Medullary lateral tegmental field mediates the cardiovascular but not respiratory component of the Bezold-Jarisch reflex in the cat

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Submitted 7 June 2005; accepted in final form 11 August 2005

Barman, Susan M., Shaun W. Phillips, and Gerard L. Gebber. Medullary lateral tegmental field mediates the cardiovascular but not respiratory component of the Bezold-Jarisch reflex in the cat. Am J Physiol Regul Integr Comp Physiol 289: R1693–R1702, 2005. First published August 11, 2005; doi:10.1152/ajpregu.00406.2005.—We determined the effects of bilateral microinjection of muscimol and excitatory amino acid receptor antagonists into the medullary lateral tegmental field (LTF) on changes in sympathetic nerve discharge (SN), mean arterial pressure (MAP), and phrenic nerve activity (PNA; artificially ventilated cats) or intrathoracical pressure (spontaneously breathing cats) elicited by right atrial administration of phenylbiguanide (PBG; i.e., the Bezold-Jarisch reflex) in dial-urethane anesthetized cats. The PBG-induced depressor response [−66 ± 8 mmHg; mean ± SE] was converted to a pressor response after muscimol microinjection in two of three spontaneously breathing cats and was markedly reduced in the other cat; however, the duration of apnea (20 ± 3 vs. 17 ± 7 s) was essentially unchanged. In seven paralyzed, artificially ventilated cats, muscimol microinjection significantly (P < 0.05) attenuated the PBG-induced fall in MAP [−39 ± 7 vs. −4 ± 4 mmHg] and the magnitude (−98 ± 1 vs. −35 ± 13%) and duration (15 ± 2 vs. 3 ± 2 s) of the sympathoinhibitory response. In contrast, the PBG-induced inhibition of PNA was unaffected (3 cats). Similar results were obtained by microinjection of an N-methyl-D-aspartate (NMDA) receptor antagonist, d(-)2-amino-5-phosphonopentanoic acid, into the LTF. In contrast, neither the cardiovascular nor respiratory responses to PBG were altered by blockade of non-NMDA receptors with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzof[l]quinoxaline-7-sulfonamide. We conclude that the LTF subserves a critical role in mediating the sympathetic and cardiovascular components of the Bezold-Jarisch reflex. Moreover, these data show separation of the pathways mediating the respiratory and cardiovascular responses of this reflex at a level central to bulbo-spinal outflows to phrenic motoneurons and preganglionic sympathetic neurons.

cardiopulmonary chemosensitive afferent; medullary lateral tegmental field; phrenic nerve activity; sympathetic nerve discharge; vagal afferent

Since the pioneering work of von Bezold and Hirt (42) and Jarisch and Henze (18), many investigators have shown that activation of chemosensitive vagal C fibers in the cardiopulmonary region (e.g., juxta-capillary region of the alveoli, ventricles, atria, great veins, and pulmonary artery) leads to a profound bradycardia, hypotension, and a brief period of apnea followed by rapid shallow breathing [see reviews by Hainsworth (17) and Verberne et al. (39)]. The Bezold-Jarisch reflex can be elicited by a variety of substances including capsaicin, serotonin, phenylbiguanide (PBG), and veratridine in cats, rabbits, and rodents (9, 11, 14, 20, 28, 33, 36, 37, 41). Although originally viewed as a pharmacological curiosity, there is a growing body of evidence supporting the view that the Bezold-Jarisch reflex is activated during certain pathophysiological conditions. For example, this reflex may be activated during myocardial ischemia and reperfusion as a result of increased production of oxygen radicals and by agents used as radiocontrast for coronary angiography (23, 31). Also, the syndrome of cardiac slowing with hypotension (vasovagal syncope) has been attributed to activation of the Bezold-Jarisch reflex (1). Finally, activation of cardiopulmonary chemosensitive receptors may be part of a defense mechanism protecting individuals from toxic chemical hazards (1).

Over the past 15 years, several studies have attempted to identify the central pathways that mediate the Bezold-Jarisch reflex (14, 28, 32, 33, 36, 37, 38, 40, 41). Studies primarily using rodents and rabbits have led to the proposal (8, 39, 41) that the central pathway responsible for the cardiovascular responses elicited by activation of cardiopulmonary chemosensitive afferents is the same as, or at least similar to, the baroreceptor pathway, which includes synapses in the nucleus tractus solitarii (NTS), caudal ventrolateral medulla (CVLM), and rostral ventrolateral medulla (RVLM). Although only a few NTS neurons have been shown to be excited by activation of both baroreceptor and cardiopulmonary chemosensitive afferents (28, 33), most CVLM neurons that are excited by stimulation of baroreceptor afferents also are excited by PBG (32, 37). Also, most putative RVLM sympathoexcitatory neurons are inhibited by activation of both baroreceptor and cardiopulmonary chemosensitive afferents (37, 40). The results of other studies also implicates a role of NTS, CVLM, and RVLM in mediating the Bezold-Jarisch reflex. Gieroba et al. (14) showed fos-positive neurons in the NTS and CVLM after intravenous administration of PBG in rats and rabbits. Furthermore, microinjection of the γ-aminobutyric acid (GABA) receptor agonist muscimol (37) or the nonspecific glutamate receptor antagonist kynurenate (36, 37, 41) into the NTS abolished the cardiorespiratory responses to right atrial administration of PBG. Blockade of excitatory amino acid (EAA) receptors in the CVLM prevented the reduction in sympathetic nerve discharge (SN) and depressor response to activation of cardiopulmonary chemosensitive afferents in rats (38, 41). Finally, blockade of GABA receptors in the RVLM markedly attenuated the sympathoinhibitory and depressor responses to PBG in rats (41). The respiratory responses to PBG were not described in these studies in which cardiopulmonary chemosensitive afferents were activated before and after microinjection of muscimol or EAA receptor antagonists in the CVLM or RVLM.

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The current study was designed to determine whether the medullary lateral tegmental field (LTF) plays an important role in mediating the Bezold-Jarisch reflex in cats. We considered this possibility in light of recent work from our laboratory (26, 27, 29) and a study by Vayssettes-Courchay et al. (37). Vayssettes-Courchay et al. (37) suggested that the cat LTF was involved in mediating this reflex because some LTF neurons with activity correlated to SND responded to right atrial administration of PBG. However, they did not determine whether chemical inactivation of the LTF blocked the cardiovascular and respiratory components of the Bezold-Jarisch reflex. Investigations from our laboratory (26, 27, 29) have shown that LTF neurons in the cat are elements of the medullary pathways that mediate baroreceptor, chemoreceptor, and vagal afferent influences on SND. Specifically, blockade of N-methyl-D-aspartate (NMDA) receptors in the LTF significantly reduced baroreceptor-mediated inhibition of SND and the cardiac-related rhythm in SND (26), whereas blockade of non-NMDA EAA receptors reduced the increases in SND produced by activation of arterial chemoreceptors and electrical stimulation of afferents in the cervical vagus nerve (27). Also, we showed that chemical inactivation of the LTF with microinjection of muscimol eliminated the component of SND time-locked to intratracheal pressure (ITP, an index of vagal lung inflation afferent activity) without disrupting the entrainment of phrenic nerve activity (PNA) to ITP (29). These data led us to postulate that the LTF was involved in mediating changes in SND elicited by activation of all afferents whose primary site of termination is in the NTS. Such afferents would include those mediating the Bezold-Jarisch reflex (17, 28, 33). Thus, in the current study, we determined the effects of chemical inactivation of the LTF (muscimol microinjection) or blockade of EAA receptors in the LTF on the cardiovascular and respiratory components of the Bezold-Jarisch reflex.

METHODS

General Procedures

Experiments were performed on 21 adult cats (2.3–4.7 kg) anesthetized with an intraperitoneal injection of a mixture of sodium diaethylbarbiturate (60 mg/kg), urethane (240 mg/kg), and monoethylurea (240 mg/kg). The protocols used in these studies were approved by the All-University Committee on Animal Use and Care of Michigan State University. Cats were placed in a stereotaxic apparatus and spinal investigation unit. A femoral artery and vein were cannulated to measure arterial pressure and to administer drugs, respectively. Another cannula (PE-50) was filled with PBG (1 mg/ml) and inserted into the right atrium via the right jugular vein. Six cats were allowed to breathe spontaneously throughout the experiment. In these cats, ITP was monitored using a volumetric pressure transducer (model PTS; Grass Instruments). The other 14 cats were paralyzed (gallamine triethiodide, 4 mg/kg iv initial dose), pneumothoracotomized, and artificially ventilated with room air. End-tidal CO2 was held near 4.6% (model 2200; Traverse Medical Monitors Cmapnometer) by adjusting the parameters of artificial ventilation, and rectal temperature was kept near 38°C with a heat lamp. In spontaneously breathing cats, the adequacy of anesthesia was indicated by the absence of a palpebral and paw pinch reflexes. In paralyzed cats, an adequate level of anesthesia was indicated by the inability of noxious stimuli (pinch, heat, surgery) to increase blood pressure or change the pattern of the frontal-parietal electroencephalogram from delta-slow waves to high-frequency, low-voltage activity (34).

As was the case in earlier studies (26, 29), we maintained mean arterial pressure (MAP) at approximately the same level throughout the experiment by adjusting the rate of an intravenous infusion of a mixture of dextan and saline. This was done to maintain a reasonably stable level of baroreceptor afferent nerve activity.

Sympathetic and Phrenic Nerve Recordings

The inferior cardiac postganglionic branch of the left stellate ganglia was exposed retropleurally by removing the head of the first rib (13), and the right phrenic nerve was isolated in the neck (29). The nerves were covered with silicone release agent (Dow Corning 7 compound). Potentials were recorded monophasically from the central ends of the cut nerves placed on platinum bipolar electrodes. The capacity-coupled preamplifier band pass was set at 30–3,000 Hz for SND and 10–1,000 Hz for PNA. Signals were then passed through a 50/60 Hz noise eliminator (Hum Bug; Quest Scientific, North Vancouver, BC, Canada). PNA was further processed with a moving averager (model MA-821RSP; CWE, Ardmore, PA) with a 100-ms time constant.

Data (1-ms resolution) 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). Datapac software (Run Technologies, Mission Viejo, CA) was used to rectify and integrate (500-ms reset) the recording of SND. The component of the integrated record of SND that was attributable to noise was measured after ganglionic blockade with hexamethonium bromide (5 mg/kg iv) at the end of the experiment. This value was subtracted from the integrated records of SND used to quantify changes in SND. As described by Orer et al. (26), spectral analysis was used to calculate power in SND and the coherence value relating SND to the arterial pulse.

Bezold-Jarisch Reflex

The Bezold-Jarisch reflex was elicited by right atrial administration of the serotonergic receptor agonist PBG (25–90 μg/kg). Because the cannula was prefilled with PBG, no flush was needed. This was done to minimize activation of atrial mecanoreceptors by volume expansion. The maximum injection volume used in these experiments was 0.28 ml. In agreement with others (11, 33, 37), the onset latency of PBG-induced changes in SND, MAP, heat rate, and respiration was <5 s. The reflex was elicited at least twice (separated by >15 min) before microinjection of drugs into the LTF to assure reproducibility of the response elicited by activation of cardiopulmonary chemosensitive afferents. In spontaneously breathing cats, we quantified the changes in MAP, the interval between heart beats, the amplitude of the rectified and integrated (500-ms reset) record of SND, the duration of the sympathoinhibitory response, and the duration of apnea (as monitored with ITP). In paralyzed and artificially ventilated cats, instead of ITP, we quantified the respiratory response to PBG by measuring changes in the amplitude of the moving average record of PNA and the duration of the reduction in PNA. The PBG-induced changes in these parameters were compared before and ~10 min after microinjection of a drug into the LTF.

Microinjections

Procedures used for exposing the brain stem and for microinjection of drugs into the LTF are the same as those described in earlier reports from this laboratory (4, 26, 27, 29). In fact, the cats in which we studied the effects of muscimol microinjection into the LTF on the Bezold-Jarisch reflex were also included in the study of the effects of chemical inactivation of the LTF on the influence of vagal lung inflation afferents on SND and PNA (29).

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 two sites in the LTF on each side of the medulla over a period of ~3 min. The micropipette was positioned in tracks located ~2 and 3 mm rostral to the obex and 2.5–2.8 mm lateral to the midline; microinjections were made bilaterally at a depth of ~3.8 mm from the dorsal surface. Multiple injections were made so that the drug would spread through the portion of the LTF that contains neurons with activity correlated to SND (2, 3, 6, 13). The most rostral injection site in the LTF is ~2 mm caudal, 1 mm medial, and ~1 mm dorsal to the region of the RVLM that contains neurons with activity correlated to SND (2, 13). The most caudal injection site in the LTF is ~1–1.5 mm medial and ~1 mm dorsal to the most rostral portion of the caudal CVLM that contains neurons with activity correlated to SND (5). According to investigators who have calculated the spread of injection of a bolus of fluid in the brain (25), the 50-nl volume of muscimol used in our experiments should spread over a radius of ~0.5 mm. Thus it is unlikely that drugs spread from the LTF to these other medullary sites shown to be involved in mediating the Bezold-Jarisch reflex in other species (38, 41). In two cats, saline was injected into LTF (same sites as other drugs). The Bezold-Jarisch reflex was not altered by these injections.

All chemicals used for microinjection into the LTF were diluted in 0.9% saline. Solutions were adjusted to a pH of 6–8 (litmus paper test) and placed in a glass micropipette (~40-μm tip diameter) that was glued (cyanoacrylate) to the needle of a 5-μl Hamilton syringe. The syringe and micropipette were mounted on a microinjection unit (model 5000; David Kopf Instruments). A 50-nl injection was made slowly (~10 s) at each medullary site by turning the calibrated micrometer on the microinjection unit. The following drugs were injected into the LTF: the GABA antagonist muscimol (0.5 mM), the competitive NMDA receptor antagonist d(-)-2-amino-5-phosphono-pentanoic acid (d-AP5; 3 mM), or the competitive non-NMDA receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[quisxaline-7-sulfonamide (NBQX; 1.25 mM). Only one drug was injected into the LTF of each cat. Muscimol, d-AP5, and NBQX were purchased from Sigma (St. Louis, MO), and drug concentrations were the same as used in past studies from this laboratory (4, 26, 27, 29). No attempt was made in the current study to test the selectivity of NBQX or d-AP5 on different classes of EAA receptors. However, the doses of d-AP5 and NBQX used were similar to those used in other studies in which their ability to block selectively NMDA or non-NMDA receptors, respectively, was demonstrated (10, 22).

**Histology**

At the end of the experiment, the brain stem was removed and fixed in 10% buffered Formalin. Frontal sections (40 μm thick) were cut and stained with cresyl violet to locate the levels of microinjection with reference to the stereotaxic planes of Berman (7). Injection sites were identified on the basis of the bottom of the tracks made with the micropipette. The histological sections in Fig. 1, right, show the tracks made with a micropipette filled with d-AP5 placed at ~2 and 3 mm rostral to the obex on the right half of the medulla in one of these cats. The dots in Fig. 1, left, show the target sites of the injections into the LTF. Examination of histological sections showed that the effective injection sites were within 300 μm of the target sites. In one experiment in which the injection of muscimol did not block the effects of PBG on SND, the injection site was determined to be in the inferior olive.

**Statistical Analysis**

Data are expressed as means ± SE. P ≤ 0.05 indicates statistical significance. A paired t-test was used to compare the PBG-induced changes in MAP, heart rate, SND, ITP, and PNA before and after microinjection of a drug into the LTF. We also used a paired t-test to compare the level of basal SND before and after microinjection of a drug into the LTF.

**RESULTS**

**Effects of Muscimol Microinjection Into the LTF on the Bezold-Jarisch Reflex in Spontaneously Breathing Cats**

Figure 2A shows an example of the Bezold-Jarisch reflex in a spontaneously breathing cat. Note that shortly after injection of PBG into the right atrium, SND was markedly reduced for ~10 s. The sympathoinhibitory response was accompanied by a 53-mmHg fall in MAP. Although not obvious on the time scale of this trace, heart rate was also reduced (i.e., the beat-to-beat interval increased from 322 to 375 ms). In addition, activation of cardiopulmonary chemosensitive afferents led to an ~24-s period of apnea (ITP recording). As shown in Fig. 2B, although activation of vagal cardiopulmonary chemosensitive afferents still induced apnea 10 min after bilateral microinjection of muscimol into the LTF, PBG administration now caused a transient, modest increase in SND and a 30-mmHg rise in MAP. The bradycardia induced by PBG was essentially eliminated; the beat-to-beat interval increased from 354 to 360 ms. Also, note that the basal level of SND was slightly decreased after chemical inactivation of the LTF as indicated by the reduction in the amplitude of the rectified and integrated (500-ms reset) signal before administration of PBG.
The data from three spontaneously breathing cats are summarized in Fig. 3 and can be described as follows. First, the depressor response produced by PBG was converted to a pressor response in two cats and was markedly reduced in one cat after chemical inactivation of the LTF. Although not included in Fig. 3 because SND recordings were made in only two of the three spontaneously breathing cats, the sympathoinhibitory response to PBG was virtually eliminated after muscimol microinjection (see example in Fig. 2).

Second, without fail, the magnitude of the PBG-induced bradycardia was attenuated after microinjection of muscimol into the LTF. Third, the duration of PBG-induced apnea was essentially the same before and after chemical inactivation of the LTF. In agreement with the results from an earlier study in our laboratory (29), muscimol microinjection into the LTF decreased the baseline interbreath interval from 2.21 ± 0.30 to 1.38 ± 0.13 s in these spontaneously breathing cats. The baseline heart beat-to-beat interval was similar (306 ± 10 vs. 311 ± 23 ms) before and after microinjection of muscimol in these cats in which MAP was maintained relatively constant (130 ± 11 vs. 135 ± 10 mmHg) by an infusion of dextran and saline.

We compared the responses elicited by right atrial administration of PBG before and after bilateral cervical vagotomy in three other spontaneously breathing cats. PBG reduced MAP by 90 ± 10 mmHg, increased the heart beat-to-beat interval by 880 ± 653 ms, and produced apnea that lasted for 16.6 ± 2.2 s before vagotomy. After vagotomy, PBG did not elicit changes in blood pressure, heart rate, or respiration.

Effects of Muscimol Microinjection Into the LTF on the Bezold-Jarisch Reflex in Paralyzed, Artificially Ventilated Cats

We studied the effects of muscimol microinjection into the LTF on the PBG-induced sympathoinhibitory and depressor responses in seven paralyzed, artificially ventilated cats. In three of these cats, we also recorded PNA. The results from this series of experiments corroborated the findings in spontaneously breathing cats. Figure 4, A and B, compares the effects of injection of PBG into the right atrium of one of these cats before and after bilateral microinjection of muscimol into the LTF, respectively. Note that ~10 min after chemical inactivation of the LTF, the PBG-induced depressor response was eliminated and the profound decrease in SND was converted to a biphasic response (a brief increase followed by a short-lasting decrease). In contrast, the PBG-induced inhibition of PNA was actually enhanced at this time.

Figure 5 summarizes the results from the seven paralyzed, artificially ventilated cats in which we determined the effects of chemical inactivation of the LTF on the Bezold-Jarisch reflex. Chemical inactivation of the LTF significantly attenuated the PBG-induced fall in MAP (P < 0.0001) and the magnitude (P = 0.0022) and duration (P = 0.0008) of the sympathoinhibitory response. In contrast, muscimol microinjection into the LTF did not markedly alter the magnitude or duration of the PBG-induced inhibition of PNA (3 cats). In this group of cats, PBG-induced changes in heart rate were minimal even before chemical inactivation of the LTF. This was likely due to block of the vagal-mediated bradycardia by the neuromuscular blocking agent gallamine triethiodide (21). The beat-to-beat interval...
Changes in SND were quantified by comparing the amplitude basal SND to 61.1/11006 analyzed, artificially ventilated cats (27, 29), microinjection of (5.6 was only modestly changed before (6.4 11% of control. In the current study, 4.5 ms) microinjection of muscimol into the LTF. PNA burst amplitude was reduced by 65 (136 11 mmHg) by adjusting the rate of an intravenous infusion of a mixture of dextran and saline. The interval between bursts of PNA was slightly decreased from 3.65 ± 0.49 to 3.30 ± 0.63 s after chemical inactivation of the LTF in the three cats in which PNA was recorded.

In agreement with other work from our laboratory on paralyzed, artificially ventilated cats (27, 29), microinjection of muscimol into the LTF significantly (P = 0.0302) reduced basal SND to 61 ± 11% of control. In the current study, changes in SND were quantified by comparing the amplitude of the rectified and integrated (500-ms reset) signals before and after microinjection of muscimol. As indicated in methods, the resting level of MAP was maintained at a similar level before and after microinjection of muscimol (128 ± 10 vs. 123 ± 9 mmHg) by adjusting the rate of an intravenous infusion of a mixture of dextran and saline. The interval between bursts of PNA was slightly decreased from 3.65 ± 0.49 to 3.30 ± 0.63 s after chemical inactivation of the LTF in the three cats in which PNA was recorded.

**Effects of Blockade of EAA Receptors in the LTF on the Bezold-Jarisch Reflex**

We studied the effects of microinjection of EAA receptor antagonists into the LTF on the Bezold-Jarisch reflex in seven paralyzed, artificially ventilated cats. As was the case with muscimol microinjections, the respiratory depressant effect elicited by right atrial administration of PBG was not affected by blockade of either NMDA or non-NMDA receptors in the LTF of the five cats in which PNA was recorded.

D-AP5. Figure 6, A and B, shows data from one of the four paralyzed, artificially ventilated cats in which we studied the effects of microinjection of D-AP5, an NMDA receptor antagonist, into the LTF on the depressor and sympathoinhibitory effects of right atrial administration of PBG. During control conditions (Fig. 6A), right atrial administration of PBG caused a fall in MAP of ~20 mmHg and SND was profoundly reduced for ~12 s. In contrast, ~10 min after bilateral microinjection of D-AP5 into the LTF (Fig. 6B), administration of the same dose of PBG did not affect MAP or SND.

Figure 7, A–C, summarizes the data from the four cats in which the PBG-induced changes in MAP and SND were compared before and after microinjection of D-AP5 into the LTF. Blockade of NMDA receptors significantly attenuated the reductions in MAP (P = 0.0189) and SND (P = 0.0048) elicited by right atrial administration of PBG. Also, the duration of the sympathoinhibition was significantly (P = 0.0286) shortened. Although not shown, PBG-induced changes in PNA were not significantly affected by microinjection of D-AP5 into the LTF. PNA burst amplitude was reduced by 65 ± 11% for 15.6 ± 3.7 s before and by 76 ± 13% for 16.8 ± 6.0 s after microinjection of D-AP5 into the LTF (n = 3).

In agreement with our past study (26), microinjection of D-AP5 into the LTF interfered with baroreceptor reflex control of SND as indicated by a significant reduction (P = 0.0089) in the coherence between SND and the arterial pulse at the frequency of the heart rate from 0.88 ± 0.07 to 0.34 ± 0.05. Nonetheless, blockade of NMDA receptors in the LTF did not significantly affect basal SND (100 ± 11% of control). This can be attributed to a shift in the power from the cardiac-related band of SND to irregular low-frequency oscillations (26). As already indicated, MAP was maintained at a similar level before (136 ± 11 mmHg) and after microinjection of D-AP5 (138 ± 8 mmHg) in these experiments.

**NBQX.**Figure 6, C and D, shows data from one of the three paralyzed, artificially ventilated cats in which we studied the effects of microinjection of NBQX, a non-NMDA receptor antagonist, into the LTF on the depressor and sympathoinhibitory effects of right atrial administration of PBG. During control conditions (Fig. 6C), right atrial administration of PBG caused an ~50-mmHg fall in MAP and a marked reduction in SND that lasted for ~11 s. These responses were essentially unchanged 10 min after bilateral microinjection of D-AP5 into the LTF (Fig. 6D).

Figure 7, D and E, summarizes the data from the three cats in which the PBG-induced changes in MAP and SND were compared before and after microinjection of NBQX into the LTF. Note that blockade of non-NMDA receptors in the LTF did not significantly affect the changes in SND or MAP produced by right atrial administration of PBG. In the two cats in which PNA was recorded, right atrial administration of PBG

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Fig. 3. Summary of the effects of chemical inactivation of the LTF on the Bezold-Jarisch reflex in 3 spontaneously breathing cats. Bar graphs show responses to right atrial administration of PBG during control and ~10 min after microinjection of muscimol (25 pmol/injection site) bilaterally into the LTF. A: changes (Δ) in mean arterial pressure (MAP). B: changes in the interval between heart beats (Beat-to-Beat Int.). C: changes in the duration of apnea.
Fig. 4. Effects of chemical inactivation of LTF on the Bezold-Jarisch reflex in a paralyzed, artificially ventilated cat before (A) and −10 min after (B) microinjection of muscimol (25 pmol/injection site) bilaterally into the LTF. Traces (top to bottom) show AP, SND, Int., and moving averaged recording of phrenic nerve activity (PNA, Mov. Avg.). Time scale, 2 s/division. Arrows mark right atrial administration of PBG (45 μg/kg).

Fig. 5. Summary of the effects of chemical inactivation of the LTF on the Bezold-Jarisch reflex in paralyzed, artificially ventilated cats. Bar graphs compare responses to right atrial administration of PBG during control and −10 min after microinjection of muscimol (25 pmol/injection site) bilaterally into the LTF. A: changes in MAP (n = 7). B: magnitude (top) and duration (bottom) of the inhibition of SND (n = 7). C: magnitude (top) and duration (bottom) of the inhibition of PNA (n = 3). *Significantly different from control response.
reduced the burst amplitude by at least 95% for 15–26 s before and after microinjection of NBQX.

Although microinjection of NBQX into the LTF did not affect the Bezold-Jarisch reflex, as expected (26), blockade of non-NMDA receptors in this region decreased basal SND (74 ± 8% of control) in these cats in which the resting level of MAP was maintained at a similar level before (133 ± 5 mmHg) and after microinjection of NBQX (130 ± 11 mmHg). Also, in agreement with the earlier study (26), the coherence value relating SND to the arterial pulse at the frequency of the heart beat was not significantly affected by microinjection of NBQX into the LTF (0.96 ± 0.01 vs. 0.85 ± 0.09).

DISCUSSION

To our knowledge, this is the first study to demonstrate directly that the LTF subserves a critical role in mediating the sympathetic and cardiovascular components of the Bezold-Jarisch reflex. Specifically, bilateral microinjection of muscimol into the LTF significantly attenuated the magnitude and duration of the sympathoinhibition and the reduction in MAP elicited by right atrial administration of PBG in spontaneously breathing as well as paralyzed, artificially ventilated cats.

In contrast to its ability to attenuate or abolish changes in SND and MAP due to right atrial administration of PBG, chemical inactivation of the LTF with muscimol did not affect the ability of activation of cardiopulmonary chemosensitive afferents to induce apnea or a reduction in PNA burst amplitude. These data demonstrate separation of the pathways mediating the respiratory and cardiovascular depressant components of the Bezold-Jarisch reflex at a level central to the bulbospinal outflows to phrenic motoneurons and preganglionic sympathetic neurons. Such also is the case for the reflex effects mediated by activation of vagal lung inflation afferents, because investigators in our laboratory (29) have shown that
microinjection of muscimol into the LTF eliminates the component of SND time-locked to ITP, an index of vagal lung inflation afferent activity, without disrupting the entrainment of PNA in a 1:1 relation to the artificial ventilation cycle.

It is unlikely that muscimol spread from the LTF to NTS to block the cardiovascular component of the Bezold-Jarisch reflex. If such spread had occurred, the respiratory changes elicited by right atrial administration of PBG should also have been eliminated. Indeed, Vardhan et al. (36) have shown that microinjection of muscimol or kynurenate into the NTS abolishes both the cardiovascular and respiratory responses to activation of cardiopulmonary chemosensitive vagal afferents.

The current study also demonstrated that NMDA receptor-mediated neurotransmission in the LTF is involved in mediating the sympathoinhibitory and depressor effects of the Bezold-Jarisch reflex. Specifically, we found that these components of the Bezold-Jarisch reflex were virtually eliminated after bilateral microinjection of D-AP5 into the LTF. In contrast, the PBG-induced reductions in SND and MAP were maintained after microinjection of the non-NMDA receptor antagonist NBQX. These findings parallel those our group (26) reported in a study on the effects of microinjection of D-AP5 and NBQX into the LTF on baroreceptor-mediated changes in SND. These data raise the possibility that the same LTF neurons mediate both the Bezold-Jarisch and baroreceptor reflexes in the cat. Some evidence in favor of this possibility exists. The cat LTF contains neurons whose basal activity is correlated to the cardiac-related rhythm (2, 3, 6, 13, 37). These neurons have been classified as putative sympahtoexcitatory and sympathoinhibitory neurons depending on their responses to baroreceptor reflex activation; the firing rate of sympathetic excitatory neurons is decreased and that of sympathoinhibitory neurons is increased during elevations in arterial pressure. Vayssettes-Courchay et al. (37) showed that the firing rate of seven of eight putative LTF sympathoexcitatory neurons was increased by PBG, whereas the firing rate of four of eight putative LTF sympathoexcitatory neurons was decreased. Future studies should be directed at determining whether the response of such LTF neurons to right atrial administration of PBG are affected by microiontophoresis of D-AP5. We have shown that microiontophoresis of D-AP5 interrupts baroreceptor influences on putative LTF sympathoexcitatory and sympahtoinhibitory neurons as indicated by the loss of their cardiac-related activity (6).

In rats and rabbits, the CVLM has been shown to mediate the baroreceptor and Bezold-Jarisch reflexes (8, 15, 16, 22, 30, 35, 39). This raises the question of whether the LTF of the cat is a functional homolog of the CVLM in these other species. There are several notable similarities between these two regions. Like the CVLM of the rat (19, 32), the LTF of the cat contains putative sympathoinhibitory neurons that are excited during activation of the baroreceptor and/or Bezold-Jarisch reflexes (3, 13, 37). In addition, blockade of NMDA receptors in both the CVLM of the rat (8, 15, 35, 38) and the LTF of the cat (Ref. 26; current study) interferes with the baroreceptor and Bezold-Jarisch reflexes. These parallels are consistent with the view that LTF sympathoinhibitory neurons in the cat serve as functional homologs of those in the CVLM of other species. However, whereas microinjection of EAA receptor antagonists or muscimol into the CVLM of rats and rabbits leads to an increase in SND and MAP as expected if one blocks the activity of sympathoinhibitory neurons (8, 15, 22, 30, 35, 38,
microinjection of NBQX or muscimol into the LTF decreases SND and MAP (4, 26, 27, 29). This difference can be explained by the fact that the LTF of the cat contains a functionally heterogeneous population of neurons involved in control of SND (2, 3, 6, 13, 37). Whereas the CVLM contains sympathoinhibitory neurons (5, 19, 32, 37), the LTF of the cat contains both sympathoinhibitory and sympathoexcitatory neurons (2, 3, 6, 13, 37). LTF sympathoexcitatory neurons are inhibited during activation of the baroreceptor and/or Bezold-Jarisch reflexes (2, 6, 13, 37), and their axons appear to terminate in the RVLM (2). Because the ratio of sympathoexcitatory neurons to sympathoinhibitory neurons in the LTF is about 2:1 (13), our group (4, 26, 27, 29) proposed that the decreases in SND produced by microinjection of muscimol or NBQX into the LTF reflect the inactivation of a greater number of sympathoexcitatory than sympathoinhibitory neurons. These LTF sympathoexcitatory neurons not only play a role in setting the basal level of SND and MAP (4, 26) but also contribute to the increases in SND and MAP produced by activation of peripheral arterial chemoreceptors and electrical stimulation of vagal afferents (27). Thus, whereas sympathoinhibitory neurons in the LTF of the cat may be the functional homologs of those in the CVLM of other species, the overall roles of these two regions in the control of SND and MAP are not identical.

The extent to which the CVLM is involved in mediating the baroreceptor and Bezold-Jarisch reflexes in the cat is unclear. Like the CVLM of other species (19, 32), the CVLM of the cat contains neurons with pulse-synchronous activity that are excited during the inhibition of SND produced by activation of arterial baroreceptors (5, 37) as well as during activation of the Bezold-Jarisch reflex (37). Also, our group (26) showed that microinjection of d-AP5 into the CVLM of the cat reduced cardiac-related power in SND and the coherence value relating SND to the arterial pulse. These data support the view that the CVLM is in the pathway mediating these reflexes in the cat. However, Gatti et al. (12) reported that muscimol microinjection into the CVLM of the cat did not induce a pressor response as might be expected if neurons in this region were contained in the baroreceptor reflex pathway. Moreover, no one has determined whether interrupting neural transmission within the CVLM of the cat interferes with the Bezold-Jarisch reflex.

Microinjection of muscimol into the LTF also essentially eliminated the fall in heart rate resulting from activation of vagal chemosensitive afferents in spontaneously breathing cats. PBG-induced bradycardia is primarily attributable to reflex-induced activation of vagal cardiac motoneurons in the nucleus ambiguus (43). Whether the failure to elicit a bradycardia by right atrial administration of PBG after microinjection of muscimol was due to a direct action of the GABA agonist on LTF neurons or spread to cardiac vagal motoneurons in the adjacent nucleus ambiguus remains to be determined. Regarding this point, the nucleus ambiguus lies ~0.5 mm from the injection sites in the LTF (24). Not surprisingly, PBG did not elicit a profound bradycardia in paralyzed, artificially respired cats, because gallamine triethiodide blocks cholinergic effects on the heart (21).

After chemical inactivation of the LTF in some cats, right atrial administration of PBG caused an increase in MAP. Verberne and Guyenet (41) reported that the sympathoinhibitory and depressor responses to administration of PBG in rats were also reversed to sympathoexcitatory and pressor responses after microinjection of the EAA receptor antagonist kynurenate into the CVLM or after microinjection of the GABA antagonist bicuculline methiodide into the RVLM. They suggested that PBG activated not only vagal chemosensitive afferents but also cardiac sympathetic afferents, the effects of the latter being manifested only after blockade of the more dominant inhibitory effects mediated by vagal afferents. It is unlikely that this is the major cause of the PBG-induced pressor response in our experiments, because right atrial administration of PBG failed to change blood pressure after bilateral vagotomy. Others also have reported that right atrial administration of PBG does not alter MAP after vagotomy (11, 20, 43). In our experiments, the reversal to a pressor response after chemical inactivation of the LTF was most pronounced in spontaneously breathing cats. In these cases, the increase in MAP may have resulted as a consequence of the PBG-induced apnea leading to activation of central and/or peripheral arterial chemoreceptors.

Perspectives

Whereas CVLM neurons in the rat and rabbit are considered to be the major link between the NTS and RVLM within the pathways mediating the sympathetic and cardiovascular responses to activation of baroreceptor and vagal cardiopulmonary chemosensitive afferents (8, 14, 15, 16, 22, 30, 35, 39, 32, 41), the current study in conjunction with other reports from this laboratory (6, 26) provide compelling evidence that the LTF plays a critical role in mediating the baroreceptor and Bezold-Jarisch reflexes in the cat. Whether sympathoinhibitory neurons in the LTF of the cat replace those in the CVLM of other species or act in conjunction with CVLM neurons to mediate these reflexes remains to be determined. However, it is clear that the functions of the LTF in the cat and the CVLM in other species are not identical. Because the LTF contains both sympathoexcitatory and sympathoinhibitory neurons, this region plays a role in the control of SND and MAP extending well beyond just mediating the baroreceptor and Bezold-Jarisch reflexes. Specifically, the LTF has been shown to be involved in setting the basal level of SND (4, 26, 27, 29) as well as in mediating changes in SND elicited by activation of arterial chemoreceptors and vagal lung inflation afferents (27, 29). Finally, our results show separation of the pathways mediating the respiratory and cardiovascular components of the Bezold-Jarisch reflex at a level between the NTS and RVLM extending well beyond just mediating the baroreceptor and Bezold-Jarisch reflexes. Whereas CVLM neurons in the rat and rabbit are considered to be the major link between the NTS and RVLM within the pathways mediating the sympathetic and cardiovascular responses to activation of baroreceptor and vagal cardiopulmonary chemosensitive afferents (8, 14, 15, 16, 22, 30, 35, 39, 32, 41), the current study in conjunction with other reports from this laboratory (6, 26) provide compelling evidence that the LTF plays a critical role in mediating the baroreceptor and Bezold-Jarisch reflexes in the cat. Whether sympathoinhibitory neurons in the LTF of the cat replace those in the CVLM of other species or act in conjunction with CVLM neurons to mediate these reflexes remains to be determined. However, it is clear that the functions of the LTF in the cat and the CVLM in other species are not identical. Because the LTF contains both sympathoexcitatory and sympathoinhibitory neurons, this region plays a role in the control of SND and MAP extending well beyond just mediating the baroreceptor and Bezold-Jarisch reflexes. Specifically, the LTF has been shown to be involved in setting the basal level of SND (4, 26, 27, 29) as well as in mediating changes in SND elicited by activation of arterial chemoreceptors and vagal lung inflation afferents (27, 29). Finally, our results show separation of the pathways mediating the respiratory and cardiovascular components of the Bezold-Jarisch reflex at a level between the NTS and phrenic motoneurons and preganglionic sympathetic neurons. It is our hope that these studies will stimulate renewed interest in the role of the LTF in mediating clinically relevant reflexes.

ACKNOWLEDGMENTS

We thank Lindsey Fink for preparation of histological sections.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grant HL-33266.

REFERENCES


