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Respiration after phrenicotomy and hydrocortisone treatment in anaesthetized rats

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Respiration after phrenicotomy and hydrocortisone treatment in anaesthetized rats. J. Nacházel, F. Paleček. ©ERS Journals Ltd 1993.

ABSTRACT: The study was designed to determine the extent to which respiratory muscle wasting, resulting from corticosteroid-induced atrophy, may affect respiration in normal rats and in rats with denervated diaphragm.

Twenty four male Wistar rats were divided into four groups: 1) controls with sham operation (SX) and vehicle injections: 2) SX with eight hydrocortisone (HC) injections (60 $mg \cdot kg^{-1} \cdot day^{-1}$ *i.m.*); 3) phrenicotomized (PX), injected with vehicle; 4) PX and HC-treated. HC treatment was started on the thirteenth day after surgery. Under urethane anaesthesia, tidal volume, respiratory rate, arterial carbon dioxide tension ($Paco_2$) and occlusion pressure were measured at rest and after 5 min of stimulated-breathing induced by added dead space 22nd day after surgery.

All HC-treated animals decreased body weight by 32% compared to untreated rats. The diaphragm weight was reduced in PX rats by 29%, and after HC by 44%, while in PX rats with HC treatment diaphragm weight decreased by only 21%. PX rats (HC-untreated) had the lowest minute ventilation and occlusion pressure. There was no difference in ventilation between control and both HC-treated groups at rest. However, ventilation in PX and HC-treated rats did not increase upon stimulation, and the occlusion pressure increased significantly only in the HC-untreated animals.

We conclude that in the rat, HC treatment did not affect resting ventilation, but it impaired ventilation performance, during increased demand, in animals handicapped by diaphragm denervation.

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It is well-known that corticoid treatment results in atrophy, especially of the less active muscles [1]. But even the active muscles, such as those responsible for breathing, can be affected. This has been shown in clinical observations [2–4], as well as in animal experiments [5–7]. Approximately one third of asthmatics treated chronically with systemic glucocorticoids have signs of muscular weakness [8]. Experimentally, MOORE *et al.* [9] found that cortisone treatment in rats resulted in an atrophy of the diaphragm, and correspondingly smaller force of its *in vitro* contractions. However, when related to muscle cross-sectional area, the diaphragm strips of cortisone-treated rats showed force comparable to those of untreated animals. FERGUSON *et al.* [10], working with rabbits, observed no change in diaphragm contractility *in vivo* after corticosteroid therapy, but endurance of the diaphragm was decreased. On the other hand, SASSON *et al.* [11] and VIIRES *et al.* [12] reported that diaphragm atrophy induced by corticosteroid treatment in rats was accompanied by a reduction of normalized force *in vitro*. However, the ventilation and gas exchange were not examined. Also, it is not known whether loading of inspiratory muscles of cortisone-treated rats would be tolerated.

The purpose of this study, therefore was to examine the effect of chronic corticosteroid administration on ventilation in rats under control conditions, and in rats in which the loading of the intercostal muscles was augmented as the result of complete denervation of the diaphragm. The rat model resembles reality in the sense that in patients with chronic obstructive pulmonary disease (COPD) who exhibit hyperinflation, the diaphragm is similarly highly ineffective [13].

Methods

Animals

The experiment was performed on 24 male rats of the Wistar strain. Their body weight at the beginning ranged from 165–222 g. They were divided randomly into four groups, and all the animals were operated on; two groups were subjected to bilateral phrenicotomy (PX) (seven animals each), and two groups underwent sham operation (SX) (five animals each). Half of the PX and half of the SX rats were given hydrocortisone (HC). Their body weight was measured at regular intervals of

2–3 days. The animals had free access to pelleted diet and water.

Phrenicotomy

Under light ether anaesthesia both phrenic nerves were cut in the lower neck region. After careful dissection, both the cervical plexuses were identified and the phrenic nerves were transected within the upper thoracic aperture. The method was described in detail previously [14]. The animals recovered without complications. The sham-operated rats were subjected to an identical procedure, except for cutting the nerves.

Hydrocortisone

Hydrocortisone acetate (Léciva, Praha, Czechoslovakia) was suspended in a 1% solution of carboxymethylcellulose, in the concentration of 60 $mg \cdot ml^{-1}$. Intramuscular injections of 60 $mg \cdot kg^{-1}$ were administered for eight consecutive days. The untreated animals received the same volume of 1% solution of carboxymethylcellulose.

Functional measurements

At the end of the experiment, the rats were anaesthetized with 1.3 $g \cdot kg^{-1}$ urethane *i.p.* A tracheal tube was inserted through a tracheostomy, at the level of the 4^{th}–5^{th} tracheal ring. An arterial catheter was introduced into the left carotid artery. Colonic temperature was measured with a thermistor probe.

To measure tidal volume and respiratory rate the rats were placed in a body plethysmograph for small animals [15]. The pressure changes were measured with a differential pressure transducer (Hewlett-Packard 270) and recorded on a strip-chart recorder (Hewlett-Packard 7786A). The tracheal occlusion pressure was measured by a water-filled tube connecting the tracheal tube with a pressure transducer (Hewlett-Packard 267 BC). To measure occlusion pressure, the tracheal tube was occluded for two breaths at the end-expiratory level. Arterial blood samples (0.1 ml) were withdrawn anaerobically from the carotid artery into heparinized capillary tubes for analysis of arterial carbon dioxide tension ($Paco_2$) on a Radiometer analyser (PHM 72). After obtaining values during quiet ventilation, breathing was stimulated by adding a dead space of 0.5 ml to the tracheal tube and ventilation and occlusion pressure measured after 5 min. Ventilatory parameters were calculated as an average of 10 breaths. Minute ventilation was taken as the product of tidal volume and respiratory rate. The variables were recalculated per 100 g of body weight.

Protocol

Twenty two days after the beginning of the experiment, during which the body weight was monitored, all of the animals appeared healthy and showed no pronounced differences in behaviour and body weight. Surgery was performed and 13 days after the operation hydrocortisone injections were started. They were given daily for eight consecutive days. Functional measurements were performed in anaesthetized animals one day after the last injection, *i.e.* on the 22nd day after the surgery. The rats were than killed with an overdose of pento-barbital. The lungs were removed and examined. The diaphragm was carefully dissected from the ribs, trimmed of tendon, fat, and connective tissues and weighed.

Statistics

Student's t-test for paired data was used to compare the difference in the same rat within a group. To compare the four groups of rats, analysis of variance with the Tukey test was applied. A $p<0.05$ was considered statistically significant.

Results

Body weight

The main effect of hydrocortisone on wasting body tissues is well-documented by the decreased body weight (fig. 1). The initially homogeneous group of animals differentiated according to the treatment: the rats receiving HC lost weight irrespective of whether they had been phrenicotomized or sham-operated, the difference was reaching statistical significance 3–6 days after the first injection. Comparing body weights before injecting the solvent or hydrocortisone with those at the end of the experiment, we obtain the relative changes of +11% and +7% for the SX and PX groups and -22% and -25% for the SX-HC treated and PX-HC treated rats.

Diaphragm weight

Phrenicotomy in untreated rats resulted in a decrease of the absolute diaphragm weight by 29% when compared with that in SX animals (fig. 2). Hydrocortisone administration alone in SX rats also decreased the weight of the diaphragm by 44% (SX-HC compared to SX in figure 2). However, the combination of phrenicotomy with hydrocortisone treatment (PX-HC) resulted in less weight loss of the diaphragm by 21% compared to HC administration alone ($p<0.01$). The relative diaphragm weight in this group of rats was significantly higher, compared with that in the groups SX, SX-HC or PX.

On postmortem examination of the lungs, gross inflammatory changes in 10 out of 12 hydrocortisone-treated rats, and in 1 out of 12 remaining animals, were seen. There were no differences in body temperature between HC-treated rats and those without medication.

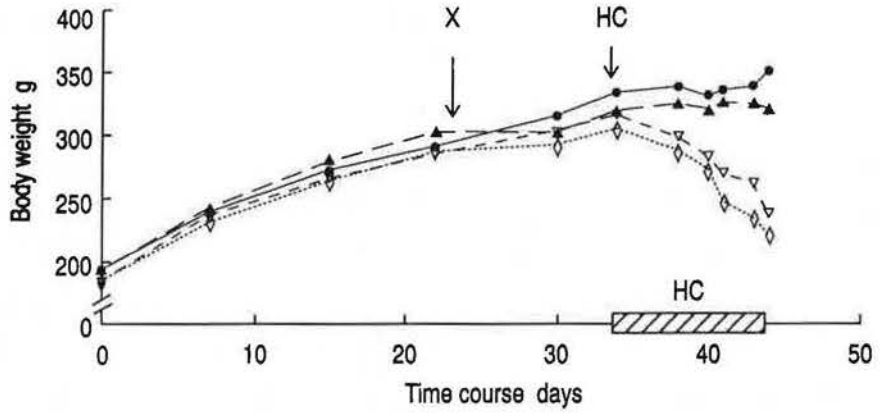

Fig. 1. – The time course of changes in body weight in untreated sham-operated (●) and phrenicotomized (▲) rats, and in hydrocortisone-treated sham-operated (▽) and phrenicotomised (◊) rats. Values represent the mean of the group. X: the time of surgery; HC: the first injection of hydrocortisone;▨: HC administered, 60 mg·kg^{-1} *i.m.*, for 8 days.

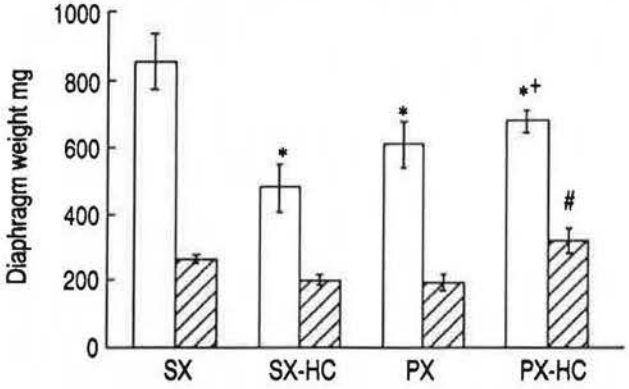

Fig. 2. – Absolute and relative weight of the diaphragm. SX: untreated sham-operated rats; PX: phrenicotomized rats; SX-HC and PX-HC: hydrocortisone treated rats. Absolute values (□): *: significantly different from the control group (SX); +: significantly different from the sham operated, hydrocortisone treated rats (SX-HC). Relative values (▨): #: significantly higher than SX, SX-HC and PX ($p<0.05$).

Table 1. – Resting breathing in urethane anaesthetized rats

	SX	SX-HC	PX	PX-HC
Breathing frequency b·min^{-1}	88 (5)	111 (6)	105 (15)	122 (29)
Tidal volume ml·100g^{-1}	0.54 (0.04)	0.51^{+} (0.07)	0.33* (0.03)	0.44 (0.1)
Minute ventilation ml·min^{-1}·100g^{-1}	47 (3)	57^{+} (9)	34* (4)	53^{+} (16)
Occlusion pressure kPa	1.76 (0.33)	1.68 (0.38)	1.17* (0.22)	1.50 (0.32)
$Paco_2$ kPa	4.03 (0.44)	4.19 (0.32)	4.91 (0.89)	5.40 (1.46)

Results are expressed as means (SD). SX: untreated, sham operated rats; PX: phrenicotomized rats; SX-HC, PX-HC: hydrocortisone treated rats. *: significantly different from the control (SX) rats. $^{+}$: significantly different from the phrenicotomized (PX) rats ($p<0.05$).

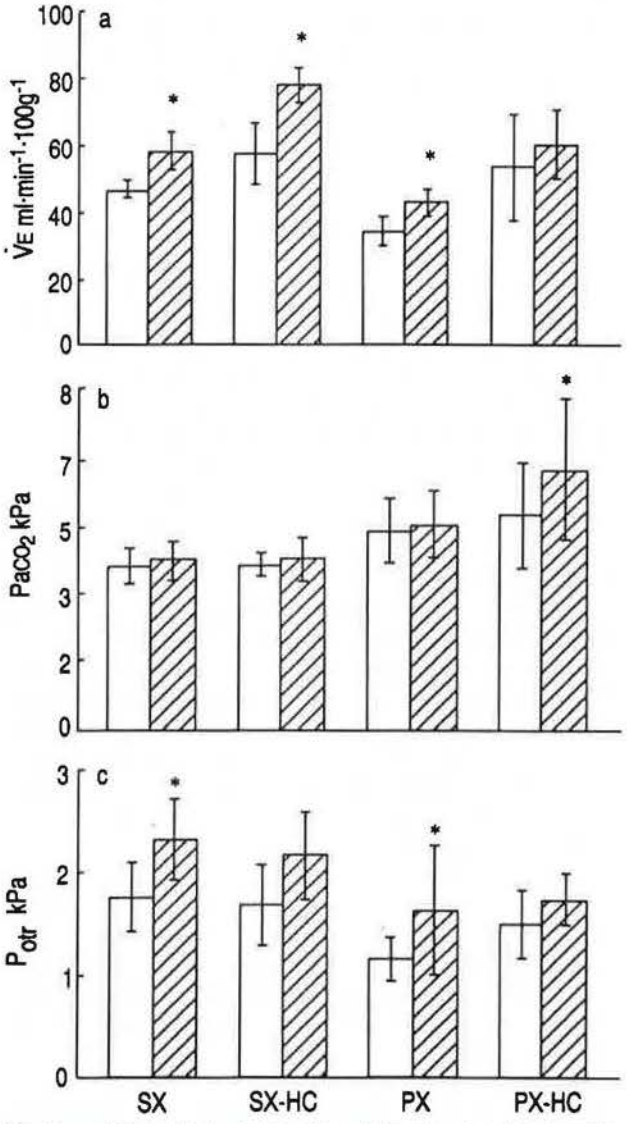

Fig. 3. – Effect of stimulation of breathing on: a) minute ventilation (V_E); b) arterial carbon dioxide ($Paco_2$); and c) occlusion tracheal pressure (Potr). Values are mean±SD. SX: untreated sham-operated; PX: phrenicotomized rats; SX-HC and PX-HC: hydrocortisone-treated rats; (□): resting breathing; ▨ : stimulated breathing; *: difference from resting values, $p<0.05$.

Ventilatory functions

One of the PX rats with HC treatment died before the final measurement. Under anaesthesia, 3 weeks after phrenicotomy, tidal volume, minute ventilation and tracheal occlusion pressure were lower in the PX rats compared to SX; $Paco_2$ and respiratory rate were not significantly different (table 1). HC treatment had no significant effect on breathing in the anaesthetized rats. No difference was observed between HC-treated rats (both the SX and PX rats) and HC-untreated rat with respect to tidal volume, respiratory rate, minute ventilation, occlusion pressure and $Paco_2$ (table 1).

Stimulation of breathing by added dead space increased tidal volume in all of the groups by 22–35% ($p<0.001$); the respiratory rate did not change significantly. In SX, PX and SX-HC rats the stimulation of breathing led to an isocapnic increase in minute ventilation (fig. 3a). In contrast, in PX rats treated with HC the addition of dead space increased $Paco_2$ without significant changes in ventilation (fig. 3b). A significant increase of the occlusion pressure occurred only in the HC-untreated animals (fig. 3c).

Discussion

The main findings in this study are that administration of high doses of hydrocortisone for eight days resulted in a loss of body weight and diaphragm weight. The HC treatment did not impair resting ventilation in either the control or the phrenicotomized rats; however, HC-treated rats with the denervated diaphragm failed to respond adequately to stimulation by added dead space.

Hydrocortisone, compared with other glucocorticoid steroids, is more musculotropic and has less other effects [16]. Nonetheless, similar wasting effects on body and diaphragm weights were observed after cortisone [9], and triamcinolone [12]. The body weight curves exhibit a difference only between HC-treated and untreated animals, without an appreciable effect of phrenicotomy. This is interpreted as a sign of atrophy of body tissues after HC. The 33% difference of body weight between controls (SX) and SX-HC-treated rats is in good agreement with the results of MOORE *et al.* [9], and of VIIRES *et al.* [12], who found a difference of 30% after a comparable time period.

The weight of the diaphragm is expected to decrease both as the result of denervation and of hydrocortisone treatment. Our results with phrenicotomy alone indicate a decrease of denervated diaphragm weight. The results with hydrocortisone treatment alone also show a decrease of diaphragm weight which is in agreement with the values of MOORE *et al.* [9], and VIIRES *et al.* [12].

The combination of HC treatment with denervation presented us with unexpected results: the diaphragm weight was significantly higher than after HC alone, which indicates that the influence of HC and phrenicotomy on the diaphragm weight is not additive. The fact that denervation-induced diaphragm wasting may be somewhat attenuated by HC treatment is surprising, and is difficult to explain, especially considering the hypothesis that an increased number of glucocorticoid receptors due to denervation may be responsible for the denervation atrophy [17, 18]. We can only speculate on the possible species differences: the rat is known to secrete mainly corticosterone, whereas hydrocortisone is much more important in dog or man [19]. We may hypothesize that the denervation atrophy in the rat is mediated by natural glucocorticoid, *i.e.* corticosterone. Hydrocortisone treatment will reduce ACTH secretion, and thus cortisone secretion, by a negative feedback mechanism; therefore, the denervation atrophy with hydrocortisone is less pronounced.

Long-term experiments with bilateral phrenicotomy have shown that some species can survive it without evident problems [20, 21]. In the rat, the only reference that we have found is that of MASKREY *et al.* [22]. Both their experience and ours [23] indicate that the rat survives the decrease of ventilation immediately after bilateral phrenicotomy. The measurements performed in this experiment on awake rats also showed an improvement of ventilation in time. Basically, there was no difference in the pattern of breathing between rats receiving HC and those without it. The results on HC-treated awake rats will be reported elsewhere.

Under urethane anaesthesia in resting conditions, the lowest ventilation, tidal volume and occlusion pressure related to body weight were found in the untreated PX rats, which is in accordance with our previous observations [23, 24]. The gross morphological changes found in 10 out of 12 HC-treated rats led us to assume that their work of breathing was increased by the lower lung compliance and increased resistance due to pneumonia. This alone had no effect on resting or stimulated ventilation, as is evident from $Paco_2$ and ventilation values, which are not different from their respective controls.

The effect of HC is evident only in PX rats during stimulation by added dead space; in spite of increasing $Paco_2$ neither ventilation nor occlusion pressure increased significantly. Rats with HC treatment alone responded to added dead space by an isocapnic increase of ventilation. Thus, there is no substantial difference between HC-treated and untreated rats without phrenicotomy, except for tachypnoea and increased minute ventilation, which may be explained by the underlying pneumonia.

The suggested clinical implications of our study are that the wasting of muscle mass during steroid treatment affects most skeletal muscles, including those involved in breathing, but that their function remains adequate for resting ventilation. The rat experiment showed that the animals with paralysed diaphragm, with decreased body mass, pneumonia (as a result of HC treatment) and higher level of resting ventilation did not respond adequately to stimulation by added dead space. The use of topical corticosteroid administration to asthmatics, eliminates the problem at least for this group of patients.

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