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Twitch potentiating effects of theophylline on rat diaphragm are enhanced by foreshortening

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Twitch potentiating effects of theophylline on rat diaphragm are enhanced by foreshortening. G. Gayan-Ramirez, N. Buts, M. Decramer. ©ERS Journals Ltd 1994

ABSTRACT: In patients with chronic obstructive pulmonary disease (COPD) and acute respiratory failure, acute hyperinflation is likely to induce foreshortening of inspiratory muscles. Since no data are available on the effects of inotropic agents at lengths below the optimal length (L_o), we compared the effects of theophylline on forty rat diaphragm bundles placed at L_o and at 70% L_o.

Twitches and tetanic stimulations were recorded before and after addition of theophylline, in concentrations of 20, 100, 200 or 400 mg·l^{-1}.

Compared with values obtained before theophylline, twitch tension (P_t) and maximal tetanic tension (P_o) of the bundles placed at L_o slightly decreased at 20 and 100 mg·l^{-1} whereas a clear increase in P_t was obtained at 400 mg·l^{-1} (15±21% (mean±SD)). In contrast, P_t of the bundles placed at 70% L_o increased with all theophylline concentrations, and vastly more than at L_o (*e.g.* at 400 mg·l^{-1}: 74±34%, $p<0.05$); whereas P_o slightly decreased, except at 400 mg·l^{-1}. Moreover, the difference between the effects at L_o and at 70% L_o increased with increasing theophylline concentrations.

We conclude that even at low, *in vivo* attainable serum levels, theophylline exerted greater positive inotropic effects on twitch tension (P_t) of rat diaphragm when foreshortened than when at optimal length.

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Since they may improve the function of respiratory muscles, methylxanthines, such as theophylline, have received considerable attention in recent years. It has been suggested that they increase the force developed by respiratory muscles in animals [1–10], and in normal subjects [11, 12], as well as in patients with chronic obstructive pulmonary disease (COPD) [13]. In these patients, hyperinflation may be extremely severe [14]. It presumably shortens inspiratory muscles, and thereby displaces them to a less advantageous position of their length-tension curve. Although adaptive changes are likely to occur in chronic hyperinflation [15], the diaphragm is still expected to be foreshortened in acute hyperinflation [14, 16].

The effects of theophylline on contractile properties of the foreshortened diaphragm have, to the best of our knowledge, never been studied. The results of such a study would be of potential conceptual interest to respiratory muscle pharmacotherapy in COPD patients, who often present with acute hyperinflation. The present study was, thus, designed to examine the effects of theophylline on contractile properties of rat diaphragm placed at optimal length (L_o), and to compare them with those obtained at 70% L_o which is a considerably shorter length, reached at total lung capacity [14].

Materials and methods

Forty male Wistar rats weighing 350–400 g were anaesthetized with sodium pentobarbital (Nembutal, 60 mg·kg^{-1} *i.p.*). Each animal was tracheostomized and mechanically ventilated with an O_2 enriched gas mixture. The diaphragm was quickly removed through a laparotomy, and immediately immersed in a cooled, oxygenated Krebs solution, containing (in mM): NaCl 137, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, KH_2PO_4 1, $NaHCO_3$ 12, glucose 6.5. Two small bundles were obtained by careful dissection parallel to the long axis of the fibres. Silk sutures were firmly tied to both ends of the bundle to serve as anchoring points.

Each bundle was then placed within the external chamber of a jacketed tissue bath, containing Krebs solution, maintained at 37°C and perfused with a 95% O_2 and 5% CO_2 mixture. The pH varied between 7.4–7.5. One end of the bundle was tied to a rigid support, while the other was fastened to an isometric force transducer, mounted on a micrometer. The muscle was placed between two large platinum stimulating electrodes.

After a 15 min thermoequilibration period, the bundles were placed at L_o, defined as the length at which peak twitch force was obtained. Maximal response was

estimated by twitch amplitude. Stimulation was delivered through a Harvard 50-5016 stimulator (Edenbridge, Kent, UK) connected in series to a power amplifier made from power one model HS24-4.8 developed by the computer technology resources center, University of Virginia (R.J. Evans, 1983). Maximal stimulation was achieved at approximately 34 V. The voltage was then increased by 20% to ensure supramaximal stimulation. Isometric force was measured with a Maywood force transducer (Maywood Ltd, Hampshire, UK). The signal was amplified and recorded on a hot pen recorder (W & W Electronics, Basel, Switzerland). Measurements were made directly from the recorder tracings.

The following measurements were then performed: 1) Twitch characteristics - two twitches were recorded at L_o to determine maximal twitch tension (P_t). The average value was used as P_t. Time to peak tension (TPT) and half relaxation time (1/2RT) were also measured; 2) Maximal tetanic force - the bundles at L_o were then stimulated tetanically at 160 Hz, during 350 ms, with supramaximal 0.2 ms voltage pulses, to obtain a clear plateau in force generation. Tetanic tension (P_o) was recorded as the maximal tension at 160 Hz.

Subsequently, one bundle was placed at 70% L_o, and was stimulated as described above.

Theophylline was added to each muscle bath to obtain a concentration of 20, 100, 200 or 400 mg·l^{-1}. After 30 min equilibration time, the same measurements (two twitches and one tetanic stimulation) were repeated for each bundle. Each pair of bundles was studied simultaneously, and 10 pairs were obtained at each concentration.

Finally, each muscle bundle was removed from the bath and its length, thickness and width were measured at L_o using a micrometer. The bundle was blotted dry and weighed. Cross-sectional area (CSA) was calculated by dividing weight by specific density (1.056) and muscle length. Twitch forces and tetanic forces were expressed per unit cross-sectional area. The twitch-tetanus ratio (P_t/P_o) was calculated for each muscle bundle. The total duration of an experiment was about one hour.

Data were collected from a total of 40 pairs of bundles with a tetanic force of at least 2 kg·cm^{-2}. Means ±SD were calculated and are represented in the text and the figures. Data obtained before theophylline were compared to those obtained after, using two-way analysis of variance. Differences between means were assessed by a subsequent Duncan test.

Results

Comparison of bundle properties at L_o

Geometric properties of the bundles. No significant differences in weight (0.024±0.01 *versus* 0.025±0.01 g) were present at L_o between the bundles further studied at L_o and those further studied at 70% L_o. There were also no differences in thickness (0.59±0.16 *versus* 0.59±0.15 mm), or CSA (0.011±0.003 *versus* 0.011±0.003 cm^2). Only a small difference in L_o (2.01±0.25 v*ersus* 2.19±0.25 cm) was observed, reaching statistical significance ($p<0.05$).

Contractile properties of the bundles. No significant differences in P_t, P_o, TPT and 1/2RT were observed at L_o between the bundles studied at L_o and those further studied at 70% L_o, for each theophylline concentration except 200 mg·l^{-1} (table 1). At this concentration, P_t of the bundles which were later shortened, was greater than that of bundles subsequently studied at L_o. Consequently, P_t/P_o was also significantly greater (table 1).

Effects of theophylline on contractile properties at L_o

Twitch characteristics. With the exception of the 200 mg·l^{-1} theophylline concentration, statistically significant differences in P_t were observed after theophylline, compared to values obtained before (fig. 1a). P_t slightly decreased after 20 or 100 mg·l^{-1} theophylline (-22±12% and -13±11%, respectively, $p<0.05$) but increased with 400 mg·l^{-1} (15±22%, $p<0.05$). TPT increased significantly with 400 mg·l^{-1} and 1/2RT with 200 mg·l^{-1} (table 2).

Table 1. – Contractile properties of bundles at L_o before theophylline

	Before 20 mg·l^{-1}		Before 100 mg·l^{-1}		Before 200 mg·l^{-1}		Before 400 mg·l^{-1}	
	L_o	L_o (70% L_o)	L_o	L_o (70% L_o)	L_o	L_o (70% L_o)	L_o	L_o (70% L_o)
P_t g·cm^{-2}	766±161	784±216	684±139	706±116	697±111	924±130*	660±123	665±197
TPT ms	22.9±5.4	20.2±4.1	23.2±5.2	20.6±2.7	23.6±4.1	20.0±0.9	17.8±3.9	19.6±1.0
1/2RT ms	41.7±6.8	38.1±8.8	43.8±6.6	42.6±3.8	40.4±8.0	38.5±6.7	41.3±11.3	38.8±7.0
P_o kg·cm^{-2}	2.96±0.45	2.96±0.76	2.69±0.46	2.71±0.38	2.76±0.47	3.03±0.34	2.51±0.34	2.51±0.49
P_t/P_o	0.26±0.03	0.27±0.03	0.26±0.05	0.26±0.03	0.25±0.02	0.31±0.03*	0.26±0.03	0.28±0.04

Note that "L_o" denotes bundles that were subsequently studied at optimal length (L_o); while "L_o (70% L_o)" denotes bundles further studied at 70% L_o. All contractile properties shown in this table are obtained at L_o in both groups. Data are presented as mean±SD. P_t: twitch tension; P_o: tetanic tension; P_t/P_o: twitch to tetanus ratio; TPT: time to peak tension; 1/2RT: half relaxation time. *: $p<0.05$ comparison at L_o between the two groups.

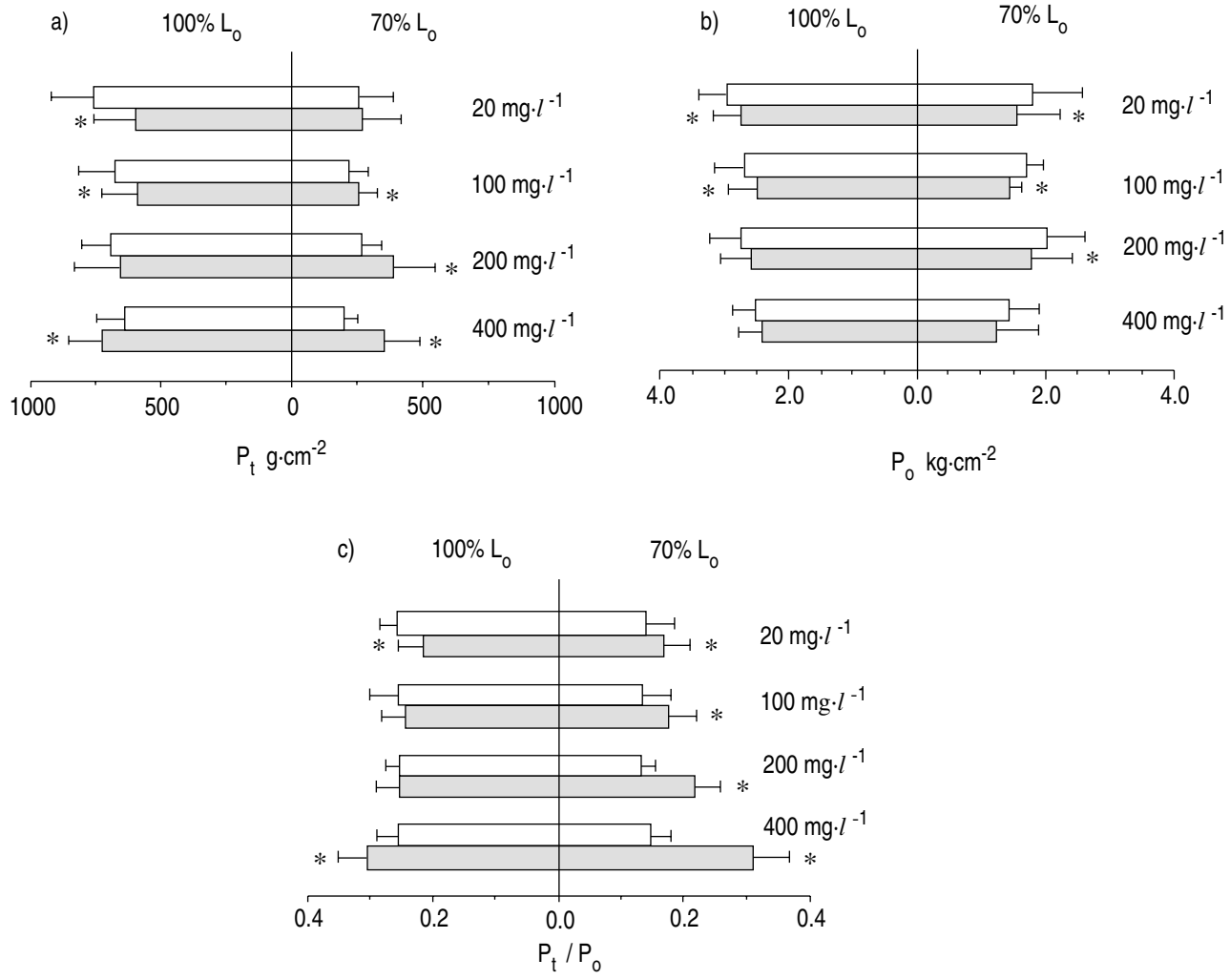

Fig. 1. – Absolute values (mean±SD) of: a) P_t; b): P_o; and c) P_t/P_o obtained at 100% L_o and 70% L_o before theophylline (open bars), at different concentrations 30 min later (hatched bars). *: $p<0.05$. L_o: optimal length; P_t: twitch tension; P_o: tetanic tension; P_t/P_o: twitch to tetanus ratio.

Table 2. – Time to peak tension (TPT) and half relaxation time (1/2RT) obtained at L_o and 70% L_o before and after each theophylline concentration

	L_o		70%L_o	
	TPT ms	1/2RT ms	TPT ms	1/2RT ms
Before	22.9±5.4	41.7±6.8	17.4±5.9	15.0±5.5
After 20 mg·l^{-1}	21.8±5.8	39.3±11.4	20.0±5.9	19.9±7.5
Before	23.2±5.2	43.8±6.6	16.9±5.7	20.6±2.8
After 100 mg·l^{-1}	20.4±4.5	48.3±6.5	18.4±4.8	29.8±7.9*
Before	23.6±4.1	40.4±8.0	16.8±1.4	19.6±9.1
After 200 mg·l^{-1}	21.9±5.7	46.2±10.7*	17.8±1.8	27.4±5.5*
Before	17.8±3.9	41.3±11.3	16.2±1.8	19.7±7.1
After 400 mg·l^{-1}	22.0±4.9*	43.2±8.7	17.8±1.9*	25.3±6.9*

Data are presented as mean±SD. *: $p<0.05$ compared to values obtained before theophylline. For abbreviations see legend to table 1.

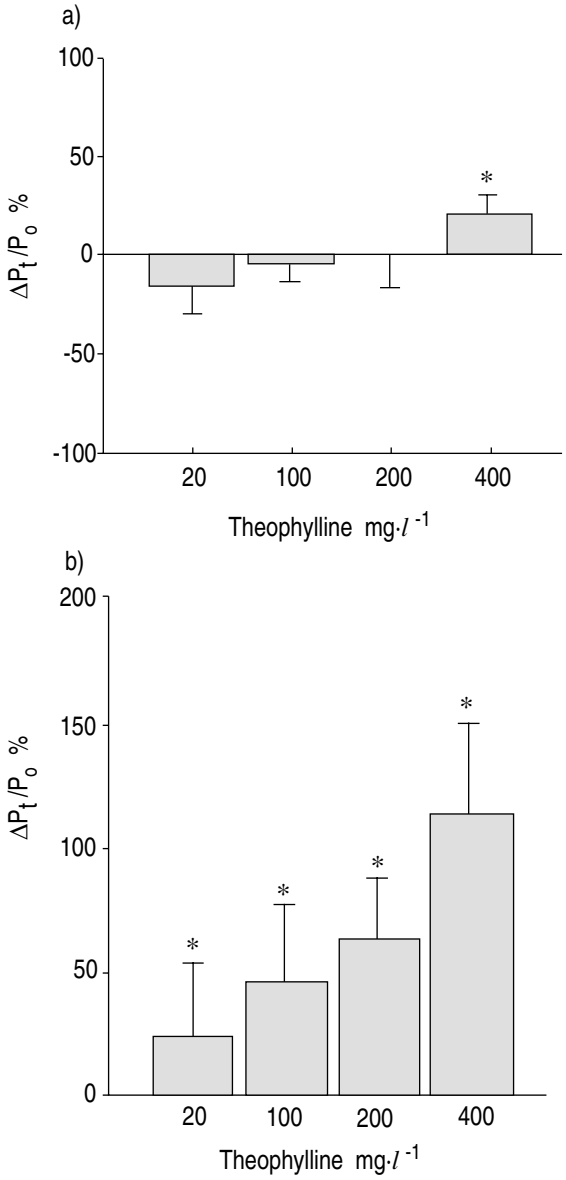

Fig. 2. – Effects of different theophylline concentrations on the twitch-tetanus ratio (P_t/P_o): a) at 100% L_o; and b) at 70% L_o. Data are presented as mean±SD, expressed as a percentage of values obtained before theophylline. *: $p<0.05$. For abbreviations see legend to figure 1.

Maximal tetanic force and twitch-tetanus ratio. Compared with data obtained before theophylline, P_o significantly decreased after 20 or 100 mg·l^{-1} theophylline (fig. 1b). Other concentrations did not affect P_o. P_t/P_o increased with 400 mg·l^{-1} theophylline and decreased with the other concentrations. The changes in P_t/P_o only reached statistical significance at 20 and 400 mg·l^{-1} ($p<0.05$) (figs. 1c and 2a).

Effects of theophylline on contractile properties at 70% L_o

Twitch characteristics. Compared with values obtained before theophylline, P_t increased with increasing theophylline concentrations (fig. 1a). This increase, thus, exhibited a clear dose-response behaviour (*e.g.* 23±23 and 74±34% at 100 and 400 mg·l^{-1}, respectively), and reached statistical significance at all concentrations except 20 mg·l^{-1}. TPT significantly increased with 400 mg·l^{-1}, and 1/2RT increased at all concentrations of theophylline, reaching statistical significance at 100, 200 and 400 mg·l^{-1} (table 2). The prolonged 1/2RT was, however, not dose-related.

Maximal tetanic force and twitch-tetanus ratio. P_o decreased at all theophylline concentrations (fig. 1b). This decrease reached statistical significance at all concentrations except at 400 mg·l^{-1}. The increase in P_t/P_o observed at each theophylline concentration was significant, and exhibited a clear dose-response behaviour (figs. 1c and 2b). This was predominantly caused by an increase in P_t.

Discussion

The present data show that the inotropic effects of different theophylline concentrations on P_t and P_t/P_o of rat diaphragm *in vitro* are vastly greater at 70% L_o than at L_o. The increase in P_t and P_t/P_o observed at 70% L_o exhibits a clear dose-response behaviour. Moreover, the difference between the effect of theophylline at 70% L_o and at L_o also increases with increasing theophylline concentrations (fig. 1 and 2).

The effects of theophylline on contractile properties of the diaphragm have been studied extensively *in vitro* in small rodents [1–8], and more recently in dogs [9, 10]. Most of these studies, performed at L_o, showed that the positive inotropic effects of theophylline occurred at considerably higher doses than those attainable in patients [2–6, 9, 10]. Our data obtained at L_o with a theophylline concentration of 400 mg·l^{-1} are in keeping with these studies. At *in vivo* attainable serum levels, the effects of theophylline are highly controversial. In rodents, several *in vitro* studies demonstrated that theophylline improved diaphragm contractility [3, 4, 7, 8], and might exert a protective effect against fatigue [7], although the latter effect was not found in a recent study [8].

At low theophylline concentrations, our data show a tendency for P_t and P_t/P_o to decrease. This may be attributed to the appearance of a small degree of fatigue in the bundles during the experiment, as further reflected by the slight decrease in P_o over time, or to a pharmacological effect of theophylline on P_o. However, as this decrease was not dose-dependent and was relatively constant, it was probably the result of fatigue. These findings are in line with previous reports that P_t/P_o decreased during chronic stimulation of cat skeletal muscle [17]. In the present study, the prolonged 1/2RT observed after theophylline may reflect a pharmacological effect of theophylline, as previously demonstrated in the fatiguing diaphragm, where theophylline was shown to accentuate the slowing of relaxation caused by fatigue [18]. The physiological significance of this phenomenon is unknown.

Our results obtained at L_o with low theophylline concentrations seem to be in apparent contradiction with previous studies in rats [7, 8]. However, such discrepancy

is likely to result from differences in methodology. KOLBECK and SPEIR [7] used a perfused, contracting intact rat diaphragm preparation, in which theophylline was added to the perfusion fluid. In this preparation, the diaphragm was supposedly placed at L_o, as evaluated by a length-tension study, although no actual length measurements were provided. As a consequence, foreshortening might have enhanced the inotropic effects of theophylline. In the study of KUEI and SIECK [8], theophylline was given by intravenous injection of aminophylline, and its effect was studied *in vitro* on innervated muscle strips. This study, however, was performed at 26°C rather than at 37°C. The extrapolation of these findings to 37°C may be problematical.

Also, in dog and human studies, contradictory results have been obtained. Several studies have demonstrated that theophylline increased diaphragm force production [9, 11–13], and decreased fatigability [11], whilst others showed no effects on diaphragmatic contractility or fatigue [10, 19, 20]. These contradictory observations may be explained by interspecies differences in muscle fibre composition, and by a different sensitivity to the drug, as demonstrated previously for caffeine [1, 21], isoprenaline, and salbutamol [22].

The effects of theophylline on contractile properties of foreshortened diaphragm have, to the best of our knowledge, never been examined before. Compared with our data obtained at L_o, theophylline-induced effects on foreshortened diaphragm are vastly greater, and increase significantly with increasing theophylline concentration. Moreover, the decrease in P_o observed during the experiment was more pronounced at 70% L_o than at L_o, which is in line with previous observations on accelerated fatigue in foreshortened muscles [23].

The alterations of muscle mechanics with changes in acute shortening are well-known in skeletal muscles. Not only the sliding filament overlap [24], but also other factors play a role. Thus, in electrically stimulated muscle fibres, extreme shortening (less than 80% L_o) inhibits activation of central myofibrils [25], and decreases shortening velocity in the central sarcomeres [26]. Indeed, because of shortening, muscle fibres were swollen, thereby compressing the T-tubular system, and impeding exit electrolyte flow [27]. As a consequence, a failure of T-tubular conduction [25] and/or of calcium release from the sarcoplasmic reticulum may occur, leading to a decreased intracellular calcium concentration [26, 28]. Muscle shortening appears, therefore, to be associated with calcium deactivation.

The mechanisms by which theophylline induces inotropic effects at optimal length remain ill-defined. Several studies performed at L_o have suggested that theophylline induced a hyperpolarization of the cell membrane [5, 29], or interacted with intracellular [30–32], or transmembrane [32, 33] calcium transfer, but not with cyclic adenosine monophosphate (cAMP) [32].

As theophylline's effects at shorter lengths have not been studied, one can only speculate on the mechanisms involved, envisioning a similar mechanism as proposed for caffeine. Indeed, like theophylline in the present study, caffeine potentiates twitch and tetanic tensions in a way inversely related to the cell length, these effects being associated with an increase in intracellular calcium concentration [26]. In addition, caffeine induces a synchronous contraction of the myofibrils at lengths below L_o [25, 26]. Thus, we speculate that the greater effects of theophylline on P_t and P_t/P_o of foreshortened diaphragm bundles might result from increased calcium release and more homogeneous activation of the foreshortened muscle fibres, as demonstrated for caffeine [25, 26]. We hypothesized that the inotropic effect of theophylline at shorter diaphragm length could either result from a direct action of theophylline on sarcoplasmic reticulum channels, or from an action on T-tubular systems. Clearly, further studies are required to unravel these mechanisms.

However, it is worthwhile to consider that the present results pertain to an *in vitro* model and results may be different *in vivo*, where blood flow, among other factors, is maintained. Indeed, theophylline is a well-known vasodilating agent [34]. Thus, it is possible that by inducing systemic vasodilation, theophylline may also increase diaphragmatic blood flow, and thereby improve diaphragmatic force. However, several studies have demonstrated that diaphragmatic blood flow failed to increase after aminophylline [35–37], even when animals were breathing against an inspiratory resistive load [36, 37], thus, showing that aminophylline has no direct effect on diaphragmatic arterioles. Moreover, we recently compared *in vivo* the effects of aminophylline on canine diaphragm stimulated at functional residual capacity (FRC) and near total lung capacity (TLC) (producing a diaphragm shortening similar to that in the present *in vitro* study), and we observed results similar to those obtained *in vitro* [38].

The present results may be significant for the treatment of COPD patients. Although severe hyperinflation decreases airways resistance in these patients, it profoundly alters respiratory muscle function, presumably by shortening inspiratory muscles. As a consequence, the tension generated by these shortened muscles decreases as a result of both geometric changes and intrinsic length-tension characteristics. Moreover, adaptive changes to shortening, as occur during chronic hyperinflation in the diaphragm [15], are unlikely to occur with acute hyperinflation. Therefore, by increasing the force generated by shortened diaphragm, theophylline might contribute to improving respiratory muscle function in patients with acute hyperinflation.

Nevertheless, such increase in force production may involve a concomitant increase in oxygen consumption and substrate utilization by the muscle, which may enhance fatigability. This remains to be studied. In any event, the variability in theophylline-induced inotropic effects on diaphragm contractility is well known [7–11, 13, 20]. Our findings suggest this variability could be related to different degrees of hyperinflation in the patients studied, or to whether hyperinflation is acute or chronic.

In conclusion, the present study demonstrates greater inotropic effects of theophylline on P_t and P_t/P_o of foreshortened rat diaphragm bundles. These effects are also observed at *in vivo* attainable serum levels and are

dose-related. Whether or not these effects are clinically significant has to be determined. Studies on the effects of theophylline on respiratory variables in patients with acute hyperinflation are warranted.

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