Regulatory Peptides 178 (2012) 76–79

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Erythropoietin acutely decreases airway resistance in the rat

Alessandro Rubini [a,*], Daniele del Monte [b], Vincenzo Catena [c]

[a] Department of Human Anatomy and Physiology, Section Physiology, University of Padova, Italy
[b] Department of Anesthesiology and Intensive Care, ULSS 15, Camposampiero (PD), Italy
[c] Department of Anesthesiology and Intensive Care, ULSS 3, Bassano del Grappa (VI), Italy

ARTICLE INFO

Article history:
Received 3 November 2011
Received in revised form 2 May 2012
Accepted 22 June 2012
Available online 2 July 2012

Keywords:
Airway resistance
End-inflation occlusion method
Erythropoietin
Rat

ABSTRACT

While some experimental data suggest that erythropoietin (EPO) influences respiratory mechanics, reports on scientific trials are lacking.
In the present work, respiratory mechanics were measured using the end-inflation occlusion method in control and EPO treated anaesthetised and positive-pressure ventilated rats. Causing an abrupt inspiratory flow arrest, the end-inflation occlusion method makes it possible to measure the ohmic airway resistance and the respiratory system elastance.
It was found that EPO induces a significant decrement in the ohmic airway resistance, not noted in control animals, 20 and 30 min after intraperitoneal EPO injection. The elastic characteristics of the respiratory system did not vary.
Hypotheses about the mechanism (s) explaining these results were addressed. In particular, additional experiments have indicated that the decrement in airway resistance could be related to an increase in nitric oxide production induced by EPO.
Spontaneous increments in plasmatic erythropoietin levels, such as those that take place in association with hypoxia and/or blood loss, appear to be related to the decrement in airway resistance, allowing pulmonary ventilation to increase without altering respiratory mechanics leading to deleterious increments in energy dissipation during breathing.

1. Introduction

Erythropoietin (EPO) is a well known stimulating agent of early erythroid precursor proliferation, but non-haematopoietic effects have also been shown. Just as indicated by the expression of EPO and its receptors in normal healthy lung tissues [1], those effects may affect the physiology of the respiratory system. EPO has, for example, been shown to augment pulmonary ventilation by increasing both the breathing rate and the tidal volume in haemodialised patients [2], and those same effects have also been detected in anaesthetised rats after intracerebroventricular injections [3].

Some investigators have, moreover, reported that EPO seems to have effects on respiratory mechanics parameters such as airway resistance. It has, in fact, been shown to inhibit carbachol-induced bronchoconstriction in mice [4], and to induce a significant increment in forced vital capacity and in peak expiratory flow rate in humans [2], both indicative of EPO induced decrements in airway resistance.

Similar effects may be the consequence of the protective influences provoked by EPO on inflamed lung tissues [5,6] by counteracting, for example, the increment in airway resistance caused by inflammatory cytokines such as IL-6 [7].

Data regarding EPO's influence on vascular smooth muscle tone indicate that it has relaxing effects [8,9], which may be connected to airway smooth muscle tone. Those effects have, in fact, been shown to be associated with increased nitric oxide (NO) production [10–12], known to reduce airway smooth muscle tone and resistance [13–16]. No studies directly investigating EPO's effects on respiratory mechanics parameters and airway resistance in healthy mammals have been published in the literature. In this study respiratory system mechanics were measured in rats using the end-inflation occlusion method before and after EPO administration. The end-inflation occlusion method makes it possible to measure both the ohmic, Newtonian airway resistance, as predicted by Poiseuille's law, and respiratory system elastance [17,18]. The aim of this study was to investigate if spontaneous variations in EPO plasma levels occurring in conditions characterised by blood loss or hypoxia can induce modifications in respiratory system mechanics parameters.

2. Materials and methods

2.1. Animals

The experiments were carried out on a total of 20 albino rats of both sexes (mean weight 307 ± 23 g., 10 males). Eight rats were

* Corresponding author at: Department of Human Anatomy and Physiology, Section Physiology, University of Padova, Via Marzolo, 3 35100 Padova, Italy. Tel.: +39 0498275310; fax: +39 0498275301.
E-mail address: alessandro.rubini@unipd.it (A. Rubini).

0167-0115/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2012.06.012

studied to investigate the effects of EPO on respiratory mechanics, six rats with similar characteristics were studied as control animals, and 6 others were studied to test the hypothesis that EPO administration increases NO production.

The experimental protocol received the approbation of the local ethical committee (CEASA, University of Padova, ref. no. 48/2011).

The animals were housed and treated in accordance with Italian law on animal experimentation (L. 116/92) and with the European Council (EC) provision 86/609/EEC.

2.2. Experimental procedure

Each rat was anaesthetised with 50 mg/100 g intraperitoneal chloralose and laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2 mm ID 5 cm long) was inserted through an incision in the second tracheal ring and firmly secured in place.

Limb electrocardiogram (ECG) probes were placed and the rat was paralysed (cisatracurium 1 mg/100 g intraperitoneally injected).

Positive end-expiratory pressure using a 10 ml/kg tidal volume and a 60/min breathing frequency (PEEP 3 cmH2O) (Rodent Ventilator 7025, Basile, Italy) was begun and maintained constantly throughout the experiment (apart from the short time necessary to measure respiratory mechanics).

Positive pressure ventilation was maintained for 5 min and respiratory mechanics were measured at that time using the end-inflation occlusion method [17,18].

The ventilator was disconnected, PEEP was discontinued, and the tracheal cannula was connected to a constant flow pump (SP 2000 Series Syringe Pump sp210iw, World Precision Instruments, USA) set to deliver a tidal volume (VT) of 3 ml with a square wave flow (F) of 4 ml/s. The rise and the fall flow time was approximately 30 ms. The pump setting was carefully checked during trial runs carried out before the experiments were begun. To avoid determinant arterial blood gas changes, the time the ventilator remained disconnected for each inflation was about 15 s. [19].

The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d, Honeywell, USA) and continually recorded (1326 Econo Recorder, Biorad, Italy). As abrupt changes in the diameters were not present in the circuit, errors in flow resistance measurements, such as those reported elsewhere [20], were avoided. The frequency response of the transducer and the pressure measuring system were tested using sinusoidal forcing and found to be flat up to 20 Hz. In accordance with data reported in the literature [21,22], that frequency response was considered adequate to avoid mechanical artefacts in pressure signal records.

Measurements were made for each rat at 10, 20, and 30 min following a 1000 U kg^{-1} rat recombinant EPO (SIGMA, St. Louis, Missouri, USA) dissolved in 100 μl PBS intraperitoneal injection. Although some data in the literature indicate that the mechanical ventilation parameters adopted here are not injurious to the respiratory system [7,16], six (a number considered sufficient for this purpose) control rats were studied to exclude the possibility that the variation noted in the experimental group was due to a time-related effect. They were administered 100 μl PBS intraperitoneally and respiratory mechanics parameters were measured following the same temporal sequence described for the experimental rats receiving EPO.

At the end of the experiments, the animals were sacrificed with a lethal intraperitoneal injection (Tanax® 0.3 ml/kg).

2.3. Data calculation

The end-inflation occlusion method was utilised to determine respiratory mechanics parameters. The static elastic pressure of the respiratory system (Pel,rs) and the sudden Newtonian resistive pressure drop at flow interruption (Pmin,rs) were measured on adequately magnified tracings (Fig. 1). Pmin,rs was calculated as the difference between Pdyn, max, the maximum value of pressure at end inflation, and P1, the pressure value immediately after the flow was interrupted (Fig. 1). The sum of Pmin,rs and of the slower, nearly exponential, pressure drop following flow interruption due to respiratory system visco-elastic behaviour, i.e. stress relaxation [17,18,21,22], was termed Pmax,rs. Our tracings made it possible to identify P1 (see Fig. 1), which separates the pressure drop due to friction related to the movement of airflow in the airway (Pmin,rs) from the visco-elastic pressure drop.

To avoid a visco-elastic pressure component in Pmin,rs, P1 values were identified by extrapolating the pressure tracings to the time the flow stopped [23].

The mean pressure data obtained from 3 to 5 inflations for each rat were used to calculate the respiratory system static elastance (Est,rs = Pel,rs/VT) and the ohmic inspiratory resistance to airflow offered by the airways and the movement of respiratory system tissues (Rmin, rs = Pmin,rs/F).

The equipment resistance, including the tracheal cannula and the standard three-way stopcock, was measured separately at a flow rate of 4 ml/s and amounted to 0.0575 $cmH_2O\ ml^{-1}\ s^{-1}$ (Req). All inflations were performed at a fixed flow rate of 4 ml/s, and Req was subtracted from the results, which thus represent intrinsic values.

2.4. Statistics

The measured and calculated values of respiratory system mechanics parameters before and after EPO resulted to be normally distributed (Smirnov–Kolmogorov test). Statistical analysis was performed (ANOVA) analysing the differences in mean values. Data are expressed as mean values ± SE (n = 8).

3. Results

The mean values of respiratory mechanics parameters before EPO administration resulted as follows: Rmin,rs = 0.14 ± 0.02 $cmH_2O\ ml^{-1}\ s^{-1}$, and Est,rs = 1.95 ± 0.07 $cmH_2O\ ml^{-1}$. The percentage variations following EPO administration in the study and control rats are outlined in Figs. 2 and 3. While there were no significant changes in the control animals, the study rats exhibited significant decrements in Rmin,rs over time. Significant variations in Est,rs were not noted in the study or control rats. Heart rate values did not vary significantly during the experiments (Table 1).

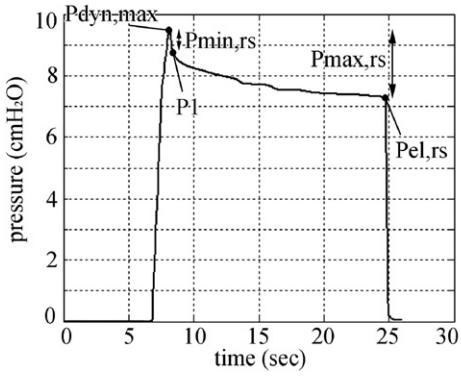

Fig. 1. Representative tracing of lateral tracheal pressure on flow interruption. The relevant pressures used for the calculations of respiratory system mechanics are indicated: maximal pressure at end inflation (Pdyn max), pressure immediately after airflow interruption (P1), static elastic pressure of the respiratory system (Pel,rs), pressure drop due to the ohmic respiratory system resistance (Pmin,rs) and total pressure drop including the effects of stress relaxation (Pmax,rs).