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Activity of the Laryngeal Abductor and Adductor Muscles during Cough, Expiration and Aspiration Reflexes in Cats

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Summary

We studied the temporal relationships and the patterns of electromyographic activities of the posterior cricoarytenoid and thyreoarytenoid muscles (laryngeal abductor and adductor), the diaphragm and abdominal muscles in anesthetized cats during mechanically induced tracheobronchial and laryngopharyngeal coughs, expiration and aspiration reflexes. The posterior cricoarytenoid muscle activity reached the maxima just before the peak of diaphragmatic activity in both types of cough and aspiration reflexes and slightly before the top of abdominal muscle activity in coughs and the expiration reflex. Thus, this muscle contributes to the inspiratory phase of coughs and aspiration reflex and also to the expulsive phase of coughs and the expiration reflex. The thyreoarytenoid muscle presented strong discharges in the compressive phase of coughs and expiration reflex (during the rising part of the abdominal muscle activity) and in the subsequent laryngoconstriction (following the diaphragmal and/or abdominal muscle activity) in all four reflexes. This muscle was also slightly activated at the beginning of the aspiration reflex. The existence of four phases of the cough reflex is also discussed.

Key words
Laryngeal muscles • Cough • Expiration and aspiration reflexes • Electromyogram • Cat

Introduction

The larynx plays an important role in the breathing, coughing, sneezing, vocalization, and many other respiratory-related reflexes. In all these behaviors the laryngeal function is based on the dynamic widening and narrowing or closure of the laryngeal lumen due to contraction of the intrinsic laryngeal muscles concerned (Korpáš and Tomori 1979, Harding 1984, Konrad *et al.* 1984, Iscoe 1988, Bartlett 1989). The vocal folds in eupnoea are abducted during the inspiration to decrease the airway resistance, and they are slightly adducted during the phase of postinspiration to brake the expiratory airflow (Bartlett *et al.* 1973, Harding 1984). On the other hand, defensive airway reflexes are characterized by vigourous, shortlasting, and highly coordinated changes in the laryngeal calibre. However, the time-related changes in activation of the particular laryngeal abductors and adductors as well as the neural factors involved in their regulation during particular respiratory reflexes are

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incompletely understood. Especially the study of differences in central inputs to the respiratory pump and laryngeal valve muscles during defensive airway reflexes (Baekey *et al.* 2001) is interesting. Thus, it is necessary to know exactly the behaviors and interrelationships of the pump and laryngeal muscles during reflex responses under control conditions. However, only a small number of studies describes this behavior of laryngeal abductors and adductors during the cough, expiration and aspiration reflexes (e.g. Stránsky 1975, Korpáš and Tomori 1979, Sant′Ambrogio *et al.* 1997, Tomori *et al.* 1998, Shiba *et al.* 1999). Moreover, both the dynamic time relations and the intensity changes in the activities of particular laryngeal muscles during airway reflexes have not been analyzed sufficiently in animal experiments. In addition, it is still not clear whether the phase of subsequent glottal constriction is an essential part of the cough and other defensive airway reflexes and if there are any differences in the activity of laryngeal muscles during the cough reflex elicited from the laryngeal and tracheobronchial regions.

The aim of this study on cats was to describe precisely the time course and pattern of electrical activity in the laryngeal abductor (posterior cricoarytenoid muscle - PCA) and laryngeal adductor (thyreoarytenoid muscle - TA) during both the tracheobronchial cough (TBC) and laryngopharyngeal cough (LPhC), as well as during the expiration reflex (ER) and aspiration reflex (AR) with time relations to the activities of the diaphragm and abdominal muscles. We ascertained the variations in laryngeal muscle activities during TBC and LPhC.

Methods

The experiments were performed on 45 spontaneously breathing adult cats (mean body weight 2.77±0.10 kg). The animals were anesthetized with sodium pentobarbitone by an initial intraperitoneal dose of 35-40 mg/kg and maintenance doses of 1-2 mg/kg/h given intravenously. The end-tidal CO_2 was continually monitored in the course of experiment by a capnograph (Capnogard, Novametrix). A supplemental dose of pentobarbitone was given when the blood pressure or respiratory rate increased spontaneously or when the eupnoeic end-tidal CO_2 value dropped below 3.8 % or in response to surgical procedures. Airway reflexes were evoked at least 20 min after the administration of an additional dose of the anesthetic. Arterial blood samples were analyzed for PO_2, PCO_2 and pH using a blood gas analyzer (AVL - Compact 2). Metabolic acidosis, when it occurred, was corrected by an intravenous administration of 8.4% sodium bicarbonate. Rectal temperature was kept at 37.5±0.5 °C using servo-controlled heating lamps. The cats received hydrocortison (VUAB, Prague) in a dose of 6 mg/kg intramuscularly 15 h before the experiment and 3 mg/kg intravenously with a single intravenous dose of atropine (0.2 mg/kg) during the first stage of anesthesia. The animals were killed by an overdose of pentobarbitone at the end of the experiment. Animal care was conformed to the guidelines accepted by the European Physiological Society.

Surgical preparation

After a tracheostomy, both ends of the dissected trachea were fixed by a polyethylene tube (external diameter: 6-8 mm). The right femoral artery and the right femoral vein were cannulated for measurements of blood pressure (BP) by an electromanometer (Tesla LDP 186) and for further intravenous injections. A cannula was also introduced into the right pleural cavity to record the pleural pressure (Ppl) by an electromanometer (Tesla LDP 165). Bipolar fine wire electrodes (diameter 0.1-0.3 mm) were introduced into the crural part of the diaphragm (DIA), to the abdominal rectus abdominis or external oblique abdominis muscles (ABD), and to the PCA and TA to record their electromyographic (EMG) activities. The electrodes were inserted under a visual control into the DIA and ABD through a short midline and a right ventrolateral abdominal incision, respectively. The electrodes to the PCA were implanted along the dorsal surface of the cricoid cartilage and the electrodes to the TA were inserted through the cricothyroid membrane. The correct positions of the electrodes in individual laryngeal muscles were confirmed on necropsy by dissecting the excised larynx. The right recurrent laryngeal nerve (RLN) was also prepared in 17 experiments for recording of electroneurographic (ENG) activity by bipolar silver hook electrodes. The transglottic pressure (Ptg), the difference between the subglottic pressure in the upper tracheal cannula and the atmospheric pressure during constant flow of heated humidified air through the upper tracheal cannula and the ventromedial pharyngostomic opening was also measured in 4 experiments. During registration the animal was kept in the prone position by means of a stereotaxic apparatus.

The TBC, LPhC, ER and AR were elicited by mechanical stimulation of the mucous membrane in the tracheobronchial (*via* the caudal tracheal cannula), the laryngopharyngeal and glottal (*via* the rostral tracheal cannula) and nasopharyngeal (*via* the pharyngostomic opening) regions, respectively, by a nylon fiber (diameter 0.2-0.5 mm).

Data analysis

The EMG and ENG activities were amplified (Iso-Dam8 Amplifier, WPI), low and high pass filtered (0.3-3 kHz) and displayed on a screen of osciloscopes (Tektronix 5223, Tesla OPD 609). The signals were recorded online together with Ppl and BP (or Ptg) at a sampling frequency of 2 or 4 kHz using a PC software (Lab View, National Instruments).

Computer-assisted processing of the recorded signals was performed. The bursts of discharges were „on line“ integrated by RC circuits and/or a moving average of raw signals was calculated „off line“ by the computer, both with a time constant of 50 ms. The durations and temporal correlations of the PCA, TA, DIA and ABD (or DIA, ABD and RLN) activities were determined from their raw and integrated EMG (ENG) and expressed in milliseconds. The values of individual integrated electrical activities (EMG and/or ENG) were calculated for each particular experimental animal separately and were expressed in relative percentages of the control as follows:

1. One hundred per cent of integrated electrical activity in DIA (in PCA or in RLN) was calculated as a mean of 5 maxima of inspiratory integrated activities in DIA (in PCA or in RLN) during quiet breathing.
2. The integrated ABD activities were calibrated by means of the Ppl and DIA activity because usually no ABD activity was detected during eupnoea. The mean value of 5 integrated electrical activities in ABD at a level of expiratory effort at a pressure of +1.0 kPa for each of 5 different reflexes was calculated. The pressure was measured from the baseline of quiet expiratory level of the Ppl. This average of the electrical signal represents a mean percentage value of analogical 5 integrated activities in DIA for the inspiratory efforts of –1.0 kPa from the same baseline.
3. No electrical activity was recorded in TA during eupnoeic breathing in most of our experiments. Thus, the EMGs of TA were quantified directly on a percentage scale of PCA.

In addition to the EMG (ENG) data, the strength of each effort in the particular airway reflexes was also determined by the maximal and minimal Ppl swings. The results are expressed as means ± S.E.M. and statistically processed by the analysis of variance (ANOVA).

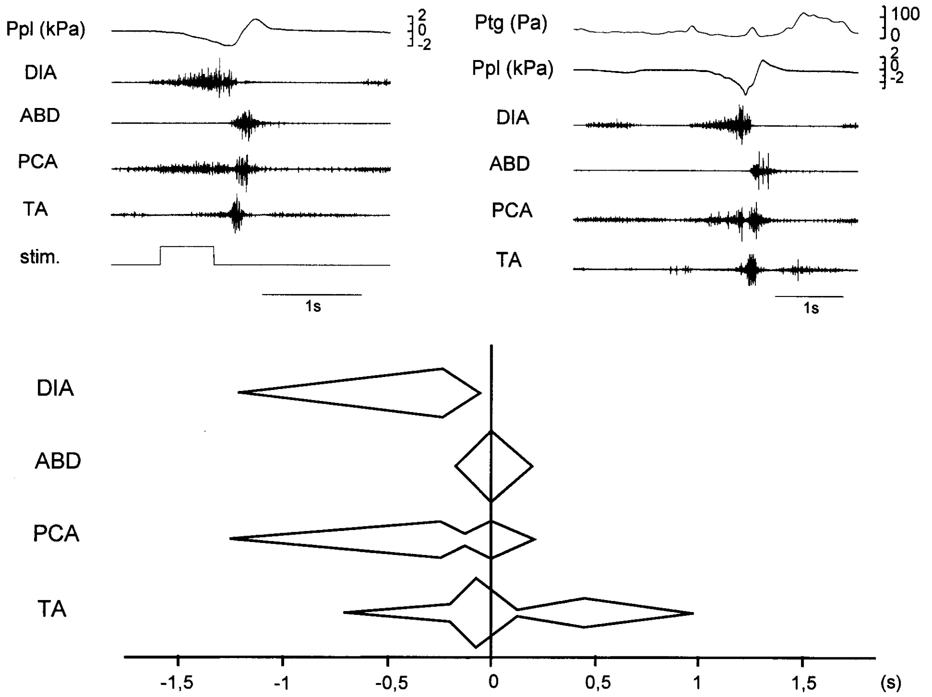
Results

The electroneurographic activity of the RLN was recorded in 17 experiments. Since the RLN comprises motoneural fibers supplying both the laryngeal abductors and adductors, the multifiber recording does not allow to classify and quantify exactly the central laryngeal motor output during particular phases of the studied reflexes. Thus, the direct recording of electrical activities in PCA and TA proved to be more suitable for the analysis of abductor and adductor laryngeal activities in our study.

Tracheobronchial cough

Mechanical probing of the tracheobronchial mucous layer during inspiration regularly evoked the tracheobronchial cough. It consisted of a strong and prolonged burst of discharges in the DIA resulting in a negative deep deflection of Ppl, which was immediately followed and partially overlapped by a burst of activity in the ABD accompanied by a strong positive swing of Ppl (Fig. 1). Evaluation of 258 TBCs in 43 cats (6 coughs with strongest expiratory effort from each animal) showed that the duration of EMG activity in DIA was 1155±40 ms. The maximal intensity of integrated electrical activity in DIA was 560±30 % and the peak of inspiratory Ppl reached –1.28±0.05 kPa. The duration of electrical discharges in ABD lasted 375±15 ms and the maximal intensity of integrated activity in ABD attained 810±80 %. The maximal value of Ppl was +1.70±0.12 kPa.

During TBC, the parameters of electrical activity in PCA were evaluated in 162 reflexes in 27 cats and those of TA were assessed in 168 reflexes in 28 cats (diagram in the lower part of Fig. 1). The discharges in PCA started 40±20 ms before the onset of electrical activity in DIA. Separate bursts of electrical activity in PCA were rarely superimposed on the „ramp“ character of PCA activity during the initial inspiration of TBC. Two marked maxima were observed at integrated electromyograms of PCA during 158 reflexes. One maximum only (the first one) was observed in 4 coughs. The positions of these maxima in the integrated EMG of PCA were 10±20 ms before the peak of DIA activity (the first maximum – peak of initial cough inspiration), and 5±5 ms before the peak of ABD activity (the second maximum – expulsive phase of cough). The maximal intensities of integrated activity in PCA reached 800±100 % (at the first peak) and 840±100 % (at the second peak). The whole electrical activity in PCA during TBC lasted 1450±50 ms.



time (ms)		intensity (%)	time (ms)		intensity (%)
-1250±40	onset of the PCA		-55±20	offset of the DIA	
-1210±40	onset of the DIA		-5±5	2. peak of the PCA	840±100
-710±50	onset of the TA		0	peak of the ABD	810±80
-250±10	1. peak of the PCA	800±100	200±10	offset of the PCA	
-240±35	peak of the DIA	560±30	205±15	offset of the ABD	
-170±10	onset of the ABD		450±30	2. peak of the TA	670±110
-80±10	1. peak of the TA	1600±250	980±60	offset of the TA	

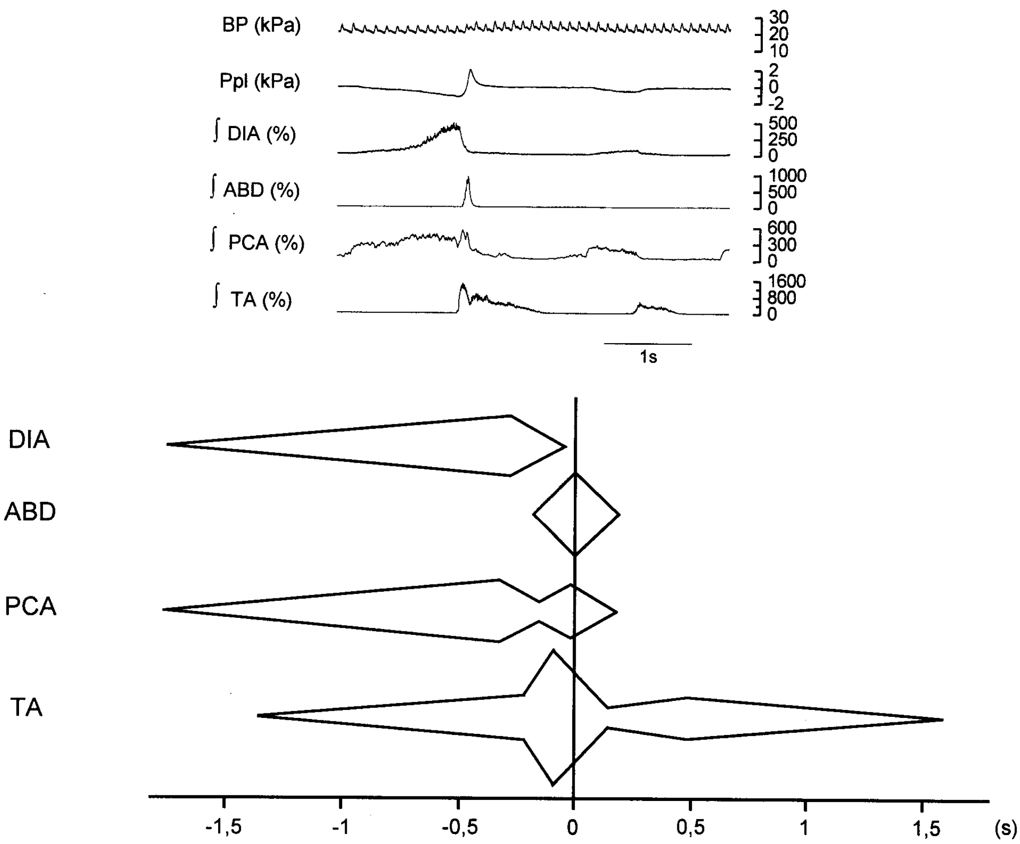
Fig. 1. *TRACHEOBRONCHIAL COUGH. Upper part: Records of the pleural pressure (Ppl), electromyographic activities in the diaphragm (DIA), abdominal muscles (ABD), posterior cricoarytenoid muscle (PCA) and thyreoarytenoid muscle (TA) with moment of stimulation (stim.) (left side) and with a record of the transglottic pressure (Ptg) (right side). Lower part: Diagram of the electromyographic activities in the DIA, ABD, PCA and TA during tracheobronchial cough indicating the time relations and the maximal intensities compared to the baseline values as explained in the Methods.*

Electrical discharges in TA started 500±50 ms after the onset of the burst in DIA. Separate bursts of activity in TA during the initial inspiration of TBC were occasionally detected. At 162 TBC two marked maxima were observed in the integrated EMG of TA. Only the first peak was found in 6 reflexes. The positions of these important maxima in integrated EMG of TA were 80±10 ms before the peak of the ABD activity (compressive phase of cough) and 245±25 ms after the offset of the bursts in ABD (subsequent laryngeal constriction). The maximal intensities of integrated activity in TA reached 1600±250 % at the first peak and 670±110 % at the second one. The duration of overall activity in TA lasted 1690±80 ms. The effects of contraction of the laryngeal abductors and adductors on the transglottic pressure are shown on Figure 1.

Laryngopharyngeal cough

Mechanical stimulation of the laryngopharyngeal mucosa evoked the LPhC. This was characterized by typical sequential changes in the electromyograms of the DIA and ABD and particular inspiratory-expiratory deflections of Ppl (Fig. 2). Evaluation of 213 coughs in 37 cats (4-6 reflexes with the strongest expiratory effort from each cat) showed that the duration of EMG activity in DIA was 1695±60 ms, the maximal intensity of integrated electrical activity in DIA reached 660±40 % of the basic level and the peak inspiratory Ppl reached -1.45±0.05 kPa. The electrical activity in ABD lasted 370±20 ms and its integrated peak intensity attained 860±100 %. The maximal value of Ppl was +1.63±0.13 kPa on the average.

During LPhC, the parameters of electrical activities in PCA and TA were evaluated in 130 reflexes in 22 cats and in 145 reflexes of 25 cats, respectively (diagram on Fig. 2, lower part). The discharges in PCA started 10±50 ms before the beginning of electrical activity in DIA. In most LPhC short and non-intensive bursts in PCA were superimposed on the regularly observed „ramp" shape of this activity in initial cough inspiration induced by stimulation. When mechanical probing was not applied to the mucous membrane, superimposed bursts of activity were not present in EMG of PCA (Fig. 2). Two important maxima were detected in integrated EMG of PCA during 112 reflexes. Only one maximum (the first one) was observed in 18 evaluated coughs. The positions of both these maxima were 40±15 ms before the peak of the DIA activity (inspiratory phase of cough) and 15±7 ms before the peak of ABD activity (expulsive phase of cough). The integrated electrical activity in PCA reached maximal relative intensity 1240±260 % (at the first peak) and 1110±170 % (at the second peak). The duration of the whole PCA activity during LPhC lasted 1930±70 ms.



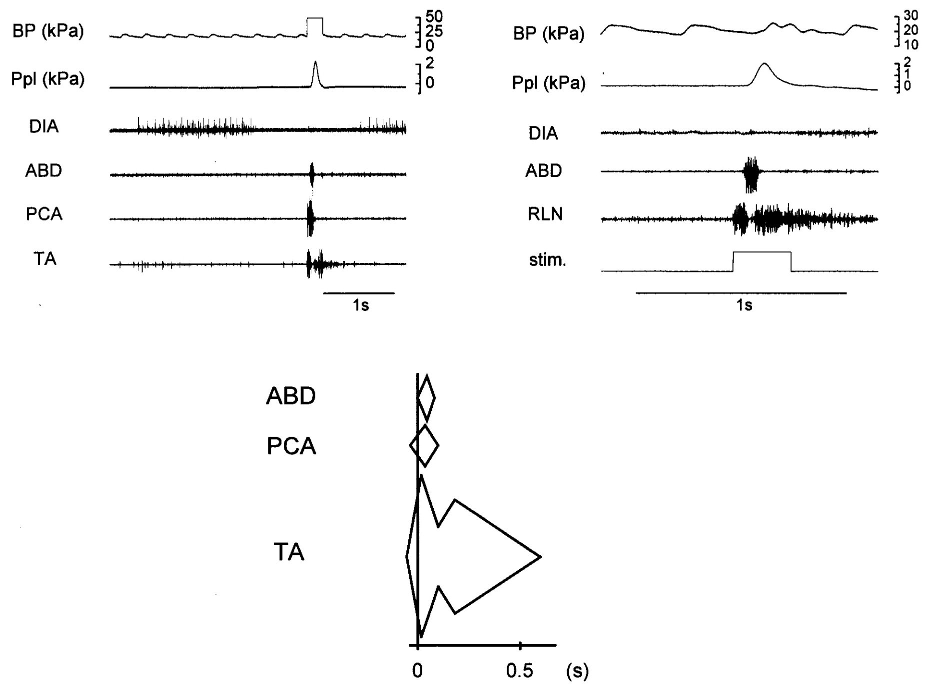
time (ms)		intensity (%)	time (ms)		intensity (%)
-1750±60	onset of the PCA		-45±20	offset of the DIA	
-1740±60	onset of the DIA		-15±7	2. peak of the PCA	1110±170
-1350±70	onset of the TA		0	peak of the ABD	860±100
-315±15	1. peak of the PCA	1240±260	180±20	offset of the PCA	
-275±50	peak of the DIA	660±40	190±10	offset of the ABD	
-180±10	onset of the ABD		480±30	2. peak of the TA	900±200
-80±10	1. peak of the TA	2700±400	1590±80	offset of the TA	

Fig. 2. *LARYNGOPHARYNGEAL COUGH. Upper part: Records of blood pressure (BP), pleural pressure (Ppl), integrated electromyographic activities in the diaphragm (∫DIA), abdominal muscles (∫ABD), posterior cricoarytenoid muscle (∫PCA) and thyreoarytenoid muscle (∫TA) during „spontaneous" laryngopharyngeal cough immediately following a period of previous reflex elicited mechanically. Lower part: Diagram of the electromyographic activities in the DIA, ABD, PCA and TA during laryngopharyngeal cough indicating the time relations and the maximal intensities compared to the baseline values as explained in the Methods.*

The discharges of electrical activity in TA started at different parts of the initial inspiration, 400±100 ms after the onset of the DIA activity on the average. During the period of stimulation, single short bursts of electrical discharges in adductor TA were regularly observed in contrast to the TBC, probably as a reaction to individual contacts of a nylon fiber with the mucous membrane. No TA activity was detected during initial inspiration of a "spontaneous" cough (Fig. 2). At 139 coughs, two marked maxima were observed in the course of integrated electrical activity of TA. The first peak was found only in 6 reflexes. The positions of these maxima in integrated EMG of TA were 80±10 ms before the peak of ABD activity (compressive phase of cough) and 290±30 ms after the end of the discharges in ABD (subsequent laryngeal constriction). The intensities at maxima reached 2700±400 % and 900±200 % of the relative intensity scale. The whole activity in TA lasted 2940±120 ms.

Expiration reflex

Mechanical stimulation of the vocal folds during expiration evoked a typical ER characterized by a short but strong burst of electrical activity in the ABD presented by a brief and forceful expiratory effort and a prompt and steep positive swing of Ppl without preceding inspiration (Fig. 3). Altogether 192 ER in 32 cats (6 strongest reflexes from each cat) were evaluated in our experiments. During the ER, the electromyographic activity in ABD lasted 84±3 ms and its maximal integrated intensity reached 470±30 %. Contraction of the expiratory pump muscles led to an expiratory effort with an average maximal value of Ppl +0.95±0.08 kPa.



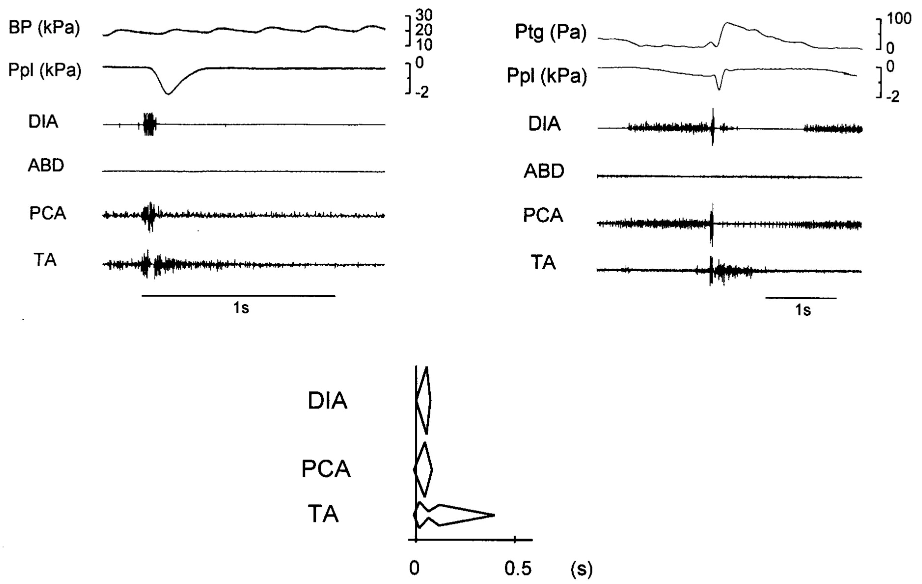
time (ms)		intensity (%)	time (ms)		intensity (%)
-42±3	onset of the TA		50±2	peak of the ABD	470±30
-28±2	onset of the PCA		84±3	offset of the ABD	
0	onset of the ABD		100±8	offset of the PCA	
10±2	1. peak of the TA	3500±500	185±10	2. peak of the TA	2500±500
27±3	peak of the PCA	900±80	628±35	offset of the TA	

Fig. 3. *EXPIRATION REFLEX. Upper part: Records of the blood pressure (BP), pleural pressure (Ppl), electrical activities of the diaphragm (DIA) and abdominal muscles (ABD) with activities in the posterior cricoarytenoid (PCA) and thyreoarytenoid (TA) muscles (left side) and with activity of the recurrent laryngeal nerve (RLN) (right side). stim - moment of stimulation (superimposed on BP trace on the left side). Lower part: Diagram of the electromyographic activities in the ABD, PCA and TA during the expiration reflex indicating the time relations and the maximal intensities compared to the baseline values as explained in the Methods.*

The electrical activities of PCA and TA were studied in 108 ER of 18 cats and in 132 ER of 22 cats, respectively (diagram on Fig. 3, lower part). The discharges in PCA preceded the discharges in ABD by 28±2 ms. The maximal intensity of the integrated activity in abductor PCA reached 900±80 % and preceded the peak of the integrated electrical activity in ABD by 23±3 ms. The duration of PCA activity was 128±8 ms. A biphasic EMG activity of TA was regularly recorded during the ER (Fig. 3, upper left part). The discharges in TA began by 42±3 ms earlier than the activity in ABD. The first maximum appeared 40±3 ms before and the second maximum 135±10 ms after the peak of ABD activity. Intensity of the integrated electrical signal of TA reached its maxima 3500±500 % and 2500±500 % respectively. Both bursts of discharges in TA together lasted 670±40 ms.

Aspiration reflex

Mechanical stimulation of the nasopharynx regularly evoked a sniff-like AR. This was characterized by a brief but large burst of electrical activity in the DIA accompanied by a short, steep and strong negative deflection of Ppl (Fig. 4). Evaluation of 288 reflexes in 37 cats (7-8 AR with highest peak values of Ppl from each animal) showed that the duration of electrical bursts in the DIA was 77±2 ms. The maximal intensity of the integrated activity reached 650±30 % of the baseline. The peak value of the negative swing in Ppl was –2.41±0.09 kPa on the average.



time (ms)		intensity (%)	time (ms)		intensity (%)
-10±3	onset of the TA		48±2	peak of the DIA	650±30
-6±2	onset of the PCA		77±2	offset of the DIA	
0	onset of the DIA		84±3	offset of the PCA	
18±2	1. peak of the TA	470±30	118±5	2. peak of the TA	390±20
42±2	peak of the PCA	1070±50	390±20	offset of the TA	

Fig. 4. *ASPIRATION REFLEX. Upper part: Records of the pleural pressure (Ppl), electrical activities of the diaphragm (DIA), abdominal (ABD), posterior cricoarytenoid (PCA) and thyreoarytenoid (TA) muscles with a record of the blood pressure (BP) (left side), and with a record of the transglottic pressure (Ptg) (right side). Lower part: Diagram of the electromyographic activities in the DIA, PCA and TA during the aspiration reflex indicating the time relations and the maximal intensities compared to the baseline values as explained in the Methods.*

The electrical activities of laryngeal muscles were assessed in 160 AR in 21 cats (diagram in Fig. 4, lower part). The discharges in abductor PCA preceded the electrical activity in DIA by 6±2 ms and the peak of integrated electrical activity in PCA preceded the top of integrated electrical activity in DIA by 6±3 ms. The maximal intensity of the integrated electrical activity in PCA reached 1070±50 % of its control value during quiet inspiration lasting 90±5 ms. The discharges in TA started 10±3 ms earlier than the bursting activity in DIA.

In 86 % of evaluated reflexes (137 AR out of 160) the electrical activity in TA consisted of two bursts. The first maximum of integrated electrical activity in TA preceded the peak of DIA activity by 30±4 ms, while the second maximum followed the top of inspiratory effort by 70±7 ms. The intensity of integrated electromyogram of TA in maxima reached 470±30 % and 390±20 % of the baseline activity in PCA during quiet inspiration. The duration of bursting activity in TA lasted 400±20 ms. The effects of contraction of intrinsic laryngeal muscles during AR is demonstrated in Fig. 4 (upper right side).

Comparison of the tracheobronchial and laryngo-pharyngeal coughs

Various parametres of the tracheobronchial (Fig. 1) and laryngopharyngeal (Fig. 2) coughs induced by mechanical stimulation were compared and the following significant differences were found.

1. Duration of the electrical activity in the DIA during TBC (1155±40 ms) was shorter than during LPhC (1695±60 ms, $p<0.001$).
2. Duration of the negative deflection of Ppl during TBC (1100±40 ms) was shorter than during LPhC (1620±60 ms, $p<0.001$).
3. The maximal intensity of the integrated electrical activity in the DIA during TBC (560±30 %) reached a lower value than during LPhC (660±40 %, $p<0.01$).
4. The first burst of electrical activity in PCA during initial inspiration of TBC (1130±50 ms) was shorter than that during LPhC (1600±60 ms, $p<0.001$).
5. The first burst of electrical discharges in TA (during the inspiratory and compressive phases) was shorter during TBC (830±50 ms) than during LPhC (1500±70 ms, $p<0.001$). The second burst in the electromyogram of TA (in the phase of subsequent constriction) was shorter in TBC (860±60 ms) than during LPhC (1440±90 ms, $p<0.001$).
6. The maximal intensity of the integrated electrical activity in TA was lower during TBC (1600±250 %) than during LPhC (2700±400 %, $p<0.02$).

During both types of coughs there was a typical sequence of events: firstly, the peak activity in PCA and DIA (inspiratory phase), peak activity in TA (compressive phase) and lastly peak activity in PCA and ABD (expulsive phase). Interestingly, the time interval from the offset of DIA activity at the end of the inspiratory phase of cough to the onset of the following inspiratory activity of the DIA was shorter in TBC (1100±50 ms) than in LPhC (2550±90 ms, $p<0.001$). In addition, we did not detect any significant differences in the mean values of maximal integrated electrical activities in ABD and the maxima of Ppl during both types of coughs.

Discussion

Cough reflex

In accordance with conclusions of several authors (Stránsky 1975, Stránsky *et al.* 1976, Korpáš and Tomori 1979, Poliaček *et al.* 1999, Shiba *et al.* 1999) the present results indicate that the complex act of coughing consists of four successive phases: a deep initial inspiration, a compressive phase, an expulsive phase and a phase of subsequent glottal constriction.

Our results confirm the former findings (e.g. Stránsky 1975, Korpáš and Tomori 1979, Leith *et al.* 1986, Jakuš *et al.* 1985, 1998, 2000, Shannon *et al.* 1997), that the *deep initial inspiration* of cough is characteristic by a strong prolonged burst of electrical discharges in the DIA resulting in a deep negative deflection of Ppl. The typical eupnoeic „ramp“ character of the EMG activity in DIA also persists during coughing, however, with a markedly higher peak level. The DIA activity was closely associated with the first burst of discharges in the laryngeal abductors. The eupnoeic „plateau“ character of the PCA activity (Iscoe 1988) was changed during the cough to a „ramp“ shape with much stronger maximal intensity. Analogously, a higher frequency of discharges in the inspiratory fibers of RLN during the initial inspiration of fictive cough compared to quiet inspiration was described in anesthetized paralyzed cats (Stránsky 1975, Stránsky *et al.* 1976). Using intracellular measurement on decerebrate paralyzed animals Shiba *et al.* (1999) showed a higher level of depolarization and activation of the inspiratory laryngeal motoneurons (ILM) in this phase than during eupnoeic inspiration. However, using a similar method, Gestreau *et al.* (2000) reported no significant change of depolarization and firing rate of ILM during the initial inspiration of cough. Dawid-Milner *et al.* (1993) and Baekey *et al.* (2001) observed higher activation of ILM in this phase of fictive cough on both decerebrate spontaneously breathing and decerebrate paralyzed and artificially ventilated cats.

In part of our experiments, there were short bursts of discharges superimposed on the „ramp“ shape of the PCA and isolated bursts of electrical activity in TA which corresponded to separate mechanical contacts with the airway in the course of initial cough inspiration. Similar electrical discharges were observed in the DIA and ABD, as was also shown by Bongianni *et al.* (1998). Transient depolarizations of expiratory laryngeal motoneurons (ELM) with electrical discharges during initial inspiration of fictive cough was lately confirmed by intracellular recording (Shiba *et al.* 1999, Gestreau *et al.* 2000) and by recording of bursting activity in ELM (Baekey *et al.* 2001).

The *compressive phase* of cough is characterized by a sudden drop of activity in the DIA and laryngeal abductor PCA accompanied by a short but very strong burst of discharges in the laryngeal adductors, e.g. in TA, and by activation of the ABD muscles. These activities resulting in sequential contractions of the laryngeal valve and respiratory pump muscles cause breaking of the inspiratory airflow and compression of air in the lungs and hence a strong rise in Ppl. In our experiments, intensive bursts in TA and synchronous inhibition of the PCA activity were regularly present during the compressive phase of cough. Correspondingly, there was a higher level of depolarization of ELM and a hyperpolarization of ILM during the compressive phase of cough (Shiba *et al.* 1999). However, Gestreau *et al.* (2000) observed only strong depolarization of ELM. A high frequency of discharges was described in ELM without any activity in fibers of ILM in anesthetized paralyzed cats (Stránsky 1975, Stránsky *et al.* 1976), and strong activation of ELM with deactivation of ILM were recorded during the compressive phase of fictive cough (Baekey *et al.* 2001).

The most important component of the cough reflex is its *expulsive phase* typical by strong activation and contraction of abdominal and other expiratory muscles with a strong positive swing of Ppl. In our experiments, active opening of the glottis due to activation of the laryngeal abductor PCA was usually observed during this phase (in 98 % of TBC and in 86 % of LPhC). Moreover, an abrupt decrease of the electrical activity in the laryngeal adductor TA was regularly present during the phase of expulsion in the EMG of the TA. In accordance with our results, similar sequential depolarization with high frequency of bursts in ILM and hyperpolarization of ELM was recorded during cough expulsion in experiments on decerebrate and paralyzed animals (Shiba *et al.* 1999). Likewise, a decrease of discharge frequency in ELMs of RLN was reported during the expulsive phase of fictive cough (Stránsky 1975, Stránsky *et al.* 1976), however, in contrast to our present findings there was only very slight activity in the inspiratory fibers. Similarly, higher activation was found in the the laryngeal tensor cricothyroid muscle than in the abductor PCA during the expulsive phase of cough in experiments on dogs (Sant′Ambrogio *et al.* 1997).

Gestreau *et al.* (2000) recorded monophasic membrane potentials in both types of laryngeal motoneurons in the caudal nucleus ambiguus during fictive cough in experiments on decerebrate and paralyzed cats. The DIA activity was accompanied by depolarization and activation of the ILM and the ABD activity with depolarization and activation of ELM. Similarly, activation of ILM only during the phase of initial inspiration and uninterrupted activity of the majority of ELM during compression and expulsion was observed by Baekey *et al.* (2001). However, in part of ELM there was a sudden decrease of discharges still in the course of lumbar nerve activity. Several ELM with continuous depolarization persisting from the beginning of compression to the end of the cough reflex were also described, but most of the ELM were inhibited during the expulsion (Shiba *et al.* 1999). Pprolonged activation of laryngeal adductors during the whole cough reflex is also possible (Dawid-Milner *et al.* 1993). Probably different experimental conditions (anesthesia – decerebration, spontaneous breathing – paralysis and artificial ventilation, mechanical – electrical stimulation, stimulation of the tracheal – laryngeal region) are the reason of such different findings concerning the laryngeal activity during the cough reflex reported in individual papers. Most experiments performed on anesthetized animals (Stránsky 1975, Stránsky *et al.* 1976, Korpáš and Tomori 1979, Tomori 1979, Sant′Ambrogio *et al.* 1997, Poliaček *et al.* 1999) and partially on decerebrate and paralyzed animals have documented a standard biphasic course in the laryngeal abductor (Shiba *et al.* 1999) and adductor (Shiba *et al.* 1999, Baekey *et al.* 2001) activities during cough. It is difficult to assess precisely the intensity of inspiratory and expiratory efforts during fictive cough in paralyzed animals. Possibly an incomplete cough response consisting only of a deep inspiration and a forced expiration (without compression) could be occasionally elicited. Szereda-Przestaszewska *et al.* (1992) also described stronger laryngeal abductor inspiratory activity and adductor postinspiratory activity during augmented breaths following i.v. administration of almitrine in cats.

The phase of *subsequent glottal constriction* was characterized by prolonged activation of laryngeal adductor TA resulting in narrowing of the laryngeal lumen with an increase in transglottic pressure (Fig. 1, top right side). An increase of laryngeal resistance in the phase of subsequent glottal constriction is also documented by activation of ELM (Stránsky *et al.* 1976, Leith *et al.* 1986) and prolonged depolarization and activation of ELM observed in the last phase of fictive cough in decerebrate paralyzed animals (Shiba *et al.* 1999, Baekey *et al.* 2001).

The cough reflex can be divided only into three phases neglecting the last phase of subsequent glottal constriction by several authors (Leith *et al.* 1986, Shannon *et al.* 1997, Satoh *et al.* 1998, Gestreau *et al.* 2000, Baekey *et al.* 2001). In the present experiments, we failed to detect the activity of TA during this phase of cough in 4 % of cough efforts. The phase of subsequent glottal constriction is evidently the most variable part of the cough and is probably due to a forced postinspiratory "braking" mechanism of eupnoeic breathing (Bartlett *et al.* 1973, Harding 1984, Bartlett 1989). Gradual attenuation and abbreviation of the subsequent constriction phase in a series of successive cough efforts comprising a cough attack or in weak cough responses as well as the presence of this phase also in the ER and AR seems to support this possibility. A similar pattern of electrical activity in TA resembling that occurring in the phase of subsequent glottal constriction of the cough was also observed during augmented breaths (Szereda-Przestaszewska *et al.* 1992). This course of TA activity was frequently detected in the present study during several breathing cycles following the cough reflex sometimes also with weak activation of the ABD. Co-activation of lumbar motoneurons and ELM atypical for eupnoeic breathing (Bianchi *et al.* 1995) was also described during reflex responses elicited by mechanical airway stimulation (Baekey *et al.* 2001) and by electrical stimulation of the superior laryngeal nerve (Shiba *et al.* 1999, Gestreau *et al.* 2000) in decerebrate and paralyzed cats. Participation of the neuronal network regulating eupnoeic expiration also in the phase of subsequent glottal constriction of the cough reflex is in accordance with the conclusion that the respiratory neurons in the rostral ventral respiratory group (region of respiratory rhythm generator in Bötzinger and pre-Bötzinger complexes) are essential for the generation of a central neuronal pattern of the cough reflex (Jakuš *et al.* 1985, Shannon *et al.* 1997, 1998).

The intensity of glottal constriction in our experiments was much stronger in ER and LPhC than in TBC and AR elicited from distant regions. This suggests that airway stimulation may trigger an elementary (regional) reflex acting as a longer-lasting protective glottal constriction, which is temporarily interrupted by advanced superposed activation of the laryngeal abductor muscles. Such a regional origin of reflex glottal constriction may facilitate both the preparatory inspiration and expulsion.

The comparison of TBC and LPhC in our study did not confirm the previous assumption that the intensity of expiratory cough efforts during TBC is higher than during LPhC, as was described in both anesthetized cats (Korpáš and Tomori 1979) and anesthetized dogs (Tatár *et al.* 1994). In our experiments, the differences in both the peak values of Ppl and maximal intensities of integrated electrical activity in ABD during TBC and LPhC were not significant. Similarly, we could not confirm the stronger inspiratory efforts than the expiratory ones during LPhC (Korpáš and Tomori 1979) in the present experiments. This was probably because the stimulation was limited to the larynx-mesopharynx but not to the nasopharynx (Tomori *et al.* 1957). However, in accordance with Korpáš and Tomori (1979) the peak value of inspiratory Ppl during initial inspiration of the LPhC was higher compared to this parameter in TBC. Likewise, in our study the integrated electrical activity of TA during the compressive phase of LPhC was higher than in the TBC. Our findings indicate that central neuronal and peripheral motor patterns of LPhC and TBC are partly different. On the other hand, the main components of cough reflex described by the time course of events: namely peak of deep inspiration – compression (TA activation) and expulsion (activation of PCA and ABD) were practically identical for both types of coughs (see results and diagrams on Figs 1 and 2).

Expiration reflex

In addition to other findings (Korpáš 1972, Stránsky 1975, Korpáš and Tomori 1979, Stránsky and Tomori 1979, Jakuš *et al.* 1985, Bongianni *et al.* 1988), the present results have shown that the ER is characterized by a strong expiratory effort resulting in fast expiratory airflow through the upper airways. In our experiments, the ER was much shorter and the maximal positive Ppl during ER reached a lower level on the average than during the cough compression.

ER consisted of three phases, similarly as had been described earlier (Korpáš and Tomori 1979, Stránsky and Tomori 1979, Poliaček *et al.* 1999). The first concerned the *compressive phase* with activation of laryngeal adductor TA and ABD resulting in a strong increase of Ppl. There was a higher frequency of discharges in ELM and an increase of laryngeal resistance during this phase of ER (Stránsky 1975, Stránsky and Tomori 1979) with a narrowing of the glottis (Korpáš and Tomori 1979). The expulsive phase of ER was characterized by large bursts of discharges in both the ABD and PCA and by simultaneous inhibition of the adductor laryngeal activity. In paralyzed animals, Stránsky and Tomori (1979) described a decrease or absence of activity in ELM during the expulsive phase of ER, but they did not detect any activity in inspiratory fibers of RLN. On the contrary, in the present report a burst of activity was regularly recorded in PCA during the expulsive phase of ER in spontaneously breathing cats. This difference may be due to different experimental conditions. Phase of the *subsequent glottal constriction* was regularly detected in our study at the end of ER. Similarly as in cough, the duration of this phase was very variable.

Aspiration reflex

AR is characterized by a short but strong inspiratory effort with a large burst of discharges in DIA and with deep negative deflection of Ppl and fast inspiratory airflow through the upper airways (Tomori and Widdicombe 1969, Nail *et al.* 1972, Stránsky 1975, Korpáš and Tomori 1979, Tomori 1979, Jakuš *et al.* 1985, Tomori *et al.* 1998). In the present experiments, large bursts of electrical activity in PCA and biphasic activity in the TA were recorded during the AR. Minimal activity in TA was associated with peak activation of the DIA and PCA.

AR could be divided into two phases. During the *inspiratory phase* of AR, a short-lasting burst of discharges was recorded in TA, which was immediately followed by very large but short bursts of electrical activities in both the PCA and DIA and by a deep negative deflection of the Ppl. A prolonged burst of electrical activity in TA was characteristic for the *expiratory phase* of AR. A decrease of the laryngeal resistance during the inspiratory phase of AR and a subsequent increase during the expiratory phase was detected in experiments on cats (Stránsky 1975, Stránsky *et al.* 1976). However, a significant increase of ELM discharge frequency was not recorded during the expiratory phase of AR. There was an opening of the glottal lumen during the inspiratory phase of the reflex and a slight glottal narrowing during its expiratory phase (Korpáš and Tomori 1979, Tomori 1979, Tomori *et al.* 1998).

To our knowledge, the short adductor TA activity occurring at the beginning of the inspiratory phase of AR has not been reported previously. However, Arita *et al.* (1994) and Oshima *et al.* (1994) described a "hiccup-like" response, which was elicited by mechanical stimulation of the dorsal pharynx or electrical stimulation of the medullary region lateral to the nucleus ambiguus in anesthetized spontaneously breathing cats. The strong but rapid inspiration was accompanied by short activation of adductor TA and initial inhibition followed by activation of abductor PCA. This time course of activities in the laryngeal muscles seems to be very similar to our measurements of electrical activities in the laryngeal abductor and adductor muscles during AR. The first slight burst of discharges in TA at the beginning of AR was not detected earlier, probably because not all ELM were activated and only a slight narrowing of the glottal lumen resulted. This hypothesis was confirmed by measurements of both the intensity of integrated electrical activity in TA and various parameters of the glottal lumen (Tomori 1979). The maximal intensity of the first peak at the beginning of AR reached a several times lower level compared to the analogous TA activities during the compressive phase of the ER or cough. Moreover, during multifiber recording of ENG activity in RLN the adductor ELM activities might be submerged in discharges of abductor ILM. Co-activation of laryngeal abductors and adductors during the inspiratory phase of AR probably have to prevent passive obstruction in the larynx during the fast inspiratory airflow (Tomori 1979).

In decerebrate paralyzed cats, Fung *et al.* (1995) and Tomori *et al.* (1998) described tonic adductor activity persisting during the whole period of nasopharyngeal stimulation, however, repeated separate contacts with the nasopharyngeal mucous elicited short-lasting but strong activations of the abductors and immediate strong inhibitions of long-lasting tonic activity in the adductor thyreoarytenoideal branch of RLN. Intracellular recording of neuronal membrane potentials showed simple (bell-shaped) course of depolarization of ILM and hyperpolarization of ELM during fictive AR elicited by mechanical nasopharyngeal stimulation (Shiba *et al.* 1999). It seems that there are very important differences between the reflex responses of decerebrate paralyzed animals and reflex responses of spontaneously breathing anesthetized animals.

Our results lead to the assumption that the first burst of discharges in TA during the AR, similarly as in other airway reflexes, can be a prompt direct response to the touch stimulation of airway mucous membrane resulting in long-lasting constriction. The second burst is probably a component of expiratory airflow regulation (augmented postinspiratory adductor activity) due to disinhibition of central mechanisms responsible for breathing control. Tomori *et al.* (1998) reported that the adductors might also be activated in some cases during preparatory introduction of a nylon fiber to the pharynx before proper nasopharyngeal contact stimulation. Indirect evidence for the reflex character of this initial constriction of glottis in the AR induced by mechanical stimulation of the nasopharynx is its absence in gasps occurring spontaneously during extreme hypoxia in decerebrate paralyzed and artificially ventilated cats. The gasp is characterized by practically identical inspiratory activity as the AR but without initial activation of the thyreoarytenoid muscle (Widdicombe 1954, Fung and St. John 1995).

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