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Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants

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Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. K.F. Rabe, H. Magnussen, G. Dent. ©ERS Journals Ltd 1995.
ABSTRACT: In addition to its emerging immunomodulatory properties, theophylline is a bronchodilator and also decreases mean pulmonary arterial pressure *in vivo*. The mechanism of action of this drug remains controversial; adenosine antagonism, phosphodiesterase (PDE) inhibition and other actions have been advanced to explain its effectiveness in asthma. Cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) are involved in the regulation of smooth muscle tone, and the breakdown of these nucleotides is catalysed by multiple PDE isoenzymes. The PDE isoenzymes present in human bronchus and pulmonary artery have been identified, and the pharmacological actions of inhibitors of these enzymes have been investigated.

Human bronchus and pulmonary arteries are relaxed by theophylline and by selective inhibitors of PDE III, while PDE IV inhibitors also relax precontracted bronchus and PDE V/I inhibitors relax pulmonary artery. There appears to be some synergy between inhibitors of PDE III and PDE IV in relaxing bronchus, and a pronounced synergy between PDE III and PDE V inhibitors in relaxing pulmonary artery. In neither tissue does 8-phenyltheophylline, a xanthine exhibiting adenosine antagonism but not PDE inhibition, cause any significant relaxation, implying that theophylline does not exert its actions through adenosine antagonism.

The close correspondence of theophylline concentrations inhibiting bronchus or pulmonary artery PDE and those causing relaxation points towards PDE inhibition as the major mechanism of action of theophylline in smooth muscle relaxation.
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For several years, theophylline (1,3-dimethylxanthine) and other xanthine derivatives have been recognized as effective agents for the treatment of reversible and chronic obstructive airways diseases [1, 2]. The introduction of slow-release formulations and the development of improved monitoring techniques for the avoidance of toxicity have led to increased use of theophylline in the clinic, whilst extensive laboratory investigations have increased our understanding of the mechanisms through which the drug exerts its actions.

Evidence is emerging that theophylline suppresses some aspects of the inflammatory response underlying asthma [3, 4], but the basis for the drug's therapeutic use in this disease, until now, has been its bronchodilatory action. Orally or intravenously administered theophylline or aminophylline improve forced expiratory volume in one second (FEV_1) in patients with chronic obstructive pulmonary disease (COPD) [2, 5–11] or asthma [1, 12, 13], whilst intravenous theophylline also protects against bronchoconstriction induced by methacholine, histamine or exercise in asthmatic patients [14, 15]. The actions of theophylline in asthmatics are clearly dose-related [12, 14], and, interestingly, the protective effect is more pronounced against histamine-induced than methacholine-induced bronchoconstriction [14]. Theophylline also decreases mean pulmonary arterial pressure [16–18], implying a relaxation both of airway and vascular smooth muscle. Various mechanisms have been proposed for this action, of which inhibition of cyclic nucleotide phosphodiesterase (PDE) is the most widely accepted. In this article, the functional role of cyclic nucleotides and PDE in regulation of smooth muscle tone are summarized, and the actions both of theophylline and of selective inhibitors of PDE isoenzymes on airway and pulmonary vascular smooth muscle are described.

Role of cyclic nucleotides in regulation of smooth muscle tone

Several neurotransmitters, hormones and autacoids, as well as such drugs as β-adrenoceptor agonists, activate cell surface receptors which, through stimulation of the enzyme adenylyl cyclase, cause increased production of cyclic adenosine 3',5'-monophosphate (cAMP). PDE, a group of enzymes catalysing the breakdown of cAMP and/or cyclic guanosine 3',5'-monophosphate (cGMP), presents a second target for regulation of intracellular cAMP levels.

In smooth muscle, cAMP activates two cyclic nucleotide-dependent protein kinases, protein kinase A ("cAMP-dependent") and protein kinase G ("cGMP-dependent") [19, 20], which phosphorylate a range of proteins involved in the induction and maintenance of contraction. Thus, intracellular calcium concentration ($[Ca^{2+}]i$) is lowered, through the increased activity of Ca^{2+}-adenosine triphosphatase (ATPase) and inhibition of Ca^{2+} channel opening, whilst large conductance Ca^{2+}-activated K^+ channels are opened and phosphatidylinositol metabolism and myosin light chain kinase activity are reduced [19]; thereby, suppressing force generation in the smooth muscle cell.

Phosphodiesterases and PDE inhibitor actions in the airways

Biochemical investigations have identified PDEs of the I, II, III, IV and V families in human bronchus and trachea [21–24]. PDE I is relatively scarce in bronchus, whilst PDE IV and cGMP-specific PDE V are the most abundant isoenzymes (fig. 1). In the trachea, PDE I represents two distinct enzymes and accounts for a large proportion of the total PDE activity [23].

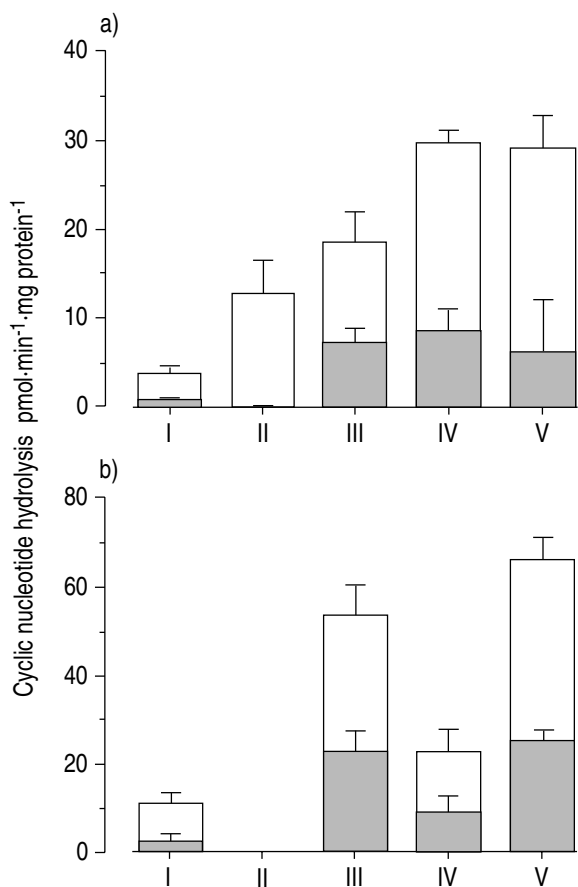Theophylline relaxes the inherent tone of human bronchial rings at similar concentrations to those which inhibit cAMP hydrolysis by PDE in homogenates of bronchial tissue (fig. 2). Theophylline appears to display no selectivity for particular PDE isoenzyme families, and the PDE inhibition curve is essentially monophasic. A similar pattern of inhibition of cAMP hydrolysis is observed with zardaverine, a dual inhibitor of the PDE III and PDE IV isoenzymes (table 1) that account for most of the cAMP-hydrolysing PDE in bronchus [22]. The potency of theophylline in relaxing bronchial smooth muscle is independent of the size of the airways [26–28], a property that is not shared by a selective PDE III inhibitor, siguazodan (see below).
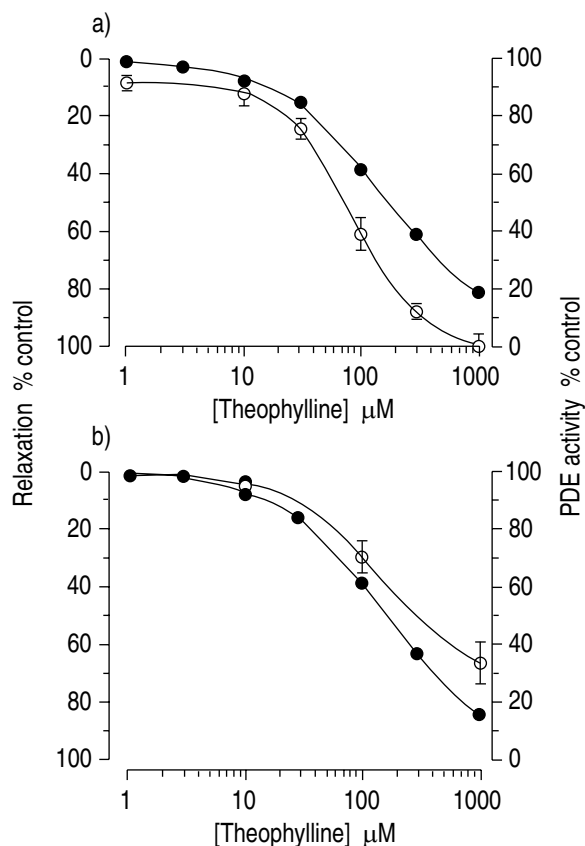

Fig. 2. – Inhibition of cyclic AMP PDE (—●—) and relaxation of smooth muscle (—○—) of: a) human bronchus; and b) pulmonary artery by theophylline. AMP: adenosine monophosphate; PDE: phosphodiesterase.

Table 1. – Isoenzyme selectivity of PDE inhibitors cited in this article

Drug	Selectivity
IBMX	Nonselective
Theophylline	Nonselective
Milrinone	III
Motapizone	III
Org 9935	III
Siguazodan	III
Org 30029	III/IV
Zardaverine	III/IV
Rolipram	IV
Zaprinast	V/I

PDE: phosphodiesterase; IBMX: isobutyl-1-methylxanthine.

Fig. 1. – Activities of PDE isoenzymes I–V in the cytosolic (▢) and particulate phases (▨) of homogenates of: a) human bronchus; and b) pulmonary artery PDE: phosphodiesterase. (Data are taken from RABE and co-workers [22, 25]).

The selective PDE III inhibitors, motapizone and Org 9935, and the PDE IV-selective inhibitor, rolipram, give biphasic curves for inhibition of PDE activity, apparently reflecting loss of isoenzyme selectivity at higher concentrations [21, 22]. Zardaverine and motapizone are also effective relaxants of bronchial smooth muscle inherent tone whilst rolipram relaxes bronchi only slightly at high concentrations (fig. 3), and the PDE V/I inhibitor, zaprinast, is also a rather weak relaxant [22]. In contrast, zaprinast is effective - and rolipram is highly effective - in relaxing bronchial smooth muscle precontracted with methacholine or histamine [21, 29] although data from another laboratory indicate that rolipram is also ineffective against carbachol-induced contractions in preparations which relax readily in response to the PDE III inhibitor, siguazodan [23]. In this latter study, it was also observed that, while siguazodan causes a tenfold potentiation of the relaxation of leukotriene D_4 (LTD_4)-precontracted bronchi to the β-adrenoceptor agonist, isoprenaline, this phenomenon occurs neither with inhibitors of PDE IV or V nor in tissues precontracted with carbachol instead of LTD_4 [23]. It has also been demonstrated that nonselective (isobutyl-1-methylxanthine (IBMX)), PDE III-selective (Org 9935), and PDE V/I-selective (zaprinast) PDE inhibitors are more potent against contractions induced by histamine than by methacholine (fig. 4) (cf. the effect of theophylline versus histamine- and methacholine-induced bronchoconstriction in vivo; described above); although, selective PDE IV (rolipram) and mixed PDE III/IV (Org 30029) inhibitors do not exhibit this differential effect [21]. Interestingly, rolipram and siguazodan exhibit synergy in relaxing carbachol-precontracted bronchi [23], confirming the earlier observation that dual inhibition of PDE III and PDE IV is a more effective mechanism for relaxation of human bronchus than selective inhibition of either enzyme [21].
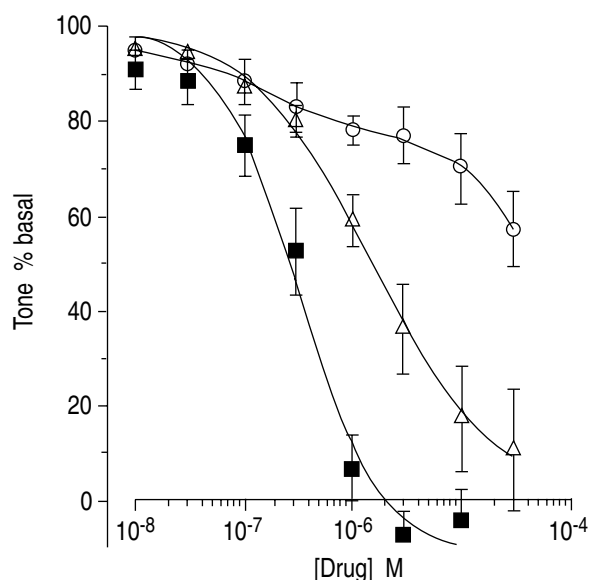

Fig. 4. – Relaxation of human bronchus, precontracted with histamine (—▲—) or methacholine (—○—), by: a) the PDE IV inhibitor rolipram; or b) the PDE III inhibitor Org9935. Results are mean±SEM. PDE: phosphodieste-rase. (Data are taken with permission from DE BOER et al. [21]).

No relaxations can be achieved with siguazodan - either alone or in combination with rolipram - in airways smaller than 2 mm in diameter [23].

Since theophylline, in addition to its PDE inhibitory activity, is an antagonist at adenosine receptors, blockade of adenosine activity has been proposed as a possible mechanism for theophylline's effects in asthma [30]. In human bronchial smooth muscle, however, 8-phenyl-theophylline - a methylxanthine adenosine antagonist with negligible PDE inhibitory actions - has no effect on inherent tone, whilst theophylline causes significant relaxation [31], implying that theophylline does not exert its action through adenosine antagonism.

The clinical effectiveness of theophylline as a bronchodilator has not, so far, been matched by PDE III/IV inhibitors or selective inhibitors of PDE IV [32], although some promising data from in vivo animal studies suggest that these drugs may have potential in asthma prophylaxis and disease modification [32–37]. One of the motives for seeking more selective PDE inhibitors for therapeutic use has been the desire to avoid the side-effects associated with high doses of theophylline. However, reappraisals of the role of theophylline in asthma therapy have drawn attention to the tendency to aim for

Fig. 3. – Effects of the mixed PDE III/IV inhibitor zardaverine (—■—), the selective PDE III inhibitor motapizone (—△—) and the selective PDE IV inhibitor rolipram (—○—) on inherent tone in human bronchus. PDE: phosphodiesterase. (Data are taken from RABE and co-workers [22]).
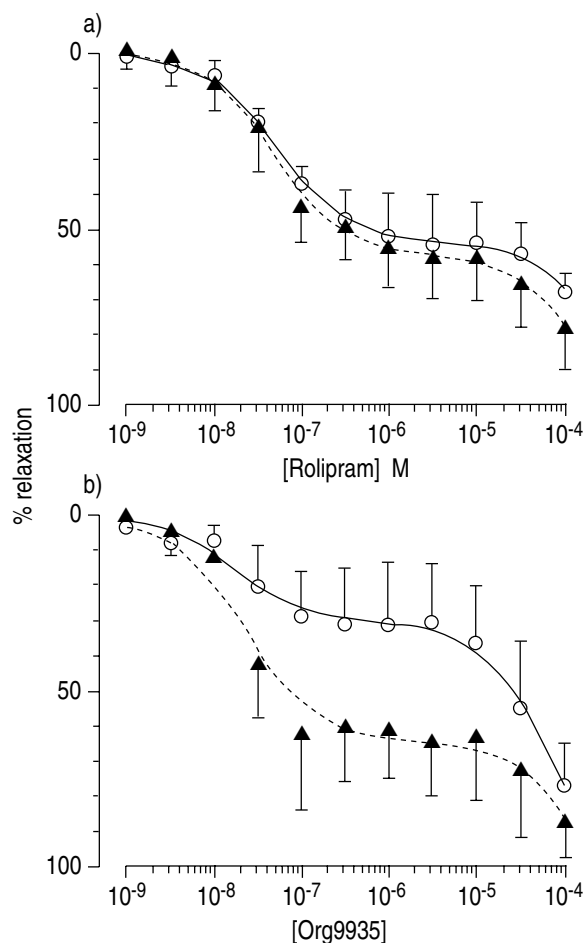
higher serum concentrations of theophylline than may be necessary [38]. The effectiveness of low doses of theophylline has been recognized for some time [14, 15], and the increased bronchodilation achieved by increasing serum theophylline concentrations far beyond 10 mg·l^{-1} is slight compared to the increased potential for side-effects [1, 38]. While new selective drugs are awaited, therefore, there may be grounds to reconsider the role of theophylline in the therapy of asthma.

Phosphodiesterases and PDE inhibitor actions in the pulmonary artery

Research into pulmonary vascular tone is comparatively scarce, and there is a limited amount of data available both on the *in vitro* regulation of artery tone and the clinical use of PDE inhibitors in pulmonary hypertension.

Biochemical investigations have identified PDEs of the I, III, IV and V families in human pulmonary artery [25]. PDE I activity is low, whilst PDE III and PDE V are the most abundant isoenzymes (fig. 1). This profile of PDE isoenzyme expression corresponds broadly to that observed in systemic blood vessels of the rat [39, 40] and human aortic smooth muscle cells [41].

Theophylline relaxes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced tone of pulmonary artery rings *in vitro* at similar concentrations to those which inhibit cAMP hydrolysis by PDE in artery homogenates (fig. 2), whilst 8-phenyltheophylline is ineffective. Although theophylline appears to be less potent in pulmonary artery than in bronchus, despite the similarity in the magnitude of inherent bronchial tone and $PGF_{2\alpha}$-induced arterial muscle contraction [22, 25], this may reflect differences in the nature of the musculature, including the fact that the total PDE activity in pulmonary artery is approximately double
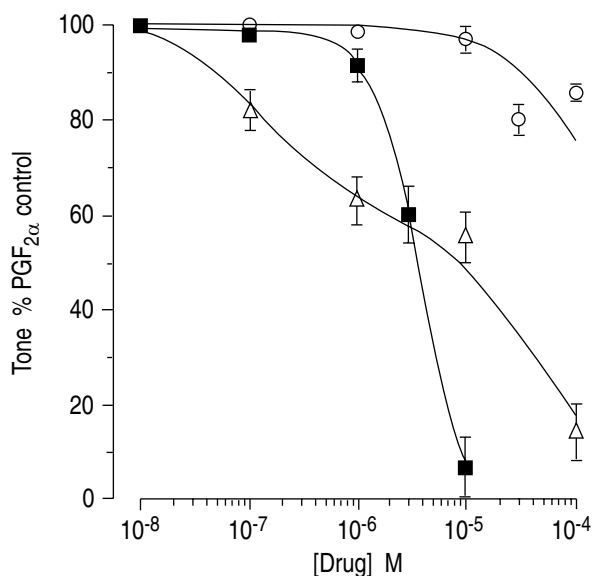

Fig. 5. – Effects of the mixed PDE III/IV inhibitor zardaverine (—■—), the selective PDE III inhibitor motapizone (—△—) and the selective PDE IV inhibitor rolipram (—○—) on inherent tone in human pulmonary artery. $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; PDE: phosphodiesterase. (Data are taken from RABE and co-workers [25]).
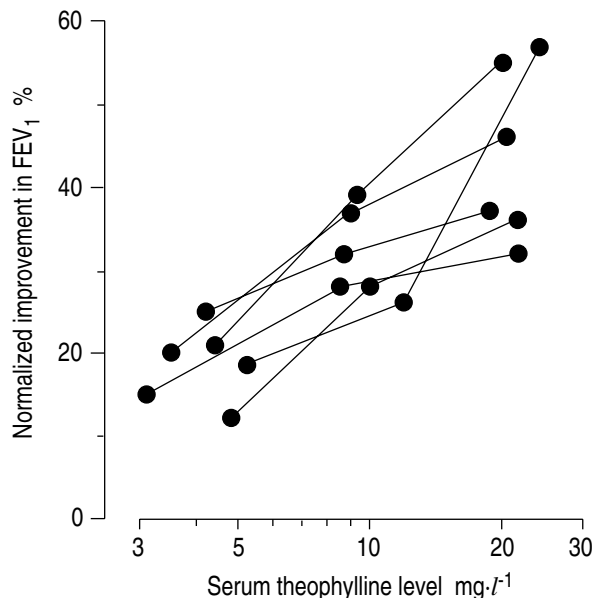

Fig. 6. – Relationship between serum theophylline concentrations and improvement in lung function in six juvenile asthmatic patients. FEV_1: forced expiratory volume in one second. (Data taken from MITENKO and OGILVIE [12]).

that in bronchus (fig. 1). As in bronchus, the PDE inhibition curve for theophylline is essentially monophasic, and a similar pattern of inhibition of cAMP hydrolysis is observed with zardaverine [25].

IBMX, zardaverine, motapizone and zaprinast all relax $PGF_{2\alpha}$-precontracted pulmonary artery rings, whilst rolipram has no significant effect at concentrations up to 100 µM (fig. 5), possibly reflecting the relatively small proportion of total PDE activity represented by PDE IV. In this tissue, there is a marked synergy between motapizone and zaprinast, a combination that relaxes precontracted arterial rings to well below basal tension [25]. Thus synergy may reflect additional inhibition of PDE III by the elevated intracellular concentrations of cGMP resulting from PDE V inhibition or a combination of inhibitory actions of protein kinases A and G. Like theophylline, selective PDE III inhibitors, such as milrinone, decrease mean pulmonary artery pressure in patients with pulmonary hypertension [42], although it remains to be seen whether PDE V inhibitors share this action.

Conclusion

In conclusion a direct relaxant effect of theophylline on bronchial smooth muscle can be demonstrated, which may underlie the clinical bronchodilator properties of this drug. The range of theophylline concentrations in which this relaxant action is exerted corresponds closely with the concentrations causing bronchodilation *in vivo* (fig. 6): a concentration of 70 µM (approximate median effective concentration (EC_{50}) for relaxation of human bronchus) is equivalent to 12.6 mg·l^{-1}, an effective bronchodilator and bronchoprotective serum concentration [12, 14]. The most likely mechanism for this action is inhibition of PDE, offering hope for the development of more potent and selective PDE inhibitors for clinical

use as bronchodilators. Similarly, theophylline and selective inhibitors of certain PDE isoenzymes cause a direct relaxation of pulmonary arterial smooth muscle, which may be of clinical use in treating the pulmonary hypertension often associated with COPD and acute asthma. Evidence has been presented for the efficacy of low doses of theophylline in improvement of lung function [15, 38] and suppression of inflammatory processes in asthma [43], which may lead to the reassessment of the drug's role in asthma therapy, whilst we await the clinical development of newer, more selective PDE inhibitors.

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