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Dual effect of (−)-N^6-phenylisopropyl adenosine on guinea-pig trachea

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1 The effect of (−)-N^6-phenylisopropyl adenosine (PIA), a metabolically stable P_1-receptor agonist, was investigated on guinea-pig isolated trachea.

2 PIA showed two opposite effects: contraction, evident at low concentrations (10^{-7} to $2-5 \times 10^{-6}$ M), and relaxation at higher doses.

3 Relaxation by PIA was antagonized in an apparently competitive manner by two antagonists of extracellular (P_1) adenosine receptors: theophylline (Theo) and 8-phenyltheophylline (PT).

4 Contraction by PIA was not inhibited by methylxanthines and was not mediated by stimulation of cholinergic or histaminergic systems.

5 Inhibitors of arachidonic acid cascade acting at different levels, i.e. indomethacin, nordihydroguaiaretic acid (NDGA) and BW 755C, all inhibited the contraction by PIA, while they potentiated the relaxation in a concentration-dependent manner. Mepacrine, an inhibitor of phospholipase A_2, inhibited the contraction by PIA, but did not affect the relaxation.

6 These results indicate that the contractile effect induced by PIA is supported by an indirect mechanism involving the release of arachidonic acid derivatives (via P_2-purinoceptor?). Thus the balance between the two opposite effects of adenosine on tracheal tone is possibly modulated by the prostaglandin turnover.

Introduction

Evidence for a purinergic inhibitory innervation of the guinea-pig and dog trachea has been obtained by several groups in recent years (Coburn & Tomita, 1973; Coleman & Levy, 1974; Richardson & Bouchard, 1975; Coleman, 1976; Farmer & Farrar, 1976; Kamikawa & Shimo, 1976; Cameron & Kirkpatrick, 1977; Christie & Satchell, 1980; Satchell & Maguire, 1983). Most of the research in this field deals with the ability of adenosine to relax airway smooth muscle. However, excitatory responses to ATP and adenosine have been observed (Coleman & Levy, 1974; Kamikawa & Shimo, 1976; Coleman, 1976).

Recently adenosine and ATP were found to exert different effects on the guinea-pig isolated trachea (Advenier *et al.*, 1982), depending on whether the trachea had previously been contracted with acetylcholine or was at resting tone. Prostaglandins appeared to be involved in this effect (Kamikawa & Shimo, 1976; Advenier *et al.*, 1982).

PIA, (−)-N^6-phenylisopropyl adenosine is a potent, metabolically stable adenosine analogue, acting as agonist on adenosine-responsive systems (Schwabe, 1981). PIA has a higher affinity for extracellular adenosine receptors; that is, the P_1-receptor according to the classification of Burnstock (1978), or the R-receptors according to Londos & Wolff (1977).

In preliminary experiments on guinea-pig isolated trachea (Fassina *et al.*, 1982) PIA showed a contractile effect at lower concentrations (0.1–2 μM) while at higher ones (5–200 μM) it caused relaxation.

The purpose of the present study was to investigate the mechanism of the dual effect of PIA on guinea-pig trachea and its relation with adenosine function on this organ.

Methods

Tracheal chains were placed in a 10 ml organ bath containing Tyrode solution at 37° and gassed with a mixture of 95% O_2 and 5% CO_2. The tracheal chains were connected to isotonic transducers (ECTA Mod. ITE-51) and a Watanabe recorder (LINEAR CORDER Mar III). The resting tension was adjusted to

0.5 g. After an equilibration period of 60 or more min, the tracheae were contracted with carbachol (0.1–0.5 μM) and relaxed with noradrenaline (1 μg ml^{-1}). After washing, the tracheae were left to reach control level tone.

Increasing concentrations of PIA were added cumulatively to the bath and left to act until a steady state tension was reached (10 to 15 min). Responses to PIA were expressed as a percentage of the maximal relaxation evoked by noradrenaline (1 μg ml^{-1}).

PIA was also tested in tracheae contracted with carbachol to 50% or to 80–90% of maximal tension; or in trachea relaxed with noradrenaline (1 μg ml^{-1} = 3.10^{-6} M).

In those experiments where the effect of inhibitors were studied, the drugs were incubated with the tissue for 30 min before adding PIA.

Drugs used (−)-N^6-phenylisopropyl adenosine (Boehringer), noradrenaline bitartrate (Sigma), 8-phenyltheophylline (Calbiochem), theophylline and nordihydroguaiaretic acid (Sigma), indomethacin (Merck, Sharp & Dohme), FPL 55712 (Sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy)-2hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate, Fisons Ltd., Loughborough), BW 755C 3-amino-1-[3-trifluoromethyl-phenyl]-2-pyrazdine, Wellcome Research Labs., Beckenham. Atabrine hydrochloride (mepacrine; Winthrop Labs., New York) was kindly donated by Drs Cristiano Staidler and V. Bollati, Winthrop, Milano.

All compounds were made up in aqueous solution when possible. Indomethacin and other drugs not water soluble, were dissolved and added to the bath in amounts of 90° EtOH or DMSO that did not affect the responses of preparations.

Statistical analysis of results

All values in the text and Figures are expressed as mean ± s.e.mean. Significance of differences in the experimental results was performed using Student's *t* test, and assumed significant at the 1% probability level.

Results

Dual effect of phenylisopropyl adenosine

PIA showed a dual effect (contraction and relaxation), depending on the concentration (Figure 1). At concentrations lower than 10^{-6} M, PIA had a slow, long lasting contractile effect. Non-cumulative assays demonstrated that these contractions are constant for 30 and more min (not shown). Relaxation occurred at higher concentrations (Figure 1). At 10^{-4} M, PIA gave a relaxation similar to the maximal response to noradrenaline or theophylline 10^{-3} M.

Effect of phenylisopropyl adenosine in the presence of carbachol and noradrenaline

The contractile effect of PIA was evident not only under resting tone, but also when tracheae had previously been contracted with carbachol 5 × 10^{-8} M. Contraction by PIA was no longer evident after contraction by carbachol 5 × 10^{-7} M, a concentration inducing 80–90% of maximal tension (not shown).

Contraction by PIA was very evident when tracheal chains had previously been relaxed with noradrenaline at maximum relaxing concentration (3 μM).

Influence of theophylline and 8-phenyltheophylline

Alkylxanthines are known to be antagonists of adenosine at the level of extracellular adenosine receptors (Burnstock, 1978; Fredholm & Persson, 1982).

The cumulative dose-response curve of PIA was shifted to the right by theophylline (Theo) and by 8-phenyltheophylline (*PT*) and the contraction was not antagonized (Figure 2).

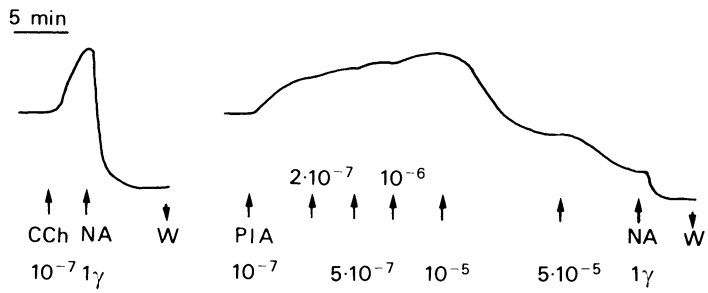

Figure 1 Dual effect of (−)-N^6-phenylisopropyl adenosine (PIA) on guinea-pig isolated trachea. Before treatment with PIA, carbachol (CCh) 10^{-7} M and noradrenaline (NA, 1 μg ml^{-1} = 3 μM) were used to test the tracheal chain reactivity.

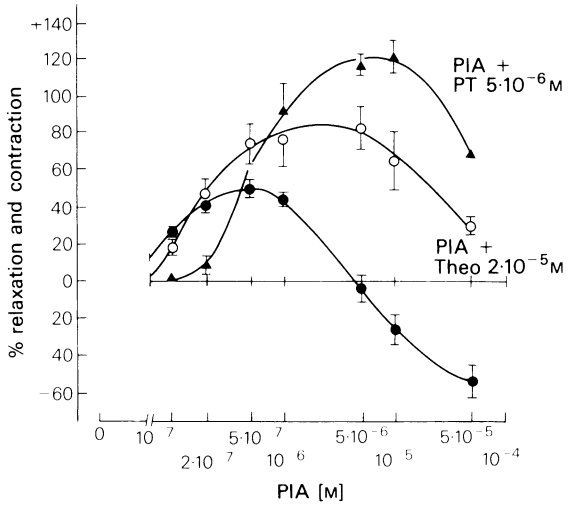

Figure 2 Effect of theophylline (Theo) and of 8-phenyltheophylline (PT) on cumulative concentration-curves for phenylisopropyl adenosine (PIA). Theo 20 μM and PT 5 μM were added 30 min before PIA. The contraction and relaxation by PIA are expressed as percentage of maximum relaxation induced by noradrenaline (3 μM). Data are the mean of 5 to 12 experiments; s.e.mean shown by vertical lines.

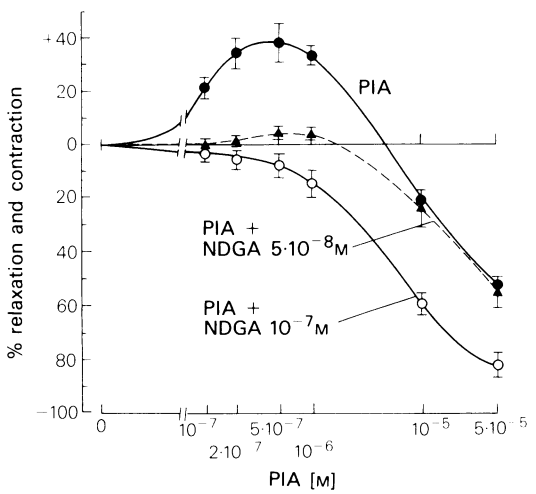

Figure 4 Cumulative concentration-response curves for phenylisopropyl adenosine (PIA) on guinea-pig tracheal chain, in the presence of nordihydroguaiaretic acid (NDGA). NDGA 0.05 μM (▲) and 0.1 μM (○) was added before PIA. The contraction and relaxation by PIA are expressed as percentage of the maximum relaxation induced by noradrenaline (3 μM). Values are the means of 12 experiments.

Theo 2×10^{-5} M decreased the relaxing component of PIA while it enhanced the contraction. In these conditions, concentrations of PIA that normally relax tracheae (10^{-5} M), had a contractile effect in the presence of Theo 2×10^{-5} M (Figure 2). It has to be pointed out that the IC_{50} of Theo versus phosphodiesterase is in the range of 2 to 10 mM (Fredholm, 1980; Scotini *et al.*, 1983). Theo 2×10^{-5} M did not affect the response to carbachol or noradrenaline.

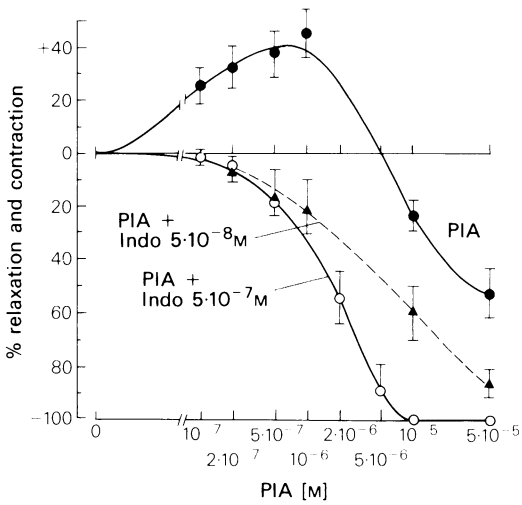

Figure 3 Cumulative concentration-response curves for phenylisopropyl adenosine (PIA) in guinea-pig tracheal chains pretreated with indomethacin (Indo) 0.05 μM (▲) and 0.5 μM (○). Indodomethacin was added 30 min before PIA. Each point represents the mean of 12 experiments; s.e.mean shown by vertical lines.

PT is more selective than theophylline as an antagonist of adenosine extracellular receptors (Smellie *et al.*, 1979; Bruns, 1981; Griffith *et al.*, 1981) and is a very poor inhibitor of PDE in peripheral tissues (Scotini *et al.*, 1983). PT 5×10^{-6} M enhanced the contraction by PIA while, like theophylline, it decreased the relaxing component (Figure 2).

The possible involvement of the cholinergic or histaminergic systems in the contractile effect induced by PIA was excluded, because pretreatment with pirylamine or atropine did not modify the contractile response to low PIA concentrations (not shown). Thus, the influence of arachidonic acid inhibitors was tested.

Effect of arachidonic acid cascade inhibitors

At 5×10^{-8} M and 5×10^{-7} M, indomethacin did not modify the muscle tension but antagonized the contractile effect of PIA and potentiated its relaxant effect in a concentration-dependent manner (Figure 3). Concentration-response curves for PIA-induced relaxation were shifted to the left by indomethacin.

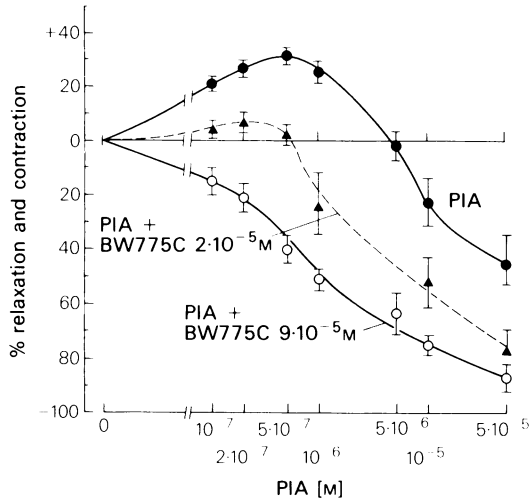

Figure 5 Cumulative concentration-response curves for phenylisopropyl adenosine (PIA) on guinea-pig tracheal chains, in the absence and presence of BW 755C. BW 755C 20 μM (▲) and 90 μM (○) was added 30 min before PIA. The contraction and relaxation by PIA are expressed as a percentage of the maximum relaxation induced by noradrenaline (3 μM). Values are the means of 10 experiments; s.e.mean shown by vertical lines.

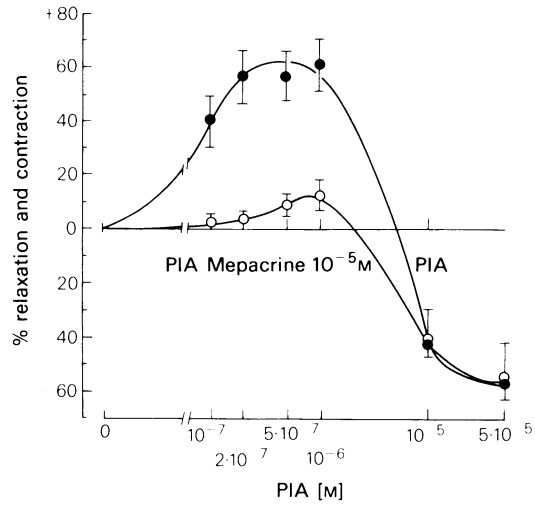

Figure 6 Cumulative concentration-response curves for phenylisopropyl adenosine (PIA) on guinea-pig tracheal chain in the absence (●) and in the presence (○) of mepacrine. Mepacrine (10 μM) was added 30 min before PIA. The contraction and relaxation by PIA are expressed as a percentage of the maximum relaxation induced by noradrenaline (3 μM). Values are the means of 4 experiments; s.e.mean shown by vertical lines.

The relaxing ED_{50} of PIA was reduced from 5×10^{-5} M to 2×10^{-6} M by indomethacin 5×10^{-7} M.

Also nordihydroguaiaretic acid (NDGA), a lipoxygenase and cyclo-oxygenase inhibitor, at very low concentrations (5×10^{-8} and 10^{-7} M) inhibited the contraction induced by PIA (Figure 4). The concentration-response curve for relaxation by PIA was shifted to the left by NDGA in a concentration-dependent manner.

BW 755C, another compound which inhibits both cyclo-oxygenase and lipoxygenase, abolished the contractile effect of PIA and potentiated the relaxation in a concentration-dependent manner (Figure 5).

Mepacrine (10^{-5} M), an inhibitor of phospholipase A_2, inhibited the contractile effect of PIA without affecting the relaxant component (Figure 6).

Indomethacin, NDGA and mepacrine, at concentrations that inhibit PIA-induced contraction, did not affect the response to carbachol or noradrenaline. Only BW 755C 10^{-5} M and 5×10^{-5} M, was able to reduce the contractile response to carbachol (not shown).

Effect of a leukotriene antagonist, FPL 55712

FPL 55712, a leukotriene antagonist in guinea-pig trachea (Armour *et al.*, 1982), was tested at concentrations ranging from 10^{-7} to 10^{-5} M. This compound did not modify the concentration-response curve to PIA (not shown), thus apparently excluding the involvement of leukotrienes in the PIA-induced contraction.

Discussion

The present results show that PIA exerts two opposite effects on guinea-pig trachea, depending upon the concentration: contraction, which is evident at low concentrations (10^{-7} to $2-5 \times 10^{-6}$ M) and relaxation at higher doses. Contraction by PIA was not antagonized by Theo and PT. This contraction was weak as compared with that induced by carbachol, slow in onset, long lasting and not accompanied by tachyphylaxis. The contraction by PIA was evident under resting tone as well as when PIA was added to low tone preparations (in the presence of noradrenaline) or to preparations partially pre-contracted by carbachol.

A contraction by adenosine and ATP on isolated tracheal muscle of guinea-pig was also observed by Advenier *et al.* (1982), but only in low tone preparations and tachyphylaxis rapidly developed. These differences between the effects of PIA and adenosine or ATP, are probably due to the fact that PIA is a

metabolically stable analogue of adenosine and a more potent agonist on adenosine-responsive systems (Schwabe, 1981).

The contractile response of trachea to PIA is not mediated by stimulation of cholinergic or histaminergic systems, as suggested by the fact that pretreatment with atropine or pirylamine did not modify this response.

All the inhibitors of the arachidonic cascade tested, i.e. indomethacin, NDGA and BW 755C, abolished the tracheal contraction induced by PIA, while they potentiated the relaxation in a concentration-dependent manner. Among these compounds the cyclo-oxygenase inhibitor, indomethacin (Vane, 1971) was the most effective blocker of PIA-induced contraction, suggesting that PIA may act by stimulating the release of prostaglandins or thromboxanes, which in turn contract the guinea-pig trachea. However, the fact that mixed inhibitors of cyclo-oxygenase and lipoxygenase, NDGA (Hamberg, 1976) and BW 755C (Higgs *et al.*, 1978; Higgs & Mugridge, 1983) as well as the inhibitor of phospholipase A_2, mepacrine (Vargaftig & Hay, 1972; Flower, 1974) also blocked PIA-induced contractions does not exclude the possibility that PIA acts in part by promoting the release of leukotrienes, which contract the trachea (Folco *et al.*, 1982; Piper & Tippins, 1980). However, contraction by PIA was refractory to FPL 55712, a leukotriene antagonist on guinea-pig trachea (Armour *et al.*, 1982). This result makes the involvement of leukotrienes unlikely. This is in agreement with the findings that adenosine and ATP (Advenier *et al.*, 1982) induce a moderate contraction of low tone guinea-pig trachea by releasing prostaglandins and/or thromboxane A_2 (TXA_2). Kamikawa & Shimo (1976) suggested that prostaglandin E_2 (PGE_2) is the prostaglandin involved in the biphasic response to ATP. But, in our experimental conditions, PGE_2 caused relaxation, while $PGF_{2\alpha}$ contracted tracheal muscle, in accordance with the literature (see review: Horton, 1979). Thus our results do not allow identification of the specific prostanoid responsible for PIA-induced contraction.

The mechanism by which BW 755C, but not other arachidonic acid cascade inhibitors, reduces the contractile effect of carbachol remains to be elucidated.

Relaxation by PIA is in accordance with the well-documented relaxant effect of adenosine in high tone tracheal preparations (Coleman & Levy, 1974; Coleman, 1976; Farmer & Farrar, 1976; Christie & Satchell, 1980; Brown & Collis, 1982).

Relaxation by PIA was antagonized by low concentrations of theophylline. This drug is an antagonist of adenosine at the level of extracellular adenosine receptors (Burnstock, 1978; Schwabe, 1981; Bruns *et al.*, 1983). 8-Phenyltheophylline, an antagonist of extracellular adenosine receptors more selective than theophylline (Smellie *et al.*, 1979; Bruns, 1981; Griffith *et al.*, 1981), was also able to antagonize PIA-induced relaxation in an apparently competitive manner. These results indicate that guinea-pig trachea possesses extracellular adenosine receptors that mediate relaxation, in accordance with previous data (Clark *et al.*, 1980; Coleman, 1980; Brown & Collis, 1982; Satchell & Maguire, 1983).

Burnstock (1978, 1979) proposed some criteria that would allow distinction between two types of purinoceptors, namely P_1 and P_2. P_1-receptors are blocked by methylxanthines, are most sensitive to adenosine; their activation leads to changes in cyclic AMP levels without affecting prostaglandin synthesis. P_2-receptors are not blocked by methylxanthines, are more sensitive to ATP, and their occupation does not affect cyclic AMP accumulation but increases prostaglandin biosynthesis. On the basis of these criteria, P_2-receptors might exist in the trachea which would mediate the methylxanthine-insensitive contractile effect of PIA, which was blocked by arachidonic acid cascade inhibitors.

In conclusion, PIA has two opposite effects, depending on dose: contraction, which is evident at low concentrations and is mediated by an indirect mechanism involving the release of arachidonic acid derivatives; relaxation at higher doses, due to a direct interaction with extracellular purine (P_1) receptors.

These findings further support the importance of purines in controlling tracheal muscle resistence, and also stress its complex mechanism. Adenosine-induced bronchoconstriction has been reported by Lefort & Vargaftig (1978) in the guinea-pig *in vivo*. Moreover, Cushley & Holgate (1983) found that adenosine is a potent bronchoconstrictor in human asthma, and stressed its specific relationship to airway reactivity. Adenosine effects may be variable and even opposite, depending on the metabolic state of the tissue and, in particular, on the turnover of prostaglandins.

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