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Hypoventilation after Acute Phrenicotomy of the Urethane Anaesthetized Rats

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Summary

The aim of this study was to explore the mechanism resulting in hypoventilation in rats with denervated diaphragm. Bilateral cervical phrenicotomy (PX) was performed in 15 male rats anaesthetized with urethane (1.3 g/kg i.p.); other 8 rats were sham operated (SX). Ventilation, Pa_{CO2} and the integrated EMG of the external intercostal muscles (iEMG) were measured before and after the surgery, at regular intervals, up to 4 hours postoperatively. During the 4 hours after PX there was a progressive decrease in minute ventilation and an increase in Pa_{CO2} compared with the control values and with that in the SX rats. The increase in Pa_{CO2} was accompanied by an increase in the peak amplitude of the iEMG to 155 ± 18 % of control values after PX and to 228 ± 33 % 4 hours later. Despite the augmented EMG activity tidal volume gradually decreased. The iEMG of the intercostal muscles, however, did not reach a maximum because the shortlasting stimulation of breathing by acute hypercapnia and hypoxia as the result of added dead space (0.5 ml) increased the iEMG still further. These results indicate that both the central and peripheral mechanisms contribute to hypoventilation in anaesthetized rats with denervated diaphragm.

Key words

Phrenicotomy – Hypoventilation – Peripheral failure – Central control

Introduction

Bilateral paralysis of the diaphragm may be followed by death in rabbits (Sant'Ambrogio *et al.* 1970), hypoventilation in cats (Sant'Ambrogio *et al.* 1970) or normal ventilation in dogs (DeTroyer and Kelly 1982, Stradling *et al.* 1987). In urethane anaesthetized rats it resulted in hypoventilation (Nacházel and Paleček 1990). This may be due to an insufficiency of respiratory muscles and/or to an attenuation of the central output. It was found in experimental animals (Aldrich and Apel 1985, Aubier et al 1981, Viires *et al.* 1984) and humans (Juan *et al.* 1984, Roussos and Macklem 1977) that a failure of the inspiratory muscles developed when the demands were increased out of proportion to their capacity. A similar situation may arise in rats after bilateral phrenicotomy when the main inspiratory muscle, the diaphragm, is denervated. The remaining inspiratory muscles are burdened and fatigue may develop. On the other hand, Bellemare and Bigland-Ritchie (1987) reported that about 50 % decline of the diaphragm force in awake human subjects during inspiratory flow loading could be attributed to a reduction of central motor drive. Such a reduction of central motor drive seems to provide a protective mechanism to prevent exhaustion of the inspiratory muscles. It is not clear, however, whether and to what extent the central inhibition plays a role in acute or chronic respiratory failure. Previous studies of phrenicotomized rats (Nacházel and Paleček 1990) provide only indirect evidence of the extent to which hypoventilation can be apportioned between a peripheral and central component of the inspiratory system.

The present experiments were designed to extend the earlier findings by recording an electromyogram from the external intercostal muscles. Specifically, the purpose of this study was to ascertain the possible participation of central vs. peripheral mechanisms of hypoventilation in anaesthetized rats after bilateral cervical phrenicotomy.

Material and Methods

Animals

Twenty-three healthy male rats of the Wistar strain weighing 250 to 320 g were anaesthetized with urethane (1.3 g/kg i.p.). The animals were intubated surgically with an endotracheal tube inserted between the 3rd and 4th tracheal rings; they were placed in the supine position and breathed spontaneously throughout

Table 1
Hypoventilation in phrenicotomized rats

		f	V_T	V'_E	Pa_{CO_2}	iEMG	V_T/t_I	t_I/t_T
		(c/min)	(ml)	(ml/min)	(kPa)	(%)	(ml/s)	
Control	SX	101 ±7	1.90 ±0.21	192 ±17	4.76 ±0.15	100 ±0	8.0 ± 0.9	0.40 ±0.02
	PX	100 ±13	1.97 ±0.27	194 ±19	4.88 ±0.27	100 ±0	7.8 ±0.8	0.41 ±0.04
X	SX	100 ±8	1.90 ±0.21	190 ±16	4.76 ±0.16	100 ±0	8.0 ±0.9	0.40 ±0.02
	PX	80 ±8	1.79 ±0.26	143 ±21	5.65 ±0.41	155 ±18	6.4 ±0.8	0.37 ±0.04
2 hours	SX	95 ±5	1.95 ±0.13	186 ±15	4.87 ±0.35	114 ±18	8.3 ±0.7	0.38 ±0.02
	PX	74 ±7	1.65 ±0.27	122 ±20	6.42 ±0.49	189 ±20	5.7 ±0.8	0.36 ±0.4
4 hours	SX	90$^+$ ±7	2.01$^+$ ±0.11	180$^+$ ±13	5.04$^+$ ±0.31	122$^+$ ±12	8.1 ±0.8	0.38 ±0.02
	PX	62* ±6	1.46* ±0.23	91* ±16	7.45* ±0.53	228* ±33	4.6* ±0.7	0.33* ±0.04

Values are means ± SD; f = frequency of breathing; V_T = tidal volume; V'_E = minute ventilation; Pa_{CO_2} = arterial partial pressure of CO_2; iEMG = integrated EMG of external intercostal muscles; V_T/t_I = mean inspiratory flow; t_I/t_T = inspiratory duty cycle; SX = sham operated rats; PX = phrenicotomized rats; Control = the control measurement; X = measurement 5 min after the operation; 2 hours, 4 hours = measurements 2 and 4 hours later. All values after phrenicotomy are statistically different from control and sham operated ($p<0.001$). $^+$ = $p<0.05$ for comparison with control values; * = $p<0.01$ for comparison with values 5 min after phrenicotomy (PX).

the experiment. The left common carotid artery was cannulated for sampling the arterial blood. The blood samples were immediately analyzed in a blood gas analyzer (Radiometer PHM 72) and the partial pressure of carbon dioxide (Pa_{CO_2}) was determined. Colonic temperature was monitored continuously with a termistor probe and maintained constant by external heating, as necessary.

Respiratory variables

The air flow was measured with a pneumotachometer connected to a differential pressure transducer (Hewlett-Packard 270); tidal volume was obtained by electronic integration of the flow signal. Both signals were displayed on the screen of a cathode ray tube and recorded on a strip chart recorder (Hewlett-Packard 322). The air flow signal was also recorded on magnetic tape. The electromyogram (EMG) was recorded with a concentric needle electrode (diameter 0.2 mm) inserted into the external intercostal muscles in the 2nd or 3rd intercostal space in the mid-axillary line. The needle electrode was fixed in the same site throughout the duration of the experiment. The electrical signal from the preamplifier was filtered, displayed simultaneously on a cathode ray tube, monitored by a loud speaker and stored on magnetic tape (EMN Tesla) for further analysis. The EMG was subsequently rectified and integrated (time constant 80 ms) and recorded on a UV-oscilloscope (Honeywell 1706 Visicorder) together with the air flow signal. To quantify the EMG activity, the peak

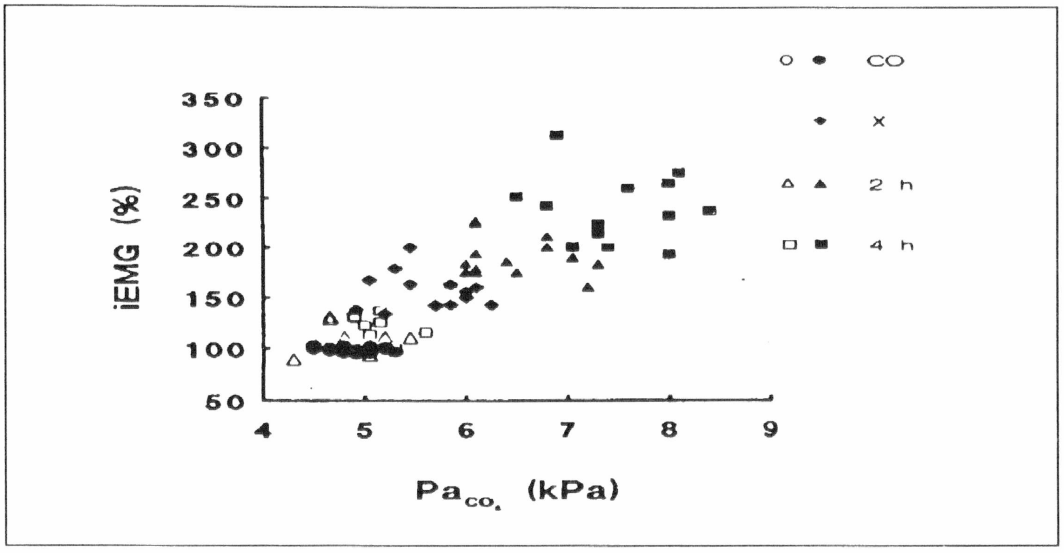

Fig. 1

Relationship between arterial partial pressure of CO_2 (Pa_{CO2}) and integrated electromyogram of external intercostal muscles (iEMG) in 8 sham-operated (open symbols) and 15 phrenicotomized rats (filled symbols). The symbols represent individual values obtained throughout the experiment (during the control measurement (CO), 5 min after PX (X), and 2 (2 h) and 4 (4 h) hours later). During this time both the iEMG and Pa_{CO2} increased.

amplitude of the integrated signal was measured. Stimulation of breathing, for a period of 5 minutes, was achieved by inserting a dead space (a tube of 0.5 ml in volume) between the tracheal cannula and the pneumotachometer.

Protocol

The first, preoperative measurements will be referred to below as "control" measurements. After the control measurements all the 23 animals were randomly divided into two groups. Bilateral phrenic nerve transection (PX) was performed in the first group of rats (15 animals). The section of the phrenic nerves was performed low in the neck where the nerves leave the cervical plexus. The completeness of phrenicotomy was verified by recording the EMG of the diaphragm; a satisfactory record was obtained before, and it was totally absent after phrenicotomy. Immediately after phrenicotomy the rib cage expanded during inspiration, but the abdomen moved paradoxically inward. Eight animals of the second group were sham operated by exposing their phrenic nerves (SX). The sham operated rats were subjected to an identical procedure except for cutting the nerves. After recording the control values the next measurements were made within 5 minutes after the operation and 2 and 4 hours later.

Data analysis

Ten consecutive breaths were analyzed and averaged in any given period. From the record, tidal volume (V_T) and the durations of inspiration (t_I), expiration (t_E) and of the cycle (tT) were measured. From these measurements the respiratory rate (f), minute ventilation (f*VT), inspiratory duty cycle (tI/tT) and mean inspiratory flow (V_T/t_I) were calculated. The peak amplitude of the integrated EMG signal (iEMG) was expressed in arbitrary units. The EMG activity values were calculated in percent of the control values. The data are presented as means ±S.D. Student's test for paired data was used to compare the difference in the same rat within a group. To compare the two groups of rats, analysis of variance with the Bonfferoni test was applied. The correlation coefficient was calculated by the method of least squares. A $p<0.05$ was considered statistically significant.

Results

Resting breathing

Bilateral cervical phrenicotomy in the urethane anaesthetized rats resulted in hypoventilation (Table 1). During the 4 hours after phrenicotomy there was a progressive decrease in minute ventilation and an increase in Pa_{CO_2} compared with the control values and with those in the SX rats. A decrease of minute ventilation was due both to a decline in tidal volume and to slowing of the respiratory rate. The inspiratory duty cycle and the mean inspiratory flow were reduced progressively (Table 1).

A linear relationship between the Pa_{CO_2} and the iEMG of external intercostal muscles was found both in the PX and SX rats; both Pa_{CO_2} and iEMG increased throughout the experiment. In PX rats the

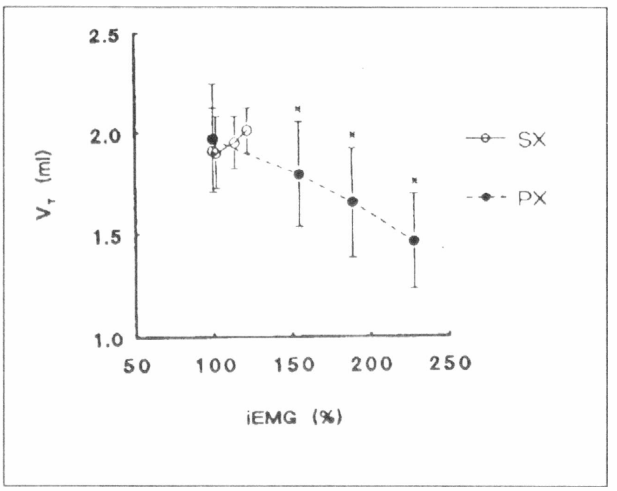

Fig. 2

Relationship between integrated EMG of the external intercostal muscles (iEMG) and tidal volume (V_T) in sham-operated (SX - open circles) and phrenicotomized rats (PX - filled circles). Values are means, bars represent standard deviations. With time the iEMG increased. The phrenicotomized and sham operated rats already differed significantly ($p<0.05$) 5 min after the operation.

increase both in Pa_{CO_2} and iEMG was larger than in the SX rats. The relationship between these parameters is demonstrated in Fig. 1 for each rat throughout the experiment. The correlation coefficient was $r=0.81$ ($p<0.0001$) for PX rats and $r=0.65$ ($p<0.001$) for SX rats.

In SX rats an increase in the integrated electrical activity of the external intercostal muscles during the duration of the experiment was associated with an increase in the tidal volume (Fig. 2). Also in the PX rats the iEMG increased continuously until the end of the experiment. However, despite the increased electrical activity in the intercostal muscles, the tidal volume gradually declined (Fig. 2).

Stimulated breathing

During the control measurement the stimulation of breathing by added dead space led to an isocapnic increase of minute ventilation ($p<0.001$). In SX rats the ventilatory response to the added dead space remained the same throughout the experiment. In contrast, in PX rats, the minute ventilation initially increased with the increase of Pa_{CO_2}. Four hours after phrenicotomy, however, the addition of dead space was followed by an increase of Pa_{CO_2} without any changes in ventilation (Fig. 3).

In both the SX and the PX rats the stimulation of breathing with added dead space was followed by a significant increase in the iEMG and tidal volume. This is showed in Fig. 4 where changes of iEMG and tidal volume are expressed as the difference between resting and stimulated breathing. In the SX rats an increase

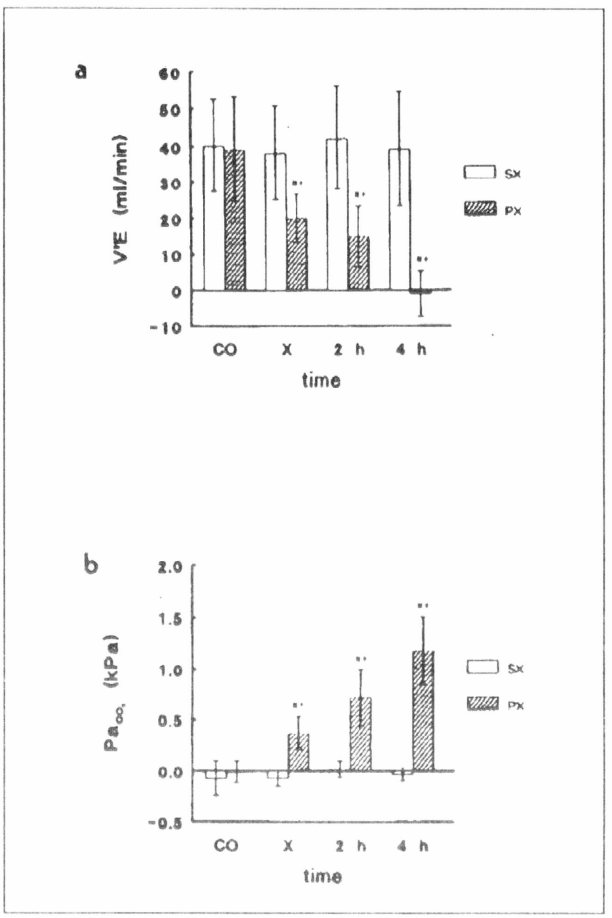

Fig. 3

Effect of stimulation with added dead space on minute ventilation (V'E) panel a), and partial pressure of CO_2 (Pa_{CO_2}) panel b). The values are expressed as differences between resting and stimulated breathing (0 indicates the resting level of breathing). Means ±S.D. are represented in sham-operated rats (SX) by open bars and in phrenicotomized rats (PX) by filled bars. CO = the control measurement; X = measurement 5 min after the operation; 2h, 4h = measurements 2 and 4 hours later. * = significantly different compared to control values (CO); + = significantly different compared with the sham-operated rats (SX) ($p<0.05$).

both in iEMG and tidal volume was the same throughout the experiment. On the other hand, the iEMG during stimulation in phrenicotomized rats increased significantly more compared to the SX rats. An increase in tidal volume of PX rats was initially the same as that in the SX rats, but four hours later the tidal volume increased significantly less.

Discussion

The main findings of the present study are as follows: 1) In the urethane anaesthetized rats there is an immediate hypoventilation (due to decreased tidal volume and rate of breathing) following bilateral cervical phrenicotomy. 2) This hypoventilation is

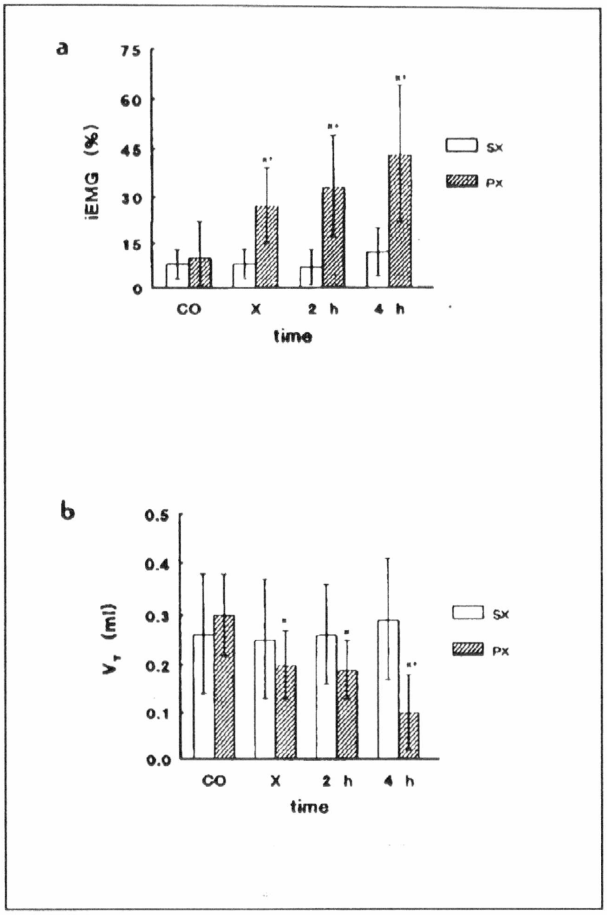

Fig. 4

Effect of stimulation with added dead space on integrated EMG (iEMG) panel a), and tidal volume (V_T) panel b). Symbols and arrangement are the same as in Fig. 3. All the values in SX and PX rats significantly increased during stimulation. * = significantly different compared to control values (CO); + = significantly different compared with the sham-operated rats (SX) ($p<0.05$).

accompanied by an increase in the iEMG of the external intercostal muscles. 3) Throughout the four hours after phrenicotomy there is a progressive increase of the iEMG of external intercostals and a progressive decrease of ventilation in spite of increasing Pa_{CO_2}. 4) Short-lasting (5 min) stimulation of breathing by acute hypercapnia and hypoxia, as the result of added external dead space, at any time after PX is followed by increased iEMG, Pa_{CO_2} and ventilation. Progressively, the increase in Pa_{CO_2} and iEMG is more and the increase in ventilation less pronounced.

The hypoventilation in complete diaphragm paralysis is not surprising. The chest wall mechanics are markedly altered so that the rib cage expansion during inspiration is partly offset by the cephalad movement of the paralyzed diaphragm. This results in smaller tidal volume in spite of increased iEMG of the inspiratory muscles, as evidenced by similar experiments in dogs (Ninane et al 1989, DeTroyer and Kelly 1982, Nochomowitz et al 1981), cats and rabbits (Sant'Ambrogio *et al.* 1970).

The controversial part of the experiments is provided by the results obtained four hours after PX:

The progressive hypoventilation can be explained by central inhibition, resulting in smaller central drive; or by further mechanical disadvantage with which the contractility of the inspiratory muscles is decreased; or by a progressive failure of the inspiratory muscles to respond by adequate contraction to their excitation.

The central inspiratory drive is strongly dependent on the level of chemoreceptor stimulation by CO_2. In phrenicotomized rats an increasing level of Pa_{CO_2} resulted in an increase in the iEMG of the external intercostal muscles (Fig. 1). These results indicate that the central inspiratory drive is augmented in phrenicotomized rats with increased levels of CO_2. Nonetheless, the central drive is not maximal, as evidenced by the increased iEMG during the stimulation of breathing by added dead space (Fig. 4).

The mechanism underlying the less than maximal inspiratory drive is uncertain. The central inhibition after fatigue could arise within the central nervous system from changes in central motoneurone excitability (Kernell and Monster 1982). Alternatively, it might follow a reflex inhibition of motoneurones by afferents from the muscles (Garland et al 1988, Bigland-Ritchie *et al.* 1986). We may also consider central depression by hypercapnia (Barbour and Seevers 1956). The prolongation of the duration of expiration and thus a shortened inspiratory duty cycle is also in accord with central inhibition.

The progressive hypoventilation could also be explained by a continuing mechanical disadvantage of the contracting inspiratory muscles of PX rats. The resting length of the active inspiratory muscles is, if anything, longer than that with the active diaphragm. This is also evidenced by the smaller functional residual lung capacity. At a measurement 4 hours later, however, the functional residual lung capacity did not change significantly, while the tidal volume decreased further (unpublished results). There does not seem to be evidence available that these mechanical conditions deteriorate with time after phrenicotomy. Actually, the work of breathing exerted on the lungs of PX rats decreased in time (Nacházel and Paleček 1990). Also the elastic properties of the lungs and of the respiratory system did not change during the four hours after phrenicotomy (unpublished results).

Since the mechanical characteristics of the respiratory system did not change significantly and the iEMG of the inspiratory muscles increased (Fig. 1) the failure of neuromuscular transmission was not responsible for the decrease in tidal volume. This could be due to an impairment of excitation-contraction coupling and/or a failure of the contractile machinery.

Tidal volume was decreasing (Fig. 2) in spite of increasing iEMG of the external intercostal muscles after phrenicotomy. This indicates that the inspiratory muscles were not able to generate sufficient force even at higher levels of excitation. Our results are similar to those of Merton (1954) who showed unchanging amplitude of the muscle action potential evoked by electrical stimulation of the ulnar nerve despite an almost complete loss of force. He concluded that fatigue was located solely in the muscle itself. Therefore, the discrepancy between the iEMG of the external intercostal muscles and tidal volume in phrenicotomized rats (Fig.2) is likely to be due to the failure of a process beyond the excitation of the muscles. Our observation of an increase in the peak amplitude of the iEMG under these conditions agrees with those of others. Aubier *et al.* (1981) reported that ventilatory failure in dogs with cardiogenic shock was associated with an increase in EMG of the respiratory muscles. Hussain *et al.* (1985) made a similar observation during septic shock, and Lockhat *et al.* (1989), likewise, in dogs whose diaphragm was loaded and underperfused.

We conclude that the hypoventilation observed in phrenicotomized rats is due to peripheral failure of the respiratory system and to submaximal – though increased – drive. The safeguard against complete exhaustion of the inspiratory muscles is achieved by an attenuation of the central respiratory drive. This arrangement may have long-term survival value.

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