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Laryngeal Patency and Expiration Reflex Following Focal Cold Block of the Medulla in the Cat

J. JAKUŠ, A. STRÁNSKY, I. POLIAČEK, H. BARÁNI

Department of Biophysics, Jesenius Faculty of Medicine, Comenius University, Martin, Slovak Republic

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Summary

The involvement of the intermediate area and Bötzinger complex (BOT) of the rostral ventral respiratory group (r-VRG) in laryngeal control and generation of the expiration reflex were studied in anaesthetized non-paralyzed cats. Focal cooling (to 20 °C) of the nucleus paraambigualis (NPA) caused changes in the frequency and timing of breathing with the concomitant rise in laryngeal resistance. Cooling of the nucleus ambiguus resulted in a consistent drop in laryngeal resistance. Alterations in timing and intensity of breathing but no changes in laryngeal patency were found during cooling of the BOT. The expiration reflex was inhibited by cooling of either the NPA or BOT. The role of these medullary regions in the control of laryngeal patency and central integration of the expiration reflex is discussed.

Key words

Respiratory control – Laryngeal resistance – Expiration reflex – Ventral respiratory group – Focal cold block of medulla

Introduction

The activity of laryngeal motoneurones and laryngeal muscles is closely related with respiration, airway protection, deglutition, vomiting (Korpáš and Tomori 1979, Grélot and Miller 1994) etc. The behaviour of the larynx depends on complex integration at several levels of the central nervous system (Bartlett 1989). The motoneurones to the intrinsic laryngeal muscles extend throughout the rostral-caudal region of the nucleus ambiguus (NA), including the retrofacial nucleus (RFN) to the upper cervical cord (Iscoe 1988). However, little is known about the inputs to the laryngeal motoneuronal pools from the well-defined respiratory regions of the dorsal and ventral respiratory groups (DRG and VRG) within the medulla. The larynx is also an extremely sensitive source of respiratory protective and defensive reflexes. Mechanical, chemical or electrical stimulation of the laryngeal mucosa elicits either apnoea and tonic laryngeal closure (Szereda-Przestaszewska and Widdicombe 1973), or coughing (Jiménez-Vargas *et al.* 1962, Boushey *et al.* 1972) and the expiration reflex (ER) (Korpáš 1972, Stránsky and Tomori 1979) with concomitant cardiovascular and bronchomotor responses (Tomori and Widdicombe 1969). Information regarding the central mechanisms which integrate the expiration reflex (ER) is incomplete (Jakuš *et al.* 1985, 1987, Bongianni *et al.* 1988, Dyachenko 1990). The present study was designed to examine the effects of focal medullary cold block on quiet breathing, laryngeal resistance and on the character of the expiration reflex evoked by mechanical stimulation of the vocal folds in cats.

Methods

The experiments were performed on twelve non-paralyzed cats of either sex (mean body weight 2.8±0.1 kg). The animals were first anaesthetized with 3 % Halothane and then with α-chloralose (Merck, 55 mg/kg) intravenously. The surgical preparation was described in detail previously (Stránsky *et al.* 1973, Jakuš *et al.* 1990). Briefly, after tracheostomy both femoral veins and a femoral artery were cannulated for further venous injections and measurements of arterial

blood pressure (BP). A cannula for recording pleural pressure (P_{pl}) was introduced into the right pleural cavity. Electrical activity of the sternal part of the diaphragm (EMG_d) was recorded with a bipolar needle electrode. The trachea was sectioned below the larynx and both ends were cannulated with tracheal tube. The caudal tube was tied to the lower cervical trachea and connected to a pneumotachograph (Commet LBL 50) to record a tracheal airflow ($\dot{V}$). The second tube was inserted below the cricoid cartilage. A wide suprahyoid pharyngostomy was performed and the epiglottis was fixed; thus, pressure above the vocal folds was equal to atmospheric pressure. A stream of humidified warm air passed through the rostral tube and larynx at a constant flow rate of 0.25–1.0 l/min. The transglottal pressure (P_{tg}) across the larynx was measured from the infralaryngeal region and referred to the atmospheric pressure. Laryngeal resistance (R_{lx}) in inspiration and expiration was calculated as the ratio of peak inspiratory or expiratory transglottal pressure to the constant laryngeal airflow. The elicitability, intensity and character of the expiration reflex were tested during the focal medullary cold blocks. The expiration reflex was elicited by mechanical stimulation of the glottal region using a nylon fibre loop (diameter 0.3 mm).

The animals were placed in a stereotactic frame. The dorsal surface of the medulla was exposed by occipital craniotomy and partial cerebellectomy. The locations of NA, NPA and BOT were determined with reference to stereotactic co-ordinates (Berman 1968) and by extracellular recordings of units with respiratory burst activity. Unilateral focal cold block of the individual respiratory nuclei was carried out on the right or left sides of the medulla by introduction of a needle thermode (T-01, Tesla) with an external diameter of 0.6 mm (Jakuš *et al.* 1988). The temperature of the thermode tip was measured using a semiconductor thermoprobe (AP-212, Tesla) and was maintained at 20 °C throughout the procedure. The temperature gradient was measured intramedullarly between the tip of the thermode and a locus 1 mm from it by means of a second thermoprobe. The gradient averaged 8–10 °C, depending on the blood supply in the given area. This method (Bénita and Condé 1972, Jakuš *et al.* 1990) allowed a selective reversible block of synaptic transmission without disturbing the impulse conduction in dendrites and neurites. Introduction alone of the thermode into the respiratory nuclei did not produce any changes in the character of baseline breathing or blood pressure. After completing the experiment, the brain stem was removed for histological verification of cooling sites, using sections 50 μm thick (Fig. 1). Arterial blood gas tensions and pH values were monitored throughout the experiments. Rectal temperature was maintained at 37 °C.

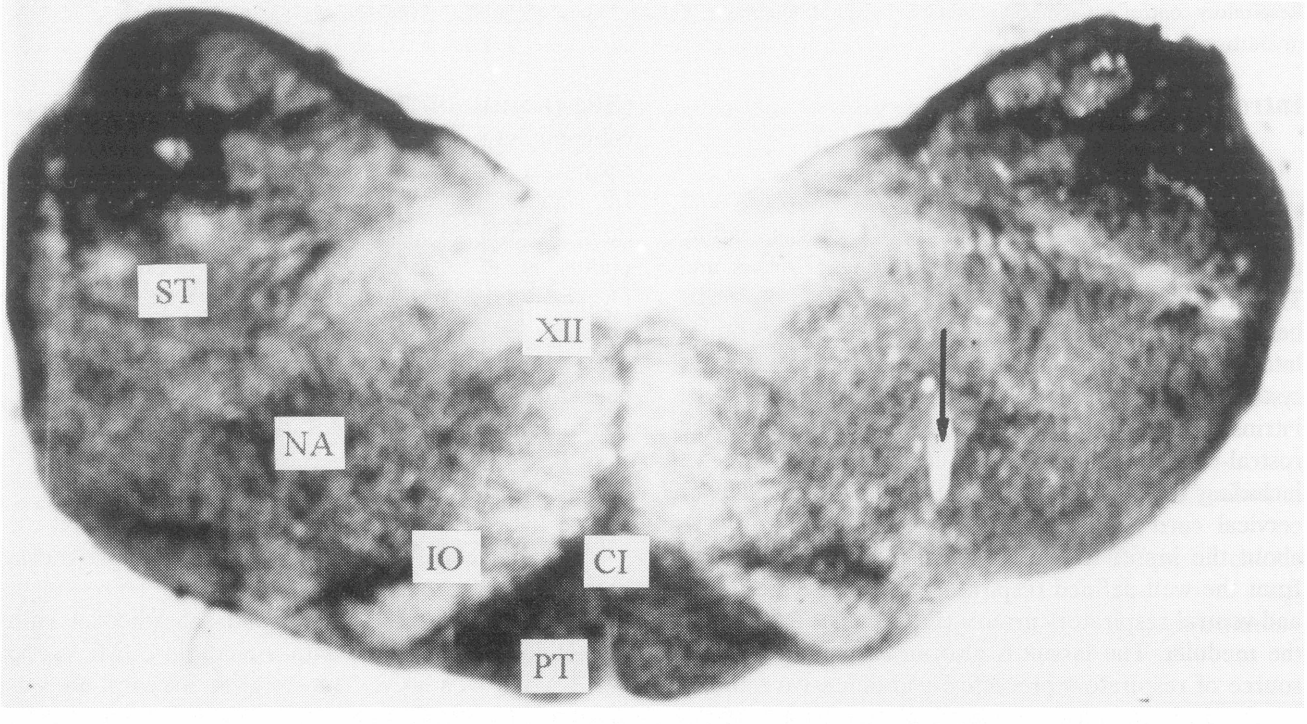

Fig. 1
Transversal medullary section demonstrating the introduction of the thermode (↓) into the region of the NA. Coordinates: + 0.5, 3.0, 3.8. Abbreviations: PT – pyramidal tract, CI – central inferior nucleus, IO – inferior olive, NA – nucleus ambiguus, ST – spinal trigeminal nucleus, XII – hypoglossal nucleus.

Table 1
Mean values of the respiratory rate (f), duration of inspiration (t_I) and expiration (t_E), inspiratory and expiratory airflows ($\dot{V}_I$ and $\dot{V}_E$), inspiratory (Ppl_I) and expiratory (Ppl_E) pleural pressures and the mean arterial blood pressure (Mean BP) under baseline conditions (Control), during cooling (Cold block) and after rewarming (Rewarming) of the respiratory nuclei (NA, NPA and BOT) to the given thermode (T) temperatures

Area	n	Conditions	T (°C)	f [min^{-1}]	t_I [s]	t_E [s]	$\dot{V}_I$ [l/min]	$\dot{V}_E$ [l/min]	Ppl_I [kPa]	Ppl_E [kPa]	Mean BP [kPa]
	7	Control	34	13.86±0.34	1.61±0.09	2.61±0.15	1.05±0.07	1.80±0.24	−0.46±0.04	−0. 16±0.04	17.5±1.1
NA	7	Cold block	20	11.00±0.95*	3.25±1.08	3.01±0.14*	1.20±0.18	1.55±0.25	−0.47±0.06	−0.15±0.04	16.1±1.1
	7	Rewarming	34	13.86±0.39	1.61±0.09	2.83±0.20	1.20±0.10	1.80±0.24	−0.51±0.05	−0.21±0.04	18.2±0.7
	9	Control	34	14.10±0.60*	1.90±0.13	2.38±0.15	1.30±0.19	2.30±0.40	−0.42±0.04	−0.21±0.05	17.6±1.3
NPA	9	Cold block	20	6.10±1.00****	7.11±1.48***	3.26±0.45*	1.25±0.22	2.25±0.42	−0.43±0.03	−0.20±0.04	−13.5±1.1*
	9	Rewarming	34	13.90±0.8####	1.72±0.15###	2.54±0.17	1.35±0.19	2.40±0.35	−0.50±0.04	−0.19±0.04	15.7±1.1
	9	Control	34	15.44±1.09****	1.68±0.13***	2.41±0.20	1.25±0.15	2.20±0.22	−0.45±0.03	−0.10±0.06	17.7±0.8
BOT*	9	Cold block	20	13.11±2.04*	2.92±0.56	2.58±0.31	1.00±0.15*	1.55±0.22	−0.40±0.03**	−0.10±0.06	11.8±1.1***
	9	Rewarming	34	15.78±1.02	1.51±0.123	2.33±0.16	1.40±0.1433	2.25±0.22	−0.47±0.023	−0.12±0.07	15.8±0.933

*Values are means ± S.E.M., n is number of tests. Significance compared with the control values: * $p<0.05$, ** $p<0.02$, *** $p<0.01$, **** $p<0.001$, compared with cold block # $p<0.05$, ## $p<0.02$, ### $p<0.01$, #### $p<0.001$.*

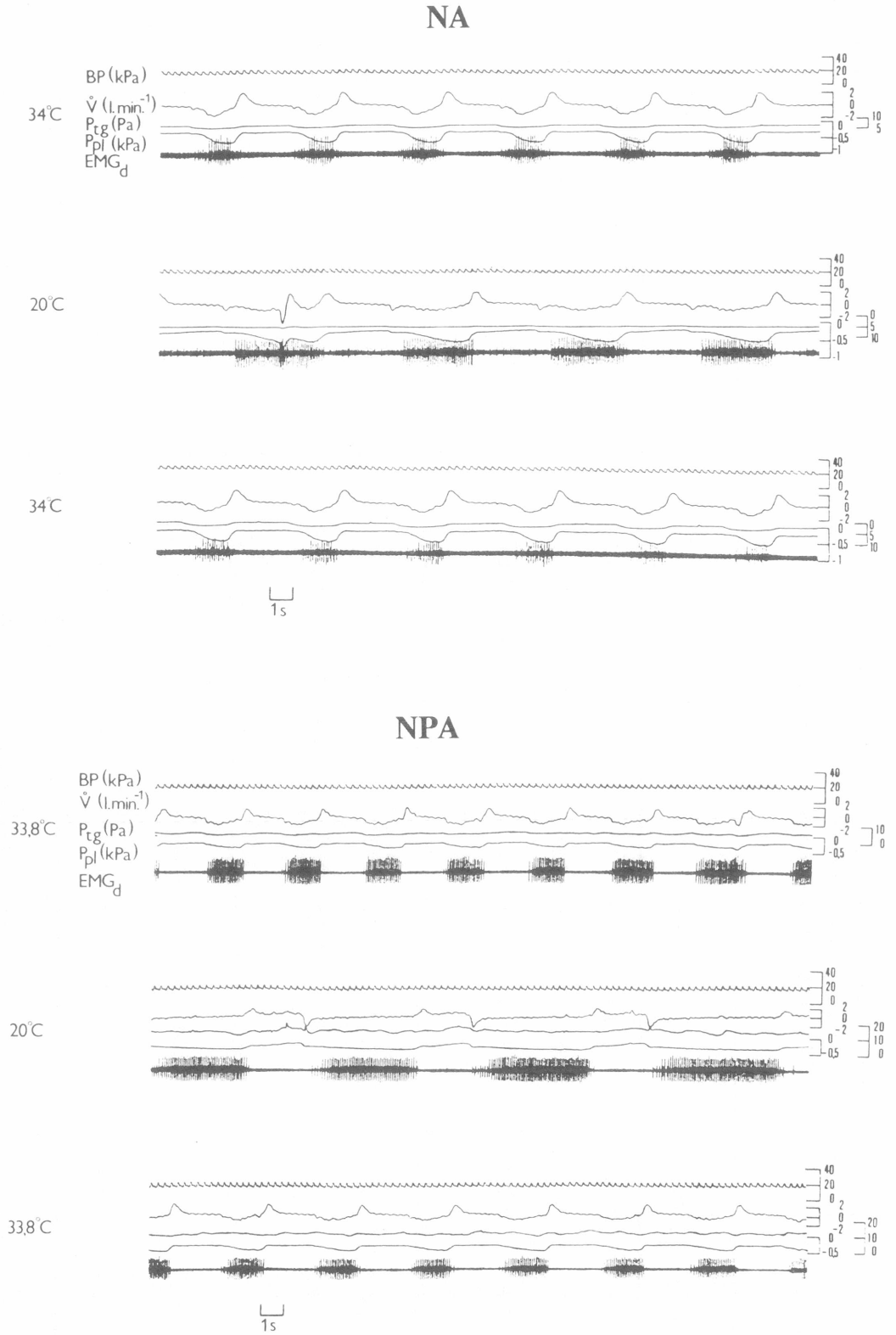

Fig. 2

Effects of focal cooling of the NA (upper part) and the NPA (lower part) on arterial blood pressure (BP), airflow ($\dot{V}$), transglottal pressure (Ptg), pleural pressure (Ppl) and electromyogram of the diaphragm (EMG_d) in control (top record), during cooling (middle record) and after rewarming (bottom record). Cooling site of the NA: +1.0 (mm rostral to the obex), L 3.5 mm (lateral to the midline), D 3.6 mm (below the dorsal surface of medulla). Cooling site of the NPA: +3.2, L 3.0, D 3.9.

Blood, pleural and transglottal pressures as well as tracheal airflow and EMG_d were monitored, filmed with a camera (OK-3, Medipan) from an oscilloscope screen (Tektronix, type 5223) and stored. The recordings were used for evaluating the respiratory frequency (f), inspiratory (t_I) and expiratory (t_E) times, derived from the duration of EMG_d during breathing (Jakuš *et al.* 1990) and from the expiratory airflow in reflexes, maximal inspiratory and expiratory tracheal airflows ($\dot{V}I_{max}$ and $\dot{V}E_{max}$) as well as maximal inspiratory and expiratory pleural pressures (P_{plImax} and P_{plEmax}). Maximal transglottal pressures in inspiration and expiration (P_{tgImax} and P_{tgEmax}) were used to calculate the inspiratory and expiratory laryngeal resistances (R_{lxI} and R_{lxE}). The obtained data were statistically evaluated by means of Student's t-test.

Results

Unilateral focal cold block of the BOT was performed in areas 3.9–5.4 mm rostral to the obex, 3.0–3.7 mm lateral to the midline and 3.7–5.5 mm below the dorsal surface of the medulla. Typically, expiratory neurones with an augmenting discharge pattern (BOT E-Aug) were identified prior to cooling. The NPA was cooled over an area 2.5–3.5 mm rostral to the obex, 2.9–3.2 mm lateral to the midline and 3.0–4.3 mm below the dorsal medullary surface. The neurones exhibited predominantly I-Aug and E-Aug burst patterns. Similarly, focal cooling of either right or left NA regions was performed (co-ordinates: +0.5–2.0 mm, 3.3–3.9 mm, 3.3–3.9 mm, respectively).

Breathing pattern

Table 1 summarizes the mean values of respiratory parameters and the mean arterial blood pressure in twelve cats before and during focal cold block in the region of NA, NPA and BOT. Following focal cold block in the area of NA (Fig. 2A) there was a slight drop in the respiratory frequency and mild increase in the duration of expiration compared with the pre-cooling baseline state. Cold block in the area of NPA (Fig. 2B) led to apnoeic breathing with a 2.3-fold drop in the respiratory frequency, mainly on account of the increased duration of inspiration, without significant changes in either pleural pressure or airflow. The inspirations during cooling of the NA and NPA were accompanied by short inspiratory efforts resembling gasps (Fig. 2A) in 43 and 44 % of cases, respectively. Cooling of the BOT caused a 1.7-fold rise in inspiratory duration with a concomitant drop in pleural pressure and inspiratory airflow values compared to the pre-cooling baseline state. In addition, different responses in breathing frequency – either slowing (in five cats) or acceleration (in two cats) were observed. None of the cold blocks in the BOT area were accompanied by gasp-like efforts. Spontaneous rewarming in the estimated nuclei to the control temperature (34 °C) led to gradual recovery to the baseline breathing parameters. A transient drop in the mean arterial blood pressure accompanied the cooling of both NPA and BOT.

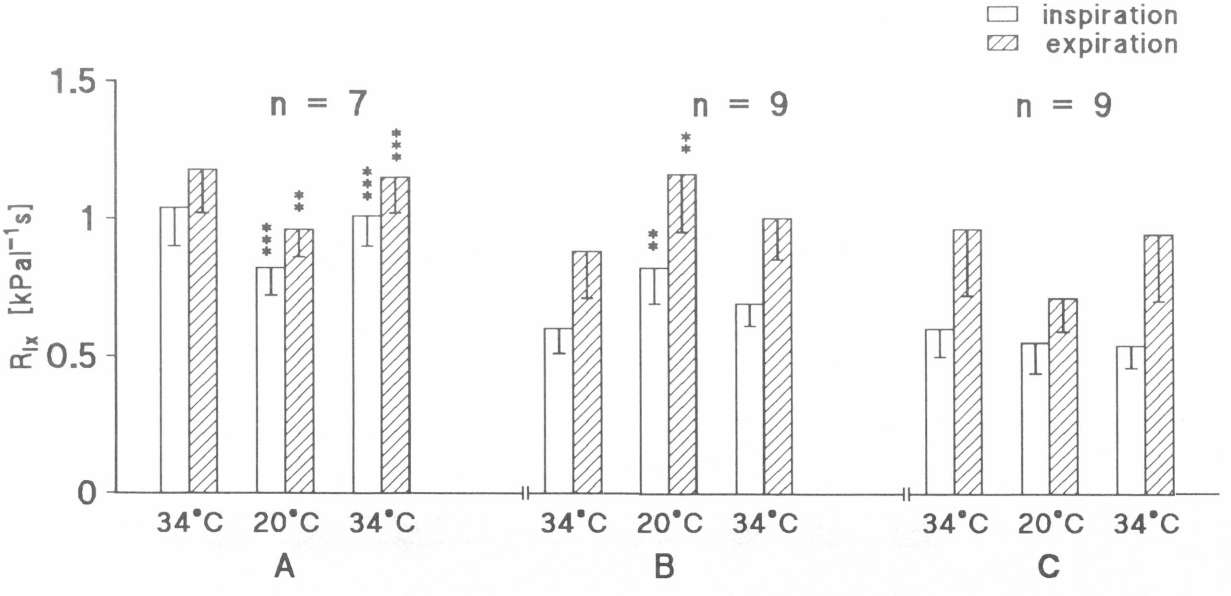

Fig. 3

*Maximal inspiratory and expiratory laryngeal resistances (R_{lx}) in control (34 °C), during (20 °C) and after (34 °C) focal cooling of the NA (A), the NPA (B) and the BOT (C). Values are means ± S.E.M., n = number of tests. Cold block values were compared with the controls; rewarming values to cold block values. ** = $p<0.02$, *** = $p<0.01$. For symbols see text.*

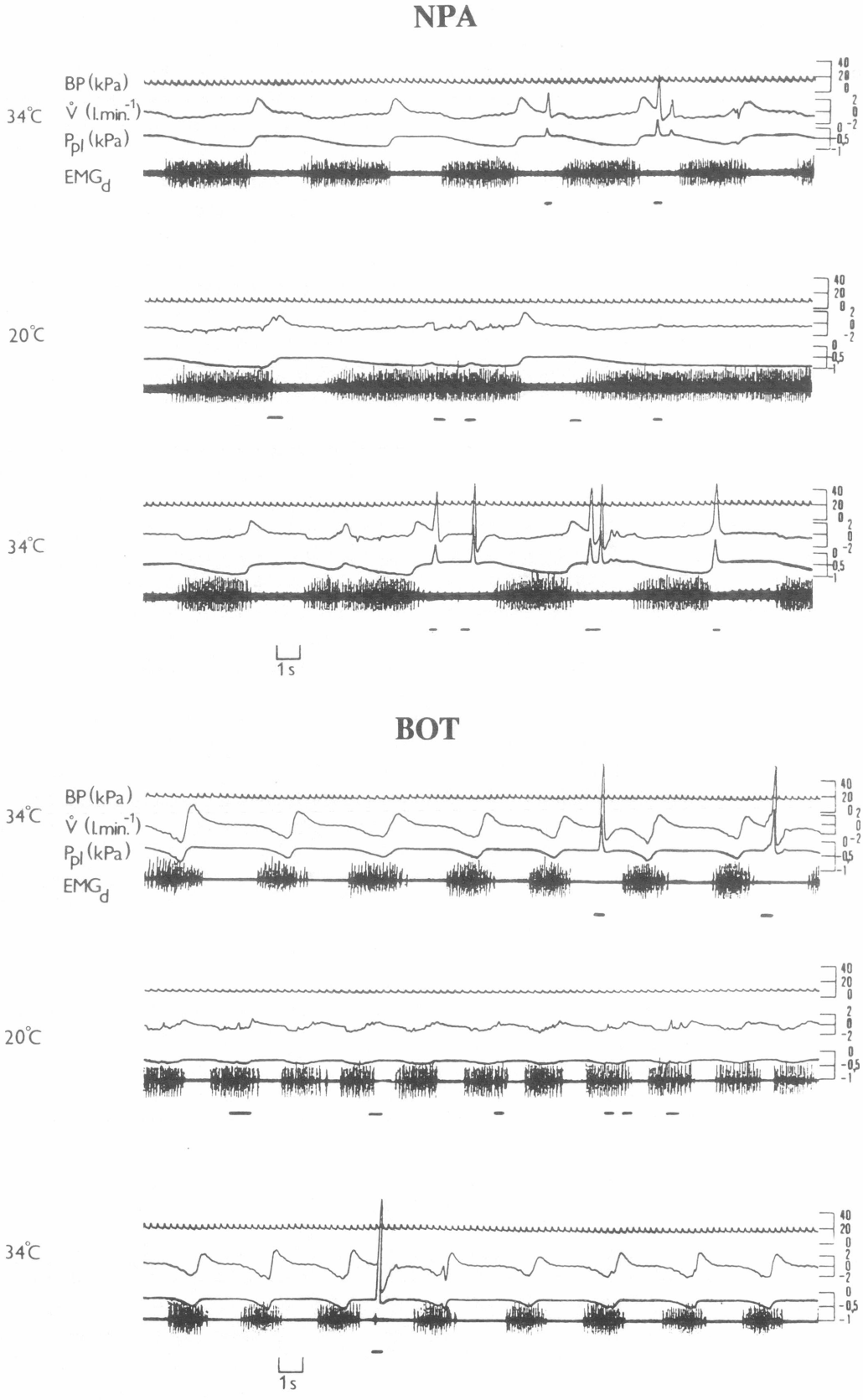

Fig. 4

Effects of focal cooling of the NPA (upper part) and the BOT (lower part) on expiratory reflex elicited by mechanical stimulation of the vocal folds. Recorded parameters: arterial blood pressure (BP), airflow ($\dot{V}$), pleural pressure (Ppl) and electromyogram of the diaphragm (EMG_d). Bars indicate the mechanical stimulation. Cooling site of the NPA: +3.5, L 3.2, D 4.2. Cooling site of the BOT: +5.1, L 3.5, D 5.2. Further description as in Fig. 1.

Table 2
Mean values for duration (t_{ER}), airflow ($\dot{V}_{ER}$) and pleural pressure (Ppl_{ER}) of expiration reflex and the expiratory variables during quiet breathing

Area	Conditions	T °C	Breathing n	t_E [s]	$\dot{V}_E$ [l/min]	Ppl_E [kPa]	Expiration reflex n	t_{ER} [s]	$\dot{V}_{ER}$ [l/min]	Ppl_{ER} [kPa]
NPA	Control	34	8	2.51±0.16	1.84±0.18	−0.22±0.04	8	0.203±0.010	7.34±0.46	0.81±0.15
	Cold block	20	8	3.04±0.24	1.81±0.21	−0.23±0.03	8	absent	absent	absent
	Rewarming	34	8	2.70±0.19	2.18±0.17	−0.23±0.03	8	0.214±0.010	6.05±0.78	0.49±0.20
BOT	Control	34	10	1.99±0.17	2.23±0.14	−0.13±0.06	14	0.184±0.020	7.15±0.36	1.71±0.16
	Cold block	20	10	1.99±0.11	1.61±0.13***	−0.13±0.06	4$	0.218±0.020*	4.80±0.25***	0.33±0.08***#
	Rewarming	34	10	1.94±0.09	2.20±0.17#	−0.15±0.05	14	0.199±0.012###	7.09±0.40###	1.75±0.16

For explanation of symbols and significance see Table 1, $ – absent in 10 of 14 animals

Laryngeal resistance

The control values for inspiratory and expiratory laryngeal resistances (1.04±0.14 and 1.18±0.16 kPa.l^{-1}.s, respectively) decreased to 0.82±0.13 and 0.82±0.1 (kPa.l^{-1}.s) during focal cold block of the NA (Fig. 3A). On the contrary, the baseline inspiratory (0.6±0.09 kPa.l^{-1}.s) and expiratory (0.88±0.17 kPa.l^{-1}.s) laryngeal resistances rose to 0.82±0.13 and 1.16±0.21 kPa.l^{-1}.s, following the cooling of the NPA (Fig. 3B). Cooling in the area of BOT did not affect the laryngeal resistance significantly (Fig. 3C). The effect of cold blocks on laryngeal resistance was reversible when the cooling was discontinued. Evaluation of blood gases and pH during cooling of the NA and BOT revealed no significant changes. Compared with the control ($PaCO_2$=3.99±0.18 kPa, PaO_2=11.01±0.48 kPa, pH=7.35±0.01) focal cold block in the area of the NPA (n=6) led to mild reversible hypercapnia (5.27±0.23 kPa, $p<0.01$), hypoxia (9.41±0.43 kPa, $p<0.05$) and acidosis (pH=7.21±0.017, $p<0.01$), testifying to moderate alveolar hypoventilation.

Expiration reflex from the vocal folds

Mechanical stimulation of the vocal folds at the beginning or during control expiration regularly evoked the expiration reflex characterized by brief, forceful expiratory effort with rapid increases in pleural pressure and expiratory airflow, without preceding inspiration. The mean values of parameters recorded in eight cats are shown in Table 2. Stimulations of the vocal folds during cooling of the NPA evoked only slight alterations in pleural pressure and airflow which could not be regarded as expiration reflexes (Fig. 4A). Cold block in the area of BOT failed to evoke any signs of the expiration reflex in 10 out of 14 tests in five cats (Fig. 4B, Table 2). However, in 4 tests on three cats the expiration reflex was present in a weak form. Rewarming of both nuclei to the control temperature restored the original pattern of the reflex observed prior to cooling.

Discussion

In this study we used the method of unilateral focal cold block of respiratory nuclei. Our former findings (Jakuš *et al.* 1990) had proven a bilateral symmetry of changes in the left and right parts of the bulbar motor output during unilateral cooling. In agreement with Budzińska *et al.* (1985 a,b) interconnections between symmetrically localized structures in two halves of the medulla are important for respiratory rhythm maintenance in the cat. This study revealed a fall in the frequency of breathing with a concomitant rise in duration of inspiration following

focal cold block of the NPA, and a drop in intensity of inspiration in the case of the BOT cold block. These findings are in accordance with the results of Budzińska *et al.* (1985 a,b,c) who also described some depression in intensity of the inspiratory motor output during cooling in the ambigual-paraambigual areas (Budzińska *et al.* 1985a). This was confirmed in our previous report (Jakuš *et al.* 1990) concerning cooling the rostral part of the nucleus retroambigualis to 15 °C. Under the present experimental conditions, cold block of the BOT led either to a fall or a rise in respiratory frequency primarily in conjunction with a mild increase in inspiratory duration. However, an increase in respiratory frequency as a consistent response to cold block of the BOT was also reported (Budzińska *et al.* 1985b). These differences in findings may be due to the extension of the blockade of functionally variable neuronal populations, using thermodes of a different size. Our present findings that focal cooling in the NA slightly altered the eupnoic rhythm of breathing might reflect the block of synaptic transmission in bulbospinal neurones located in close proximity to the NA. During cooling in the NA and the NPA areas, but not the BOT region, short inspiratory gasp-like efforts were observed during inspirations. These could be related to partial disinhibition of the gasp generator in the lateral tegmental field, probably owing to removal of some pontine influences (St. John 1990).

In our experiments, the larynx was excluded from the breathing circuit, but it remained neurally intact. The maximal baseline values of laryngeal resistance varied among animals, but the range of variability resembled that found previously using a similar method (Stránsky *et al.* 1973). Introduction of the thermode into the NA area increased the baseline values of laryngeal resistance. This is most likely due to mechanical damage of the medullary tissue which contains a dense population of laryngeal motoneurones with lower thresholds (Iscoe 1988). One of the major findings of this study was that focal cooling in the NPA consistently increased the values of both the inspiratory and expiratory laryngeal resistance, in spite of mild hypercapnia and hypoxia (see Results), which regularly have opposite effects (Glogowska *et al.* 1974, Iscoe 1988). Budzińska *et al.* (1985c) reported CO_2-dependent enhancement of rhythmic activity in expiratory pump muscles in response to focal cold block of the NPA. They suggested that the effects are due to disinhibition of the neural mechanisms governing the threshold and intensity of expiratory motor output. It remains to be established whether these neural mechanisms influence the expiratory laryngeal motoneurones similarly, or if the increase in laryngeal resistances is also mediated by a blockade of inhibitory inputs from BOT expiratory neurones to laryngeal motoneurones (Jiang and Lipski 1990), or if other sources are involved. There were no significant changes in laryngeal resistance compared to the controls, during focal cooling of the BOT. Since the BOT E-Aug neurones have an inhibitory effect on expiratory laryngeal motoneurones (Jiang and Lipski 1990), cold block of the BOT may increase the activity of laryngeal adductors. The opposite effect (decrease in R_{lx}) could be mediated by the cold blockade of post-inspiratory neurones located in the area of the RFN (Bianchi *et al.* 1988) which may control laryngeal adductors. Since we did not find any significant increase in laryngeal resistance during cold block of the BOT, the involvement of post-inspiratory RFN neurones in laryngeal motor control could be assumed.

Unlike the cough or sneeze, the expiration reflex starts directly, without initial inspiration, with a short burst of activity in the lumbar motoneurones followed by a prompt and large increase in pleural pressure and expiratory airflow. These are taken to demonstrate the active nature of expiration (Korpáš and Tomori 1979). Another important feature of this reflex is that it can be elicited more easily, when stimuli are applied during the expiratory rather than the inspiratory phase of the respiratory cycle (Korpáš and Tomori 1979, Nishino and Honda 1986). The changes in laryngeal motoneurone activity and laryngeal resistance during the ER, were reported previously by Stránsky and Tomori (1979). Little information is available, however, about the central mechanisms integrating the expiration reflex . Our earlier studies (Jakuš *et al.* 1985, 1987) showed that the pontine and upper medullary regions might be involved. The recent work of Bianchi *et al.* (1988) demonstrated excitatory effects of superior laryngeal nerve (SLN) stimulation on inspiratory and expiratory neurones found in the BOT and the RFN. This is in agreement with our finding, showing that the cold block of the BOT greatly increased the ER threshold. On the contrary, Bongiani *et al.* (1988) reported inhibition of the BOT E-Aug neurones. However, they also noted excitation in a majority of bulbospinal expiratory neurones of the caudal part of VRG during the expiration reflex, evoked by SLN stimulation. Our cold block experiments also revealed that the intermediate part of the VRG containing primarily inspiratory neurones is important for the maintenance of the expiratory reflex. Nevertheless, it cannot be excluded that cooling in the NPA area may have reached some neurones in the lateral tegmental field which, according to Dyachenko (1990), may form the central arc of the expiration reflex.

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Dr. J. Jakuš, Department of Biophysics, Jesenius Faculty of Medicine, Comenius University, Malá Hora 4, 037 54 Martin, Slovak Republic.