

RESEARCH ARTICLE

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## The effects of nifedipine on respiratory mechanics investigated by the end-inflation occlusion method in the rat

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### ABSTRACT

**Context:** Calcium channel blockers may theoretically exhibit relaxing effects not only on vascular smooth muscle but also on airway smooth muscle.

**Objective:** To investigate possible effects of nifedipine on respiratory mechanics in the rat.

**Methods:** Respiratory system mechanical parameters were measured by the end-inflation occlusion method in the rat *in vivo* before and after the intraperitoneal administration of nifedipine.

**Results:** We found that nifedipine affects respiratory mechanics, inducing a reduction of airway resistance and of respiratory system elastance, probably because of a relaxing action on airway and parenchymal smooth muscle cells.

**Conclusion:** Should these results be further confirmed by human investigations, a possible role of nifedipine in pharmacological respiratory system's diseases treatment may be suggested.

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### KEYWORDS

End-inflation occlusion method; nifedipine; rat; respiratory system mechanics

### Introduction

Nifedipine is a well-known dihydropyridin calcium channel blocker, mostly utilised for the clinical treatment of hypertension and angina. The calcium channel blocking action determinates a relaxing effect on the smooth muscle cells of the vascular walls. A working hypothesis was suggested about possible relaxing effects on the airway smooth muscle cells too, which would lead to potential beneficial consequences on the clinical respiratory system pharmacology.

A number of investigators studied this aspect in the past, and found interesting results. For example, both *in vitro*<sup>1–4</sup> and *in vivo*<sup>5–8</sup> experimentations on various mammalian species, including humans, showed a relaxing action on airway smooth muscle and/or a related decrement of airway resistance.

These results were interpreted as promising data for a potential clinical use of the drug in patients deserving a pharmacological treatment for both cardiovascular and a respiratory medical problems. The purpose of this report is to confirm these data by experiments performed on the rat utilising the end-inflation occlusion method which was never applied before to investigate this aspect. Beside the assessment of the ohmic resistive properties of the respiratory system, the method allows to measure the stress relaxation-linked inflation pressure dissipation, and to investigate the elastic properties too (see Methods), so that the effects of nifedipine on the respiratory system visco-elastic properties and static elastance may be investigated, which were not measured in the past.

### Materials and methods

#### Animals

The experiments were carried out on 8 albino Wistar rats of both sexes (4 males, mean weight  $404 \pm 19$  g, 4 females, mean weight

$284 \pm 7$  g). 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. The experimental protocol received the approval of the Italian national authority for animal experimentation.

#### Experimental procedure

Each rat was anaesthetized with 50 mg/kg intraperitoneal Zoletil<sup>®</sup> and laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2 mm i.d., 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 (cis-atracurium 1 mg/100 g intraperitoneally injected).

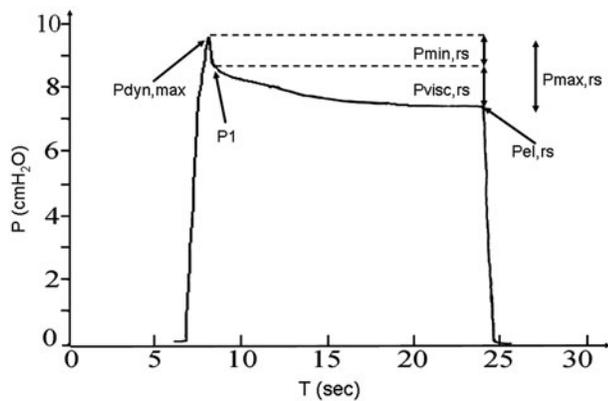
Positive pressure ventilation with room air using a 8 ml/kg tidal volume and a 60/min breathing frequency (PEEP 3 cm H<sub>2</sub>O) (Rodent Ventilator 7025, Basile (VA), Italy) was begun and maintained constantly throughout the experiment (apart from the short time necessary to measure respiratory mechanics, see below).

Positive pressure ventilation was maintained for 5 min and respiratory mechanics were then measured using the end-inflation occlusion method<sup>9–16</sup>. 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, Sarasota, FL) 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. The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d, Honeywell, IN)

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**Figure 1.** Example of tracing recorded upon constant flow inflation arrest. The maximum pressure achieved at end inflation ( $P_{\text{dyn,max}}$ ), the pressure drop due to frictional forces in the airway ( $P_{\text{min,rs}}$ ) and the overall resistive pressure drop ( $P_{\text{max,rs}}$ ), including  $P_{\text{min,rs}}$  and the nearly exponential pressure dissipation due to viscoelasticity ( $P_{\text{visc,rs}}$ ), are shown. P1: pressure value immediately after flow arrest,  $P_{\text{el,rs}}$  elastic static pressure.

and continually recorded (1326 Econo Recorder, Bio-Rad (MI), Italy).

The above-described experimental procedure was applied before ( $t_0$ ) and repeated 5, 10 and 20 min ( $t_1$ ,  $t_2$  and  $t_3$ , respectively) after an intraperitoneal injection of nifedipine (5 mg/kg dissolved in saline).

#### Data calculation

The end-inflation occlusion method was utilised to determine respiratory mechanics parameters<sup>9–16</sup>. The static elastic pressure of the respiratory system ( $P_{\text{el,rs}}$ ) and the sudden Newtonian resistive pressure drop at flow interruption ( $P_{\text{min,rs}}$ ) were measured on adequately magnified tracings (Figure 1).  $P_{\text{min,rs}}$  was calculated as the difference between  $P_{\text{dyn,max}}$ , the maximum value of pressure at end inflation, and P1, the pressure value immediately after the flow was interrupted (Figure 1). The sum of  $P_{\text{min,rs}}$  and of the slower, nearly exponential, pressure drop following flow interruption due to respiratory system visco-elastic behaviour, i.e. stress relaxation, was termed  $P_{\text{max,rs}}$ . Our tracings made it possible to identify P1 (see Figure 1), which separates the pressure drop due to friction related to the movement of airflow in the airway ( $P_{\text{min,rs}}$ ) from the successive visco-elastic pressure drop ( $P_{\text{visc,rs}}$ ).

The Newtonian, “ohmic”, component of airway resistance may be thought as the airway resistance occurring at infinite breathing frequency, when the time allowed for the pressure drop due to visco-elastic phenomena approaches zero. Instead, the additional visco-elastic component defines an higher resistance value which depends on a complete stress relaxation-linked pressure drop, theoretically occurring at zero breathing frequency. Accordingly, in real conditions *in vivo*, the actual resistance value will be a function of the breathing frequency, depending on the visco-elastic phenomena slow time course.

The mean pressure data obtained from 3 to 5 inflations for each rat were used to calculate the respiratory system static elastance ( $Est,rs = P_{\text{el,rs}}/VT$ ), and the total inspiratory resistance ( $R_{\text{max,rs}} = P_{\text{max,rs}}/F$ ), including the “ohmic” inspiratory resistance to airflow offered by the airways and the movement of respiratory system tissues ( $R_{\text{min,rs}} = P_{\text{min,rs}}/F$ ), and the visco-elastic inspiratory resistance due to stress relaxation ( $R_{\text{visc,rs}} = R_{\text{max,rs}} - R_{\text{min,rs}}$ ).

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  $0.0575 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$  ( $Req$ ). All

inflations were performed at a fixed flow rate of 4 ml/s, and  $Req$  was subtracted from the results, which thus represents intrinsic value.

#### Statistics

To verify any statistically significant effect of nifedipine, a Student’s *t*-test for paired data was applied. Data are expressed as mean values  $\pm$  SE.

#### Results

The results are depicted in Table 1, together with the pertinent statistical indexes of the differences. It was found that nifedipine administration caused a statistically significant decrement of  $Est,rs$  at  $t_1$  (almost significant at  $t_2$  and  $t_3$ ) and of  $R_{\text{min,rs}}$  at  $t_2$  (almost significant at  $t_3$ ). No statistically significant effects of nifedipine were found on  $R_{\text{max,rs}}$  and  $R_{\text{visc,rs}}$ . Heart rate mean values exhibited a trend indicating a time-related decrement.

#### Discussion

##### Experimental procedure

Modelling the respiratory system as consisting of two distinct compartments, the end-inflation occlusion method has been widely used to study respiratory mechanics in experimental animals<sup>9–16</sup>. Ideally, the inflation flow should stop instantaneously, but this is practically impossible to achieve. However, a procedure has been proposed to correct for this technical limitation<sup>17</sup>.

In this procedure, pressure tracings are manually extrapolated to account for the time that is necessary to completely halt the inspiratory flow, thereby minimising the error<sup>17</sup>. This procedure was employed to analyse the inflation pressure tracings in the current study and, similarly to what previously reported<sup>18</sup>, the corrections resulted almost negligible.

The mechanical ventilation settings used in these experiments imply a tidal volume even lower than that described as “non-injurious” in the literature<sup>14,19</sup>. In particular, “non-injurious” ventilation lasting the time requested for the present experiments has been shown to induce no alterations of respiratory system mechanics<sup>14,19</sup>. The results reported here, therefore, were not influenced by the injurious effects that longer term mechanical ventilation *per se* might exert.

The mean heart rate values we observed are similar to those previously reported in anaesthetised rats<sup>12–14,20,21</sup>, and suggest that the conditions of the animals during the experimental procedure were generally stable.

The mean values of respiratory system mechanics parameters reported here (Table 1) are comprised in the range of those previously measured by the same techniques in rats by various authors working in different laboratories<sup>9–16,18,22</sup>. The presently measured respiratory mechanics parameters have been widely investigated previously<sup>16–19,22</sup>, and represent the most useful data in order to assess a possible respiratory system mechanical impairment.

The presently used nifedipine dosage may be considered rather high with respect to oral commonly used dosages in humans (see below). Intraperitoneal nifedipine administration is a well-established experimental procedure<sup>23–25</sup>.

##### The effects of nifedipine on respiratory mechanics

Data depicted in Table 1 show that nifedipine affects respiratory system mechanical parameters, inducing statistically significant

**Table 1.** Mean values ( $\pm$ SE,  $n = 8$ ) of respiratory mechanics parameters as measured before (t0) and 5 (t1), 10 (t2) and 20 (t3) min after nifedipine intraperitoneal administration (5 mg/kg).

	t0	t1	t2	t3
Est,rs (cmH <sub>2</sub> O/ml)	1.79 $\pm$ 0.12	1.36 $\pm$ 0.08	1.54 $\pm$ 0.13	1.59 $\pm$ 0.11
		$p < 0.05$	$p = 0.09$	$p = 0.09$
Rmax,rs (cmH <sub>2</sub> O/ml s <sup>-1</sup> )	0.41 $\pm$ 0.05	0.52 $\pm$ 0.11	0.39 $\pm$ 0.04	0.41 $\pm$ 0.03
		n.s.	n.s.	n.s.
Rvisc,rs (cmH <sub>2</sub> O/ml s <sup>-1</sup> )	0.26 $\pm$ 0.035	0.39 $\pm$ 0.1	0.28 $\pm$ 0.02	0.31 $\pm$ 0.02
		n.s.	n.s.	n.s.
Rmin,rs (cmH <sub>2</sub> O/ml s <sup>-1</sup> )	0.15 $\pm$ 0.025	0.13 $\pm$ 0.025	0.11 $\pm$ 0.016	0.1 $\pm$ 0.014
		n.s.	$p < 0.05$	$p = 0.06$
Heart rate (beats/min)	515 $\pm$ 29	445 $\pm$ 22	404 $\pm$ 23	356 $\pm$ 24
		$p < 0.01$	$p < 0.01$	$p < 0.01$

Est,rs: respiratory system elastance, Rmax,rs, Rvisc,rs, Rmin,rs: respiratory system total, visco-elastic, and ohmic resistances. Statistical indexes are also reported.

decrements of Est,rs and Rmin,rs, while no significant effects were observed on Rvisc,rs and Rmax,rs.

The observed reduction in Rmin,rs after nifedipine confirms data previously reported by other authors who, however, never applied the end-inflation occlusion method. A bronchodilator effect of nifedipine was observed *in vivo* in guinea pig by Fanta et al.<sup>5</sup> These data were reported some years after the observation *in vitro* of a dose-dependent reversal of intrinsically existing tone in both tracheal spirals and parenchymal strips, associated with the inhibition of carbachol and histamine induced bronchoconstriction<sup>1</sup>. Nifedipine was also shown to inhibit *in vivo* the antigen<sup>6</sup>, methacholine<sup>7,8</sup>, and histamine<sup>8</sup> induced bronchoconstrictions, and *in vitro* carbachol<sup>2,3</sup> and interleukin-6<sup>4</sup> induced airway smooth muscle contractions. Taken together, older and present data show that the smooth muscle cells relaxing effect of nifedipine is well evident not only in vascular walls but also in airway, suggesting a potential therapeutical indication of this drug in the clinical management of airway diseases, most of all if associated with cardio-vascular diseases necessitating the administration of a calcium channel blocker. On the other end, nifedipine did not exhibit significant effects on the respiratory system visco-elastic properties, as reflected by Rvisc,rs measurements, at least in the rat.

The second important result we obtained concerns the significant nifedipine-induced reduction of Est,rs. This means that, beside a reduction of the inspiratory pressure the respiratory muscle have to develop to win the ohmic airway resistance, nifedipine permits a reduction of the elastic static component of the inspiratory workload also. It may be remembered that a similar observation (for the measurement of the dynamic respiratory system compliance) has been already described<sup>1</sup>, and accordingly we suggest that nifedipine may reduce the constriction of peripheral airway and lung contractile tissues<sup>26</sup>, in that reducing the elastic forces opposing alveolar distension.

The observed reduction in both Rmin,rs and Est,rs may be further explained by remembering that nifedipine was shown to exhibit natriuretic action<sup>27</sup>. In fact, it was shown that a modification in circulating blood volume may affect respiratory mechanical parameters<sup>28</sup>, in such a way that a diuretic effect, together with the associated reduction in circulating blood volume, may reduce both Rmin,rs and Est,rs.

Alveolar ventilation was kept constant throughout the experiments, apart from the short time needed for constant flow inflations. Thus, it seems unlikely that blood gases changes could have contributed to the observed effects of nifedipine<sup>29</sup>.

Obviously, our results need further confirms, most of all if a clinical application will be hypothesised, and direct human experimentations are needed. In this regard, it is of interest a relatively

old paper reporting detrimental effects of nifedipine therapy cessation on asthmatic symptoms<sup>30</sup>. It is also possible that in mammals other from the rat the effectual nifedipine dosage may be lower than presently used, which is much higher than that currently applied in humans. We adopted the presently used dosage in order to assure evident pharmacological effects, and the same dosage was previously adopted by other in the rat<sup>24</sup>.

The time-related decrement of heart rate we observed is probably due to the deepening of anaesthesia level, but the related decrement of sympathetic tone should cause an increment in bronchoconstrictor activity, hence in airway resistance.

In conclusion, our results indicate that calcium channel blocking activity by nifedipine implies effects on respiratory mechanics also, at least in rats, inducing significant decrements of both Est,rs and of Rmin,rs. To what extent these findings will find practical and clinical applications remain to be determined by further investigations on human subjects.

## Disclosure statement

The authors report no conflicts of interest.

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