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CONTRIBUTION OF PROSTAGLANDINS AND THROMBOXANES TO THE ADENOSINE AND ATP-INDUCED CONTRACTION OF GUINEA-PIG ISOLATED TRACHEA

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1 In *in vitro* experiments adenosine 5'-triphosphate (ATP) and adenosine were found to exert different effects on the guinea-pig isolated trachea depending on whether the trachea had previously been contracted with acetylcholine (ACh) (6.6×10^{-6} M) or was at resting tone.

2 ATP and adenosine (10^{-5} and 10^{-3} M) were equipotent in relaxing the precontracted guinea-pig trachea, since concentrations of 1.09 ± 0.35 and 0.39 ± 0.16 mM respectively reduced by 25% the ACh-induced contraction.

3 ATP and adenosine (10^{-5} and 10^{-4} M) caused a moderate contraction of the guinea-pig trachea under resting tone. This effect was antagonized by inhibitors of cyclo-oxygenase (indomethacin 10^{-6} M, aspirin 0.3×10^{-3} M and 3×10^{-3} M) and of thromboxane synthetase (nictindole 10^{-7} M, imidazole 5×10^{-5} M), which suggests an indirect mechanism of action with release of arachidonic acid derivatives.

Introduction

Adenosine 5'-triphosphate (ATP) and adenosine have been shown to exert a relaxant effect on a variety of guinea-pig isolated lung preparations (Coleman & Levy, 1974; Coleman, 1976; Farmer & Farrar, 1976; Christie & Satchell, 1980). This effect is strongly potentiated by dipyridamole (Coleman & Levy, 1974), but whether P_1- or P_2-purinoceptors (Burnstock, 1978), are involved, remains uncertain (Coleman, 1980; Clard, Small & Turnbull, 1980). *In vivo*, both ATP and adenosine may produce bronchoconstriction in guinea-pigs, and this has been correlated with effects on platelets (Lefort & Vargaftig, 1978).

In preliminary experiments on guinea-pig isolated trachea we were able to demonstrate that the effects of ATP and adenosine differ according to the initial tone of that organ: they contract the trachea under resting tension but relax the trachea previously contracted with acetylcholine (ACh). The purpose of the present study was to investigate the mechanism of this dual effect. The results suggest that the contraction induced by ATP and adenosine is due to release of arachidonic acid derivatives.

Methods

Guinea-pig trachea in vitro

Tracheal spirals containing 2 to 4 cartilaginous rings were obtained from male guinea-pigs (250–350 g) anaesthetized with urethane (1.25 mg/kg) and were equilibrated under an initial tension of 1.20 g in Tyrode solution at 37°C gassed with O_2. Tension was measured isometrically with a Ugo Basil strain gauge and was displayed on a Ugo Basil channel pen recorder. The initial tension ensured that after a 1.50 h equilibration period the resting tension was between 0.3 and 0.7 g. Under these conditions responses to agonists were reproducible and maximal (Stephens, 1970).

The composition of the Tyrode solution was (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, Na_2HPO_4 0.4 and glucose 5.5. Preparations were contracted to maximal tension with acetylcholine (ACh) 6.6×10^{-5} M and tested for maximal relaxation with isoprenaline (Iso) 3.9×10^{-6} M.

Experimental procedure

In a first series of experiments tracheal spirals were contracted with ACh (6.6×10^{-6} M) to 80–90% of maximal tension. Incremental concentrations of purines were then added to the bath, either alone or in the presence of dipyridamole or dipyridamole plus aspirin or indomethacin. Responses were expressed as concentrations (mM) which reduced the ACh-induced contraction by 25%.

In a second series of experiments, tracheal spirals were first tested twice at 15 min interval with ACh (6.6×10^{-5} M), then allowed to resume resting ten-

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sion (376 ± 69.5 to 646 ± 126 mg). Incremental concentrations of purines were added to the bath. Owing to the development of tachyphylaxis, only one series of purine concentrations was used for each tracheal preparation. ATP or adenosine was added alone (controls) or after one of the pretreatment agents, which was introduced 20 min before one or other of the purines. In order to control experimental conditions, experiments were performed against incremental concentrations of ACh. Responses were expressed as a percentage of the maximal response to ACh. In these experiments changes in resting tension produced by the pretreatment agents were not significantly different from control values (0.533 ± 0.170 g).

Drugs

The drugs used were: adenosine (Merck, Darmstadt), ATP (Sigma, Philadelphia), mepyramine maleate, atropine sulphate, methysergide bimaleate, phentolamine methane sulphonate (Regitine, Ciba), indomethacin, aspirin as lysine acetylsalicylate, nictindole, imidazole, theophylline as sodium anisate, dipyridamole, nifedipine and FPL 55712 (sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy) -2-hydroxypropoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate).

With the exception of phentolamine, which was used as a proprietary injectable solution (Regitine, Ciba), all substances were in powder form and were dissolved in Tyrode solution. However, indomethacin, nictindole and dipyridamole were dissolved and added to the bath in amounts of 90° ethanol that did not affect the responses of the preparations.

Statistical analysis of results

All values in the text and tables are expressed as mean ± s.e.mean, Statistical analysis of the results was performed using Student's *t* test.

Results

The effects of adenosine and ATP on the guinea-pig isolated trachea were different when the trachea was previously contracted with ACh (6.6×10^{-6} M) to 805 ± 76.8 mg above resting tone and when it was under resting tension (Figure 1).

Tracheal spirals precontracted with ACh were moderately relaxed by ATP and adenosine. The concentrations (mM) of these two substances producing a 25% relaxation of the trachea contracted with ACh were not significantly different (1.09 ± 0.35 and 0.39 ± 0.16 respectively). Relaxation was considerably potentiated by dipyridamole (Figure 2 and Table 1), but relaxation in the presence of dipyridamole was unaffected by aspirin or indomethacin. No tachyphylaxis occurred.

Tracheal spirals under resting tension were contracted by ATP and adenosine in concentrations of 10^{-5} and 10^{-4} M (Figure 1 and Table 2). The effect was modest compared with the maximal ACh-induced contraction, and tachyphylaxis occurred. In concentrations of 10^{-3} M, the contraction induced by ATP and adenosine was less pronounced (+ 6.2 ± 2.6% and + 2.2 ± 2.6% respectively of the maximal ACh-induced contraction).

The influence of various agents on the action of ATP and adenosine on the trachea under resting tension was investigated (Table 2). Mepyramine, atropine, methysergide, phentolamine and FPL 55712 had no significant effect. On the other hand, indomethacin, aspirin, nictindole, imidazole, theophylline, nifedipine or dipyridamole significantly reduced the action of ATP and adenosine in concentrations of 10^{-4}M. When ATP and adenosine concentrations were 10^{-5} M, the reduction caused by these agents was not always sufficient to reach statistical significance.

Indomethacin (10^{-6} M) or aspirin (0.3 and 3×10^{-3} M) did not modify the effects of incremental doses of ACh (10^{-7} to 6.6×10^{-5} M), neither did they significantly modify the intrinsic tone of the trachea. In our experiments, the intrinsic tone was particularly low, as it was unmodified by theophylline (10^{-6} and 10^{-5} M) and was only lowered by 9.9 ± 2.6% from an initial value of 588 ± 69 mg ($P < 0.01$, $n = 12$) when isoprenaline (3×10^{-6} M) was added to the bath.

Discussion

Adenosine and ATP exert two opposite effects on the isolated tracheal muscle of guinea-pig: relaxation or contraction.

Relaxation only occurs with precontracted (high tone) preparations and is well documented (Coleman & Levy, 1974; Coleman, 1976; Christie & Satchell, 1980). ATP and adenosine have comparable activities, and their action is considerably reinforced by dipyridamole (Coleman, 1976; Farmer & Farrar, 1976; Christie & Satchell, 1980), an agent reported to inhibit adenosine uptake (Kolassa, Pfleger & Rummel, 1970).

Contraction can be demonstrated when ATP or adenosine are added to low tone preparations in concentrations of 10^{-5} or 10^{-4} M. In this, as in the relaxant effect, ATP and adenosine appear to be equipotent. The ATP- or adenosine-induced contraction is moderate when compared with that induced by ACh. Contrary to the relaxant effect, the

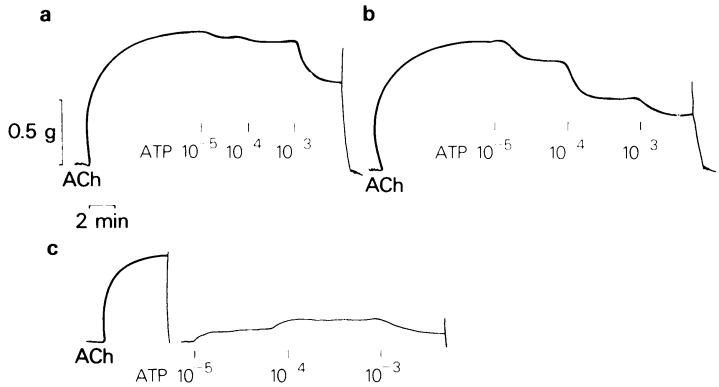

Figure 1 Action of ATP (10^{-5} to 10^{-3} M) on guinea-pig isolated trachea. (a) and (b) after prior contraction with acetylcholine (ACh) 6.6×10^{-6} M in the absence (a) or presence (b) of dipyridamole 10^{-6} M. (c) Another preparation, control effect of ACh alone (6.6×10^{-6} M) then, after washout, of ATP.

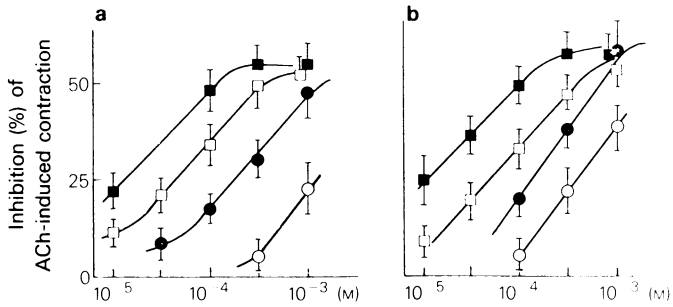

Figure 2 Dose-response curves of the effects of (a) ATP and (b) adenosine on guinea-pig isolated trachea in the absence or presence of dipyridamole. (○) Control; dipyridamole 10^{-7} M (●); 10^{-6} M (□) and 10^{-5} M (■). Each point represents the mean value; s.e. means are shown by vertical bars. Experiments were performed on groups of at least 4 preparations.

Table 1 Concentrations of ATP and adenosine required to inhibit by 25% the ACh-induced contraction of isolated guinea-pig trachea

	ATP (mM)	*Adenosine* (mM)
Control	1.09 ±0.35	0.39 ±0.16
Dipyridamole 10^{-7} M	0.17 ±0.042	0.13 ±0.06
Dipyridamole 10^{-6} M	0.043±0.025	0.05 ±0.039
Dipyridamole 10^{-5} M	0.012±0.007	0.011±0.005
Dipyridamole 10^{-6} M plus aspirin 3×10^{-3} M	0.045±0.015	0.054±0.009
Dipyridamole 10^{-6} M plus indomethacin 10^{-6} M	0.039±0.017	0.045±0.008

Table 2 Influence of various pretreatments on the contractile action of ATP and adenosine on guinea-pig isolated trachea under resting tension

Pretreatment (M)	n	*ATP* 10^{-5} M	*ATP* 10^{-4} M	n	*Adenosine* 10^{-5} M	*Adenosine* 10^{-4} M
Controls	15	9.5 ± 2.3^{b}	18.3 ± 3^{c}	14	8.0 ± 2.1^{b}	19.6 ± 2.8^{c}
Mepyramine (10^{-6})	3	8.6 ± 1.8^{a}	15.0 ± 2.0^{a}	3	6.0 ± 4.8	15.6 ± 3.7
Atropine (10^{-6})	3	9.0 ± 2.0^{a}	13.3 ± 3.3	3	9.3 ± 2.4	16.6 ± 4.3
Methysergide (10^{-8})	3	13.0 ± 5.1	16.2 ± 4.1	3	5.0 ± 3.2	16.3 ± 1.9^{a}
Phentolamine (10^{-6})	3	14.0 ± 4.5	20.1 ± 4.6^{a}	3	10.3 ± 1.8^{a}	18.1 ± 2.5^{a}
Indomethacin (10^{-6})	5	1.2 ± 0.7^{e}	2.5 ± 2.9^{e}	5	6.2 ± 3.9	$11.2 \pm 2.3^{b,d}$
Aspirin (0.3×10^{-3})	5	2.8 ± 2.2^{d}	$7.6 \pm 2.5^{a,d}$	4	2.1 ± 0.9^{d}	6.0 ± 2.1^{e}
Aspirin (3×10^{-3})	4	3.0 ± 2.0^{d}	1.7 ± 1.8^{f}	4	0.1 ± 1.1^{e}	0.7 ± 0.8^{f}
Nictindole (10^{-7})	4	0.8 ± 0.6^{e}	2.1 ± 1.7^{f}	4	0.6 ± 0.6^{e}	1.7 ± 0.8^{f}
Imidazole (5×10^{-5})	4	7 ± 2.4	8 ± 3.6^{d}	4	5.0 ± 1.7	$4.0 \pm 1.2^{a,f}$
FPL 55712 ($1{,}7 \times 10^{-6}$)	6	8.7 ± 2.5^{a}	12.4 ± 4.1^{a}	6	4.1 ± 2.4	14.7 ± 2.5^{b}
Theophylline (10^{-6})	4	3.8 ± 3.2	6 ± 4.5^{d}	4	3.2 ± 1.1	9.5 ± 5.7
Theophylline (10^{-5})	5	3 ± 1.4^{d}	$5.4 \pm 1.9^{a,e}$	5	3.6 ± 1.5	4.2 ± 1.6^{f}
Nifedipine (10^{-6})	6	3.8 ± 2.4	5.3 ± 2.4^{e}	6	4.1 ± 2.4	4.7 ± 2.5^{e}
Dipyridamole (10^{-6})	3	-7.6 ± 2.1^{f}	$-28.6 \pm 2.3^{b,f}$	3	$-11.3 \pm 2.2^{a,f}$	$-28.1 \pm 1.5^{b,f}$

The results are expressed as percentage of maximal contraction to ACh. Significant contraction: $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$.
Significant difference from controls: $^{d}P<0.05$; $^{e}P<0.01$; $^{f}P<0.001$.

contractile effect is accompanied by tachyphylaxis, a phenomenon already reported in connection with ATP-induced contraction of rabbit detrusor muscle (Andersson, Husted & Sjögren, 1980). We found that in concentrations of 10^{-3} M the ATP- or adenosine-induced contraction of the guinea-pig isolated trachea was less pronounced, probably because it was the resultant of the indirect contractile and direct relaxant effects of these substances.

A dual action of this kind (i.e. dependent upon the tone of the preparation) has been described with ATP in experiments on isolated strips of guinea-pig

stomach fundus (Baer & Frew, 1979) and with prostaglandin E in experiments on guinea-pig isolated trachea (Lambley & Smith, 1975).

Our study of the influence of various agents on the contractile effect of ATP and adenosine on low tone guinea-pig tracheal preparations has thrown some light on its mechanism. Direct stimulation of histamine, ACh, or 5-hydroxytryptamine receptors and of α-adrenoceptors can be excluded, since this effect was not modified by mepyramine, atropine, methysergide and phentolamine respectively.

On the other hand, inhibition by indomethacin and aspirin suggests that the contractile effect of ATP and adenosine is indirect and mediated by arachidonic acid derivative produced under the influence of cyclo-oxygenase. Moreover, the inhibition exerted by nictindole and imidazole, which are known to inhibit thromboxane synthetase specifically (Gryglewski, Zmuda, Korbut, Krecioch & Bieron, 1977; Nijkamp, Moncada, White & Vane, 1977; Hitchcock, 1980; Alabaster, 1980), suggests that it is predominantly mediated by thromboxane A_2 (TXA_2). Leukotriene production does not seem to be involved, since no inhibition of ATP- or adenosine-induced contraction was observed with FPL 55712, an agent that inhibits the effects of leukotrienes (Augstein, Farmer, Lee, Sheard & Tattersall, 1973; Sheard, Lee & Tattersall, 1977) produced under the influence of lipoxygenase (Samuelsson, Goldyne, Granström, Hamberg, Hammarström & Malmsten, 1978).

Another result of these experiments was to show that ATP and adenosine do not have any additional contractile effects on ACh-induced contraction of isolated guinea-pig trachea, since the dose-response relaxation curves of ATP and adenosine were not modified by indomethacin or aspirin.

Production of prostaglandins and/or TXA_2, described here under the influence of ATP and adenosine, has been observed with bradykinin in the anaesthetized guinea-pig *in vivo* or in the perfused lung of guinea-pig *in vitro* (Palmer, Piper & Vane, 1973; Greenberg, Osman, O'Keepe & Antonaccio, 1979). Working on guinea-pig isolated trachea with aspirin 0.3×10^{-3} M or indomethacin 1.6 μM or indomethacin 1.6 μM, Orehek, Douglas & Bouhuys (1975) found that: (1) aspirin or indomethacin lowered intrinsic tone; (2) indomethacin significantly reduced the effect of low doses ($> 10^{-6}$ M) of ACh, and (3) indomethacin or aspirin potentiated the effects of high doses of ACh.

In our experiments neither indomethacin nor aspirin had any effect on resting tone nor on the action of ACh (10^{-7} to 6.6×10^{-5} M). Our results are comparable to those obtained by Brink, Grimaud, Guillot & Orehek (1980) on human bronchial muscle. In this model, indomethacin did not significantly alter the basal tone of the preparations nor the histamine or ACh concentration-effect curves; only high concentrations (17 μM) of indomethacin depressed the maximal response of human bronchial muscle to histamine.

These discrepancies may be tentatively explained by differences in equipment and techniques. In the experimental procedure adopted by Orehek *et al.* (1975), spirally cut tracheae were equilibrated under an initial tension of 8 g, and the basal tone after rest was 4.5 to 5 g. In the experiments described by Brink *et al.* (1980), the initial load was 3 g and basal tone after equilibrium was approximately 2.5 g. The corresponding values in our experiments were 1.2 and 0.5 g respectively. These differences are dependent on the apparatus used, which is set to produce optimal responses (Stephens, 1970). The production of cyclo-oxygenase may possibly depend on intrinsic tone, and it is remarkable that in our experiments prostaglandins and/or TXA_2 release was observed with ATP or adenosine but not with ACh.

Finally, it must be added that ATP is capable of releasing arachidonic acid at other sites, including the isolated detrusor muscle in rabbits (Dean & Downie, 1978; Andersson *et al.*, 1980) and the uterus (Moritoki, Takei, Kasai, Matsumura & Ishida, 1979), ileum and taenia coli (Kamikawa, Serizawa & Shimo, 1977; Burnstock, Cocks, Paddle & Staszewska-Barczac, 1979) in guinea-pig.

It is difficult to determine whether P_1- or P_2-purinoceptors (Burnstock, 1978) are initially involved in the contractile effect of ATP or adenosine. The fact that ATP and adenosine are equipotent is in favour of a P_2-type effect, whereas inhibition by theophylline would suggest a P_1-type effect. However, since we were unable to establish dose-response curves because of tachyphylaxis and of the relaxant effect of theophylline it is impossible to decide whether the inhibitory effect of theophylline was due to its specific spasmolytic activity or to antagonism at purinoceptor level, as has been noted in connection with its spasmolytic effect on the guinea-pig trachea (Coleman, 1980).

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