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Cough and Laryngeal Muscle Discharges in Brainstem Lesioned Anesthetized Cats

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Summary

Experiments were carried out to determine whether there are separate drives from the selected neuronal networks of the brainstem affecting the discharge patterns of laryngeal and respiratory pump muscles during cough. Twenty-four non-decerebrate spontaneously breathing cats anesthetized with sodium pentobarbitone were used. Microinjections of kainic acid into the lateral tegmental field of the medulla, medullary midline or pontine respiratory group eliminated the cough evoked by mechanical stimulation of the tracheobronchial and laryngopharyngeal mucosa. These stimuli, in most cases, provoked irregular bursts of discharges in the posterior cricoarytenoid and thyroarytenoid laryngeal muscles (or they had no effect on them). No pattern of laryngeal muscle activities following lesions resembled the laryngeal cough response. Lesions of the target regions did not result in any apparent changes in the eupnoeic pattern of laryngeal activity. Neurons of the medullary lateral tegmental field, raphe nuclei and the pontine respiratory group seem to be indispensable for the configuration of the central cough motor pattern. However, these neurons do not appear to be essential for the discharge patterns of laryngeal motoneurons during eupnoea. The residual laryngeal „cough" responses are probably mediated by an additional motor drive.

Key words

Cough • Laryngeal abductor • Laryngeal adductor • Kainic acid brainstem lesion • Cat

Introduction

Cough is a complex defensive reflex of the airway evoked by stimulation of receptors in the tracheobronchial or laryngopharyngeal mucosa. The diaphragm and various muscle groups of the chest wall, and abdominal and cervical muscles are engaged in this important airway reflex. Laryngeal abductor and adductor muscles regulating the resistance posed by the larynx contribute to the effectiveness of the cough reflex (Korpáš and Tomori 1979, Tomori 1979). Laryngeal abductors (posterior cricoarytenoid muscles; PCA) are strongly activated during the inspiratory phase of cough. The enlarged laryngeal lumen lowers laryngeal resistance

and enables a deep initial inspiration by inspiratory pump muscles. During the compressive phase of cough the narrowing or complete closure of the larynx results from the sudden drop in PCA activity coupled with a short-lived and vigorous excitation of the laryngeal adductors (e.g. thyroarytenoid muscles; TA). The increased activity of expiratory pump muscles then leads to compression of air in the lungs. Opening of the glottis due to relaxation of the laryngeal adductors and excitation of PCA in the expulsive phase enables prompt expiratory airflow, which eliminates the irritants from the airways. Subsequently, there is an increase in glottal constriction and resistance due to activation of laryngeal adductors, described as the fourth phase of a cough (Stránsky 1975, Stránsky *et al.* 1976, Shiba *et al.* 1999, Poliaček *et al.* 2003).

The cough motor pattern is produced by the complex neuronal networks localized in the brainstem. Neurons of the dorsal and ventral medullary respiratory groups participate in the central integration of cough (Jakuš *et al.* 1985, Oku *et al.* 1994, Gestreau *et al.* 1996, Bongianni *et al.* 1998, Shannon *et al.* 1998, Mutolo *et al.* 2002) including the regulation of the laryngeal cough response (Shiba *et al.* 1999, Baekey *et al.* 2001). In the first model of cough motor pattern generator presented by Shannon *et al.* (1997, 1998), the respiratory Central Pattern Generator (CPG) was suggested to exert a substantial generator influence. Thus, it seems that the respiratory rhythm generating network can also produce the central cough pattern. Nevertheless, the neuronal circuits which produce cough and possibly other defensive airway reflexes indicate their multilevel organization (Gestreau *et al.* 1997, Bolser *et al.* 1999, Baekey *et al.* 2004, Jakuš *et al.* 2004, Poliaček *et al.* 2004). Respiratory neurons located outside the medullary respiratory groups as well as non-respiratory neurons may also be involved in generation of the cough reflex (Gestreau *et al.* 1997, Xu *et al.* 1997, Jakuš *et al.* 1998, 2000, Poliaček *et al.* 2004). It was shown in our earlier studies that no signs of cough were detected either in electromyograms (EMGs) of diaphragm and abdominal muscles or in pleural pressure recordings following lesions of the medullary midline raphe nuclei (RN; Jakuš *et al.* 1998), the medullary lateral tegmental field (LTF; Jakuš *et al.* 2000) or the pontine respiratory group (PRG; Poliaček *et al.* 2004). However, a residual response which could resemble the cough pattern remained present in a number of electro-neurograms of the recurrent laryngeal nerve during stimulation in post-lesioned animals (Jakuš *et al.* 1998, 2000).

According to our knowledge, no data are available addressing the response of laryngeal abductors and adductors during cough following any type of brainstem lesions. In these experiments we analyzed the characteristics of electrical discharges in PCA and TA during mechanical tracheobronchial and laryngopharyngeal stimulation after kainic acid lesions performed in the LTF, RN and PRG. The present study offers the hypothesis that the neurons located in the above mentioned regions contribute differently to laryngeal activation during the cough reflex. We speculate that these contributions are distinct from the effects mediated by the rostral ventral respiratory group neurons (region of the respiratory CPG). We also postulated a different impact of the lesions on laryngeal muscle and respiratory pump muscle motor outputs. In addition, we determined the pattern of PCA and TA activities during quiet ventilation prior to and following the lesions.

Methods

The methods presented here have been previously described (Jakuš *et al.* 1998, Poliaček *et al.* 2003). Briefly, 24 spontaneously breathing adult cats (2.7±0.2 kg) anesthetized with sodium pentobarbitone (an initial dose of 35-40 mg/kg i.p.) were used in this study. Supplementary doses of the anesthetic were injected (in the range of 1-2 mg/kg/h i.v.) contingent on the response to applying pressure to a limb joint and on the breathing frequency, heart rate and blood pressure (BP). End-tidal CO_2 (ET CO_2) was continuously monitored with a capnograph (Capnogard, Novametrix). Airway stimulation was applied at least 20 min after the administration of an additional dose of the anesthetic. Body temperature was maintained at 37.5±0.5 °C by external heating lamps. Hydrocortison (VUAB, Prague in a dose 6 mg/kg i.m. 15 h before and 3 mg/kg i.v. at the beginning of the experiment) and a single i.v. dose of atropine sulphate (0.2 mg/kg) were administered to prevent brain edema and to reduce salivation, respectively. On completion of the study, the animals were euthanized by an overdose of pentobarbitone. Animal welfare conformed with the guidelines accepted by the European Physiological Society.

During the general surgical preparation cannulae were placed in both ends of the dissected trachea and in the right pleural cavity in order to record the pleural pressure (Ppl) by an electromanometer (Tesla LDP 165). A femoral artery and vein were catheterized to monitor arterial blood pressure with an electromanometer (Tesla LDP 186) and for administration of drugs, respectively.

Bipolar fine wire electrodes were introduced into the crural part of the diaphragm (DIA), into the rectus abdominis or external oblique abdominis muscles (ABD) and into the PCA and TA in order to record EMG activities. The recording sites of the electrodes within laryngeal muscles were confirmed at necropsy by dissection of the larynx. The animals were placed in a stereotaxic frame. The dorsal surface of the brainstem was exposed by occipital craniotomy and partial cerebellectomy.

Mechanical stimulation of the tracheobronchial and laryngopharyngeal regions (by a nylon fibre 0.2-0.5 mm in diameter) continuing for 1-3 s was used to elicit cough and laryngeal responses during pre- and post-lesioned states.

Chemical lesions of neurons in the brainstem were produced by uni- or bilateral microinjections of the excitotoxin kainic acid (Sigma, 2.0 mg/ml) dissolved in phosphate buffered saline (pH=7.4). Kainic acid produces functional inactivation of the cell bodies within 30 min while sparing the axons (Coyle *et al.* 1978, Schwarcz *et al.* 1978). The neurotoxin was applied 1) to the LTF (30-100 nl, AP: 2.2-2.5 mm rostral to obex, 2.2-2.5 mm lateral to midline, depth: 3.0-3.3 mm below the dorsal surface) by means of glass micropipettes (tip diameter: 0.02-0.05 mm), 2) to the region of medullary midline (200-300 nl, two injections were made in one track, AP: 2 mm, depth: 1.5 and 2.5 mm) and 3) to the PRG (150-200 nl, AP: 10.2-10.7 mm, lateral: 4.0-4.3 mm, depth: 2.7-3.0 mm) with a Hamilton syringe (tip needle diameter of 0.3 mm). The solution was saturated with Fast Green dye to localize its spreading.

The EMG activities were amplified (Iso-Dam8 Amplifier, WPI), low and high pass filtered (0.3-3 kHz). The signals were recorded „on line" together with Ppl and BP using a PC software (Lab View, National Instruments). The bursts of discharges were integrated by RC circuits and/or a moving average of raw signals was calculated „off line" by the computer (both with the time constant of 50 ms).

Computer-assisted processing of the recorded signals was performed. The durations and temporal correlations of the PCA, TA, DIA and ABD activities were determined from their raw and integrated EMG signals during quiet breathing and during both types of cough. The values of individual integrated electrical activity were expressed (for each animal separately) in relative percentage units of the control (Poliaček *et al.* 2003). One hundred per cent of integrated electrical activity in PCA was calculated as a mean of five maxima of inspiratory integrated activity in the PCA during quiet breathing. No TA activity was detected during eupnoic breathing in most of our experiments. The maximal values of integrated electrical activity in the TA during eupnoea, when it occurred, were not significantly different from those of PCA. Thus, the EMG of the TA was quantified directly by means of per cent scale of the PCA (for each individual animal).

The parameters of the eupnoic breathing were averaged for each animal separately from five standard respiratory cycles during the control and post-injection states. Similarly, the variables of each quantified reflex response were determined under both experimental conditions.

The final results are expressed as means ± S.E.M. Analysis of variance (ANOVA) was used to determine the statistical significance of the differences ($p<0.05$ was considered significant).

Results

In our former studies, the effects of kainic acid lesions of the medullary raphe midline, medullary LTF and PRG on eupnoic ventilation and the electrical discharges in DIA and ABD during the cough reflex were described (Jakuš *et al.* 1998, 2000, Poliaček *et al.* 2004). In the current paper we report the effect of the lesions on the shape and sequence of burst of discharges in the PCA and TA.

Histological verification of lesioned sites in the brainstem transversal sections (a slice thickness: 0.1 mm) showed that the injections mainly infiltrated the following areas (Fig. 1): 1) the medullary midline (Jakuš *et al.* 1998): the nuclei raphe obscurus and pallidus and surrounding medial reticular formation (Berman 1968) – AP: 0-4 mm, bilateral: 0-1.0 mm, depth: 0.5-3.5 mm, 2) the region of LTF (Jakuš *et al.* 2000): the ventromedial part of LTF including the ventral reticular nucleus and adjacent medial and lateral reticular formation of the medulla (Berman 1968, Petrovický 1980) – AP: 1.6-3.4 mm, lateral: 1.6-2.7 mm, depth: 2.7-3.7 mm, and 3) PRG region: the whole area of PRG and adjacent parts of the pontine reticular formation (Poliaček *et al.* 2004) – AP: 9-12 mm, lateral: 3.5-5.5 mm, depth: 1.5-3.5 mm.

Stimulation of the tracheobronchial or laryngopharyngeal mucosa under control conditions regularly evoked the cough reflex**.** Cough was characterized by a strong and prolonged burst of discharges in DIA immediately followed (with some overlapping) by a forceful burst of activity in ABD. The

former resulted in a negative deep deflection of Ppl, the latter was accompanied by a strong positive swing of Ppl (Fig. 2). Typical biphasic electrical activities of the laryngeal abductors and adductors during cough were reported previously (Poliaček *et al.* 2003). Following each individual lesion the stimulation provoked neither EMG, nor Ppl responses characteristic of the cough reflex (Figs 2 and 3). Similarly, during tracheobronchial stimulation there was no response in 70 % of PCA and in 68 % of TA EMG recordings following LTF lesions, in 93 % and 86 % after RN lesions and in 78 % and 77 % in PRG-lesioned cats, respectively. Remaining stimulations provoked several irregular single bursts of brief discharges in PCA and TA. However, these patterns were clearly distinct from the electrical activities of PCA and TA observed in the control tracheobronchial coughs (Fig. 2). No characteristic cough sequence in laryngeal muscle activation was detected. In addition, the onset of PCA activity preceded TA discharges by 0.5±0.1 s in control tracheobronchial cough, while after any type of the lesion both PCA and TA residual responses appeared almost simultaneously ($p<0.001$). Electrical activities of PCA and TA during laryngopharyngeal stimulation after all types of lesions consisted of a single or of several series of irregular relatively high short-lived and variably prolonged bursts of discharges (Fig. 3). These patterns of responses were apparently different from those observed during a control laryngopharyngeal cough (Fig. 3). The laryngeal muscles were activated almost simultaneously in response to the stimulation following each lesion, whereas in the standard pattern of laryngopharyngeal cough the onset of PCA activity preceded TA cough response by 0.4±0.1 s ($p<0.001$).

During quiet ventilation two patterns of electrical activity in PCA were detected (Table 1). In 20 out of 24 cats (83 %) under control conditions the integrated inspiratory activity of a „plateau“ type in the PCA began at 80±10 ms prior to the activation of the DIA. It diminished in the postinspiratory period and broke up at 80±40 ms before the offset of this phase. „Continuous“ electrical activity during the whole respiratory cycle was observed in 4 animals (17 %). Kainic acid lesions (LTF, RN or PRG) did not change the character of integrated PCA electrical activity in 22 animals (92 %) (Table 1). Following kainic acid microinjection into the RN, the „continuous“ control pattern of PCA activity was altered to the „plateau“ type in one cat (4 %). The lesion at the PRG region changed the „plateau“ type of PCA activity into a „continuous“ one in one animal (4 %).

Statistical analysis of temporal correlations between PCA and DIA electrical activities during control and post-lesioned eupnea revealed no significant differences. Thus, the changes in PCA activity provoked by each lesion corresponded to the analogous alterations in the DIA breathing pattern.

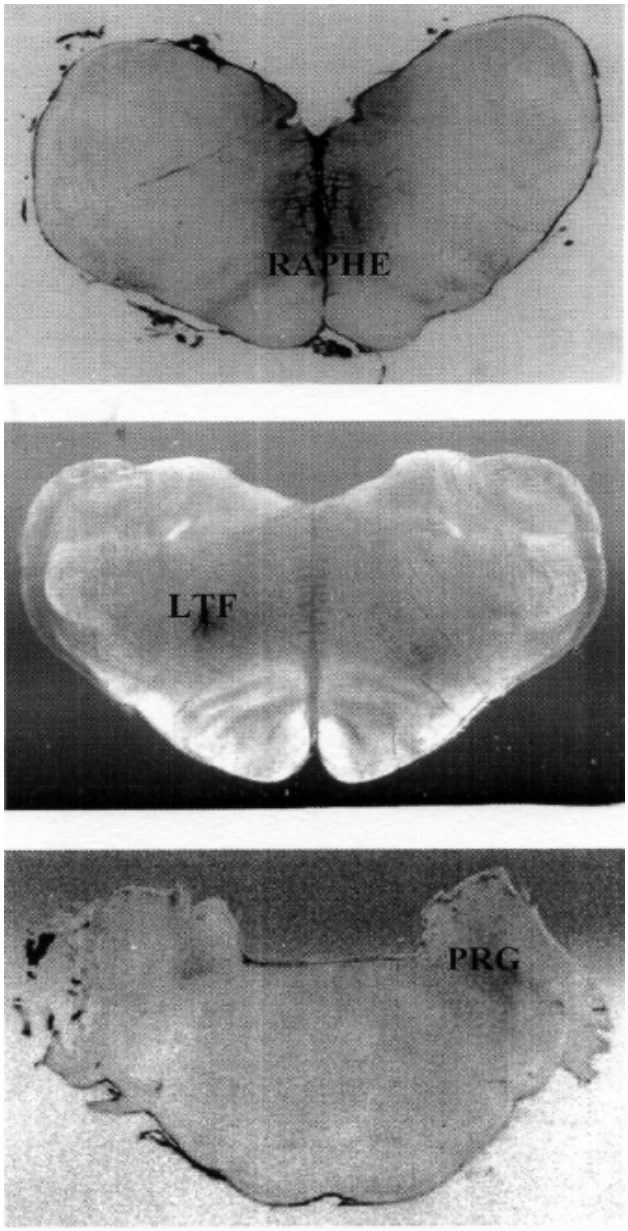

Fig. 1. Histological verification of lesioned sites. Transverse medullary section at 1.0 mm rostral to the obex showing a kainic acid lesion in the region of the medullary raphe (RAPHE; upper). Medullary section at 2.8 mm rostral to the obex indicating an unilateral lateral tegmental field lesion (LTF; middle). Transverse pontine section 10.5 mm rostral to the obex showing bilateral kainic acid lesions in the region of the pontine respiratory group (PRG; lower).

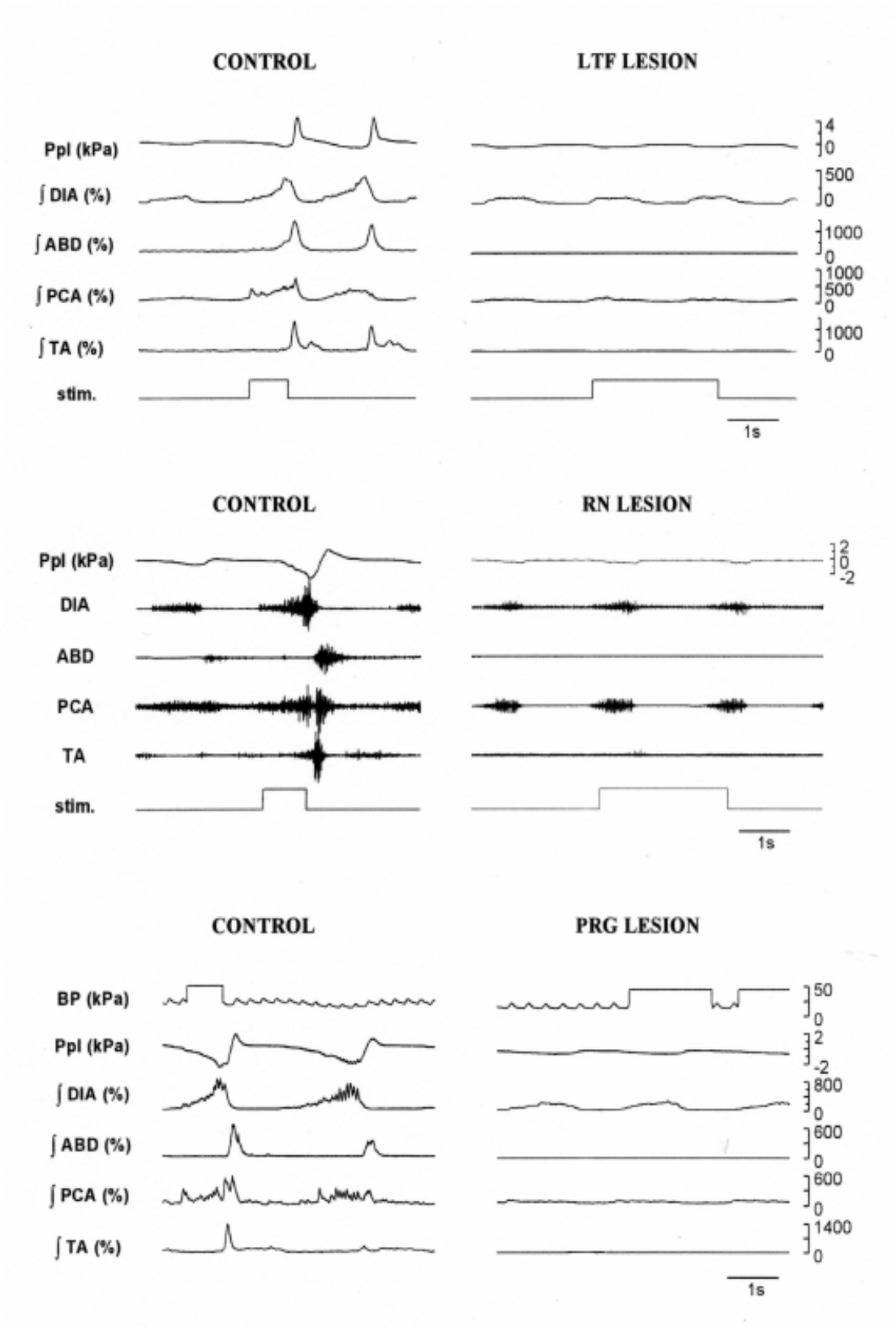

Fig. 2. Tracheobronchial stimulation. Mechanical stimulation of the tracheobronchial tree induced the tracheobronchial cough (on the left). No signs of the cough reflex were recorded following kainic acid microinjections (on the right) into the lateral tegmental field of medulla (LTF; upper), the medullary raphe nuclei (RN; middle) or the pontine respiratory group (PRG; lower). Responses of posterior cricoarytenoid and thyroarytenoid muscles in all post-lesioned states showed a low intensity of discharges or no activity modification. BP, blood pressure; Ppl, pleural pressure; DIA, ABD, PCA, and TA, electromyograms of the diaphragm, abdominal muscles, posterior cricoarytenoid, and thyroarytenoid muscles; stim., periods of stimulation (in the lower panels superimposed on BP tracings); ∫, integrated electromyogram.

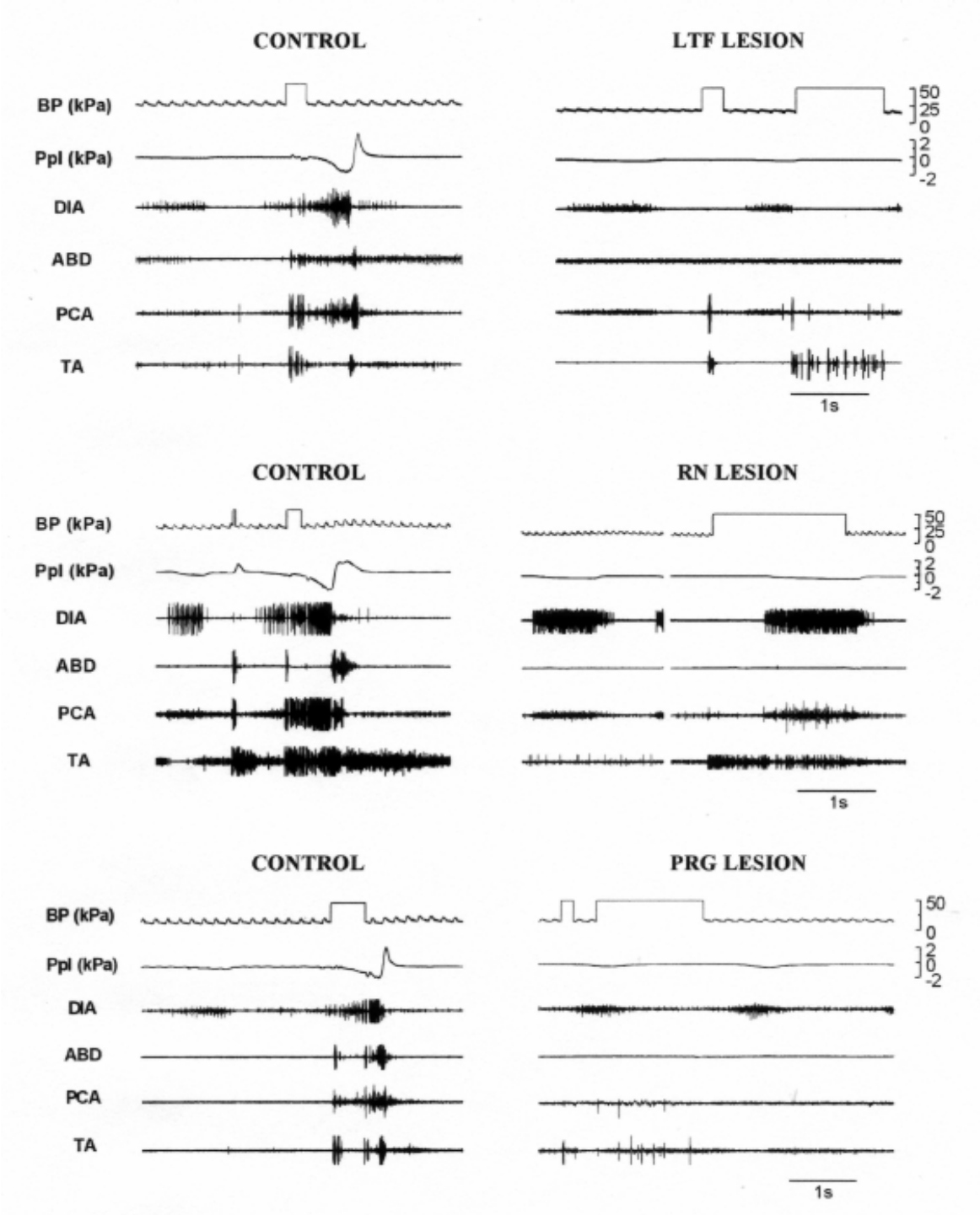

Fig. 3. Laryngopharyngeal stimulation. Mechanical stimulation of the laryngopharyngeal mucosa elicited the laryngopharyngeal cough (on the left). No signs of cough reflex were recorded after kainic acid microinjections (on the right) into the LTF (upper), RN (middle) or PRG (lower). Responses of the PCA and TA in all post-lesioned states consisted of the irregular bursts of discharges. For symbols see Fig. 2. The interruption of traces in the RN LESION recordings represents a time interval of four seconds.

The TA did not show any electrical activity during control eupnoa in 17 out of 24 cats (71 %). Bursts of discharges in postinspiratory (E1) phase (partially overlapping the expiratory (E2) phase of the respiratory cycle) was detected in 4 animals (17 %). „Continuous" type of electrical activity during the whole respiratory cycle was present in 3 cats (12 %) (Table 1). The pattern of TA activity was preserved in 21 animals (88 %) following any of the lesions. However, formerly silent TA showed electrical activity during the E1-E2 periods in 2 cats after LTF lesions and in one cat following the PRG lesion (Table 1).

Table 1. Pattern of posterior cricoarytenoid muscle (PCA) and thyroarytenoid muscle (TA) activities in quiet breathing prior to and following kainic acid microinjections.

	PCA activity		TA activity		
	I-plat	Cont	none	E1-E2	Cont
Pre-LTF lesion (n=9)	8	1	8	0	1
Post-LTF lesion (n=9)	8	1	6	2	1
Pre-RN lesion (n=7)	5	2	4	1	2
Post-RN lesion (n=7)	6	1	4	1	2
Pre-PRG lesion (n=8)	7	1	5	3	0
Post-PRG lesion (n=8)	6	2	4	4	0

I-plat, number of cats with „plateau“ shape of inspiratory integrated electrical activity; Cont, number of cats with „continuous“ (and constant) integrated electrical activity during the whole respiratory cycle; none, number of cats with no activity; E1-E2, number of cats with discharges during the postinspiratory and expiratory periods. LTF, lateral tegmental field of the medulla; RN, medullary raphe nuclei; PRG, pontine respiratory group; n, number of animals.

Discussion

Our paper provides novel information on the central regulation of intrinsic laryngeal muscles during the cough reflex. To our knowledge, this is the first report on the effects of kainic acid lesions of the brainstem on the activity of laryngeal abductor and adductor muscles during cough.

Following lesions of RN, LTF or PRG areas, when the *cough* reflex is mechanically unelicitable from both tracheobronchial and laryngopharyngeal regions, we expected some residual responses in PCA and TA resembling those during control coughs (Jakuš *et al.* 1998, 2000). However, the laryngeal muscle responses regularly consisted of shorter or longer irregular bursts of discharges in PCA and TA during laryngopharyngeal stimulation. Similar responses in less than one third of cases, as well as a lack of response in more than two thirds of cases were observed during tracheobronchial stimulation. These patterns of laryngeal activities do not correspond to control laryngeal cough responses. Furthermore, the breathing pattern was rarely and only in a slightly modified form by cough-related stimulation following the kainic acid lesions. Thus, it seems obvious that in lesioned animals the central motor pattern of cough was not produced, even in a residual form, although the respiratory CPG apparently remained unaffected by kainic acid microinjections. We interpret these findings as indicating the withdrawal of important facilitatory inputs necessary for the transformation of the respiratory rhythm generation into the cough motor pattern. Bolser *et al.* (1999) proposed the presence of a regulatory element in the cough CPG that they termed „gate“. Central antitussive drugs (in a proper dose) reduce the number of coughs in the cough trial as well as the intensity of cough expiratory efforts without a significant change of the duration or inspiratory effort of the reflex. Thus, the „gating“ mechanism may have an important role in the respiratory/cough CPG for producing coughs, as well as facilitates expiratory pre-motoneuronal pool to produce high intensity expiratory efforts (Bolser *et al.* 1999, Bolser and Davenport 2002). We propose that the cough „gates“ structures may anatomically include the neurons in RN, LTF and PRG.

Shiba *et al.* (1999) discussed the disproportion between the activity of expiratory laryngeal motoneurons and the discharges of respiratory neurons in the Bötzinger complex (area of the respiratory CPG) with monosynaptic connection with laryngeal motoneurons during the expulsive phase of coughing (Oku *et al.* 1994, Bongianni *et al.* 1998, Shannon *et al.* 1997, 1998). These neurons could control laryngeal functions during eupnoeic ventilation. It seems that despite the crucial role of the respiratory CPG in the generation of the cough motor pattern, at least in the expulsive phase of the reflex, the expiratory laryngeal motoneurons may receive an excitatory drive from other yet unknown brainstem neurons. In our experiments, the post-lesioned stimulations elicited reflex responses in laryngeal muscles, whereas the cough motor pattern was not produced. These responses corresponded neither to cough, nor to eupnoic laryngeal activities. Furthermore, apnoa induced by laryngeal stimulation is accompanied by the forceful adductor activity with a complete closure of the laryngeal lumen (Korpáš and Tomori 1979). These findings also indicate the existence of supplementary neuronal circuits responsible for the laryngeal regulation, distinct from the control of the larynx in eupnoa and cough. Our investigation has reported a smaller number of laryngeal responses elicited from the tracheobronchial region. Direct laryngeal stimulation elicited more pronounced responses in the PCA and the TA, than did the stimuli from the remaining parts of the airways. In our previous study, reflexes elicited by laryngeal stimulation were associated with higher peaks of integrated PCA and TA activity (Poliaček *et al.* 2003). This could be

explained by the fact that the larynx contains a far higher density of sensory receptors (Widdicombe 1998).

Results of the present paper confirmed previous findings that: during quiet ventilation the inspiratory electrical activity of the PCA started by several tens milliseconds earlier than the discharges in the DIA or the phrenic nerve (Iscoe 1988, Bartlett 1989). In the majority of cases (Table 1), integrated PCA activity had a „plateau" shape in keeping with data reported in conscious man (Brancatisano *et al.* 1986) and in anesthetized animals (Bartlett *et al.* 1981, Sant'Ambrogio *et al.* 1985, Harding *et al.* 1986, Zhou *et al.* 1989). We confirmed that laryngeal adductors are usually silent during eupnea, which is consistent with results obtained in decerebrate or conscious animals (Holmes and Remmers 1989, Praud *et al.* 1992, Roulier *et al.* 2003). On the other hand, laryngeal adductors were described to display either postinspiratory or tonic activity during the whole respiratory cycle in conscious man (Kuna *et al.* 1988), as well as in awake (England *et al.* 1985, Harding *et al.* 1986) and anesthetized animals (Brancatisano *et al.* 1987).

The changes in PCA and TA electrical activities following all types of kainic acid lesions corresponded to modified ventilatory patterns, which had been described in our previous papers (Jakuš *et al.* 1998, 2000, Poliaček *et al.* 2004). In the present results, the character of PCA activity was preserved, except the transformation of the continuous PCA activity into the inspiratory one after RN lesion in one cat only. In conclusion, this indicates that the contribution of the raphe midline neurons on the laryngeal motor pattern during eupnea is notably weak. Lesions in the LTF provoked E1-E2 activity of TA only in two cats. Thus, the LTF appears to play a submissive role in the eupneic control of the larynx, notwithstanding the existence of functional interconnections between LTF neurons and the medullary motor nuclei including the nucleus ambiguus (Holstege *et al.* 1977, Stocker *et al.* 1997). Standard types of PCA and TA eupneic activities under both the pre- and post-PRG-lesioned conditions appeared to be under considerable direct influence of PRG neurons. This is in accordance with the effects of chemical and electrical stimulation of the Kölliker-Fuse nucleus (KF) in decerebrate cats. Both these stimuli evoked a minor response in recurrent laryngeal nerve, attributed to a small number of direct connections between the KF and the laryngeal motoneurons (Kuna and Remmers 1999). On the other hand, application of neuroanatomical techniques have demonstrated frequent, predominantly ipsilateral interconnections between pontine nuclei (nucleus parabrachialis medialis and KF) and motor nuclei of the medulla (Denavit-Saubié and Riche 1977). Furthermore, electrical or chemical (microinjections of glutamate) stimulation of the subdivisions of PRG induces laryngeal constriction or dilatation in spontaneously breathing anesthetized rats. Both these responses are closely associated with the respiratory responses: constriction of the larynx with an inspiratory inhibition, glottal widening with an inspiratory facilitation (Lara *et al.* 2002).

The evidence presented here indicates that the neurons in the RN, LTF and PRG may affect the activity of intrinsic laryngeal muscles indirectly. We believe that the effects can be mediated through the respiratory/cough CPG and in part by the hitherto not observed pools of neurons.

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