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Theophylline dilates rat diaphragm arterioles via the prostaglandins pathway

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1 We investigated by intravital microscopy in rats, the *in vivo* direct effects of theophylline on the diameters of second and third order diaphragm arterioles.

2 Theophylline (1–100 μM) dilated second and third order diaphragm arterioles significantly, and with an amplitude which was not statistically different from the one obtained with adenosine (1–100 μM). Enprofylline (1–100 μM), a theophylline analogue with poor adenosine-receptor antagonism but with similar or higher phosphodiesterases inhibition properties than theophylline, also dilated diaphragm arterioles, causing however, a significantly smaller dilatation than theophylline.

3 Neither the A1 adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX, 50 nM), nor the A_2 adenosine receptor antagonist 3,7-dimethyl-1-proparglyxanthine (DMPX, 10 μM) reduced significantly theophylline-induced arteriolar dilatation.

4 Theophylline (100 nM) abolished adenosine-induced arteriolar dilatation.

5 The dilatation induced by theophylline was unchanged by the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine (NNA, 300 μM).

6 Theophylline-induced arteriolar dilatation was abolished by the prostaglandin synthesis inhibitors mefenamic acid or indomethacin (20 μM).

7 These findings show that theophylline induced a significant dilatation of diaphragm arterioles via the release of prostaglandins.

Keywords: Diaphragm; arterioles; theophylline; enprofylline; adenosine receptors; CPX; DMPX; nitric oxide; prostaglandins

Introduction

Theophylline is an ancient bronchodilator drug with renewed relevance in the treatment of patients with asthma and chronic obstructive pulmonary disease because it presents some additional beneficial effects which have been recently discovered (Jenne, 1995; Weinberger & Hendeles, 1996). Among these effects, an improvement in function of diaphragm has been extensively studied (Aubier *et al*., 1981; Murciano *et al*., 1989). This improvement has been related to a direct effect of the drug on muscle fiber contractility (Aubier *et al*., 1983; Oron *et al*., 1993). In addition, the improvement may be related to the hemodynamic effects of this agent since theophylline has a direct vasodilator action observed *in vitro* in vessels from different organs such as the heart and lungs (Kalsner *et al*., 1975; Persson *et al*., 1983; Sjogren & Edvinsson, 1987; Rabe *et al*., 1995). However, data obtained *in vivo* in different microcirculatory preparations of skeletal muscles showed that theophylline induced either arteriolar constriction (Proctor, 1984; Koller *et al*., 1991) or only a very slight dilatation (Morff & Granger, 1983). Furthermore, to our knowledge no data are available in the current literature concerning the direct effects of theophylline on diaphragm microcirculation.

Conclusive data regarding the mechanism(s) whereby theophylline induces vasodilation are still limited. Theophylline inhibits phosphodiesterase (PDE) isoenzymes. This phenomenon increases the intracellular concentration of cyclic nucleotides in airway and vascular smooth muscle, with ensuing smooth muscle relaxation (Rabe *et al*., 1995). Theophylline also binds to the receptors of the endogenous vasodilator adenosine, acting in general as an adenosine antagonist in several cell types and tissues including the skeletal microcirculation (Londos & Wolff, 1977; Caussade & Cloarec, 1993). As regards adenosine receptors, several *in vitro* and *in vivo* studies performed in rat brain parenchyma (Ngai & Winn, 1993), rabbit peripheral skeletal muscle (Gustafsson *et al*., 1990) and hamster skin (Stojanov & Proctor, 1989), have shown that arteriolar vasodilator effect of adenosine was mediated exclusively through the A_2 adenosine receptor. In contrast, we have demonstrated recently that the effects of adenosine on rat diaphragmatic arterioles involve essentially the A_1 adenosine receptor (Danialou *et al*., 1997), a finding which is in agreement with a recent study from Bryan & Marshall (1996) in the rat hind limb. Thus, it seems difficult to directly extrapolate to the diaphragm the data obtained in other territories about the interaction between theophylline and adenosine receptors. Finally, interaction of theophylline with the endothelial autacoids nitric oxide (NO) and prostaglandins could also explain the vascular effects of theophylline since both molecules have been implicated in the regulation of basal arteriolar tone in the diaphragm and other skeletal muscles (Koller & Kaley, 1990; Kaley *et al*., 1992; Boczkowski *et al*., 1994).

Therefore, the first aim of the present study was to investigate the *in vivo* direct effects of theophylline on arteriolar diameters of diaphragm by topical application of this agent on the muscle surface. The second aim of this study was to study the mechanism(s) underlying the effect of theophylline on diaphragm arterioles. We specifically investigated the role of inhibition of PDE, the type of interaction of theophylline with adenosine receptors, and the role of the endogenous autacoids NO and prostaglandins.

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Methods

Animals

Fifty-three male albino rats (159±10 g) of the Sprague-Dawley strain were obtained from Charles River France Inc. All rats were housed individually, acclimatized to a 12 h light dark cycle, and maintained on Purina rat chow and tap water ad libitum for a 5 day period before experimental set up.

The animals were anesthetized by an intraperitoneal injection of 50 mg/kg sodium pentobarbital and placed in a supine position on a rodent operating table (Harvard Apparatus, MA, U.S.A.). This table was placed over a machining table (Wolfcraft T No 3060, Germany) to allow the animal preparation to be moved in two directions.

Following tracheotomy, the animals were mechanically ventilated (FIO_2 of 50%) with a rodent ventilator (Ugo Basile Apparatus, Italy). The left carotid artery was cannulated for continuous measurement of systemic arterial blood pressure with a Statham P23XL transducer (Spectramed Ltd., Coventry, U.K.). A second catheter was placed in the right jugular vein to administer 5 ml kg^{-1} of sterile physiological solution (NaCl 0.9%) in order to compensate for insensible liquid losses during the surgical procedure. Rectal temperature was continuously monitored with a thermistor and maintained constant at 37°C by a heat lamp and a heating pad (Harvard Apparatus, MA, U.S.A.).

Preparation of the diaphragm

The diaphragm preparation has been previously described in detail (Boczkowski *et al*., 1990; Danialou *et al*., 1997).

Briefly, a bilateral thoracotomy avoiding the sternum was performed in the fifth intercostal space. The diaphragm was carefully separated from the lungs and from the mediastinal tissues. Then, the abdomen was opened by a midline laparotomy which was followed by a transversal incision in order to expose and to place the diaphragm in a perpendicular position relative to the body of the animal. The animal was placed in the Trendelenburg position.

Irrigation of the abdominal side of the diaphragm began immediately after exposure with a modified Krebs-Henseleit solution containing (in mM) NaCl 118, KCl 5.9, $CaCl_2.^{2H}{}_{20}$ 2.5, $MgSO_4.7H_20$ 0.5, $NaHCO_3$ 26 and glucose 10. This solution was maintained at a constant temperature of 37.5°C. By bubbling the solution with a 6% C02-94% N2 gas mixture, the pH, pO_2 and pCO_2 of this solution were fixed at 7.41±0.06, 22±1.6, and 41±0.4 T, respectively. Pancuronium bromide, 40 μM (Pavulon), was added to this solution to prevent muscle fasciculation. This dose of pancuronium had no effects on microcirculatory parameters. (Faber & Harris, 1981).

The muscle was transilluminated with a fiberoptic cool light microprobe gently introduced into the thorax via the left thoracotomy. The costal microcirculation of diaphragm was visualized by observing the abdominal side of the muscle with an articulated optic microscope (Leitz Inc., Germany) whose objective was placed in position parallel to the area of the muscle under observation. The image, magnified by a 20× long-distance objective, was projected into a CCD video camera (Sony DXC-101P) connected to a videotape recorder (Sony VO-9600 P) and a video monitor (Sony PVM-1371 QM).

Microvascular anatomy

We studied changes in diameter of the arteriolar branches arising from the internal mammary and intercostal arteries. Second and third arteriolar orders were analysed according to the number of bifurcations proximal to the studied arterioles, as previously described (Zweifach & Lipowski, 1984). Depending on the geometry of the part of the network studied, one to three arterioles were examined in each animal.

We selected a clearly distinguishable arteriolar network. Arteriolar diameters were measured by playback analysis of the video record, using the technique of Intaglietta & Tompkins (1973) and an image shearing monitor (IPM 303 and 908, San Diego, U.S.A.).

Experimental protocol

Five sets of experiments were performed. Each set of experiments included one or several different groups of animals. In the following paragraphs N refers to the number of animals, and n to the number of arterioles studied in each group.

The general schedule of all experiments was the following: after surgery, a 20–30 min period was allowed to reach a steady state of arteriolar tone and then baseline diameters were measured. Then, a cumulative concentration-response curve of arteriolar diameter in the diaphragm to an agent was performed by diluting the drug in the Krebs solution and by superfusing it in a stepwise fashion in a range of concentrations going from 1 μM to 100 μM. Individual concentrations were given until the monitored arteriolar diameter remained stable for 2 min and then the diameter of the selected arteriole was measured. Even with the lowest drug concentrations, steady state diameters occurred within 1 min.

Effects of theophylline, adenosine and enprofylline

This set of experiments was carried out to compare on diaphragm arterioles the effects of theophylline, adenosine and enprofylline, a theophylline analogue with poor adenosine-receptor antagonism but with similar or greater phosphodiesterases inhibition properties than theophylline (Bergstrand, 1980; Ukena *et al*., 1985). After measuring baseline arteriolar diameters, the animals were allocated into 3 groups ($N=8$ for each group) in accord to performance of concentration-response curves to theophylline, adenosine and enprofylline.

Interaction of theophylline with adenosine receptors

Effect of adenosine receptor antagonists on theophylline-induced dilatation of diaphragm arterioles This series of experiments was carried out to evaluate the effect of adenosine-receptor subtype antagonists on theophylline-induced dilatation of diaphragm arterioles. After measuring baseline arteriolar diameters, the animals were allocated to two distinct experimental groups according to performance of concentration-response curves to theophylline in the presence of either 50 nM of 1,3-dipropyl-8-cyclopentylxanthine (CPX, $N=5$ animals), considered as a specific antagonist of the A_1 adenosine receptor (A_1AR) (Daly *et al*., 1985; Bruns *et al*., 1987; Peet *et al*., 1990), or 10 μM of 3,7-dimethyl-1-proparglyxanthine (DMPX, $N=5$), considered as a specific antagonist of the A_2 adenosine receptor (A_2AR; Ukena *et al*., 1986). It must be noted that in a previous study we verified the specificity of both CPX and DMPX in the present experimental model, at similar concentrations to those used in the present study (Danialou *et al*., 1997). Indeed, CPX has been shown to be selective for A_1AR since it attenuated significantly the diaphragmatic arteriolar dilatation caused by R-PIA (a A_1AR agonist) whereas it did not modify the dilatation caused by

DPMA (a A_2AR agonist). In the same way, DMPX was shown to be selective for A_2AR because it significantly attenuated the diaphragmatic arteriolar dilatation caused by DPMA whereas it did not modify the dilatation caused by R-PIA.

It should be noted that in preliminary experiments we found that both CPX and DMPX, applied at the concentrations used in the present study, induced a small and significant diaphragmatic arteriolar constriction. This constriction amounted, in second and third order vessels, near 8 and 7% respectively for CPX and 6 and 3% of baseline diameter respectively for DMPX.

Effect of adenosine in the presence of theophylline Since theophylline has been reported to be an inhibitor of adenosine-induced arteriolar dilatation in non-respiratory skeletal muscle microcirculations (Morff & Granger, 1983; Mohrman & Heller, 1984; Proctor, 1984; Koller *et al*., 1991), this set of experiments was performed to evaluate whether or not the same phenomenon existed in diaphragm arterioles. After measurement of baseline arteriolar diameters, a concentration-response curve to adenosine was performed in the presence of 100 nM of theophylline, a concentration that did not it self modify arteriolar diameter ($N=4$ animals).

Role of NO in arteriolar dilatation induced in the diaphragm by theophylline

This series of experiments was carried out to determine the effect of Nω-nitro-L-arginine (NNA, $N=5$), a very potent and specific inhibitor of NO synthesis *in vitro* (Gross *et al*., 1990; Hecker *et al*., 1990; Buga & Ignarro, 1992) and *in vivo* (Mügge *et al*., 1991; Benyo 1992; Hussain *et al*., 1992), on arteriolar dilatation induced by theophylline. After measuring baseline diameter of the selected arterioles, the diaphragm was superfused with Krebs solution containing NNA (300 μM). After a 20 min period, the diameter of the selected arterioles was measured, and a concentration-response curve to theophylline was obtained. Superfusion of NNA was maintained during the whole experiment.

In a previous study we demonstrated that NNA, at the concentration used in the present experiments (300 μM), selectively inhibits synthesis of NO in diaphragm arterioles (Boczkowski *et al*., 1994).

Role of prostaglandins on arteriolar dilatation induced in the diaphragm by theophylline

This series of experiments was carried out to evaluate the effect of two structurally different specific inhibitors of prostaglandin synthesis: mefenamic acid or indomethacin (Flower & Vane, 1974), on arteriolar dilatation induced by theophylline. After measuring baseline diameter of the selected arterioles, the animals were randomly allocated into two groups in accord to superfusion of the diaphragm with Krebs solution containing 20 μM of either mefenamic acid or indomethacin ($N=5$ for each group). After a 20 min period, the diameter of the selected arterioles was measured, and a concentration-response curve to theophylline was obtained. Superfusion of mefenamic acid or indomethacin was maintained throughout the experiment.

Drugs

Pancuronium bromide, adenosine, theophylline, enprofylline, NNA and mefenamic acid were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.). Indomethacin was obtained from Merck, Sharp & Dohme Chibret Products (France). CPX and DMPX were obtained from Research Biomedicals Inc. (Natick, Mass, U.S.A.). Pancuronium bromide, adenosine, theophylline, NNA, indomethacin and norepinephrine were directly dissolved in the Krebs solution. Mefenamic acid was dissolved in 1 mg/ml sodium carbonate and then diluted to the final concentration with the Krebs solution. CPX and DMPX were made up at a concentration of 100 μM in 0.2% dimethylsulphoxide (DMSO, Sigma Chemical Co, St. Louis, MO, U.S.A.). Further dilutions of this drug were made in the Krebs solution as required. Final DMSO concentration in the bath did not exceed 0.2%. This concentration of DMSO had no effect on the responses of diaphragm arterioles to adenosine receptor antagonists. Drug solutions were prepared fresh daily.

Data analysis

Data are reported as means $\pm$ s.e.m. Comparison between arteriolar diameters at baseline and after superfusion of inhibitors in the different groups of experiments was performed by one way analysis of variance. Comparisons of the effects of theophylline, adenosine and enprofylline and comparison of the effects of theophylline in the presence or absence of the different antagonists and inhibitors, was performed by comparing the concentration-response curves by using two-way analysis of variance for repeated measurements (Winer, 1971) considering one 'grouping' factor (i.e. factor group) and one 'within' factor (i.e. factor concentration). Two by two comparisons between the concentration-response curves were made only when the overall comparison was significant. Significance level was fixed at 5%.

Results

The experimental interventions did not affect systemic blood pressure, which was stable over the course of the different experiments and was within a range of 95–120 mmHg. Mean baseline arteriolar diameter for second and third order arterioles were 39.02 ± 2.43 μm and 26.45 ± 1.54 μm respectively. These diameters were not significantly different among the different groups and sets of experiments.

Effect of theophylline, adenosine and enprofylline

Figure 1 shows second and third order arteriolar response of diaphragm to topically applied theophylline, adenosine or enprofylline. Theophylline induced a significant and dose-dependent arteriolar dilatation (factor 'dose' $P<0.001$ for second and third order arterioles). A similar response was observed with adenosine. In both arteriolar orders, dilatation induced by theophylline was not statistically different from adenosine. Enprofylline also induced a significant and dose-dependent arteriolar dilatation (factor 'dose' $P<0.001$ for second and third order arterioles). However, this dilatation was significantly smaller than the one induced by theophylline ($P<0.05$ *versus* theophylline for second and third order arterioles).

Interaction of theophylline with adenosine receptors

Effect of adenosine receptor antagonists on theophylline-induced dilatation of diaphragm arterioles Figure 2 shows the effects of the A_1AR antagonist CPX and the A_2AR antagonist DMPX on arteriolar dilatation induced by theophylline in the

diaphragm. Neither agents significantly changed the concentration-response curves of second and third order diaphragm arterioles to theophylline.

Effect of adenosine in the presence of theophylline Figure 3 shows the effects of 100 nM of theophylline on adenosine-induced dilatation of diaphragm arterioles. In the presence of theophylline, dilatation induced by adenosine was significantly reduced as compared to the absence of the molecule ($P<0.01$ for second and third order arterioles).

Role of NO in arteriolar dilatation induced in the diaphragm by theophylline

Figure 4 shows the effects of NNA on arteriolar dilatation induced by theophylline in the diaphragm. NNA superfusion caused a significant reduction in baseline diameters of second and third order arteriolar diameters (Table 1). However, NNA did not significantly change the concentration-response curves of second and third order diaphragm arterioles to theophylline when expressed as % of new baseline.

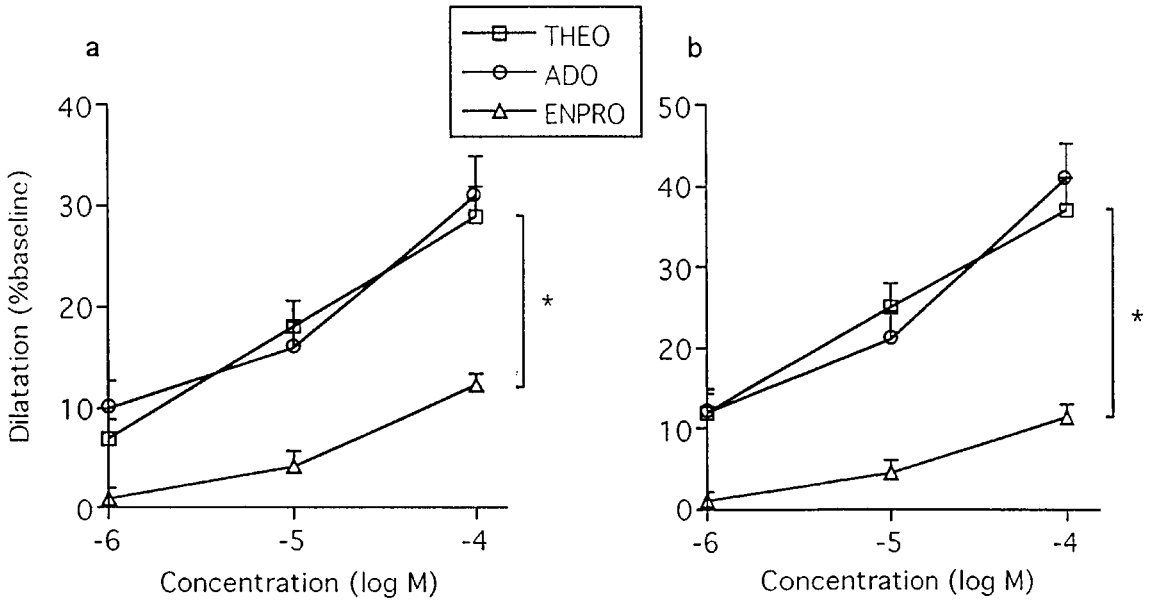

Figure 1 Concentration-response curves of (a) second and (b) third order diaphragm arterioles to theophylline (THEO), to adenosine (ADO) and to enprofylline (ENPRO). Data are expressed as mean±s.e.m. $N=8$ animals and $n=10-25$ arterioles per group respectively. Theophylline induced a significant and dose-dependent arteriolar dilatation which was similar to that of adenosine. Enprofylline also induced a significant and dose-dependent arteriolar dilatation. However, this dilatation was significantly smaller than the one induced by theophylline (*$P<0.05$).

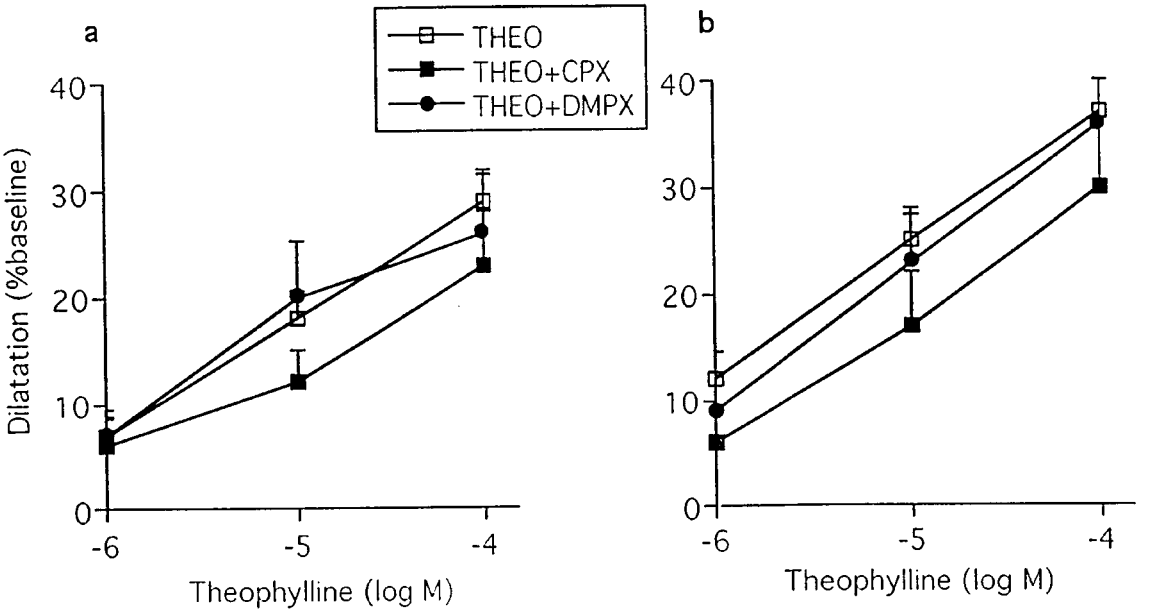

Figure 2 Concentration-response curves of (a) second and (b) third order diaphragm arterioles to theophylline (THEO) in the absence or in the presence of CPX (A_1AR selective agonist) or DMPX (A_2AR selective agonist). Data are expressed as mean±s.e.m. $N=5$ animals and $n=10$ arterioles per group respectively. Note that CPX and DMPX did not significantly modify theophylline-induced vasodilation of either order of diaphragm arterioles.

Role of prostaglandins in arteriolar dilatation induced in the diaphragm by theophylline

Figure 4 shows the effects of mefenamic acid and indomethacin on second and third order arteriolar dilatation induced by theophylline in the diaphragm. Both mefenamic acid and indomethacin superfusion caused a significant and similar reduction of baseline second and third order arteriolar diameters (Table 1).

Moreover, both agents inhibited significantly and similarly the arteriolar dilatation induced by theophylline ($P<0.01$ for each agent for second and third order arterioles). Indeed, no arteriolar significant dilatation, expressed as a % change in diameter from the new baseline, was observed when theophylline was applied in the presence of mefenamic acid or indomethacin.

Discussion

The main results of this study are: (1) Theophylline (1–100 μM) dilated second and third order diaphragm arterioles

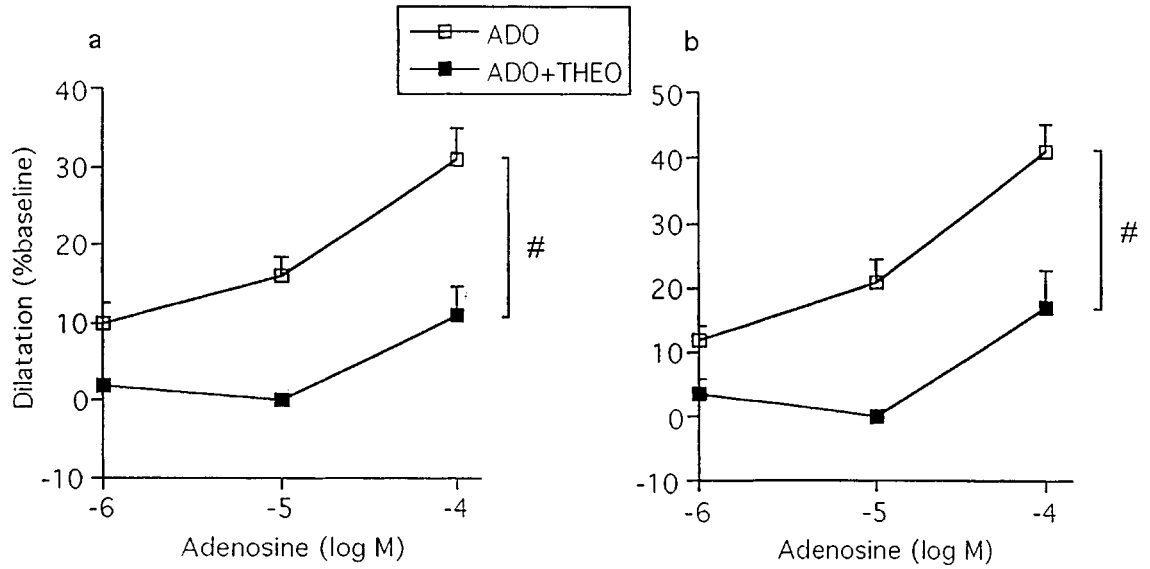

Figure 3 Concentration-response curves of (a) second and (b) third order diaphragm arterioles to adenosine (ADO) in the absence or in the presence of 100 nM of theophylline (THEO). $N=4-8$ animals, and $n=8$ arterioles per group respectively. Note that theophylline significantly inhibited adenosine-induced vasodilation of both orders of diaphragm arterioles (#$P<0.01$).

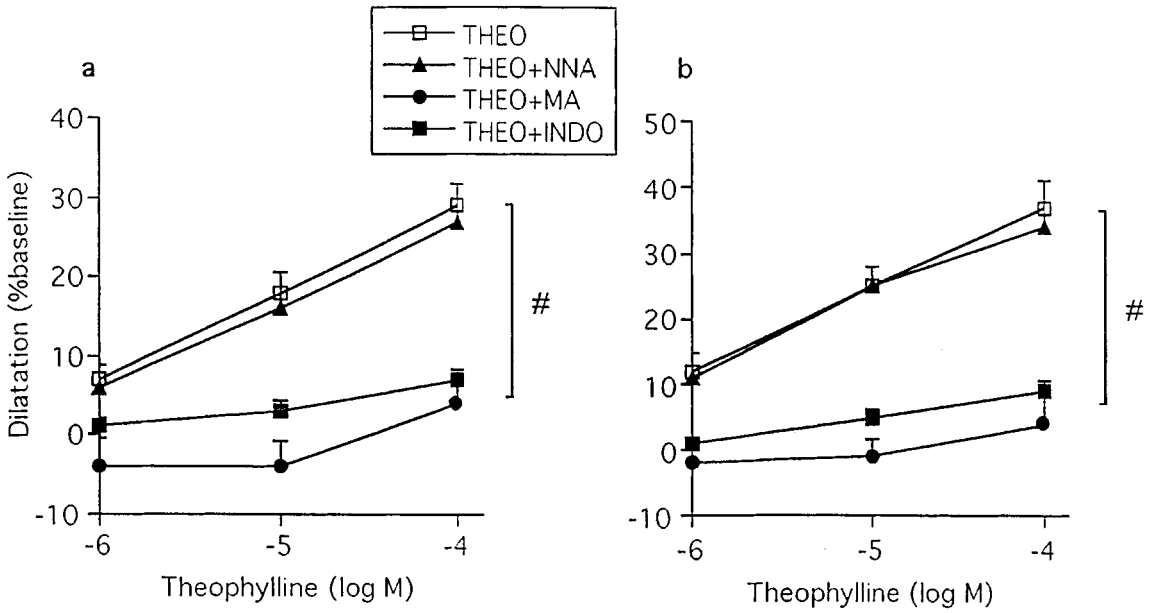

Figure 4 Effect of the NO synthesis inhibitor NNA and the prostaglandin synthesis inhibitors mefenamic acid (MA) and indomethacin (INDO) on the concentration-response curves of second and third order diaphragm arterioles to theophylline. The change in diameter induced by theophylline after these agents is presented as a % change from the new baseline. Data are expressed as mean$\pm$s.e.m, $N=5$ and $n=10$ arterioles per group respectively. NNA did not significantly modify theophylline-induced vasodilation of both order diaphragm arterioles. By contrast, both mefenamic acid and indomethacin significantly reduced arteriolar dilatation induced by theophylline in the diaphragm (# $P<0.01$).

Table 1 Diaphragm arteriolar diameter after superfusion of NAA, mefenamic acid indomethacin

	Second order arterioles	*Third order arterioles*
Diameter after NNA (% of original baseline)	84.4±2.9	83.2±3.1
Diameter after mefenamic acid (% of original baseline)	92.2±2.3	96.2±1.4
Diameter after indomethacin (% of original baseline)	92.2±2.3	94.1±1.4

Data are expressed as mean±s.e.m. $N=5$ and $n=10$ arterioles per group respectively. Diameters were significantly smaller after NNA than after mefenamic acid or indomethacin ($P<0.05$ respectively).

significantly, and with an amplitude which was not statistically different than the one obtained with adenosine; (2) Enprofylline also dilated diaphragm arterioles causing, however, a significantly smaller dilatation than theophylline; (3) Neither the A_1AR antagonist CPX nor the A_2AR antagonist DMPX reduced significantly theophylline-induced arteriolar dilatation; (4) Theophylline (100 nM) inhibited adenosine-induced arteriolar dilatation; (5) The dilatation induced by theophylline was unchanged by the NO synthesis inhibitor NNA; (6) Theophylline-induced arteriolar dilatation was abolished by the prostaglandin synthesis inhibitors mefenamic acid or indomethacin. To our knowledge, this is the first report showing that theophylline induces a significant dilatation of diaphragm arterioles *in vivo*. This dilatation amounted, for 100 μM of theophylline, 30% and 40% of baseline diameters for second and third order vessels respectively. It is noteworthy that this concentration of theophylline corresponds to the recommended theophylline serum concentration in treatment regimes (5–15 μg ml^{-1}).

Despite the fact that theophylline has been shown to induce vasodilatation in *in vitro* experiments (Kalsner *et al*., 1975; Hedqvist *et al*., 1978; Persson *et al*., 1983; Sjogren & Edvinsson, 1987; Rabe *et al*., 1995), the present results contrast with the effects of theophylline observed *in vivo* in the microcirculation of non-respiratory skeletal muscles. Indeed, with the exception of one study showing a very weak arteriolar dilatation (3–4% of baseline diameter for a concentration of 100 μM of theophylline; (Morff & Granger, 1983), several other studies found either no change in diameter (Mohrman & Heller, 1984), or an arteriolar constriction ranging from 17–30% of basal diameter (Proctor, 1984; Koller *et al*., 1991).

Different experimental conditions, such as the basal diameter of the studied vessels, could explain the discrepancy between our results and those reported in the non-respiratory skeletal muscles. Indeed, a small diameter could magnify the dilator effect of theophylline as a result of a change in the length-tension relationship of the arteriolar smooth muscle. However, we think that this is unlikely because, except for one study in which diameters were larger than in the present study (Morff & Granger, 1983), diameters in the present preparation were in the same range as in the cited studies in which theophylline did not induce arteriolar dilatation (Mohrman & Heller, 1984; Proctor, 1984; Koller *et al*., 1991). Furthermore, the dilatation induced by adenosine in the present model was very close to that observed in other muscular microvascular preparations (Morff & Granger, 1983; Vicaut & Hou, 1993).

It is thus likely that the difference between our results and those reported in other muscular microcirculations reflects heterogeneity in theophylline induced effects among different vascular beds. Several mechanisms could explain this phenomenon. One could be related to inhibition of PDE isoenzymes. Indeed, theophylline increases the intracellular concentration of cyclic nucleotides in airway and vascular smooth muscle by inhibiting PDE-mediated hydrolysis of these nucleotides, with ensuing smooth muscle relaxation (Rabe *et al*., 1995). In order to investigate the part of theophylline-induced dilatation which could be related to inhibition of PDE, we studied the changes in diaphragm arteriolar diameter induced by enprofylline, a theophylline analogue with poor adenosine-receptor antagonism but with similar or higher PDE inhibition properties than theophylline (Bergstrand, 1980; Ukena *et al*., 1985). Enprofylline dilated diaphragm arterioles. However, since this dilatation was significantly smaller than the one induced by theophylline, it is unlikely that inhibition of PDE is the only mechanism involved in theophylline-induced arteriolar dilatation in the diaphragm.

An interaction of theophylline with adenosine receptors in diaphragm arterioles might be another mechanism explaining theophylline-induced arteriolar dilatation. Theophylline is generally considered to be a non-specific adenosine receptor antagonist. However, some investigators have shown that theophylline is more selective for the A_1AR than for the A_2AR (Caussade & Cloarec, 1993), and recently we have demonstrated that adenosine-induced arteriolar dilatation in the diaphragm mainly involves the A_1AR (Danialou *et al*., 1997). It therefore seemed possible that at high concentrations theophylline acts as a partial A_1AR agonist, and that this could explain the dilatation of diaphragm arterioles observed in the present study. This hypothesis is, however, unlikely because the dilatator effect of theophylline was neither inhibited by the A_1AR not by the A_2AR antagonists, demonstrating that these receptors were not involved in theophylline-induced arteriolar dilatation in the diaphragm. It should be noted however that a slight reduction, amounting to 20–30% of theophylline-induced dilatation, was observed in the presence of the A1Ar antagonist CPX. Although this reduction was not statistically significant, a small participation of A_1AR in theophylline-induced arteriolar dilatation can not completely be ruled-out. It is interesting to note that even though the arteriolar dilatation induced by theophylline did not seem to be explained by stimulation of the A_1AR or A_2AR, it is very likely that theophylline was bound to the adenosine receptors in diaphragm arterioles, as demonstrated by the inhibition of adenosine-induced arteriolar dilatation by a non-dilator concentration of theophylline (Figure 3).

Finally, interaction of theophylline with the endothelial autacoids NO and prostaglandins might explain theophylline-induced arteriolar dilatation in the diaphragm since both substances have been implicated in the regulation of basal tone in skeletal muscle arterioles (Koller & Kaley, 1990; Kaley *et al*., 1992). The absence of effect of NNA on theophylline-induced second and third order arteriolar dilatation in the diaphragm showed that NO was not involved in the arteriolar responses to theophylline in the diaphragm. By contrast, mefenamic acid and indomethacin produced a complete inhibition of theophylline-induced arteriolar dilatation in the diaphragm. Since both agents increased basal arteriolar tone, it could be argued that the suppression of theophylline-induced dilatation was related to this increase in smooth muscle vascular tone. However, this possibility can be ruled out since NNA induced a larger increase in arteriolar tone than mefenamic acid and indomethacin, but did not modify the dilatation caused by theophylline. Rather, the complete inhibition of the theophylline-induced arteriolar dilatation by

mefenamic acid and indomethacin, which are two structurally different inhibitors of prostaglandin synthesis, clearly demonstrates a major role of prostaglandins.

To our knowledge, this is the first study showing a predominant role of prostaglandins in theophylline-induced vasodilation. This result is in contrast with *in vitro* data from precontracted human placental arteries showing that indomethacin did not modify theophylline-induced vasodilation (Nielsen-Kudsk, 1985). However, it is in agreement with data showing that theophylline stimulates prostaglandin synthesis in the guinea-pig uterus (Naderali & Poyser, 1994) and in the rat kidney (Caussade & Cloarec, 1993). The mechanism(s) by which prostaglandins mediated theophylline-induced arteriolar dilatation in the rat diaphragm is not clear. There is the possibility that theophylline stimulates local release of vasodilator prostaglandins in diaphragm arterioles by inhibiting PDE isoenzymes. However, evidence from the literature strongly suggest that PDE inhibition does not modulate the release of prostaglandins (Whorton *et al*., 1985; Schröder *et al*., 1992). Moreover, this conclusion is consistent with the present experiments with enprofylline, which showed that inhibition of PDE did not play a major role in theophylline-induced arteriolar dilatation. Alternatively, the interaction of theophylline with adenosine receptors could be postulated as another mechanism of an increase in the release of prostaglandins. However, the purinoreceptors involved in the release of prostaglandins from endothelial and smooth muscle cells are of the type P2, which mediate ATP-evoked effects (Demolle *et al*., 1988; Robertson *et al*., 1990), rather than of the type P1, which mediate adenosine-evoked effects. Finally, theophylline may increase the release of prostaglandins via an increase in intracellular calcium. The release of prostanoids in endothelial cells from large and small vessels is closely linked to the intracellular calcium concentration (Hyslop & De Nucci, 1993) and this phenomenon has been shown to be modulated by theophylline (Kolbeck *et al*., 1979).

In conclusion, this study showed that theophylline, at concentrations in the range of the recommended therapeutic plasma levels, significant dilated diaphragm arterioles via an effect on prostaglandin synthesis. This phenomenon has important potential implications because it may shed some light on the mechanisms involved in the beneficial effect of theophylline in the performance of diaphragm muscle.

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