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Parasympathetic neural control of canine tracheal smooth muscle

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The middle segment of the trachea is innervated by the recurrent laryngeal and pararecurrent nerves. This study determined the pathway that mediated descending commands to the tracheal smooth muscle. Animals used were seven paralyzed and tracheostomized dogs. Tracheal contraction induced either by apnea, mechanical stimulation of the tracheal bifurcation or hypercapnia was always composed of tonic and rhythmic components. The rhythmic contraction developed in synchrony with rhythmic bursts on phrenic nerve activity (PNA). The respiratory-related bursts were also observed on the recurrent laryngeal nerve activity (RNA) and pararecurrent nerve activity (ParaRNA). During apnea there was no tonic activity neither on RNA or PNA, whereas ParaRNA had both tonic and rhythmic activities. Bursts on RNA preceded to correspondent PNA-bursts by 90 ± 13 ms. In contrast, ParaRNA-burst always developed later than PNA-burst and it started at almost the same time as that of tracheal rhythmic contraction. During mechanical stimulation of the trachea or CO_2-loading, though RNA did not include tonic component, ParaRNA had tonic activity during tracheal tonic contraction. These findings suggested that rhythmic and tonic contractions of the trachea were mediated through the pararecurrent nerve but not through the recurrent laryngeal nerve.

Key words : respiration, recurrent laryngeal nerve, pararecurrent nerve, neurogram

INTRODUCTION

There are three major neural pathways from the medurally neurons to the airway smooth muscle. They are the cholinergic, adrenergic and non-adrenergic non-cholinergic nervous system [1]. Among them, the cholinergic nervous system is the principal excitatory system. It is also known that contraction of airway smooth muscle has both rhythmic and tonic components. The rhythmic contractions of the tracheal smooth muscle are synchronized with the rhythm of respiration [2, 3, 4]. Commands for contraction of middle part of the trachea, either rhythmic or tonic, are mediated through the recurrent laryngeal nerve [3]. The recurrent laryngeal nerve of the dog is further separated as main stem of the recurrent laryngeal nerve and pararecurrent nerve [5, 6, 7]. Both nerves project fibers to the middle part of the trachea. However, in humans, there is no apparent evidence for the existence of the pararecurrent nerve; thus parasympathetic innervation of the airways is dependent on the vagus nerve and recurrent laryngeal nerve [7].

Widdicombe [8] analyzed activities of nerve fibers to the trachea in cat and dog. He identified pararecurrent nerve as “tracheal nerve” in the cat but failed to discriminate it in the dog. There were numerous discharge units on the pararecurrent nerve fibers. These activities included inspiratory, expiratory and continuous units. However, he did not refer these activities to function of the pararecurrent nerve.

There are scarce physiological evidences

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that suggested that pararecurrent nerve conveyed commands for such rhythmic and tonic contractions of tracheal smooth muscle. In the present study we measured integrated activities of recurrent and pararecurrent nerve bundles instead of unit activities. This type of analysis is more suitable to identify functions of each nerve.

The objectives of the present study were; to determine the efferent pathway to the middle segment of the canine tracheal smooth muscle (either recurrent laryngeal or pararecurrent nerve); and to examine the neural mechanism which control the rhythmic and tonic contractions of the tracheal smooth muscle.

SUBJECTS AND METHODS

This study was approved by the Animal Ethics Committee of the Tokai University School of Medicine. Seven beagle dogs were deeply anesthetized with a short-acting barbiturate (Thyamiral, 5-10 mg/kg), and decerebration was achieved by transection of the midbrain at precolicular level and tracheostomy was made at low neck. The dogs were paralyzed with pancuronium bromide (1 mg/kg, i.v., every 30 min) and mechanically ventilated with 100 % oxygen. An arterial catheter was implanted at right femoral artery to measure arterial pressure and to sample arterial blood. Blood gasses were frequently measured and pH and PCO_2 of arterial blood was normalized by either changing ventilation or intravenously administering sodium bicarbonate.

As shown in Fig. 1, middle six to eight segments of the trachea were exposed. Left side of membraneous portion of the trachea was fixed to a rod with silk strings and right side of it was tied to an isometric force transducer (Nihon Kohden TB611T). Flow of

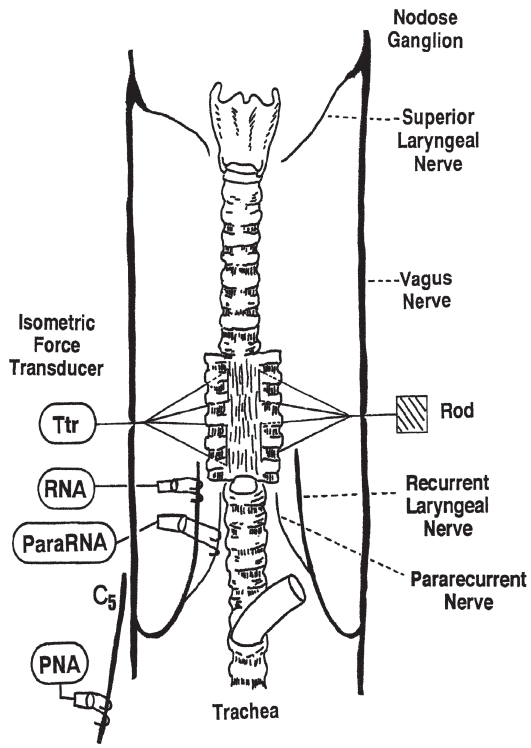

Fig. 1 Illustration showing an experimental setup for the tracheal preparation and recording of the tracheal tension and some neurogram
Left side of membraneous portion of the trachea was fixed to a rod with silk strings. Tension of membranous part of the trachea (Ttr) was measured with isometric forced transducer. Unilateral phrenic, recurrent laryngeal and pararecurrent nerves were exposed and transected. Proximal end of each nerve was hooked on bipolar recording electrodes. RNA; recurrent laryngeal nerve activity, ParaRNA; pararecurrent nerve activity, PNA; phrenic nerve activity at C_5.

mechanical ventilation ($\dot{V}$) was continuously measured with hot-wire flowmeter connected to the tracheal tube. Right C_5 phrenic nerve was exposed and transected. Proximal end of the phrenic nerve was hooked on bipolar recording electrodes. Right recurrent laryngeal and pararecurrent nerves were also exposed and transected. The proximal ends of them were hooked on pairs of bipolar recording electrodes. The measured parameters were; tension of middle segment of the trachea (Ttr), integrated phrenic nerve activity (∫PNA), integrated recurrent laryngeal nerve activity (∫RNA), integrated pararecurrent nerve activity (∫ParaRNA), and flow of mechanical ventilation ($\dot{V}$). By technical reason, simultaneous recordings of both RNA and ParaRNA were not done. Recordings were performed during the following four conditions. Mechanical ventilation, transient apnea induced by disconnecting ventilator, mechanical stimulation of the tracheal bifurcation, transient hypercapnia induced by bolus administration of 100 % CO_2 into the inspiratory circuit of mechanical ventilation.

RESULTS

As shown in Fig. 2, the tracheal smooth muscle rhythmically contracted during mechanical ventilation. These spontaneous rhythmic tracheal contractions were in synchrony with the bursts on ∫PNA and ∫RNA and both of them developed at middle inspiratory through early expiratory phase. When the ventilator was disconnected, tracheal tension immediately started to rise. This increase in Ttr was characterized by a base-line shift toward the sustained or tonic contraction. Rhythmic contractions were still observed superimposing on the tonic contraction. These rhythmic contractions on Ttr trace had the same rhythm as those of ∫PNA and ∫RNA. Although tracheal contraction had tonic and rhythmic components, there was no tonic component neither on ∫RNA or ∫PNA trace. Bursts on ∫RNA were always accompanied by those on ∫PNA. RNA-burst significantly (with paired *t*-test) preceded to PNA-burst by 90 ± 13 ms (mean ± SD).

Figure 3 is the record during mechanical

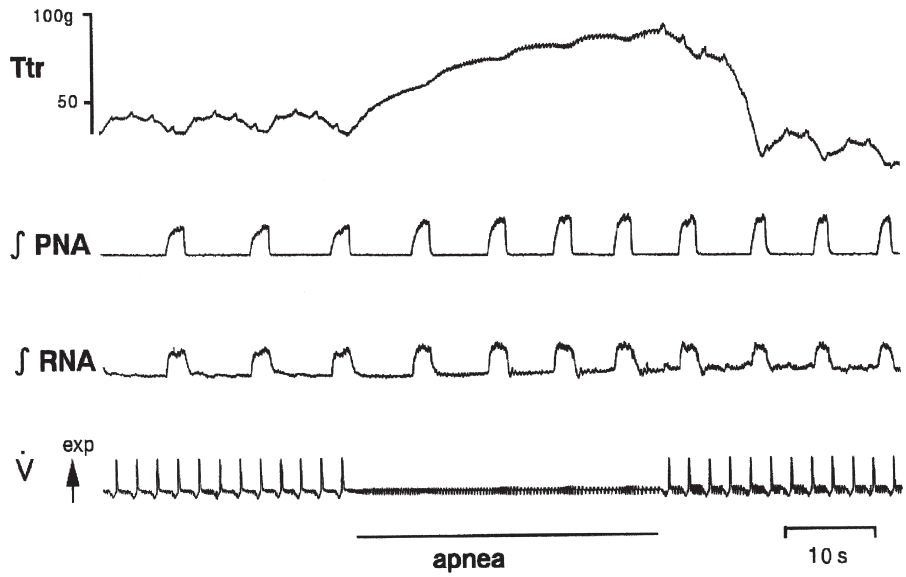

Fig. 2 Recurrent nerve activity during transient apnea
During mechanical ventilation, spontaneous phasic contraction of the trachea was observed along with phrenic bursts. The transient apnea caused Ttr to increase immediately after the onset of apnea and sustained during apneic period, i.e., the tonic contraction. Rhythmic contractions superimposed on the tonic contraction and they were synchronized with phrenic bursts. There was no tonic activity on ∫RNA. Ttr; tension of membranous part of the trachea, ∫PNA; integrated phrenic nerve activity at C_5, ∫RNA; integrated recurrent laryngeal nerve activity, $\dot{V}$; respiratory flow.

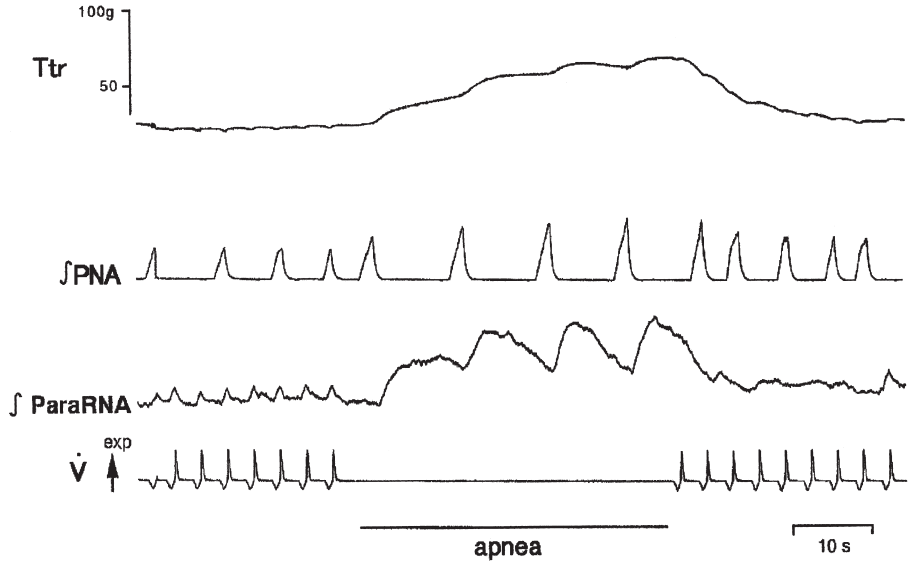

Fig. 3 Pararecurrent nerve activity during transient apnea
Transient apnea caused both tonic and rhythmic contractions of the trachea. The tonic contraction was also accompanied by an increase in sustained ∫ParaRNA. ∫ParaRNA; integrated pararecurrent nerve activity. Other abbreviations are the same as in Fig. 2.

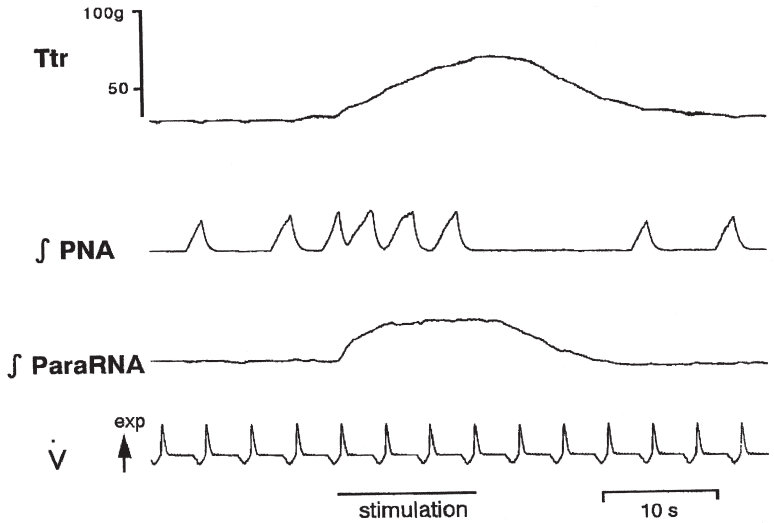

Fig. 4 Response to mechanical stimulation of the tracheal bifurcation where contains rich mechanoreceptor
Mechanical stimulation of the tracheal bifurcation elicited the tonic contraction of the trachea and augmented ∫PNA. The tonic contraction of Ttr was accompanied by a tonic activity on ∫ParaRNA. Abbreviations are the same as in Fig. 3.

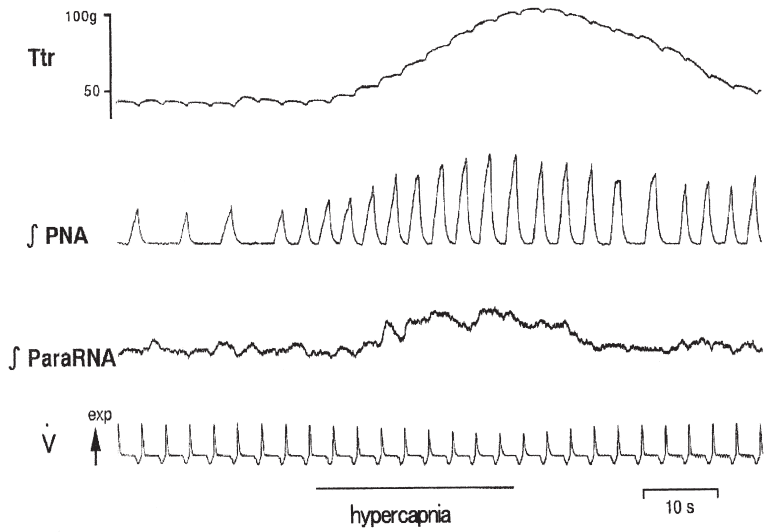

Fig. 5 Response to transient administration of CO_2
CO_2 load induced tonic contractions of the trachea and rhythmic contractions were enhanced. Both types of contraction were accompanied by increases in rhythmic and tonic activities on ∫ParaRNA. Abbreviations are the same as in Fig. 3.

ventilation showing the relationship between Ttr and ∫ParaRNA. In this trace spontaneous tracheal contraction was not apparent during mechanical ventilation. When ventilator was disconnected, tracheal tonic contraction developed and this tonic contraction was superimposed with rhythmic contractions. In contrast to ∫RNA shown in Fig. 2, ∫ParaRNA had both tonic and rhythmic activities. The rhythmic activities on ∫ParaRNA had the same rhythm as that on ∫PNA. Although the burst on RNA developed slightly earlier than corresponding PNA-burst, as seen in Fig. 3, ParaRNA-burst always developed later than PNA-burst and it started at almost the same time as that of tracheal rhythmic contraction. Quantitative analysis of ParaRNA-bursts was not possible because rhythmic ParaRNA was not separated from tonic ParaRNA activity.

As shown in Fig. 4, mechanical stimulation of the tracheal bifurcation induced tonic contraction of the tracheal smooth muscle. In this condition, PNA-burst developed more frequently and their amplitudes slightly augmented. However, individual PNA-bursts did not fuse and never assumed continuous activity. In contrast, ParaRNA-activity developed continuously and it sustained throughout the tracheal contraction. No continuous activity was observed on ∫RNA in the experiment of tracheal stimulation (not shown in the figure).

Figure 5 shows the responses to transient hypercapnia. CO_2-load induced tonic contraction of the tracheal smooth muscle and rhythmic contraction became less apparent. Tracheal tonic contraction was accompanied by both tonic and rhythmic activities on ∫ParaRNA. There was no continuous activity on ∫RNA during hypercapnia (not shown in the figure).

DISCUSSION

Although anatomical studies demonstrated that middle segment of canine trachea was innervated from the recurrent and pararecurrent laryngeal nerves. Electric stimulation studies of each nerves revealed that the pararecurrent but not recurrent nerve was responsible for the contraction of middle or caudal part of the trachea [6, 7]. These studies suggested that the pararecurrent nerve included efferent fibers to the tracheal smooth muscle. The present study added the evidence that descending commands to canine tracheal smooth muscle were mediated through the pararecurrent nerve. This speculation is based upon the following observations; 1) although both ∫RNA and ∫ParaRNA had respiratory-related rhythmic

activities, only ∫ParaRNA developed tonic activity while the trachea was tonically contracted, 2) rhythmic ParaRNA-bursts and tracheal rhythmic contraction developed simultaneously, while rhythmic RNA-bursts appeared significantly earlier than tracheal rhythmic contractions.

Tonic activity

In the present study, tracheal tonic contraction was provoked by three methods; apnea, mechanical stimulation of the trachea and hypercapnia. The tracheal contraction to either of the stimulus has been reported to be eliminated by transection of vagus trunks at the neck [3, 9]. Thus, these responses are generated by neurons in the central architecture and are mediated through vagus trunks. The vagus trunk projects to the middle trachea via the recurrent and pararecurrent nerves. During the tracheal tonic contraction, ∫ParaRNA developed tonic activity but ∫RNA lacked tonic one. Brown *et al*. [6, 10] measured RNA when tonic contraction of the tracheal smooth muscle was induced by intravenous NaCN injection. They found that ∫ParaRNA developed tonic activity during tracheal tonic contraction and this finding was quite similar to our observation on ∫ParaRNA. We further compared activity on ∫RNA to that on ∫ParaRNA and found that ∫RNA lacked tonic activity during the contraction. This finding suggested that signals for tracheal tonic contraction were mediated through the pararecurrent nerve but not through the recurrent laryngeal nerve. This speculation is compatible with the results of electric stimulation studies of each nerve [6, 7].

Rhythmic activity

Michel *et al*. [11] recorded activity of the tracheal ganglion in the cat. They reported that the ganglion cells fired during mid-inspiration through early expiration or during expiration. This firing pattern is consistent with contraction profile of the trachea. The rhythmic tracheal contraction disappeared after cervical vagotomy [3, 4]. Thus, tracheal rhythmic contractions were also mediated through vagus nerves. Several investigators have reported the respiartory-related rhythmic activity on ∫RNA [2]. Brown *et al*. [6] measured activity of the pararecurrent nerve of the dog. Although they did not mention about rhythmic activity, in Fig. 5 of their paper rhythmic activity on ∫ParaRNA was observed and this activity was synchronized with phrenic burst in the same figure. These observations assure existence of respiratory-related rhythmic activities on ∫ParaRNA and ∫PNA.

The respiratory-related rhythmic activities on ∫RNA and ∫ParaRNA were synchronized with tracheal rhythmic contraction. Although RNA-burst developed slightly earlier than PNA-burst, ParaRNA-burst always developed later than PNA-burst. ParaRNA-burst started at almost the same time as that of tracheal rhythmic contraction. Since contraction of the tracheal smooth muscle started at mid-inspiratory or late inspiratory phase [3], the phase relationship between ParaRNA-burst and RNA-burst suggested that tracheal contraction was controlled through the pararecurrent nerve.

Cell bodies of parasympathetic neurons projecting to tracheal ganglion cells are believed to be located in the near vicinity of nucleus ambiguous or retrofacial nucleus, while the bronchus, i.e., the intrathoracic airway, is innervated by efferent axons from the dorsal motor nucleus of the vagus [12, 13]. Rhythmic contractions of the trachea seem to be generated by the physiological process related to the central respiratory rhythm, but the relation between these neurons and respiratory-related neurons remains to be investigated. The physiological role of the rhythmic contraction of the trachea is also unknown. Mechanical dilation of the trachea is produced during mid to late inspiratory phase. Therefore, it is reasonable to speculate that the active rhythmic contractions of trachea is restricted to this phase [5, 14, 15].

An anatomic study has shown that the recurrent laryngeal nerve supplies the muscles of the larynx [5]. The bursts on ∫RNA developed slightly but significantly earlier than phrenic burst. The plausible role of RNA-bursts is to open vocal cord during inspiration. This speculation may be reasonable because vocal cord should be open before inspiratory phase is started.

In conclusion, contraction of the middle part of tracheal smooth muscle, either it is rhythmic or tonic, is mediated through pararecurrent nerve fibers but not through recurrent laryngeal nerve fibers.

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