Br. J. Pharmac. (1980), **70**, 512–514

PURINE RECEPTORS IN THE TRACHEA: IS THERE A RECEPTOR FOR ATP?

J. CHRISTIE & D.G. SATCHELL

Department of Zoology, University of Melbourne, Parkville, 3052, Australia

In guinea-pig trachea adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-phosphate (AMP), adenosine and adenine were similarly potent in causing relaxation of the smooth muscle. This is in contrast to gut where ATP and ADP are 30 times more potent than adenosine. Studies with dipyridamole suggest that in trachea, as in gut, nucleotides are rapidly metabolized to adenosine. A polyphosphate modified analogue of ATP, the α,β-methylene isostere, which resists degradation to adenosine was inactive in trachea although it is a potent relaxant in gut. This result may suggest that the intact ATP molecule is also inactive in the tracheal preparation: i.e. ATP acts only via its adenosine metabolite implying that receptors for adenosine but not ATP are present in the tissue.

Introduction Purines are potent in causing relaxation of smooth muscle especially in gut (Satchell & Burnstock, 1975) and the airways (Coleman, 1976). Although both these tissues share a common embryological origin the potencies of the purines differ at the two sites. Adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP) were 30 times more potent than adenosine 5'-phosphate (AMP) and adenosine in the guinea-pig taenia coli (Satchell & Burnstock, 1975) and this may be related to the suggestion of separate receptors for ATP and adenosine in the tissue (Burnstock, 1978). In the guinea-pig trachea the potency of ATP was not greater than that of adenosine (Coleman, 1976; Farmer & Farrah, 1976).

In gut, studies of receptor potencies of adenine nucleotides and adenosine are complicated by rapid metabolism of the nucleotides to adenosine and rapid uptake of the latter into the tissue. These effects are significant even during the 30 s contact time of the purines with the preparation. This accounts for the finding that dipyridamole, which inhibits adenosine uptake into the tissue, potentiated responses to ATP as well as adenosine (Satchell, Lynch, Bourke & Burnstock, 1972).

Dipyridamole potentiates the inhibitory effects of adenosine on the guinea-pig tracheal smooth muscle also (Coleman & Levy, 1974). This drug has been used in the present experiments to determine whether exogenously applied adenine nucleotides are rapidly broken down to adenosine in this tissue.

Experiments were also carried out with adenosine 5'-α,β-methylenetriphosphate (AOPCPOP) and 5'-adenylyl methylenediphosphonate (AOPOPCP). These compounds, especially the former, exhibit resistance to metabolic degradation to adenosine (see Satchell & Maguire, 1975) and were used as a measure of the activity of the intact nucleoside triphosphate on the tissue. Thus these experiments were designed to test whether ATP is likely to exert its action as the intact triphosphate *per se* or whether its effect is due solely to its adenosine metabolite. The results are considered in relation to the probable action of adenine nucleotides and adenosine via one or separate receptors.

Methods Tracheae were dissected from guinea-pigs of either sex weighing 500 to 1000 g. Transverse strips were dissected containing 2 to 4 cartilaginous rings and suspended in a modified Krebs solution (Maguire & Satchell, 1979). The solution was aerated with 95% O_2 and 5% CO_2 and maintained at 36°C. Tension (0.5 g) was applied to each preparation. Muscle activity was registered isometrically by means of Grass FT03 force transducers coupled to a Riken Denshi SP-H3B polygraph with appropriate amplification.

Preparations were allowed to equilibrate for 60 min before exposure to drugs. Doses of purines were added to the bath cumulatively and results plotted as the mean ± s.e. of effective concentrations required to give a certain percentage of maximum response. Responses are expressed as a percentage of the maximum response to noradrenaline. Responses to each dose (in both control and treated preparations) were determined in preparations from at least 5 animals.

Purines were obtained from E. Merck Darmstadt except for adenosine 5'-α,β-methylenetriphosphate and 5'-adenylyl methylenediphosphonate which were obtained from P.L. Biochemicals Inc., Milwaukee, U.S.A.

Results ATP, ADP, AMP and adenosine all caused concentration-dependent relaxations. Relaxations were always slow to develop taking at least 5 min to reach maximum. On washing, preparations generally failed to return to baseline. The concentration-effect curves to ATP, ADP, AMP, adenosine, adenine and AOPOPCP were similar (Figure 1). AOPCPOP was inactive up to concentrations of 10^{-3} M.

Dipyridamole (5×10^{-7} M) caused a marked potentiation of inhibitory responses to ATP, ADP, AMP and adenosine. Concentration-effect curves for

0007-1188/80/120512-03 $01.00

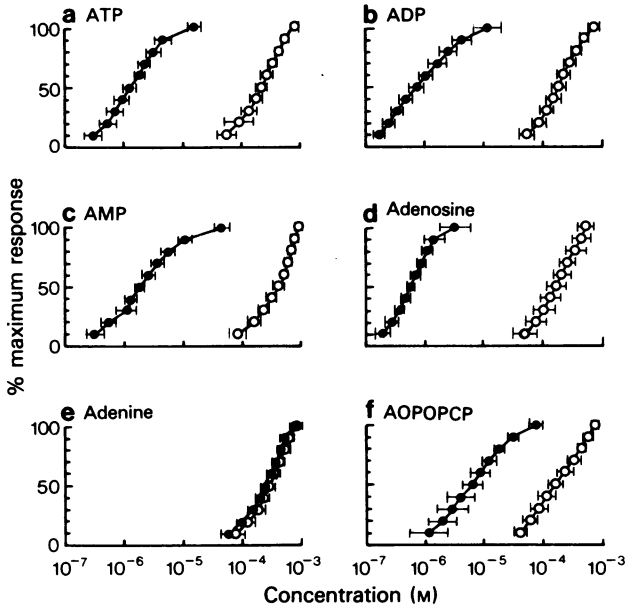

Figure 1 Cumulative log-dose response curves to (a) ATP; (b) ADP; (c) AMP; (d) adenosine; (e) adenine; and (f) 5'-adenylyl methylenediphosphonate (AOPOPCP); (○) control; (●) after incubation for 30 min with dipyridamole (0.5 μM). Horizontal lines show s.e. means.

each compound were shifted to the left by more than two orders of magnitude (Figure 1). The concentration-effect curve to adenine remained unaffected by treatment with dipyridamole while that to AOPOPCP was shifted to the left in its presence, although the shift was not as great as that to the naturally occurring purines.

Although AOPCPOP was inactive on untreated preparations, responses which were no greater than 30% of the maximal response to adenosine were observed in the presence of dipyridamole (5×10^{-7} M). This result could have been due to contamination of AOPCPOP with adenosine since chromatography of a sample revealed a faint spot at an R_F value comparable with that of authentic adenosine.

Discussion ATP, ADP, AMP, adenosine and adenine all caused similar concentration-dependent relaxations of guinea-pig tracheal strips similar to those described by Farmer & Farrar (1976). The finding that dipyridamole caused a significant and marked potentiation of inhibitory responses to ATP, ADP AMP and adenosine is consistent with the results of similar experiments on the guinea-pig taenia coli where the potentiation by dipyridamole was claimed to be due to rapid breakdown of the nucleotides to adenosine and enhanced action of the adenosine metabolite (Satchell & Maguire, 1975). The failure of dipyridamole to potentiate responses to adenine is also consistent with these findings.

The results suggest that adenine nucleotides are broken down to adenosine during their contact with the tracheal strip. They do not indicate whether inhibitory responses to exogenously applied nucleotide on untreated preparations were due to the adenosine metabolite alone, to the combined actions of intact nucleotide and adenosine metabolite or to intact nucleotide alone. The latter possibility is less likely and could only occur where the amount of adenosine formed was sufficient to cause a response in the presence of dipyridamole but insufficient to cause a response in its absence. Moreover, the studies with the naturally occurring purines provide no information as to whether the intact nucleotides (if indeed they do act without degradation) act via a separate receptor to that on which adenosine acts.

The finding that responses to AOPOPCP which is reported to resist degradation by ATPase (Moos, Alpert & Myers, 1960) were potentiated by dipyridamole is consistent with the findings on the guinea-pig taenia coli (Maguire & Satchell, 1979). Both the taenia coli and the trachea of the guinea-pig are likely to possess an adenosine triphosphate pyrophosphohydrolase which could cause AOPOPCP to be metabolized to adenosine by removal of the intact methylene diphosphonate moiety (see Maguire & Satchell, 1979).

AOPCPOP was more potent than AOPOPCP on the guinea-pig taenia coli (Satchell & Maguire, 1975)

and the responses to this compound were not potentiated by dipyridamole, leading to the proposal that this compound was not broken down to adenosine (Maguire & Satchell, 1979). In the present experiments this compound was inactive and the small relaxations recorded in the presence of dipyridamole were probably the results of traces of adenosine contaminant. The findings that this stable ATP analogue was inactive on the guinea-pig trachea could be taken to mean that the intact ATP molecule is also inactive in this preparation; that exogenously applied ATP exerts inhibitory actions via a metabolite only and that, unlike gut, no receptor for ATP is present in the trachea of the guinea-pig.

While this suggestion is likely, it should be interpreted with caution because the possibility remains that the replacement of an anhydride oxygen by a methylene group could abolish all agonist potency although AOPCPOP was a potent relaxant in the taenia coli where receptors for ATP are present (Maguire & Satchell, 1975).

Further experiments are necessary to test this view and to determine whether intact ADP and AMP molecules are also inactive.

References

BURNSTOCK, G. (1978) *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach.* ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

COLEMAN, R.A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. *Br. J. Pharmac.* **57,** 51–57.

COLEMAN, R.A. & LEVY, G.P. (1974). A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. *Br. J. Pharmac.* **52,** 167–174.

FARMER, J.B. & FARRAR, D.G. (1976) Pharmacological studies with adenine, adenosine and some phosphorylated derivatives on guinea-pig tracheal muscle. *J. Pharm. Pharmac.* **28,** 748–752.

MAGUIRE, M.H. & SATCHELL, D.G. (1979). The contribution of adenosine to the inhibitory actions of adenine nucleotides on the guinea-pig taenia coli: Studies with phosphate-modified adenine nucleotide analogs and dipyridamole. *J. Pharmac. exp. Ther.* **211,** 626–631.

MOOS, C., ALPERT, N.R. & MYERS, T.C. (1960). Effects of a phosphonic acid analog of adenosine triphosphate on actomyosin systems. *Archs. Biochem. Biophys.* **88,** 183–192.

SATCHELL, D.G. & BURNSTOCK, G. (1975). Comparison of the inhibitory effects on the guinea-pig taenia coli of adenine nucleotides and adenosine in the presence and absence of dipyridamole. *Eur. J. Pharmac.* **32,** 324–328.

SATCHELL, D.G., LYNCH, A. BOURKE, P. & BURNSTOCK, G. (1972). Potentiation of the effects of exogenously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridamole. *Eur. J. Pharmac.* **19,** 343–350.

SATCHELL, D.G. & MAGUIRE, M.H. (1975). Inhibitory effects of adenine nucleotide analogs on the isolated guinea-pig taenia coli. *J. Pharmac. exp. Ther.* **195,** 540–548.

(*Received July 17, 1980.*)