Central GABAergic Modulation of Physostigmine-Induced Airway Constriction in Male Ferrets: A Bilateral Dorsal Motor Nucleus Microinjection Study

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Summary

The dorsal motor nucleus of the vagus (DMV) is a key brainstem structure involved in autonomic integration and vagal regulation of thoracic organs. This study examined whether localized GABA microinjection into the DMV modulates cholinergically induced airway mechanical responses in anesthetized male ferrets. Bilateral stereotaxic microinjections of GABA (10 mM; 50-70 nl) were performed in the DMV (AP -12.6 mm, ML 0 mm, DV -7.6 mm). Airway mechanics were evaluated by measuring tracheal segment pressure (Ptseg), lung resistance (RL), and dynamic compliance (Cdyn) at baseline, following intravenous physostigmine (100 µg/kg), and after GABA administration. Physostigmine significantly increased Ptseg and RL ($P<0.01$), indicating cholinergically mediated airway constriction. GABA microinjection attenuated these effects and significantly reduced Cdyn ($P<0.05$). Under controlled mechanical ventilation, these findings support a central inhibitory influence of DMV GABAergic signaling on vagally mediated airway responses during cholinergic activation. This study provides mechanistic proof-of-concept evidence for central modulation of airway mechanics and highlights the role of brainstem inhibitory circuits in autonomic respiratory regulation.

Key words

Dorsal vagal motor nucleus GABA microinjection • Physostigmine • Airway smooth muscle • Tracheal pressure • Cholinergic regulation

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Introduction

The understanding of airway constriction mechanisms has advanced considerably over the past several decades, reflecting progress not only in pulmonary physiology but also in the neurobiological regulation of breathing [1,2]. Airway caliber is controlled by a complex interplay between mechanical forces, airway smooth muscle tone, and autonomic neural inputs, with central modulation increasingly recognized as a critical determinant [3,4]. Neurotransmitter systems regulate airway resistance through excitatory and inhibitory signals that converge within brainstem autonomic networks and modulate peripheral parasympathetic control of airway tone [5]. Among these systems, the vagus nerve plays a central role due to its dual function in integrating visceral sensory information and mediating parasympathetic efferent output to the lungs [6,7]. Functional interactions of vagal pathways within brainstem nuclei are therefore essential for the control of airway tone and reflex bronchoconstriction [8].

Within the brainstem, the dorsal motor nucleus of the vagus (DMV) represents a key site of autonomic integration [9]. Located in the medulla oblongata, the DMV contains parasympathetic preganglionic neurons that provide vagal innervation to thoracic and abdominal organs [7,10]. It receives afferent sensory input primarily from the nucleus tractus solitarius and projects efferent fibers influencing intrathoracic airway function, thereby playing an important role in the modulation of respiratory

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activity [11,12]. Accumulating evidence indicates that the DMV functions not merely as a relay nucleus, but as an active modulatory center in which multiple neurotransmitter systems interact to regulate airway mechanics [13]. Dysregulation within this nucleus has been associated with exaggerated airway reflexes in experimental models [14].

Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system, plays a critical role in regulating neuronal excitability within the DMV [15]. Through activation of ionotropic GABAa and metabotropic GABAb receptors, GABA hyperpolarizes neurons, suppresses action potential firing, and modulates synaptic transmission [16]. Experimental studies indicate that GABAergic modulation within brainstem autonomic nuclei can attenuate excessive reflex responses associated with airway constriction, suggesting an inhibitory influence on vagal efferent output [17-19].

In contrast, cholinergic neurotransmission represents a dominant excitatory influence on airway tone [20]. Acetylcholine released from parasympathetic nerve terminals induces bronchoconstriction *via* activation of muscarinic receptors on airway smooth muscle [21]. Pharmacological manipulation of this pathway provides a well-established experimental approach for probing airway physiology [22]. Physostigmine, a reversible acetylcholinesterase inhibitor, increases synaptic acetylcholine by preventing its enzymatic degradation and reliably evokes bronchoconstriction and increases airway resistance in several animal models, including ferrets [6]. These properties make physostigmine a useful experimental tool for examining inhibitory counter-regulatory mechanisms within central autonomic circuits [23].

Interactions between cholinergic and GABAergic signaling within the DMV are of particular interest, as they reflect the balance between excitatory drive and inhibitory control in a nucleus that directly governs vagal efferent output [24]. Although the DMV contains relatively few intrinsic interneurons, it receives dense GABAergic input from the nucleus tractus solitarius [25]. While exaggerated airway reflexes following cholinergic stimulation have been well documented, the extent to which localized inhibitory signaling within the DMV modulates these responses remains incompletely defined [26]. In particular, the effects of targeted GABA microinjection into the DMV on specific physiological parameters such as pressure in the tracheal segment (Ptseg), lung resistance (RL), and dynamic compliance (Cdyn) have not been systematically quantified [13,27].

Accordingly, the present study was designed to examine the effects of bilateral GABA microinjection into the DMV area on physostigmine-induced airway constriction in ferrets. Using physostigmine-evoked responses as a reproducible cholinergic challenge, the GABA intervention was assessed specifically in this context. By integrating stereotaxic microinjection with physiological measurements of Ptseg, RL, and Cdyn, this study aims to characterize the role of inhibitory neurotransmission within the DMV area in modulating cholinergically driven airway responses.

Materials and Methods

Animal and ethical approval

Experiments were performed on male adult European ferrets (Mustela putorius furo; n=6), weighing 700-900 g. The sample size was determined a priori based on previous investigations using comparable autonomic and respiratory physiological endpoints, where repeated-measures designs yielded large effect sizes, supporting adequate statistical power (≥80 %) while adhering to the principle of reduction in animal experimentation. All experimental procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council). Only male animals were used to minimize variability related to hormonal fluctuations. Ferrets were selected because they represent a well-established model for studying respiratory neurophysiology and airway control. Animals were obtained from Marshall Farms (North Rose, NY, USA) and housed at the University Animal Resource Center under controlled environmental conditions, with free access to food and water.

Surgical preparation and anesthesia

Non-survival experiments were conducted using a within-subject repeated-measures design. General anesthesia was induced and maintained with α-chloralose (70 mg/kg, i.p.), with supplemental doses (5 mg/kg, i.v.) administered as needed. Adequate depth of anesthesia was confirmed by the absence of motor or cardiovascular responses to noxious stimulation.

Polyethylene catheters were placed in the common carotid artery for arterial pressure monitoring and in the jugular vein for intravenous drug administration. A catheter inserted into the femoral artery was used to obtain arterial blood samples (0.2 ml) for

blood gas analysis using an ABL™5/BPH™5 analyzer (Radiometer Medical A/S, Copenhagen, Denmark).

Ventilation and physiological monitoring

A tracheal window was created in the cervical trachea, and a tracheostomy tube was inserted into the caudal trachea and connected to a mechanical ventilator (Rodent Ventilator Model 683, Harvard Apparatus). Animals were ventilated with a tidal volume of 8 ml/kg at a respiratory rate of 55-60 breaths/min. Ventilator frequency was adjusted to maintain arterial $PaCO_2$ between 32 and 35 mmHg. The rostral portion of the tracheal window was left open to permit subsequent instrumentation.

Neuromuscular blockade was achieved with gallamine triethiodide (4 mg/kg, i.v.; Sigma-Aldrich, Cat. No. G8134), with additional doses administered hourly. To suppress peripheral chemoreceptor drive and minimize baseline cholinergic tone, animals were ventilated with hyperoxic gas (100 % O_2), as previously described [6]. Body temperature was monitored continuously using an esophageal probe and maintained at 38-39 °C with a thermostatically controlled heating pad.

Experimental design and pharmacological interventions

Each animal served as its own control. After establishing a stable basal tracheal tone of approximately 7-9 cm H_2O, experiments were conducted in a fixed sequence of three conditions, separated by washout periods of at least 30 min:

1. baseline following intravenous saline (0.2 ml),
2. cholinergic stimulation induced by physostigmine (100 µg/kg body weight, i.v.; Sigma-Aldrich, Cat. No. P1911) infused over 1 min, and
3. bilateral microinjection of GABA into the DMV.

During surgical exposure of the carotid sheath, care was taken to preserve the integrity of the vagal nerve and its blood supply. Functional integrity of vagal cholinergic outflow was confirmed by comparing tracheal smooth muscle responses to lung deflation and hyperoxic hypocapnia before and after surgical preparation.

Stereotaxic microinjection and histological verification

Animals were positioned prone in a stereotaxic frame (Model 900LS; David Kopf Instruments). After skull exposure, a small burr hole (~1 mm) was drilled above the target region, and the dura was carefully opened. Bilateral stereotaxic microinjections of GABA (10 mM; 50-70 nl per side; Sigma-Aldrich, Cat. No. A2129) were targeted to the DMV using established coordinates (anteroposterior: -12.3 to -12.5 mm from bregma; mediolateral: 0 mm; dorsoventral: -7 to -9 mm from the brain surface).

Injection sites were verified *post hoc* by Pontamine Sky Blue dye deposition and histological examination of coronal brainstem sections. Animals were transcardially perfused with phosphate-buffered saline followed by 4 % paraformaldehyde. Brains were removed, post-fixed, and sectioned coronally (100 µm) for microscopic confirmation of bilateral DMV localization (Fig. 1A, B and C).

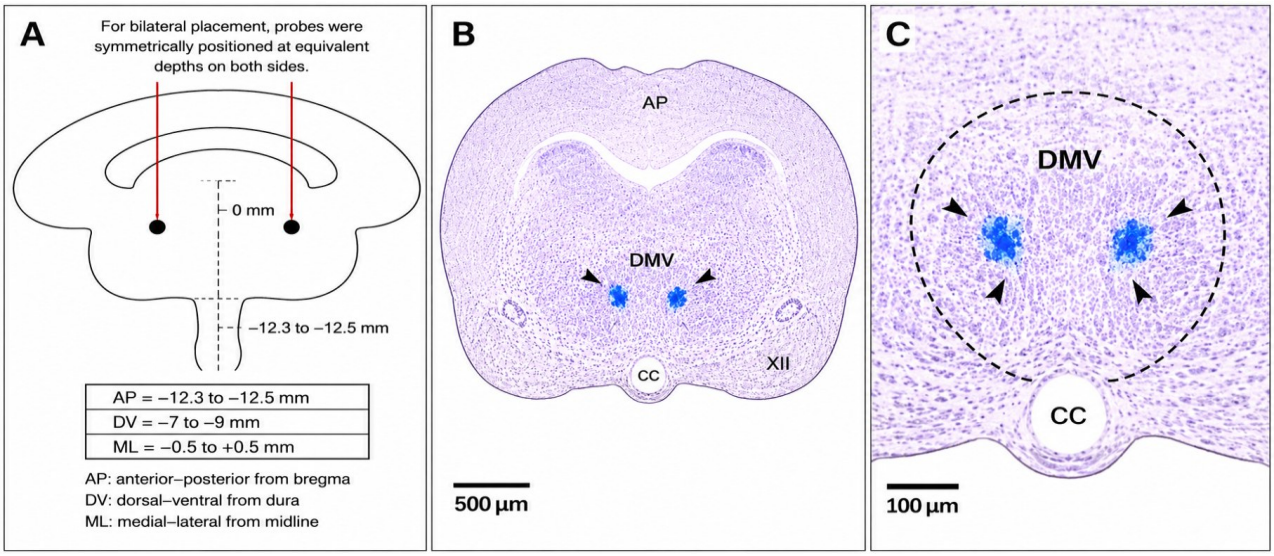


Fig. 1. Histological verification of bilateral DMV microinjections. (**A**) Stereotaxic targeting of the dorsal motor nucleus of the vagus for bilateral placement. (**B**) Coronal medullary section showing bilateral injection sites identified by Pontamine Sky Blue deposits. (**C**) Higher-magnification view of the injection region within the DMV. AP, area postrema; XII, hypoglossal nucleus; CC, central canal. Arrowheads indicate injection sites. Scale bars: 500 µm (B), 100 µm (C).

Measurement of lung mechanics

A whole-body plethysmograph was sealed using latex collars and coupling gel. Airflow was measured with a pneumotachograph connected to a differential pressure transducer (±1.4 cm H_2O; DP250, Validyne Engineering). Inflation pressure was recorded *via* a second transducer (TRD 5700, Buxco Electronics). The plethysmograph was calibrated by injecting and withdrawing 10 ml of air, and flow calibration was verified using a precision flow meter.

Signals were acquired using an ACQ-7700 interface and Universal XE signal conditioner (Buxco Electronics). Tidal volume and lung mechanics were calculated by integrating airflow and pressure signals using Biosystem XA software (version 2.7 r2, Buxco Electronics). Lung resistance (RL) and dynamic compliance (Cdyn) were computed from these signals.

Measurement of tracheal pressure

A balloon-tipped catheter (3 cm length) was inserted into the rostral tracheal segment and inflated with 1.0-1.2 ml saline. Tracheal pressure (Ptseg) was measured using a calibrated pressure transducer (0-45 cm H_2O). Basal tracheal tone was established by inducing hyperoxic hypocapnia through gradual increases in ventilatory rate, as previously described Haxhiu *et al.* [5]. A basal pressure of 8-10 cm H_2O was used as the reference level for all experiments.

Research protocol

RL, Cdyn, and Ptseg were assessed under baseline conditions and during reflex activation induced by lung deflation and transient asphyxia (30-45 s interruption of ventilation). Following intravenous physostigmine administration, airway responses were allowed to stabilize before bilateral GABA microinjection (typically within 5-10 min). Measurements were obtained under stable physiological conditions, and the repeated-measures design demonstrated consistent within-animal responses across the experimental protocol. Reflex responses were reproducible before each intervention.

Data analysis

Five consecutive respiratory cycles were analyzed during control conditions and at the peak of cholinergic responses. Data are presented as mean ± SEM, with individual animal values shown and connected to reflect within-subject comparisons. Statistical analysis was performed using one-way repeated-measures ANOVA followed by Tukey's *post hoc* test. Statistical significance was set at $P<0.05$.

Results

Localization of microinjection sites

Bilateral microinjections of GABA were localized within the DMV, as illustrated in Figure 1A. Stereotaxic coordinates of the injection sites were anteroposterior -12.6 mm, mediolateral approximately 0 mm, and dorsoventral -7.6 mm relative to bregma. Blood pressure and arterial blood gas variables were monitored throughout the experiments to ensure physiological stability but were not analyzed as primary outcome measures.

Effects of physostigmine and GABA on Ptseg

Intravenous administration of physostigmine (100 μg/kg body weight) produced a marked increase in Ptseg compared with baseline values (Fig. 2A, B). Peak Ptseg values reached 35.94 cm H_2O. Following bilateral microinjection of GABA into the DMV, Ptseg values decreased, with mean values of 12.28 cm H_2O.

Quantitative analysis demonstrated that physostigmine significantly increased Ptseg relative to baseline ($P<0.001$). GABA microinjection did not significantly differ from baseline values. A significant difference was observed between physostigmine and GABA conditions ($P<0.001$, one-way repeated-measures ANOVA with Tukey's *post hoc* test).

Effects on RL

Changes in lung resistance are shown in Figure 3C, D. Physostigmine administration resulted in a significant increase in RL compared with baseline ($P<0.01$). Bilateral GABA microinjection into the DMV did not significantly alter RL relative to baseline. Comparison between physostigmine and GABA conditions revealed a significant difference ($P<0.05$).

Effects on Cdyn

Dynamic compliance values are summarized in Figure 4E, F. Physostigmine administration did not significantly change Cdyn compared with baseline. In contrast, bilateral GABA microinjection into the DMV was associated with a significant increase in Cdyn relative to baseline ($P<0.05$). A significant difference was also observed between physostigmine and GABA conditions.

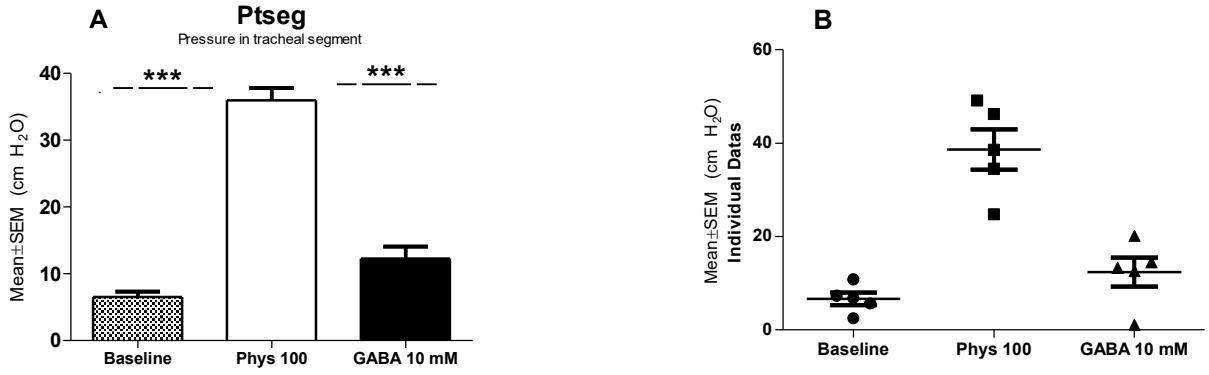


Fig. 2. Pressure in the tracheal segment (Ptseg) (**A, B**), at baseline, after intravenous physostigmine (Phys100, 100 µg/kg), and following bilateral GABA microinjection into the dorsal motor nucleus of the vagus (GABA 10 mM). Data are presented as mean ± SEM (A) with individual animal values (B). Statistical analysis was performed using one-way repeated-measures ANOVA followed by Tukey's *post hoc* test. * P<0.05, ** P<0.01, *** P<0.001.

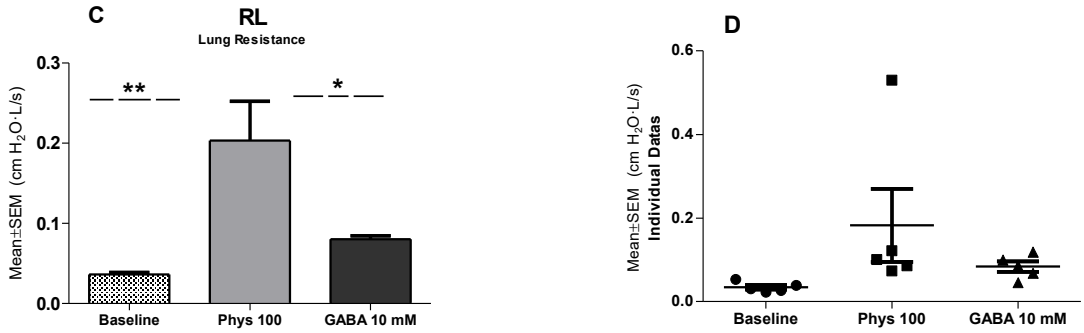


Fig. 3. Lung resistance (RL) at baseline, after intravenous physostigmine (Phys100, 100 µg/kg), and following bilateral GABA microinjection into the dorsal motor nucleus of the vagus (GABA 10 mM). Data are presented as mean ± SEM (**C**) with individual animal values (**D**). Statistical analysis was performed using one-way repeated-measures ANOVA followed by Tukey's *post hoc* test. * P<0.05, ** P<0.01, *** P<0.001.

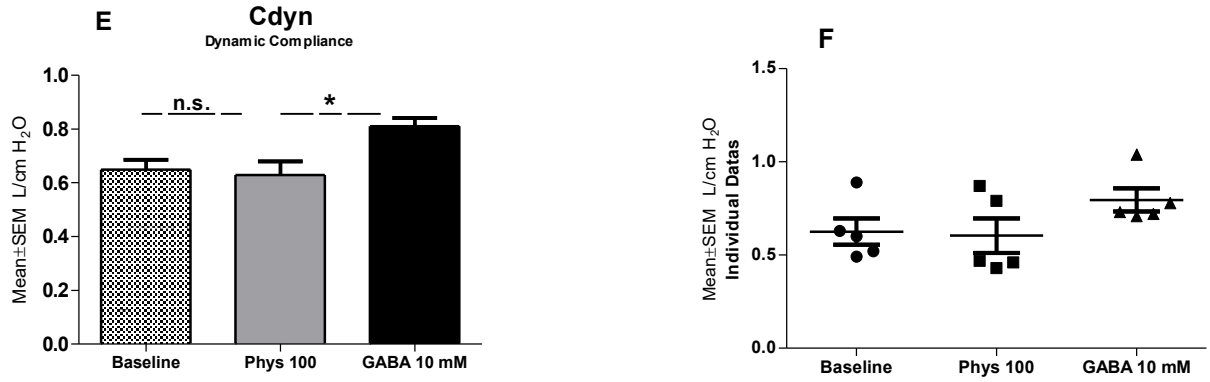


Fig. 4. Dynamic compliance (Cdyn) at baseline, after intravenous physostigmine (Phys100, 100 µg/kg), and following bilateral GABA microinjection into the dorsal motor nucleus of the vagus (GABA 10 mM). Data are presented as mean ± SEM (**E**) with individual animal values (**F**). Statistical analysis was performed using one-way repeated-measures ANOVA followed by Tukey's *post hoc* test. n.s., not significant; * P<0.05.

Summary of results

Across all measured parameters, physostigmine and GABA microinjection produced distinct effects on Ptseg, RL, and Cdyn. Physostigmine significantly increased Ptseg and RL, whereas GABA microinjection was associated with lower Ptseg values and altered Cdyn. Statistical comparisons consistently demonstrated significant differences between physostigmine and GABA conditions.

Discussion

The present study examined the effects of targeted GABA microinjection into the DMV on airway responses evoked by systemic cholinergic stimulation. The principal finding is that bilateral GABA microinjection into the DMV was associated with attenuation of physostigmine-induced changes in tracheal pressure and lung mechanics, indicating that inhibitory neurotransmission within this nucleus modulates cholinergically driven airway responses. These findings support the concept that excitatory and inhibitory neurotransmitter systems interact within brainstem autonomic nuclei to shape vagal output to the airways.

Vagal pathways play a central role in the regulation of airway defensive reflexes and respiratory sensory processing, emphasizing the importance of brainstem autonomic circuits in airway physiology [28].

GABA is the major inhibitory neurotransmitter in the central nervous system and plays an essential role in regulating neuronal excitability within autonomic circuits. In the present study, bilateral microinjection of GABA into the DMV was associated with reduced Ptseg values and altered dynamic compliance relative to the physostigmine condition. Although the present data do not allow direct assessment of neuronal firing, the observed physiological changes are consistent with reduced activity of vagal preganglionic neurons. Previous studies have demonstrated that activation of GABAa and GABAb receptors in medullary autonomic nuclei hyperpolarizes neurons and limits excitatory synaptic transmission, thereby modulating cardiorespiratory reflexes [3,29]. Because neuronal activity was not directly recorded and receptor-specific antagonists were not employed, the present findings should be interpreted as evidence of physiological modulation of vagally mediated airway responses rather than direct proof of specific GABAa or GABAb dependent neuronal mechanisms. The current findings extend this framework by demonstrating that localized GABAergic intervention within the DMV influences airway-related physiological parameters during a defined cholinergic challenge.

Cholinergic excitation and physostigmine response

Systemic administration of physostigmine produced marked increases in tracheal pressure and lung resistance, consistent with enhanced cholinergic signaling. Physostigmine inhibits acetylcholinesterase activity, thereby increasing synaptic acetylcholine availability and amplifying parasympathetic output [30,31]. The robust elevation in Ptseg observed following physostigmine administration is in agreement with previous reports describing cholinergically mediated airway constriction in the ferret and other experimental models. These observations confirm that physostigmine provides a reliable and reproducible experimental paradigm for studying centrally mediated cholinergic influences on airway mechanics.

While intracerebral cholinergic administration represents a valuable complementary experimental approach for investigating central cholinergic mechanisms, the present study was specifically designed to evaluate whether localized GABAergic modulation within the DMV can counteract a physiologically relevant cholinergic challenge; nevertheless, future studies employing direct intracerebral cholinergic stimulation, receptor-specific antagonists, and dose-response analyses will be important to further define the underlying neural mechanisms.

Interaction between inhibitory and excitatory signaling

The contrasting effects of physostigmine and GABA across respiratory parameters highlight the dynamic balance between excitatory cholinergic and inhibitory GABAergic influences within the DMV [32]. While physostigmine enhanced airway pressure and lung resistance, GABA microinjection produced opposing effects by attenuating these responses. Particularly, GABA microinjection significantly reduced lung resistance compared with the physostigmine condition, indicating that inhibition within the dorsal motor nucleus of the vagus suppresses cholinergically mediated airway constriction. Because GABA microinjection restored RL toward baseline values without evidence of direct peripheral intervention, these findings suggest that its primary site of action is the central modulation of vagal motor output rather than a direct effect on airway smooth muscle. Together, these findings are consistent with the view that the DMV functions as an integrative center in which inhibitory inputs regulate the expression of excitatory autonomic drive to the airways.

Although the DMV contributes to parasympathetic efferent control of intrathoracic airways, it operates within a broader brainstem network that includes the nucleus ambiguous, which provides major cholinergic innervation to upper airway and laryngeal musculature and plays a prominent role in respiratory rhythm generation [33]. The present findings therefore likely reflect modulation within a distributed autonomic

network rather than isolated effects confined exclusively to a single nucleus.

Study limitations

The saline-only and GABA-only baseline microinjection controls were not included in the experimental sequence. The primary objective of the study was to assess GABAergic modulation specifically in the context of physostigmine-evoked cholinergic excitation; nevertheless, the absence of these controls limits conclusions regarding baseline effects of microinjection alone. Importantly, previous studies from our laboratory and others have demonstrated that saline microinjection into the DMV does not alter airway mechanics and that GABA application under baseline conditions produces minimal effects on resting airway tone [6,34,35,36]. These observations suggest that the inhibitory effects observed in the present study primarily reflect modulation of an activated cholinergic state rather than nonspecific procedural and potential order effects. Although injections were histologically confirmed within the DMV, potential spread to neighboring medullary nuclei, including the nucleus ambiguous, cannot be entirely excluded.

The experiments were conducted under anesthesia and neuromuscular blockade, conditions that are necessary for precise physiological measurements but may influence central respiratory control. Accordingly, extrapolation of these findings to awake or freely behaving conditions should be made with caution.

Physiological relevance

Within these constraints, the present findings contribute to a growing body of evidence indicating that inhibitory neurotransmission within brainstem autonomic nuclei plays a key role in shaping vagal output to the airways. The reciprocal interaction between cholinergic excitation and GABAergic inhibition observed here underscores the importance of central integration in maintaining stable airway function during physiological and pharmacological perturbations. Only male animals were used to reduce potential variability related to hormonal influences and that future studies should evaluate possible sex-dependent differences in DMV-mediated airway regulation.

Conclusions

In summary, bilateral GABA microinjection into the DMV modulated physostigmine-evoked changes in tracheal pressure and lung mechanics in anesthetized, mechanically ventilated ferrets.

The present findings demonstrate that localized GABA delivery within the DMV modulates airway mechanical responses elicited by cholinergic activation. These results extend current understanding of inhibitory signaling within brainstem vagal circuits.

Conflict of Interest

There is no conflict of interest.

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