Regulation of the chemosensory control of breathing by Kölliker-Fuse neurons

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Damasceno RS, Takakura AC, Moreira TS. Regulation of the chemosensory control of breathing by Kölliker-Fuse neurons. Am J Physiol Regul Integr Comp Physiol 307: R57–R67, 2014. First published April 23, 2014; doi:10.1152/ajpregu.00024.2014.—The Kölliker-Fuse region (KF) and the lateral parabrachial nucleus (LPBN) are involved in the maintenance of cardiorespiratory control. Here, we evaluated the involvement of the KF region and the LPBN in cardiorespiratory responses elicited by chemoreceptor activation in unanesthetized rats. Male Wistar rats (280–330 g; n = 5–9/group) with bilateral stainless-steel guide cannulas implanted in the KF region or the LPBN were used. Injection of muscimol (100 and 200 pmol/100 nl) in the KF region decreased resting ventilation (1,140 ± 68 and 978 ± 100 vs. saline: 1,436 ± 155 ml·kg⁻¹·min⁻¹), without changing mean arterial pressure (MAP) and heart rate (HR). Bilateral injection of the GABA-A antagonist bicuculline (1 mmol/100 nl) in the KF blocked the inhibitory effect on ventilation (1,418 ± 138 vs. muscimol: 978 ± 100 ml·kg⁻¹·min⁻¹) elicited by muscimol. Muscimol injection in the KF reduced the increase in ventilation produced by hypoxia (8% O₂) (1,827 ± 61 vs. saline: 3,179 ± 325 ml·kg⁻¹·min⁻¹) or hypercapnia (7% CO₂) (1,488 ± 277 vs. saline: 3,539 ± 374 ml·kg⁻¹·min⁻¹) in unanesthetized rats. Bilateral injection of bicuculline in the KF blocked the decrease in ventilation produced by muscimol in the KF during peripheral or central chemoreflex activation. Bilateral injection of muscimol in the LPBN did not change resting ventilation or the increase in ventilation elicited by hypoxia or hypercapnia. The results of the present study suggest that the KF region, but not the LPBN, has mechanisms to control ventilation in resting, hypoxic, or hypercapnic conditions in unanesthetized rats.

chemoreflex; cardiorespiratory responses; Kölliker-Fuse; lateral parabrachial nucleus

It is well established that the parabrachial complex, located in the dorsal lateral pons, can be subdivided into several distinct subnuclei, including the lateral parabrachial nucleus (LPBN) and the Kölliker-Fuse (KF) region (21). Neurons of the parabrachial complex are shown to play an important role in the processing and relaying of somatosensory and visceral sensory information. The LPBN and the KF are considered important to respiratory control because they contain postinspiratory neurons, which are important for the transition between inspiration and expiration, as well as for maintaining respiratory rhythm (40, 46, 48).

The role of pontine areas in modulating the transition between inspiration and expiration was first demonstrated by Markwald (35) in 1887 and later by Stella (58) in 1939. Both studies showed that dorsolateral pontine transitions were able to change a normal breathing pattern to apneusis, a breathing pattern characterized by an increase in inspiratory time (8, 11, 12, 17, 18, 20, 24, 25, 35, 49, 58). The apneusis following pontine transection is dependent on the absence of vagal input from the periphery by a presynaptic inhibition-gated respiratory modulation of pontine activity (20). In addition, experiments from our laboratory showed that bilateral inactivation of the KF caused apneustic breathing in vagotomized rats (10a).

Given the profound influence of the pons on the medullary respiratory network, neuroanatomical evidence demonstrates that the parabrachial/KF complex receives projections from the retrotrapezoid nucleus and the caudal regions of the nucleus of the solitary tract, suggesting a possible role of pontine areas in the chemoreflex (28, 44, 53). Studies using c-Fos-immunoreactive expression as a marker of neuronal activity showed intense activation of the KF region after hypoxia, hypercapnia, or stimulation of the carotid sinus nerve (3, 5, 29, 61). Studies in cats and rats showed that after bilateral lesions of the parabrachial and the Kölliker-Fuse complex, the breathing increase during hypoxia or hypercapnia was attenuated (29, 38, 55–57).

However, most of the experiments showing the role of the pontine regions in viscerosensory control consider the parabrachial complex as a unique brain structure involved in different physiological functions, including breathing and autonomic regulation. Therefore, the main goal of this study was to determine the involvement of the KF and the LPBN in the resting respiratory rhythm and cardiorespiratory responses elicited by chemoreceptor activation in unanesthetized rats. To test this possibility, we evaluate the role of the KF or LPBN on cardiorespiratory control under a baseline condition and during exposure to hypoxia or hypercapnia in unanesthetized rats.

METHODS

Animals

Animal use was in accordance with the guidelines approved by the University of São Paulo Animal Care and Use Committee. All experiments were conducted using male Wistar rats weighing 280–330 g. A total of 42 rats were used for the experiments, and all efforts were made to minimize animal discomfort and the number of animals used.

Surgery and Anesthesia

Rats were anesthetized with a mixture of ketamine (80 mg/kg body wt) and xylazine (7 mg/kg body wt ip). Depth of anesthesia was assessed by the absence of the corneal and hindpaw withdrawal reflexes. Additional anesthetic was administered as necessary (20% of the original dose, administered intraperitoneally). The following procedures were performed under aseptic conditions. The rats were placed prone on a Kopf 900 stereotaxic apparatus. Body temperature
was kept close to 37°C with a servo-controlled heating pad. A 1.5-mm diameter hole was drilled bilaterally into the occipital plate to the parieto-occipital suture. Bilateral stainless-steel cannulas were implanted into the KF using the following coordinates: 8.9 mm caudal to bregma, 2.3 mm lateral to the midline, and 5.3 mm below dura mater. In another group of rats, bilateral stainless-steel cannulas were implanted into the LPBN using the following coordinates: 9.4 mm caudal to bregma, 2.0 mm lateral to the midline, and 4.1 mm below dura mater. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. Rats received postoperative boluses of ampicillin (30,000 IU) given intramuscularly and a subcutaneous injection of the anaglesic Ketoflex (ketoprofen 1%, 0.03 ml/rat) and were then placed in a heated environment (37°C) until consciousness was regained before being returned to a clean home cage. Rats were housed in the Department of Physiology vivarium for a week before experimentation. During this time, rats gained weight normally and appeared unperturbed by the guide cannula.

Pulsatile arterial pressure, mean arterial pressure (MAP), and heart rate (HR) were recorded in unanesthetized freely moving rats, as previously described (16, 39). Briefly, one day before the experiments, under intraperitoneal injection of ketamine combined with xylazine anesthesia, a polyethylene tube (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta through the femoral artery. The cannula was tunneled subcutaneously to the back of the rats to keep them unrestrained and freely moving.

In Vivo Recordings of Physiological Variables

Twenty-four hours after arterial cannulation, when the rats had adapted to the environment of the recording room, the arterial catheter was connected to a pressure transducer (MLT844; ADInstruments, Sydney, NSW, Australia) coupled to a preamplifier (Bridge Amp, ML221; ADInstruments) that was connected to a PowerLab computer data acquisition system (PowerLab 16/30, ML880; ADInstruments). Respiratory rate (fR, breaths/min) and tidal volume (VT, ml/kg) in conscious, freely moving rats were measured by whole body plethysmography, as previously described in detail (16, 36). All experiments were performed at room temperature (24–26°C). In brief, freely moving rats were kept in a Plexiglas recording chamber (5 liters) that was flushed continuously with a mixture of 79% nitrogen and 21% oxygen (95% N2 and 5% CO2) before measurements of baseline arterial pressure and ventilation were taken. Hypoxia was induced by lowering the O2 concentration in the inspired air to a level of 8% for 10 min. Hypercapnia was induced by titrating CO2 into the respiratory mixture (5% CO2 in sterile saline) and bicuculline (GABA-A antagonist) [Sigma; 1 nmol in propylene glycol/water (2:1)] were injected (100 nl in each side) bilaterally using 5-μl Hamilton syringes connected by polyethylene tubing (PE-10) to the injection needle, which was 1.5 mm longer than the guide cannulas implanted into the brain (conscious rats).

Histology

At the end of the experiments, rats were deeply anesthetized with pentobarbital sodium, and a 2% solution of Evans blue was injected into the KF and the LPBN. Saline followed by 10% buffered formalin (pH 7.4) was perfused through the heart. The brains were removed, fixed in 10% formalin for at least 2 days, frozen, cut coronally into 40-μm sections and stained with Nissl. The sections were analyzed using a Zeiss Axioskop 2 microscope (Oberkochen, Germany) to confirm the location of the injections into the KF and the LPBN, according to the atlas of Paxinos and Watson (42).

Statistics

A statistical analysis was performed with SigmaStat version 3.0 (Jandel, Point Richmond, CA). The results are presented as means ± SE. Paired t-tests and a one- or two-way ANOVA followed by the Newman-Keuls multiple-comparisons test was performed as appropriate (P < 0.05). Significance was set at P < 0.05.

RESULTS

GABAergic Mechanisms in the KF Contribute to Respiratory Drive in Unanesthetized Rats

In the first series of experiments, we hypothesized that the KF region is important for maintaining normal breathing under resting conditions in unanesthetized rats. The typical injection sites in the KF region are shown in Fig. 1B. Briefly, injections were located in the dorsolateral region of the ventral pons, lateral to the superior cerebellar peduncle. It is important to point out here that our injections were located in the rostral half of the KF region, where the catecholaminergic cells of the A7 region are located (data not shown).

Bilateral injection of muscimol (100 and 200 pmol/100 nl) in the KF decreased baseline tidal volume (12.3 ± 0.8 and 11.2 ± 1 vs. saline: 14.7 ± 0.6 ml/kg; P < 0.01), breathing rate (92 ± 0.6 and 87 ± 3 vs. saline: 102 ± 5 breaths/min; P < 0.05), and minute volume (1,140 ± 68 and 978 ± 100 vs. saline: 1,436 ± 155 ml·kg⁻¹·min⁻¹; P < 0.001) (Fig. 1A–E). Bilateral injection of muscimol in the KF did not affect baseline MAP (109 ± 3 and 108 ± 3 vs. saline: 106 ± 4 mmHg; P > 0.05) and HR (318 ± 11 vs. saline: 328 ± 9 bpm; P > 0.05) (Fig. 1, J–K).

Bilateral inhibition of muscimol in the KF region induced a slight increase in inspiration time (Ti) (275 ± 5 and 281 ± 4 vs. saline: 249 ± 6 ms; P < 0.05) and a substantial increase in expiration time (Te) (576 ± 15 and 592 ± 24 vs. saline: 452 ± 19 ms; P < 0.05) (Fig. 1, D and G). Bilateral injection of muscimol also reduced the peak of inspiratory flow (3.3 ± 0.3 and 3 ± 0.5 vs. saline: 4.6 ± 0.5 ml/s; P < 0.05) and the peak of expiratory flow (2.3 ± 0.9 and 1.6 ± 0.4 vs. saline: 5.7 ± 0.7 ml/s; P < 0.01) (Fig. 1, H and I).

Bilateral injection of bicuculline (1 nmol/100 nl) in the KF was able to block the decrease in tidal volume (14 ± 1 vs. muscimol: 11.2 ± 1 ml/kg; P < 0.05), breathing rate (100 ± 3 vs. muscimol: 87 ± 3 breaths/min; P < 0.05), minute volume (1,418 ± 138 vs. muscimol: 978 ± 100 ml·kg⁻¹·min⁻¹; P < 0.001), the peak of inspiratory flow (5 ± 1 vs. muscimol: 3 ± 0.5 ml/s; P < 0.01) and the peak of expiratory flow (6.3 ± 1.3 vs. muscimol: 1.6 ± 0.4 ml/s; P < 0.001) produced by bilateral injections of muscimol (200 pmol/100 nl) in the KF (Fig. 1, C–E; H, I). Bilateral injections of bicuculline in the KF were
also effective at blocking the increase in inspiration time (253 ± 4 vs. muscimol: 281 ± 4 ms; P < 0.05) and expiration time (463 ± 13 vs. muscimol: 592 ± 14 ms; P < 0.05) produced by muscimol injection in the KF (Fig. 1, F and G).

Effects of Bilateral Inhibition of the KF Region on Peripheral Chemoreflex Activation in Unanesthetized Rats

Hypoxia (8% O2, balanced with N2) for 10 min produced hypotension (93 ± 4 vs. normoxia: 106 ± 4 mmHg; P < 0.05), tachycardia (423 ± 12 vs. normoxia: 329 ± 9 bpm; P < 0.05), increase in tidal volume (23 ± 2.4 vs. normoxia: 14 ± 1.8 ml/kg; P < 0.05), breathing rate (136 ± 5 vs. normoxia: 99 ± 6 breaths/min; P < 0.05), and minute volume (3,179 ± 325 vs. normoxia: 1,402 ± 103 ml·kg⁻¹·min⁻¹; P < 0.05) (Fig. 2, A–F). Bilateral inhibition of the KF region reduced the increase in tidal volume (15 ± 1 vs. saline: 23 ± 2.4 ml/kg; P < 0.05), breathing rate (115 ± 3 vs. saline: 136 ± 5 breaths/min; P < 0.05), minute volume (1,827 ± 61 vs. saline: 3,179 ± 335

Fig. 1. Respiratory and cardiovascular responses to bilateral injection of muscimol in the KF in unanesthetized rats. A: typical recording showing arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), and airflow (inspiration: upward deflection; expiration: downward deflection) after bilateral injections of saline or muscimol (200 pmol/100 nl) in the Kölliker-Fuse (KF). B: diagram showing the location of injections in the KF region. Dark gray circles represent injections of muscimol (100 pmol/100 nl); solid circles show injections of muscimol (200 pmol/100 nl), and light gray circles show injections of a combination of bicuculline + muscimol. The open circles represent injections located outside the region of the KF. Photomicrograph showing a typical site of a bilateral injection in the KF region (arrows). Changes in tidal volume (C), breathing rate (D), minute volume (E), inspiration time (F), expiration time (G), peak inspiratory flow (H), peak expiratory flow (I), mean arterial pressure (J), and heart rate (K) in unanesthetized animals that received bilateral injection of bicuculline followed by bilateral injection of muscimol in the KF region. Mo5, motor nucleus mesencephalic; spc, superior peduncle cerebellar. Scale bar = 1 mm in B. *Significantly different from saline (P < 0.05). n = 5–9 rats/group.
ml·kg⁻¹·min⁻¹; P < 0.05) and heart rate (380 ± 9 vs. saline: 423 ± 12 bpm; P < 0.05) elicited by hypoxia (Fig. 2, A–F). Bilateral injection of muscimol in the KF was not able to change the hypotension elicited by hypoxia in unanesthetized rats (95 ± 4 vs. saline 93 ± 4 mmHg; P > 0.05).

Previous injections of bicuculline (1 nmol/100 nl) in the KF abolished the effects of muscimol (200 pmol/100 nl) on tidal volume (18.5 ± 0.6 vs. muscimol: 15 ± 1 ml/kg; P < 0.05), breathing rate (137 ± 8 vs. muscimol: 115 ± 3 breaths/min; P < 0.05), minute volume (2.527 ± 137 vs. muscimol: 1.827 ± 61 ml·kg⁻¹·min⁻¹; P < 0.05) and tachycardia (427 ± 21 vs. muscimol: 380 ± 9 bpm; P < 0.05) elicited by hypoxia in unanesthetized animals (Fig. 2, C–F).

Effects of Bilateral Inhibition of the KF Region on Central Chemoreflex Activation in Unanesthetized Rats

Hypercapnia (7% CO₂, 21% O₂, balanced with N₂) for 10 min increased tidal volume (22 ± 2 vs. normoxia: 14 ± 1.4 ml/kg; P < 0.05), breathing rate (159 ± 5 vs. normoxia: 97 ± 3 breaths/min; P < 0.05), and minute volume (3,130 ± 427 vs. normoxia: 1,408 ± 274 ml·kg⁻¹·min⁻¹; P < 0.05) (Fig. 3, A, D–F). Hypercapnia did not change MAP (106 ± 8 vs. normoxia: 103 ± 4 mmHg; P > 0.05) and HR (322 ± 14 vs. normoxia: 324 ± 13 bpm; P > 0.05) (Fig. 3, B and C).

Bilateral injection of muscimol in the KF reduced the increase in tidal volume (11.4 ± 1.4 vs. saline: 22 ± 2 ml/kg; P < 0.01), breathing rate (121 ± 10 vs. saline: 159 ± 5 breaths/min; P < 0.05) and minute volume (1,488 ± 277 vs. saline: 3,539 ± 374 ml·kg⁻¹·min⁻¹; P < 0.05) elicited by hypercapnia in unanesthetized animals (Fig. 3, A, D–F).

Bilateral injection of bicuculline in the KF was able to abolish the effect of muscimol on tidal volume (20 ± 4 vs. muscimol: 11.4 ± 1.4 ml/kg; P < 0.05), breathing rate (148 ± 5 vs. muscimol: 121 ± 10 breaths/min; P < 0.05), and minute volume (2,997 ± 537 vs. muscimol: 1,488 ± 277 ml·kg⁻¹·min⁻¹; P < 0.05) elicited by hypercapnia in unanesthetized animals (Fig. 3, D–F).
Lateral Parabrachial Nucleus Does Not Contribute to Respiratory Drive in Unanesthetized Rats

The typical sites of injections in the LPBN in unanesthetized rats are shown in Fig. 4A. As shown by the dye, most of the injections were into the central lateral and dorsolateral portions of the LPBN. Some of the injections spread to the brachium (superior cerebellar peduncle), or slightly ventral to this structure, reaching the dorsal portions of the medial parabrachial nucleus (MPBN) (Fig. 4A). There was no difference in the effects of whether injections were restricted to the LPBN or also spread to the brachium and dorsal portions of the MPBN (data not shown).

Bilateral injections of muscimol (200 pmol/100 nl) in the LBPN produced no change in baseline tidal volume (13.8 ± 1.6 vs. saline: 14.4 ± 1.6 ml/kg; P > 0.05), breathing rate (102 ± 4 vs. saline: 99 ± 6 breaths/min; P > 0.05) and minute volume (21 ± 1.3 vs. saline: 23 ± 2.3 ml/kg; P > 0.05) elicited by hypercapnia in unanesthetized animals that received bilateral injection of saline or bicuculline followed by saline or muscimol in the KF region. *Significantly different from baseline (P < 0.05); n = 5–7 rats/group.

Effects of Bilateral Inhibition of the LPBN Region on Chemosensory Control of Breathing and Blood Pressure in Unanesthetized Rats

Peripheral chemoreceptor activation. Bilateral injection of muscimol (200 pmol/100 nl) in the LPBN did not affect the peak of inspiratory flow (5.8 ± 0.6 vs. saline: 5.3 ± 0.3 ml/s; P > 0.05) and the peak of expiratory flow (8.5 ± 0.7 vs. saline: 8.9 ± 1 ml/s; P > 0.05) (Fig. 4, E and F).

Bilateral inhibition of the LPBN also produced no changes in inspiration time (Ti) (253 ± 6 vs. saline: 255 ± 4 ms; P > 0.05) (Fig. 4, E and F). Injection of muscimol did not affect the peak of inspiratory flow (5.8 ± 0.6 vs. saline: 5.3 ± 0.3 ml/s; P > 0.05) and the peak of expiratory flow (8.5 ± 0.7 vs. saline: 8.9 ± 1 ml/s; P > 0.05) (Fig. 4, G and H).

Bilateral injections of muscimol in the LPBN increased baseline MAP (119 ± 2 vs. saline: 104 ± 2 mmHg; P < 0.05) without changing HR (333 ± 13 vs. saline: 336 ± 24 mmHg; P > 0.05) (Fig. 4, I and J).

Fig. 3. Respiratory and cardiovascular responses elicited by central chemoreflex stimulation in unanesthetized rats with combined injections of bicuculline and muscimol in the KF region. A: typical recording showing AP, MAP, HR, and airflow (inspiration: upward deflection; expiration: downward deflection) after bilateral injections of saline or muscimol (200 pmol/100 nl) in the LPBN during activation of central chemoreceptors (hypercapnia: 7% CO₂). Changes in MAP (B), HR (C), tidal volume (D), breathing rate (E), and minute volume (F) produced by hypercapnia in unanesthetized animals that received bilateral injection of saline or bicuculline followed by saline or muscimol in the KF region. *Significantly different from baseline (P < 0.05); n = 5–7 rats/group.
hypoxia (8% O₂) in unanesthetized animals (Fig. 5, B–E). Bilateral injection of muscimol in the LBPN reduced the hypotension (96/110 ± 3 vs. saline: 86/110 ± 4 mmHg; P < 0.05) elicited by hypoxia (Fig. 5A).

Central chemoreceptor activation. Muscimol injected bilaterally in the LPBN did not change the increase in tidal volume (23 ± 1 vs. saline: 24 ± 2 ml/kg; P > 0.05), breathing rate (134 ± 9 vs. saline: 136 ± 4 breaths/min; P > 0.05), and minute volume (3,124 ± 78 vs. saline: 3,088 ± 88 ml·kg⁻¹·min⁻¹; P > 0.05) elicited by hypercapnia (7% CO₂) in unanesthetized animals (Fig. 6, C–E). Injections of muscimol in the LPBN also produced no changes in MAP (103 ± 3 vs. saline: 101 ± 2 mmHg; P > 0.05) and HR (327 ± 16 vs. saline: 325 ± 11 bpm; P > 0.05) (Fig. 6, A and B).

Effects of Muscimol Injected Outside the KF on MAP, HR, and Breathing Parameters

Injections located outside the KF region often reached the brachium (n = 2), the ventrolateral part of the pons, without reaching the A5 region (n = 3), and the dorsolateral region of the parabrachial nucleus (n = 4) (Fig. 1B).

Bilateral injections of muscimol outside the KF region produce no significant changes in the baseline MAP (110 ± 7 vs. saline: 108 ± 5 mmHg; P > 0.05), HR (338 ± 16 vs. saline: 344 ± 26 bpm; P > 0.05), tidal volume (13.4 ± 1.6 ml/kg; P > 0.05), breathing rate (98 ± 5 vs. saline: 99 ± 7 breaths/min; P > 0.05) and minute volume (1,313 ± 41 vs. saline: 1,326 ± 37 ml·kg⁻¹·min⁻¹; P > 0.05). Injections of muscimol located outside the KF did not change the cardiorespiratory effects elicited by peripheral or central chemoreceptors activation in unanesthetized rats (data not shown).

DISCUSSION

The present results show that neurons located in the KF region contribute to breathing coordination in unanesthetized rats. Pharmacological nonselective inhibition of the KF region with the GABA-A agonist muscimol leads to a considerable increase in the duration of expiratory time, suggesting a pos-
sible blockade of postinspiratory neurons, which could be responsible for the inspiratory/expiratory switch-off in mammals (19). Additionally, we show that inhibition of KF neurons reduced the respiratory response to peripheral and central chemoreceptor activation, suggesting that neurons located in this pontine region are important in the chemosensory pathway. We also found that a pharmacological blockade of LPBN did not change breathing or the chemoreflex.

A previous study reported the involvement of the parabrachial complex controlling breathing by central or peripheral chemoreflex activation (38). However, there are important issues that must be taken into consideration to interpret these results. In our study, we performed experiments with bilateral injections of the GABA agonist muscimol to study the acute effects elicited by inhibition of those neurons, while the previous study performed chemical lesions to study the chronic effects. We report a slowing of resting breathing, while the previous study did not report changes in resting breathing with chronic lesions of the parabrachial complex. Another important issue is that we observed changes in the respiratory response to chemoreflex activation only by eliminating the KF region and not the LPBN, suggesting that these nuclei have different roles in cardiorespiratory control during chemoreflex activation. In addition, we also measured MAP and showed that the inhibition of the KF did not change resting MAP, while LPBN inhibition produced an increase in resting MAP. On the basis of the differences in experimental design, site of intervention, and results, we interpret our data as showing that neurons in the KF, but not LPBN, are key elements in the neural control of breathing in unanesthetized rats.

**Kölliker-Fuse Neurons and Eupneic Breathing**

A classic study established that the dorsal pons plays a critical role in breathing patterns (34). Subsequently, it was demonstrated that a group of neurons located in the dorsolateral pons, in a region called Kölliker-Fuse (or KF), could be involved in breathing patterns (2). Several studies suggested that ablations in the KF region produced similar apneusis, as reported previously (14, 59). However, a recent study showed that bilateral lesions of the KF were able to produce apnea and not apneusis (51). Depending on which dorsolateral region is affected, there may be different types of physiological responses. The dorsolateral pontine region is a complex region that contains different neural phenotypes that are activated by a variety of stimuli, including pain (4), immune challenges (23), anorexia (45), nausea (41, 62), water intake (22, 62), cardiovascular control (37), and breathing (7). The common feature of these neurons is that they respond to pathophysiological challenges and, thus, may be an important region for triggering behavioral, motor, and autonomic adjustments.

We showed that inhibition of the KF region, but not the LPBN, decreased resting ventilation in unanesthetized rats. We also noted an increase in expiratory time, which could suggest a possible inhibition in postinspiratory neurons that are responsible for the inspiratory/expiratory switch-off. The coordinated
activity of respiratory neurons located in the ventral respiratory column is dependent on a complex system involving inputs from other nuclei, such as KF neurons (33). Electrophysiological recordings revealed many respiratory related neurons within the KF and to a lesser extent in the adjacent medial and lateral parabrachial nuclei (9–11, 15, 54). This evidence could indicate that KF neurons are the key elements that maintain eupneic breathing in unanesthetized rats.

Kölliker-Fuse Region and Chemosensory Control of Breathing

There are few reports elucidating the function of the pontine respiratory group in the chemosensory control of breathing (50, 52). We showed that bilateral inhibition of KF, but not the LPBN, reduced the ventilatory response elicited by chemoreflexes in unanesthetized rats. This reduction was mainly due to a decrease in the evoked tidal volume. Our findings suggest for the first time that the parabrachial complex, which includes more than 10 distinct subnuclei surrounding the superior cerebellar peduncle, has distinct functions in cardiorespiratory control. The role of the parabrachial complex in cardiorespiratory control is based on several excitatory and inhibitory pathways that target brain stem networks, as well as cranial and spinal motor neurons (52, 54, 63, 64). Regarding the chemical control of breathing, there is evidence that the KF region has important connections with brain stem areas that are involved in central and peripheral chemoreception (44). Additionally, the KF region also receives inputs from second-order neurons within the nucleus of the solitary tract, i.e., neurons that receive the first synapse of the viscerosensory afferents in the brain stem, including those related to peripheral chemoreceptor afferents (52). It is also important to notice here that the injections of muscimol bilaterally in the KF region decrease tidal volume immediately. The reduction in tidal volume may induce changes in blood gases, which could affect the magnitude of the respiratory responses to hypoxia or hypercapnia. We did not measure blood gases after inactivation of the KF region, but we believe that the reductions in the respiratory responses to hypoxia or hypercapnia are due to the inhibition of the KF region and not by the changes in blood gases. Our data showed a reduction in the respiratory response and not an increase in breathing elicited by hypoxia or hypercapnia. Future studies are necessary to better understand whether the respiratory response to hypoxia or hypercapnia could be affected by the changes in blood gases after blockade of the KF region.

We did not observe a reduction in the ventilation induced by hypercapnia or hypoxia after bilateral inhibition of the LPBN, suggesting that this subnucleus of the parabrachial complex is not involved in chemoreflexes. The LPBN could influence breathing indirectly via its projection to the limbic forebrain and/or hypothalamus (13). Another function of pontine respiratory neurons, especially in the LPBN, is the regulation of upper airway patency via connections with hypoglossal and laryngeal motor neurons, but this information remains to be fully investigated (13).

Parabrachial Complex and Cardiovascular Regulation

Several studies have suggested the involvement of different subnuclei of the dorsolateral pons in the chemoreflex pathways.
(26, 27, 29, 37). However, the effective role of the KF or the LPBN in the cardiovascular response of the chemoreflex, particularly in unanesthetized animals, has not been properly evaluated. It is interesting to note that our hypoxia protocol elicited a reduction in arterial pressure. Acute hypoxia in conscious rats produces a moderate depressor response (31, 32). In addition, LPB cells receive convergent baroreceptor and chemoreceptor inputs (27, 52), and LPB neurons respond to decreases in arterial pressure (37). Therefore, we evaluated the effect of decreases in arterial pressure during hypoxia and the role of parabrachial complex neurons, especially in the KF and LPBN regions. We noted that bilateral injection of muscimol into the LPBN, but not into the KF region, produced a significant reduction of the hypotension induced by peripheral chemoreflex activation. A very elegant study showed that hypotension, but not hypertension, caused c-Fos activity in the LPBN, but the KF nucleus was not activated after blood pressure change (37). Our interpretation is that neurons of the NTS, the primary site in the brain stem that receives chemoreceptors inputs, project massively to the LPBN and to a lesser degree to the KF region. Neurons located in the LPBN express VGLUT2 mRNA and, thus, are presumed to be glutamatergic neurons, i.e., excitatory neurons. These neurons project to the forebrain regions that are involved in arterial pressure regulation (6, 27, 37).

Apparently, the KF region is not involved in arterial pressure responses elicited by hypoxia. In contrast, a previous study reported that systemic hypoxia results in activation of neurons in the KF region that project to the ventrolateral medulla (VLM) (29). However, there are important issues that must be taken into consideration to interpret these results. First, they used rabbits as an experimental model, while we used rats. The differences in species may explain the different cardiovascular effects; i.e., the former study showed an increase in arterial pressure, while we observed hypotension after the hypoxic challenge. The literature already showed that the inhibition of oxygen consumption for thermogenesis caused by a hypoxia challenge is driven by a reflex whose primary objective is to reduce body temperature, because this will reduce overall metabolism and curtail further oxygen consumption throughout the body (47). Second, given the importance of the KF neurons in respiratory regulation and the fact that this nucleus projects to neurons in the ventrolateral medulla (especially the ventral respiratory group), it is possible that the double-labeled neurons in the KF region found by Hirooka and colleagues are primarily involved in regulating the respiratory but not the cardiovascular responses to hypoxia. Additionally, the “cardiovascular” neurons in the VLM, the C1 neurons, are also involved in control of breathing (1, 30). Moreover, the KF region appears to have a modulatory role in heart rate control, as inhibition of the KF reduced the tachycardia induced by hypoxia. There is evidence that the KF has connections to the nucleus ambiguus and the dorsal motor nucleus of the vagus (52, 60), which are crucial regions involved in cardiac activity; however, future studies are necessary to evaluate the role of the dorsolateral pontine region in HR control.

Conclusion

In summary, the results of the present study demonstrate distinct cardiorespiratory functions of neurons located within the KF or the lateral portion of the PBN in unanesthetized adult rats. Inhibition of the KF region reduced the increase in breathing elicited by central and peripheral chemoreceptor activation, while the LPBN was involved in cardiovascular regulation elicited by peripheral chemoreflex activation. These results are consistent with data from other studies demonstrating that the parabrachial complex is a major brain area that plays an important role in the processing and relaying of somatosensory and viscerosensory information (21).

Perspectives and Significance

The multifunctional significance of the KF neurons is consistent with its extensive connections with many brain structures involved in respiratory and autonomic regulation. Our results reveal that the KF neurons are involved in the chemosensory control of breathing, while the LPBN neurons are related to cardiovascular regulation in conscious rats. Our results show differences in these dorsolateral pontine regions in the control of physiological functions and suggest that two neighboring regions in the pons could distinctly control the cardiovascular vs. respiratory response to chemosensory signals. More studies are necessary to improve the understanding of the dorsolateral pontine regions in respiratory and autonomic regulation during chemoreceptor activation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.S.D. and T.S.M. performed experiments; R.S.D. and T.S.M. analyzed data; R.S.D., A.C.T., and T.S.M. interpreted results of experiments; R.S.D. and T.S.M. prepared figures; R.S.D., A.C.T., and T.S.M. drafted manuscript; R.S.D., A.C.T., and T.S.M. edited and revised manuscript; R.S.D., A.C.T., and T.S.M. approved final version of manuscript; A.C.T. and T.S.M. conception and design of research.

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