked responses before (1), 10 min (2), and 45 min after microinjection of d-AP5 (3). Traces here and in Figs. 3–5 show computer-averaged (bin width, 1 ms) responses in the inferior cardiac nerve to 50 8-ms trains of 3 pulses (333 Hz; 1.5 ms) applied once every 2 s. Stimulus intensity was 1.5 mA in both A and B. Vertical calibration, 45 μV (A) and 25 μV (B).

Table 1. Onset latencies and times to peak of sympathoexcitatory responses elicited by electrical stimulation of peripheral afferents and brain stem sites

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>CN Onset latency, ms (n)</th>
<th>CN Time to peak, ms</th>
<th>VN Onset latency, ms (n)</th>
<th>VN Time to peak, ms</th>
</tr>
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<tbody>
<tr>
<td>Vagus nerve</td>
<td>142.9±4.2 (n=15)</td>
<td>176.5±4.9</td>
<td>166.1±4.0 (n=14)</td>
<td>201.3±5.2</td>
</tr>
<tr>
<td>Trigeminal nerve</td>
<td>74.1±3.3 (n=13)</td>
<td>104.6±3.3</td>
<td>90.8±2.2 (n=14)</td>
<td>125.1±3.5</td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>78.1±3.0 (n=13)</td>
<td>109.8±3.2</td>
<td>95.0±6.1 (n=4)</td>
<td>135.2±6.7</td>
</tr>
<tr>
<td>PAG</td>
<td>64.2±3.3 (n=11)</td>
<td>96.9±4.3</td>
<td>85.6±7.0 (n=5)</td>
<td>124.4±8.7</td>
</tr>
<tr>
<td>Posterior hypothalamus</td>
<td>63.7±3.7 (n=10)</td>
<td>99.0±2.8</td>
<td></td>
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</tbody>
</table>

Values are means ± SE of onset latency and time to peak amplitude of the averaged excitatory response elicited by 50 trains of 3 pulses applied once every 2 s; all values are for responses before microinjection of a drug into the lateral tegmental field (LTF). CN, cardiac nerve; PAG, periaqueductal gray; VN, vertebral nerve.
increased by 22\(\pm\)5 ms and time to peak of the response was increased by 13\(\pm\)5 ms (\(P = 0.0491\)). The onset latency and time to peak of the vertebral nerve excitatory response were also significantly increased by 15\(\pm\)4 ms (\(P = 0.0235\)) and 12\(\pm\)2 ms (\(P = 0.0139\)), respectively. In the example shown in Fig. 1A, the onset latency was increased from 140 to 149 ms, and time to peak of the excitatory response was increased from 162 to 174 ms.

We studied the effects of bilateral microinjection of D-AP5 into the LTF on the excitatory response to vagal afferent stimulation in four cats. As summarized in Fig. 2A, microinjection of this NMDA receptor antagonist significantly decreased the excitatory response in the inferior cardiac nerve (\(P = 0.0130\); \(n = 4\)) and tended to decrease the excitatory response in the vertebral nerve (\(P = 0.0580\); \(n = 3\)). Within 1 h after microinjection of D-AP5 into the LTF, the excitatory responses in the inferior cardiac and vertebral nerves had returned to 105\(\pm\)13 and 103\(\pm\)16\% of control, respectively. In each of these experiments, the excitatory response and period of reduced activity were decreased in parallel by microinjection of D-AP5. In the example shown in Fig. 1B, the vagal-mediated excitatory response in the inferior cardiac nerve was reduced to 66\% of control 10 min after microinjection of D-AP5 (compare traces 1 and 2) and recovered to 106\% of control 45 min later (trace 3).

Muscimol was microinjected bilaterally into the LTF of six cats in which we recorded vagal afferent-induced responses in both the inferior cardiac and vertebral nerves; two of these cats had recovered from the effects of microinjection of NBQX at the time that muscimol was injected into the LTF. As summarized in Fig. 2A, microinjection of the GABA agonist significantly (\(P < 0.0001\)) decreased the excitatory responses in both nerves. In contrast to the effects produced by microinjection of NBQX, the positive potential after the excitatory response in both nerves was more consistently (5 of 6 cats) decreased by the GABA receptor agonist. In the other case, the period of reduced activity was only slightly reduced despite the near elimination of the excitatory response in both nerves. Figure 1A, 4 shows an example of the ability of this drug to abolish the vagal afferent-induced response in the inferior cardiac nerve.

There were only two cats in which there was still a detectable excitatory response in the inferior cardiac and vertebral nerves after muscimol microinjection into the LTF. We pooled the data from the two nerves to compare latencies before and after microinjection of the GABA agonist. The onset latency was increased by 18\(\pm\)4 ms and time to peak of the excitatory response was increased by 9\(\pm\)4 ms.

Although all three drugs suppressed the vagal afferent-mediated excitatory response, they did so to a significantly different extent. Muscimol microinjection into the LTF caused a significantly greater decrease in the excitatory response in the inferior cardiac nerve than did either NBQX (\(P = 0.0443\)) or D-AP5 (\(P = 0.0001\)). Also, NBQX caused a significantly greater decrease in the excitatory response in the inferior cardiac than did D-AP5 (\(P = 0.0046\)). Similar differences were noted for the vertebral nerve. Bilateral microinjection of saline

![Fig. 2. Summary of effects of bilateral microinjection of drugs into the LTF on the excitatory responses in inferior cardiac (CN) and vertebral nerves (VN) elicited by stimulation of peripheral afferents and supramedullary sites. A–E: changes in the amplitude of the excitatory response (expressed as % of control response) elicited by electrical stimulation of vagal (A), trigeminal (B), and sciatic nerve afferents (C), periaqueductal gray (PAG; D), and posterior hypothalamus (E), respectively, 10 min after microinjection of NBQX, D-AP5, or muscimol (indicated below the x-axis). A dashed line runs through 100% of control as a reference point. Data are means \(\pm\) SE. *Statistically different from control. The number of experiments for each bar graph is stated in the text.](http://www.ajpregu.org/issue/286/2/0576/PDF/fig2.png)
into the LTF of two cats did not affect the response to vagal stimulation.

We stimulated the vagus nerve at two or more intensities ranging from 0.5 to 1.5 mA before and after microinjection of drugs into the LTF in 11 cats. The amplitude of the response in the inferior cardiac nerve elicited by the lowest stimulus intensity was 63 ± 5% of that of the response elicited by the highest stimulus intensity. However, the degrees of inhibition of the evoked responses were essentially identical (26 ± 7 and 29 ± 9% of control, respectively). Likewise, the amplitude of the excitatory response in the vertebral nerve elicited by the lowest stimulus intensity was 60 ± 7% of that of the response elicited by the highest stimulus intensity; yet, the degree of inhibition of the evoked response was similar for both stimulus intensities (35 ± 14 and 37 ± 12% of control, respectively).

Trigeminal-sympathetic reflex. We compared the response to trigeminal nerve stimulation before and 10 min after bilateral microinjection of NBQX into the LTF of six cats. Without exception, the excitatory responses in the inferior cardiac nerve and vertebral nerves were greater after the injection. In fact, in two cats, there was no response in the inferior cardiac nerve before microinjection, but one became evident after the injection. The grouped data in Fig. 2B showing a significant increase (P = 0.0250; n = 4) in the excitatory response in the inferior cardiac nerve do not include the results from these two cats; the trigeminal nerve-induced excitatory response in the vertebral nerve was also significantly increased (P = 0.0320; n = 6). Within 1 h after microinjecting NBQX, the responses in the inferior cardiac and vertebral nerves had returned to 115 ± 16 and 125 ± 11% of control, respectively. Figure 3A shows an example of the marked facilitation of the trigeminal nerve-induced excitatory response in the inferior cardiac nerve 10 min after microinjection of NBQX into the LTF (compare traces 1 and 2). Note that the positive potential after the excitatory response was also enhanced. Within 30 min after the injection, the response had begun to recover (Fig. 3A, 3).

We tested the effects of microinjecting d-AP5 into the LTF on the trigeminal afferent-induced excitatory response in both sympathetic nerves of five cats. As shown by the composite data in Fig. 2B, microinjection of this NMDA receptor antagonist did not significantly affect the response in either the inferior cardiac or vertebral nerve.

We tested the effects of microinjecting muscimol bilaterally into the LTF on the trigeminal nerve-induced excitatory response in six cats, including two cats after they had recovered from the effects of NBQX microinjection. As summarized in Fig. 2B, microinjection of muscimol significantly enhanced the excitatory response in the inferior cardiac (P = 0.0134; n = 6) and vertebral nerves (P = 0.0409; n = 4). Figure 3B shows an example of the reversible effects of muscimol on the excitatory response in the inferior cardiac nerve; the onset latency and time to peak of the excitatory response decreased from 83 to 72 ms and from 118 to 99 ms, respectively. On a group basis, the onset latency of the trigeminal afferent-induced excitatory response in the inferior cardiac and vertebral nerves was significantly decreased by 16 ± 6 ms (P = 0.0270) and 14 ± 1 ms (P = 0.0051), respectively, by microinjection of muscimol. The effects of microinjection of muscimol into the LTF...
on the trigeminal-sympathetic reflex were not significantly
different from those produced by microinjection of NBQX.

Sciatic-sympathetic reflex. In nine cats, we compared the
excitatory response in the inferior cardiac nerve to electrical
stimulation of sciatic nerve afferents before and 10 min after
bilateral microinjection of NBQX into the LTF. As summa-
rized in Fig. 2C, there was a tendency for enhancement of this
response after microinjection of NBQX; however, on a group
basis, the change was not statistically significant. Nonetheless,
in four cats, the excitatory response was increased to 182 ±
29% of control after microinjection of NBQX into the LTF and
returned to near-control level (123 ± 9% of control) within 1 h.
In the example shown in Fig. 4A, the excitatory response was
increased to 280% of control (compare traces 1 and 2) and
returned to 128% of control within 45 min (trace 3). The
increase in the excitatory response in these cats was not due to
a generalized increase in excitability because the response to
PAG or posterior hypothalamic stimulation in the same cats
was not affected by microinjection of NBQX into the LTF (see
below, Supramedullary stimulation).

Figure 4B shows an example in which microinjection of
NBQX into the LTF did not alter the sciatic-sympathetic reflex
(compare traces 1 and 2); however, subsequent microinjection
of NBQX into the RVLM reduced the excitatory response
(trace 3). Microinjection of NBQX into the RVLM signifi-
cantly reduced the sciatic nerve-induced excitatory response in
the inferior cardiac nerve of three cats to 43 ± 12% of control
\(P = 0.0447\).

We tested the effects of bilateral microinjection of muscimol
into the LTF on the sciatic-sympathetic reflex in five cats,
including one of the cats in which NBQX had been injected
into the LTF 1 h earlier. As was the case with NBQX, there
was a tendency for the response to be facilitated after micro-
injection of muscimol. Nonetheless, on a group basis, the
change was not statistically significant (Fig. 2C) even though
the excitatory response was increased to 189 and 175% of
control in two cats. Subsequent microinjection of muscimol
into the RVLM reduced the excitatory response to 15 and 60%
of control in two cats.

The vertebral nerve response to sciatic nerve stimulation was
recorded in two of the cats in which NBQX was microinjected
into the LTF and in two of the cats in which muscimol was
microinjected. Data from these four experiments were pooled.
The excitatory response in the vertebral nerve was unchanged
10 min after microinjection of these drugs into the LTF (94 ±
5% of control). There were no instances of facilitation of the
excitatory response in the vertebral nerve although the inferior
cardiac nerve response was enhanced in one of the experiments
after microinjection of NBQX into the LTF and in one of the
experiments in which muscimol was microinjected.

Because the general neuronal depressant muscimol (31) did
not significantly affect the sciatic nerve-induced sympathoex-
citation when microinjected into the LTF, we did not test the
effects of d-AP5 on this response.

Supramedullary stimulation. The PAG stimulus-induced ex-
citatory response was compared before and 10 min after
bilateral microinjection of NBQX into the LTF of eight cats.
As summarized in Fig. 2D, this procedure did not significantly
alter the excitatory response in the inferior cardiac \(n = 8\) or
vertebral nerve \(n = 4\). Muscimol was microinjected bilat-

dFig. 4. Effects of bilateral microinjection of
NBQX into the medulla on the sciatic-sym-
pathetic reflex. **A1**–**A3**: before, 10 min, and
30 min after microinjection into LTF, re-
spectively. **B1**–**B3**: before, 10 min after mi-
croinjection into LTF, and 10 min after mi-
croinjection into rostral ventrolateral me-
dulla (RVLM), respectively. Left sciatic
nerve afferents were stimulated at an inten-
sity of 2.0 mA in **A** and **B**. Vertical calibra-
tion, 20 \(\mu\)V (**A**) and 30 \(\mu\)V (**B**).
ally into the LTF of four cats, including two in which NBQX had been injected ~1 h earlier. Blockade of LTF neuronal activity with the GABA agonist also did not significantly affect the PAG-induced excitatory response in the inferior cardiac (Fig. 2D) or vertebral nerve (87 and 104% of control; n = 2). Figure 5A shows data from one of the cats in which the response to PAG stimulation was similar before (trace 1) and after microinjection of NBQX (trace 2) and then muscimol (trace 3) into the LTF. The similarity in the evoked responses in traces 1-3 points to the reproducibility of the response over a 90-min period.

When NBQX was microinjected into the RVLM 1 h after an injection had been made in the LTF, the PAG-evoked excitatory response in the inferior cardiac nerve was reduced to 47 ± 15% of control (n = 3). The PAG-evoked excitatory response was reduced to 18 and 24% of control in the two cats in which muscimol was microinjected bilaterally into the RVLM after the same drug had been injected into the LTF.

We only recorded from the inferior cardiac nerve in experiments in which we stimulated the posterior hypothalamus. Figure 5B shows data from one of the six cats in which the hypothalamic-induced excitatory response was compared before (trace 1) and 10 min after (trace 2) bilateral microinjection of NBQX into the LTF. Note that the evoked response was similar before and after microinjection of this drug. As summarized in Fig. 2E, bilateral microinjection of NBQX into the LTF did not significantly affect the excitatory response in the inferior cardiac nerve (n = 6). In three of these cats, NBQX was subsequently microinjected bilaterally into the RVLM; this procedure significantly reduced the excitatory response to 37 ± 7% of control (P = 0.0119). An example of the effects of microinjection of NBQX into the RVLM on the hypothalamic-induced response is shown in Fig. 5B, 3.

As summarized in Fig. 2E, bilateral microinjection of muscimol into the LTF of four cats also did not significantly change the hypothalamic-induced excitatory response in the inferior cardiac nerve. An example is presented in Fig. 5C, 1 and 2. In two of these cats, subsequent microinjection of muscimol into the RVLM reduced or eliminated (0 and 35% of control) the sympathoexcitatory response. Figure 5C, 3 shows the case in which the response to posterior hypothalamic stimulation was eliminated by microinjection of the GABA agonist into the RVLM.

In seven cats, we stimulated the PAG at two or three intensities ranging from 0.2 to 1.5 mA; and in six cats, we stimulated the posterior hypothalamus at different intensities ranging from 0.3 to 1.5 mA. The lowest stimulus intensity applied to the PAG and hypothalamus elicited responses in the inferior cardiac nerve whose amplitudes were 67 ± 7 and 63 ± 9% of those elicited with the highest stimulus intensity; these responses were also unchanged by microinjection of NBQX or muscimol into the LTF. The data quantified in Figs. 2, D and E, refer to the responses elicited with the highest stimulus intensity (1.0–1.5 mA) in each experiment.

Fig. 5. Effects of bilateral microinjection of NBQX or muscimol into the medulla on the evoked responses to electrical stimulation of supramedullary sites. A1–A3: PAG stimulation (1.0 mA) before, 10 min after microinjection of NBQX into LTF, and 10 min after subsequent microinjection of muscimol into LTF, respectively. B1–B3: posterior hypothalamic stimulation (1.5 mA) before, 10 min after microinjection of NBQX into LTF, and 10 min after subsequent microinjection of NBQX into RVLM, respectively. C1–C3: posterior hypothalamic stimulation (1.0 mA) before, 10 min after microinjection of muscimol into LTF, and 10 min after subsequent microinjection of muscimol into RVLM, respectively. Vertical calibration, 100 μV (A), 30 μV (B), and 25 μV (C).
Effects of Microinjection of EAA Receptor Antagonists or Muscimol into the LTF on Responses Elicited by Arterial Chemoreceptor Activation and High-Frequency PAG Stimulation

We recorded SND and MAP responses to intravenous injection of NaCN in 16 cats with intact carotid sinus, aortic depressor, and vagus nerves. In each of these experiments, at least two injections separated by \(\sim 15\) min were made in order to ensure reproducibility of the response. The administration of NaCN increased vertebral SND in each of 13 cases; however, inferior cardiac SND was increased in eight cases and was unchanged in five cases. As reviewed by de Burg Daly (14), others have also reported either an increase or no change in inferior cardiac SND during activation of arterial chemoreceptors. We sectioned the carotid sinus, aortic depressor, and vagus nerves at the end of two experiments. After this procedure, injection of NaCN no longer increased SND or MAP. In two other cats in which the baroreceptor nerves had been sectioned before the start of the experiment, an injection of NaCN did not change SND or MAP.

Figure 6 shows data from one of the eight cats in which we tested the effects of bilateral microinjection of NBQX into the LTF on the arterial chemoreceptor reflex. In Fig. 6A, vertebral and inferior cardiac SND and arterial pressure began to increase several seconds after administration of NaCN; these responses were attenuated 10 min after microinjection of NBQX (Fig. 6B). Although not shown, 1 h later arterial chemoreceptor reflex activation again produced a marked increase in SND and MAP. As summarized in Fig. 7A, microinjection of NBQX into the LTF significantly attenuated the increases in inferior cardiac SND \((P = 0.0381; n = 4)\), vertebral SND \((P = 0.0027; n = 7)\), and MAP \((P = 0.0008; n = 8)\) elicited by arterial chemoreceptor activation. In contrast, the injection did not significantly change the inferior cardiac nerve \((n = 5)\), vertebral nerve \((n = 3)\), or MAP \((n = 4)\) responses to short periods \((5–10\) s) of high-frequency \((25\) Hz) PAG stimulation (Fig. 7B).

As summarized in Fig. 7, A and B, microinjection of muscimol into the LTF of six cats significantly reduced the arterial chemoreceptor reflex-induced increases in inferior cardiac SND \((P = 0.0145; n = 3)\), vertebral SND \((P = 0.02781; n = 4)\), and MAP \((P = 0.0088; n = 6)\) but not the increases in inferior cardiac SND \((n = 5)\), vertebral SND \((n = 3)\), or MAP \((n = 6)\) produced by high-frequency PAG stimulation. Three of these cats had received an injection of NBQX and recovered from its effects before microinjection of muscimol. In the example shown in Fig. 8, A and B, the arterial chemoreceptor-induced increases in vertebral SND and MAP were essentially abolished by microinjection of the GABA agonist into the LTF. In contrast, the PAG stimulus-induced responses in the same cat were not changed (Fig. 8, C and D). The effects of muscimol microinjection on arterial chemoreceptor reflex-induced changes in SND and MAP were not significantly different from those produced by microinjection of NBQX.

Fig. 6. Attenuation of the sympathoexcitatory response to activation of arterial chemoreceptors after microinjection of NBQX into the LTF. A and B: before and 10 min after bilateral microinjection of NBQX into LTF. Traces (top to bottom) are arterial pressure (AP; mmHg), vertebral nerve activity (VNA), integrated VNA, inferior cardiac nerve activity (CNA), and integrated CNA. NaCN \((100\ \mu\text{g/kg iv})\) was administered at the arrow. Vertical calibrations, 50 V (VNA) and 35 V (CNA); reset interval for integration, 500 ms; time scale, 5 s/division.
Figure 9 shows data from one of the cats in which we tested the effects of bilateral microinjection of D-AP5 into the LTF on the responses to arterial chemoreceptor activation (A, B) and high-frequency (25 Hz) PAG stimulation (C, D). Neither response was affected by microinjection of this drug. As summarized in Fig. 7A, microinjection of D-AP5 into the LTF did not significantly affect the ability of intravenous injection of NaCN to increase vertebral SND ($n=3$), inferior cardiac SND ($n=3$), or MAP ($n=5$). The high-frequency PAG stimulus-induced increases in inferior cardiac SND ($n=4$) and MAP ($n=5$) were also not significantly affected (Fig. 7B); in the two cats in which PAG stimulation increased vertebral SND, the injection did not alter the response.

DISCUSSION

A major new finding of the current study is that the medullary LTF plays a critical role in mediating sympathoexcitatory effects produced by arterial chemoreceptor activation and electrical stimulation of afferents in the cervical vagus nerve. The increases in inferior cardiac and vertebral SND produced by these stimuli were profoundly attenuated by bilateral microinjection of either NBQX or muscimol into the LTF. In contrast, microinjection of D-AP5 into the LTF had no effect on the chemoreceptor reflex and only modestly, albeit significantly, reduced the vagal-sympathetic reflex. Thus we propose that non-NMDA receptor-mediated activation of LTF sympathoexcitatory neurons plays an important role in eliciting inferior cardiac and vertebral nerve responses to activation of arterial chemoreceptors and vagal afferents. In contrast, LTF sympathoexcitatory neurons are not major elements of pathways mediating increases in inferior cardiac or vertebral SND produced by electrical stimulation of PAG, posterior hypothalamus, or afferents in the sciatic nerve and supraorbital branch of the trigeminal nerve. However, there was a marked enhancement of the trigeminal afferent-induced excitatory response, and in some cases the sciatic nerve-induced sympathoexcitation, after microinjection of NBQX or muscimol into the LTF. Thus we propose that the LTF also contains tonically active sympathoinhibitory neurons that modulate transmission in trigeminal-sympathetic and sciatic-sympathetic reflex pathways.

One might argue that microinjection of EAA receptor antagonists or muscimol into the LTF reduced the magnitude of the responses elicited by vagal afferent stimulation or arterial chemoreceptor reflex activation because the injectate spread to portions of the nucleus of the tractus solitarius (NTS) where these afferents terminate (10, 36, 44, 47). This is unlikely to be the case for two reasons. First, our earlier work (33) showed that microinjection of EAA receptor antagonists into the LTF caused markedly different effects on SND than when injected into the NTS. Specifically, microinjection of NBQX into the
LTF significantly reduced the basal level of SND while preserving baroreceptor reflex control of SND, whereas blockade of non-NMDA receptors in the NTS abolished the cardiac-related rhythm in SND and baroreceptor reflex-induced sympathoinhibition but did not significantly change the basal level of SND. Moreover, microinjection of d-AP5 into the LTF, but not into the NTS, eliminated baroreceptor reflex control of SND (33). Second, Vardhan et al. (44) showed that to attenuate the pressor response produced by activation of arterial chemoreceptors, it was necessary to block both non-NMDA and NMDA receptors in the NTS; however, in the current study we found that microinjection of NBQX but not d-AP5 into the LTF suppressed the NaCN-induced increases in SND and MAP.

Fig. 8. Bilateral microinjection of muscimol into the LTF attenuates the response to arterial chemoreceptor reflex activation but not to high-frequency (25 Hz) PAG stimulation. A and C: before microinjection. B and D: 10 min after microinjection. Traces show AP (mmHg) and integrated VNA (500-ms reset interval). NaCN (100 µg/kg iv) was administered at the arrow in A and B. PAG was stimulated at 1.5 mA for the duration of the bar in C and D. Time scale, 5 s/division.

Fig. 9. Bilateral microinjection of d-AP5 into the LTF does not affect the responses to arterial chemoreceptor reflex activation or high-frequency (25 Hz) PAG stimulation. A and C: before microinjection. B and D: 10 min after microinjection. Sequence of traces are as in Fig. 8.
It is also unlikely that the injectate spread from the LTF to the RVLM. First, whereas microinjection of NBQX or muscimol into the LTF blocked sympathoexcitation produced by stimulation of sciatic afferents, PAG, and posterior hypothalamus, microinjection of these drugs into the LTF did not depress these responses as should have occurred if the injectate spread to the RVLM. Second, it has been reported that blockade of NMDA receptors, but not non-NMDA receptors, in the RVLM prevented the increase in MAP produced by activation of arterial chemoreceptors (28, 43).

In the current study, we noted the reverse pattern when EAA receptor antagonists were microinjected into the LTF. That is, the increases in SND and MAP produced by arterial chemoreceptor activation were reduced by blockade of non-NMDA receptors but were unaffected by blockade of NMDA receptors.

Microinjection of NBQX or muscimol into the LTF significantly decreased basal SND to about two-thirds of control. This raises the question whether the depression of the vagal-induced excitatory response and chemoreceptor reflex-induced sympathoexcitation produced by microinjection of these drugs merely reflected disfacilitation due to the decrease in basal SND. This possibility seems remote for two reasons. First, in the same cats in which the vagal-sympathetic reflex was diminished by microinjection of these drugs into the LTF, sympathoexcitation elicited by short trains of pulses applied to sciatic or trigeminal afferents were not inhibited. Moreover, in other cats in which basal SND was reduced, excitatory responses elicited by electrical stimulation of the PAG or posterior hypothalamus were unchanged by microinjection of NBQX or muscimol into LTF. Second, the increase in SND and MAP produced by high-frequency PAG stimulation was unaffected by microinjection of NBQX or muscimol in the same cats in which the sympathoexcitatory response to activation of arterial chemoreceptors was significantly attenuated.

Comparison of the effects of microinjection of drugs into the LTF on the responses produced by intravenous NaCN and vagal afferent stimulation point to differences in neuronal processing within this medullary region for these two sympathoexcitatory reflexes. Microinjection of NBQX but not D-AP5 significantly reduced the magnitude of the increases in SND and MAP produced by NaCN. Moreover, microinjection of the generalized neuronal depressant muscimol (31) into the LTF was no more effective than NBQX in attenuating the sympathoexcitatory response to chemoreceptor activation. Thus activation of non-NMDA receptors, but not NMDA receptors, in the LTF is involved in mediating arterial chemoreceptor reflex-induced increases in SND and MAP. In contrast, microinjection of either NBQX or D-AP5 into the LTF reduced vagal-mediated sympathoexcitation, suggesting that both EAA receptor subtypes contribute to this response. Nonetheless, the contribution of non-NMDA receptors is more important than that of NMDA receptors because microinjection of NBQX produced a significantly greater attenuation of the vagal afferent-induced sympathoexcitation than D-AP5. Whether combined blockade of NMDA and non-NMDA receptors would reduce the vagal afferent-mediated excitatory response to the same extent as muscimol microinjection remains to be determined. In this regard, microinjection of muscimol into the LTF caused a significantly greater attenuation of vagal-induced sympathoexcitation than that produced by NBQX.

The study by Langhorst et al. (29) also is consistent with the view that LTF neurons are involved in mediating increases in SND produced by arterial chemoreceptor activation. They showed that neurons in this region of the medulla increased their firing rate in response to intravenous NaCN, and some of these neurons had cardiac-related activity and were inhibited during a rise in arterial pressure, suggesting that they were sympathoexcitatory neurons.

Our data do not contradict the findings of others that microinjection of EAA receptor antagonists into the RVLM or destruction of RVLM catecholaminergic neurons prevents the changes in SND and MAP produced by activation of vagal afferents or arterial chemoreceptors (27, 28, 35, 42, 43, 48). The axons of LTF sympathoexcitatory neurons do not project directly to spinal preganglionic sympathetic neurons but appear to influence SND via a connection in the RVLM (3). On this basis, we propose that both LTF and RVLM neurons are contained in the pathway responsible for mediating the sympathoexcitatory responses elicited by activation of vagal and chemoreceptor afferents. Our data also do not rule out the possibility that a direct pathway from the NTS to the RVLM plays a role in mediating the chemoreceptor reflex, as has been proposed for other species (14). In this regard, the sympathoexcitatory response was markedly reduced, but not abolished, by microinjection of muscimol into the LTF.

We did not attempt to identify the types of vagal afferents whose electrical activation induced sympathoexcitation. High-intensity stimulation of the cervical vagus nerve would be expected to activate a mixture of myelinated and unmyelinated afferent fibers from a variety of mechanoreceptors and chemoreceptors in the heart, aorta, lungs, and abdomen (10, 47). The relatively long latencies of the sympathoexcitatory responses imply that unmyelinated vagal afferents were activated (36). Whether sympathoexcitatory responses elicited by activation of all types of vagal afferents are relayed through the LTF is not known. Additional studies are needed to evaluate the importance of the LTF in mediating sympathoexcitation induced by selective activation of cardiac, pulmonary, and abdominal vagal afferents.

Independent of the afferent nerve or supramedullary site activated, short trains of three pulses elicited a stereotypical response that included an initial excitation followed by a period of reduced activity (positive potential). In some cases, the positive potential elicited by vagal afferent nerve stimulation persisted despite marked attenuation of the preceding excitatory response (see Fig. 1A for an example). This implies that the positive potential elicited by vagal afferent stimulation was not simply due to a recovery process of postganglionic neurons following their activation (22) or postexcitatory feedback inhibition at a central site. In either case, the positive potential should have been diminished in parallel with the initial excitation. It seems more likely that the positive potential reflected a period of active inhibition due to stimulation of vagal afferents different from those responsible for the excitatory response. Unlike NBQX, muscimol microinjection into the LTF consistently eliminated or reduced in parallel the excitatory response and positive potential elicited by vagal afferent stimulation. Thus the pathway responsible for that portion of the positive potential presumed to be due to active inhibition also contains a synapse in the LTF.
As demonstrated by others (1, 17, 25, 32, 34, 39), electrical stimulation of trigeminal or sciatic afferents elicits sympathoexcitatory response. These responses are generally attributable to activation of nociceptive fibers (1, 34). A consistent finding of the current study was the marked enhancement of the sympathoexcitatory response elicited by electrical stimulation of trigeminal nerve afferents after microinjection of either NBQX or muscimol, but not n-AP5, into the LTF. These results suggest that the LTF contains tonically active sympathoinhibitory neurons that modulate transmission in the trigeminal-sympathetic reflex pathway and that the discharges of these LTF neurons are non-NMDA receptor mediated. LTF neurons can also intercept transmission within the sciatic-sympathetic reflex pathway because, in some cases, the sciatic nerve-induced activation of inferior cardiac SND was also enhanced by blockade of non-NMDA receptors in the LTF or by inhibiting LTF neuronal activity with the GABA agonist.

Whereas the excitatory responses to stimulation of trigeminal and sciatic nerve afferents were enhanced after microinjection of NBQX or muscimol into the LTF, such was not the case for the responses elicited by stimulation of the PAG or posterior hypothalamus. One might argue that this difference was due to the fact that the responses produced by supramedullary stimulation were much greater in magnitude to begin with than those elicited by trigeminal and sciatic afferent stimulation. If this was the explanation, then it should have been possible to enhance submaximal responses elicited by low-intensity stimuli applied to the PAG or posterior hypothalamus. However, the sympathoexcitatory responses elicited by relatively low-intensity (0.2–0.5 mA) stimuli applied to supramedullary sites were unaffected by microinjection of NBQX or muscimol into the LTF.

There are anatomic and electrophysiological data indicating that the LTF receives inputs directly or indirectly from PAG, posterior hypothalamus, and sciatic nerve (2, 13, 24, 49). In fact, LTF neurons with activity correlated to SND are activated by electrical stimulation of the posterior hypothalamus (2) and sciatic afferents (24). Thus we sought to determine if activation of EAA receptors on LTF neurons is involved in eliciting sympathoexcitatory responses to electrical stimulation of these inputs. However, we found that neither blockade of non-NMDA receptors in the LTF nor a generalized blockade of LTF neuronal activity with muscimol (31) prevented the increase in SND elicited by stimulation of PAG, posterior hypothalamus, or sciatic nerve afferents. Thus LTF neurons apparently are not essential for eliciting increases in SND from these sources. On the other hand, microinjection of either NBQX or muscimol into the RVLVM reduced the excitatory responses to stimulation of PAG, posterior hypothalamus, and sciatic nerve afferents. These data support the work of others who have shown that the RVLVM is involved in mediating the sympathoexcitatory response to electrical stimulation of these inputs (11, 25, 32, 39, 41).

In summary, the current study has revealed two new roles for the LTF in the control of SND in the cat. One, LTF neurons are elements of vagal- and arterial chemoreceptor-sympathoexcitatory reflex pathways. Activation of these LTF sympathoexcitatory neurons is primarily non-NMDA receptor mediated. Two, the LTF also contains tonically active neurons that suppress transmission in trigeminal-sympathetic and somato-sympathetic reflex loops. The tonic activity of these neurons is also, at least in part, dependent on non-NMDA receptors. These data, together with our past work (6, 8, 33) demonstrating critical roles of the LTF in mediating baroreceptor reflex control of SND and in setting the basal level of SND, establish this region as a key element in the central sympathetic network. It is well-known that vagal, chemoreceptor, and baroreceptor afferents terminate in the NTS (10, 36, 44, 47). This raises the possibility that, at least in the cat, the LTF is an obligatory link in all reflex pathways in which the primary afferent terminates in the NTS. Future studies are needed to address this novel hypothesis.

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