Br. J. Pharmac. (1980), **69**, 359–366

PURINE ANTAGONISTS IN THE IDENTIFICATION OF ADENOSINE-RECEPTORS IN GUINEA-PIG TRACHEA AND THE ROLE OF PURINES IN NON-ADRENERGIC INHIBITORY NEUROTRANSMISSION

R.A. COLEMAN

Department of Pharmacology, Glaxo Group Research (Ware) Limited, Ware, Hertfordshire SG12 0DJ

1 To test the possibility that adenosine receptors exist within the trachea of the guinea-pig, an attempt has been made to identify a compound with adenosine antagonist activity in this tissue.

2 Quinidine, phentolamine, phenoxybenzamine, 2-2′-pyridylisatogen tosylate (PIT) and caffeine were tested for antagonism of spasmolytic responses to adenosine, adenosine 5′-triphosphate (ATP) and adenine on the guinea-pig isolated trachea.

3 Quinidine (10 and 25 μg/ml), phentolamine (10 and 30 μg/ml) and phenoxybenzamine (10 μg/ml) had little or no effect on response to adenosine, ATP and adenine. PIT (21 μg/ml) potentiated responses to adenosine, ATP and adenine by an unexplained mechanism.

4 Caffeine (25 μg/ml) partially relaxed the trachea and inhibited spasmolytic responses to both adenosine and ATP, but not to adenine, isoprenaline, aminophylline or prostaglandin E_2 (PGE_2).

5 A number of compounds related to caffeine (xanthine, hypoxanthine, theophylline and theobromine) were tested for adenosine antagonist activity. Xanthine (300 μg/ml) and hypoxanthine (300 μg/ml) did not relax the trachea or antagonize spasmolytic responses to adenosine. Both theophylline (10 μg/ml) and theobromine (30 μg/ml) partially relaxed the trachea; theophylline, but not theobromine, antagonized spasmolytic responses to adenosine.

6 pA_2 values for caffeine and theophylline as antagonists of adenosine were 4.3 and 4.7 respectively. However, the slopes of the Schild plot regressions were significantly less than 1.0 for both compounds.

7 Four compounds, adenine, AH 8883, M30966 and ICI 63197, which like caffeine and theophylline, have phosphodiesterase inhibitory activity were tested for adenosine antagonist activity in the trachea. Adenine and AH 8883 had no effect and M30966 and ICI 63197 caused significant potentiation.

8 The effects of caffeine and theophylline were also investigated on the non-adrenergic inhibitory response to nerve stimulation (NAIR). Both caffeine (100 μg/ml, $n = 4$) and theophylline (30 μg/ml, $n = 4$) enhanced the NAIR (20 Hz) while virtually abolishing matched responses to exogenous adenosine.

9 The results support the existence of adenosine receptors in the guinea-pig trachea.

Introduction

Adenine, adenosine and adenine nucleotides relax tracheal smooth muscle of the guinea-pig by mechanisms not involving β-adrenoceptors (Coleman & Levy, 1974; Coleman, 1976a; Farmer & Farrar, 1976). Although adenine is thought to act by inhibition of the enzyme, phosphodiesterase, an adenosine receptor has been postulated to account for the tracheal relaxant properties of adenosine and adenine nucleotides (Farmer & Farrar, 1976). The demonstration of an antagonist which selectively inhibits adenosine-induced relaxations of guinea-pig trachea would provide further evidence for the presence of adenosine receptors in this tissue. The present study was therefore undertaken in an attempt to identify such a compound.

Five compounds with reported purine antagonist activity in a variety of other tissues were tested: they were quinidine (Madinaveitia & Raventos, 1949; Wayne, Goodwin & Stoner, 1949; Bowman & Hall, 1970, Burnstock, Campbell, Satchell & Smythe, 1970), phentolamine (Rikimaru, Fukushi & Suzuki, 1971; Satchell, Burnstock & Dann, 1973) phenoxybenzamine (Nayler, Price & Lowe, 1967), 2-2′-pyridylisatogen tosylate (Spedding, Sweetman & Weetman, 1975) and caffeine (Ther, Muschaweck & Hergott, 1957; Nicholas & Walaszek, 1963; de Gubareff & Sleator, 1965).

In addition to providing further evidence for the presence of adenosine receptors in the guinea-pig trachea, such an antagonist may help in resolving the

0007-1188/80/070359-08$01.00

question of whether a purine is the neurotransmitter in the non-adrenergic inhibitory nerves present in this tissue (Coburn & Tomita, 1973; Coleman & Levy, 1974; Richardson & Bouchard; 1975, Coleman, 1976a).

Methods

Guinea-pig isolated tracheal tube preparations

All preparations were mounted in 10 ml organ baths containing physiological salt solution maintained at 37°C and bubbled with air (Coleman, 1976b). The salt solution had the following composition (g/l): NaCl 6.92, $NaHCO_3$ 2.1, KCl 0.35, $MgSO_4.7H_2O$ 0.15, KH_2PO_4 0.16, glucose 2.0 and $CaCl_2.6H_2O$ 0.28. The salt solution was in contact with both inner and outer surfaces of the trachea. Intra-luminal pressure (1 mmHg = 1.333 mbar) was monitored continuously. Preparations were either unstimulated or electrically stimulated.

Unstimulated preparations Guinea-pigs of either sex, weighing 300 to 400 g, were killed by a blow on the head and the tracheas excised. Two tracheal tube preparations were made from each animal by dividing the trachea halfway along its length. Each portion was mounted on a tracheal holder as described by Coleman (1976a). A high level of resting tone was induced in the trachea by repeated dosing of the preparations with a concentration of noradrenaline (0.1 μg/ml) which caused maximal relaxation, followed by washout and exposure of the tracheal lumen to atmospheric pressure (Coleman & Farmer, 1971). In experiments where drugs were tested for antagonist activity, agonist cumulative concentration-effect curves were repeated until sensitivity was constant, the antagonist was then added to the bathing solution and a 30 min contact time allowed before the agonist curve was repeated. After each concentration-effect curve, noradrenaline (0.1 μg/ml) was added to the bathing solution to determine the maximum relaxation obtainable. Responses to the spasmolytic drugs were expressed as percentages of the noradrenaline maximum. In determination of antagonism, values for concentration-ratio were calculated by dividing the EC_{40} for the agonist in the presence of the antagonist by that in its absence

$$\left(\text{concentration ratio} = \frac{EC_{40}\ \text{treated}}{EC_{40}\ \text{control}}\right).$$

The EC_{40} value is defined as that concentration of agonist causing a relaxation equal to 40% of the noradrenaline maximum. EC_{40} values were used because 50% relaxation was not achieved with all drugs.

All experiments were carried out in the presence of the adenosine uptake blocking drug, dipyridamole (1 μg/ml). This concentration of dipyridamole causes parallel shifts to the left of adenosine and adenosine 5'-triphosphate (ATP) concentration-effect curves of the order of 50 fold (Coleman, 1976a). Dipyridamole was used to prevent any possible effects of the antagonists on adenosine uptake from masking antagonistic activity, and to enhance the potency of adenosine and ATP in order to facilitate the quantification of any antagonism.

Electrically-stimulated preparations Preparations were set up as described above, but the tracheal electrode described by Farmer & Coleman (1970) was used instead of the tracheal holder. The preparations were stimulated through intraluminal and extraluminal platinum wire electrodes with square wave alternating pulses of 2 ms duration, supramaximal voltage and variable frequency.

All experiments with electrically stimulated preparations, unless otherwise stated, were carried out in the presence of atropine (0.1 μg/ml) and propranolol (1 μg/ml) to abolish cholinergic excitatory and adrenergic inhibitory nervous responses respectively (Coleman & Levy, 1974). Inhibitory responses to electrical stimulation were measured as mmHg fall in tone and no attempt was made to relate them to noradrenaline maxima. Antagonism of the inhibitory responses is defined simply as a reduction in response amplitude.

Drugs

The following drugs were used: acetyl β-methylcholine chloride (methacholine, Koch–Light), adenine (B.D.H.), adenosine (Koch–Light), adenosine 5'-triphosphate (ATP, B.D.H.), 2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-s-triazalo[1,5-a]pyrimidine (ICI 63197, ICI), 2-amino-5-methyl-7-propyl-imidazo-[1,5-a]*as*-triazin-4 (3H) (AH 8883, Glaxo), 2-amino-7-methyl-5-propyl-syn-triazolo[2,3-c]-pyrimidine (M 30966, ICI), aminophylline (Glaxo), atropine sulphate (B.D.H.), caffeine (B.D.H.), dipyridamole (Persantin, Boehringer Ingelheim), guanethidine sulphate (Ciba), hypoxanthine (B.D.H.), (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop), noradrenaline (Winthrop), phenoxybenzamine hydrochloride (SKF), phentolamine mesylate (Ciba), propranolol hydrochloride (ICI), prostaglandin E_2 (PGE_2, NEN), 2-2'-pyridylisatogen tosylate (Sunderland Polytechnic), quinidine (Hopkin & Williams), theobromine (Sigma), theophylline (B.D.H.), xanthine (Sigma).

Phenoxybenzamine was dissolved in distilled water. All other drugs were dissolved in either the physiological salt solution or 0.9% w/v NaCl solution

(saline). Ascorbic acid (200 μg/ml) was present in solutions of isoprenaline.

Statistical analysis

Values quoted in the text and tables are geometric means with 95% confidence limits in parentheses.

Where pA_2 values and regression slopes have been quoted, the value n is the number of determinations of concentration-ratio at each concentration of antagonist. Values for P were determined by the paired t test.

Results

Effects of antagonists on responses to purine agonists

The effects of quinidine, phentolamine, phenoxybenzamine, 2-2'-pyridylisatogen tosylate (PIT) and caffeine were tested on inhibitory responses to adenosine, ATP and adenine. Quinidine (10 and 25 μg/ml), phentolamine (10 and 30 μg/ml) and phenoxybenzamine (10 μg/ml) were without effect against the spasmolytic actions of any of these purines. PIT (21 μg/ml) had no antagonistic effects and actually caused a 2 to 3 fold enhancement of responses to adenosine, ATP and adenine. Further experiments revealed that PIT (21 μg/ml) also produced 2.8 (1.1 to 7.2, $n = 5$) fold enhancement of PGE_2 responses. Caffeine (25 μg/ml) reduced the level of tone of the trachea and also significantly ($P < 0.001$) antagonized responses to both adenosine and ATP, giving concentration ratios of 3.7 (2.7 to 5.1, $n = 4$) and 2.4 (1.6 to 3.7 $n = 4$) for adenosine and ATP respectively.

Specificity of action of caffeine

The effects of caffeine (25 μg/ml) on isoprenaline, aminophylline and PGE_2 were investigated. Caffeine potentiated the action of isoprenaline, causing a 1.7 (1.4 to 2.0) fold displacement to the left of the isoprenaline concentration-effect curve. It had no effect on curves to either aminophylline or PGE_2.

Effect of other xanthine derivatives on responses to adenosine

As caffeine selectively antagonized responses to adenosine, some related compounds, xanthine, hypoxanthine, theophylline and theobromine, were also tested as antagonists of adenosine (Table 1).

Xanthine and hypoxanthine had little effect on spontaneous tone of the trachea in concentrations up to 300 μg/ml. Theophylline (3 to 100 μg/ml) and theobromine (10 to 300 μg/ml), resembled caffeine in causing concentration-dependent relaxations of the trachea. The relaxant potency of theophylline was about twice that of caffeine and theobromine.

Theophylline (3, 10 and 30 μg/ml) and caffeine (10, 30 and 100 μg/ml) caused concentration-dependent parallel shifts to the right of adenosine concentration-effect curves. Theobromine (10, 30 and 100 μg/ml), xanthine (300 μg/ml) and hypoxanthine (300 μg/ml) were all without effect. The data obtained with caffeine and theophylline were used to calculate pA_2 values for the two compounds by the method of Arunlakshana & Schild (1959). The results were: caffeine pA_2 4.3 (4.1 to 4.7), regression slope 0.66 (0.39 to 0.92) ($n = 4$ to 6); theophylline pA_2 4.7 (4.6 to

Table 1 Guinea-pig isolated tracheal tube: the effects of caffeine, theophylline, theobromine, xanthine and hypoxanthine on concentration-effect curves to adenosine in the presence of dipyridamole (1 μg/ml)

Compound	*Concentration (μg/ml)*	*Number of experiments*	*Adenosine concentration-ratio*	*95% confidence limits* $\frac{EC_{40}\text{ treated}}{EC_{40}\text{ control}}$
Caffeine	3	6	1.0	(0.7–1.5)
	10	9	2.2	(1.7–2.9)
	25	4	3.7	(2.7–5.1)
	30	5	3.0	(2.4–3.8)
	100	6	6.2	(4.0–9.6)
Theophylline	1	4	1.4	(1.0–2.0)
	3	5	1.9	(1.7–2.2)
	10	8	3.7	(2.8–4.8)
	30	5	5.5	(3.3–8.9)
Theobromine	10	5	0.8	(0.6–1.2)
	30	5	0.7	(0.4–1.2)
	100	8	0.7	(0.5–1.0)
Xanthine	300	6	1.1	(0.7–1.7)
Hypoxanthine	300	6	0.8	(0.6–1.1)

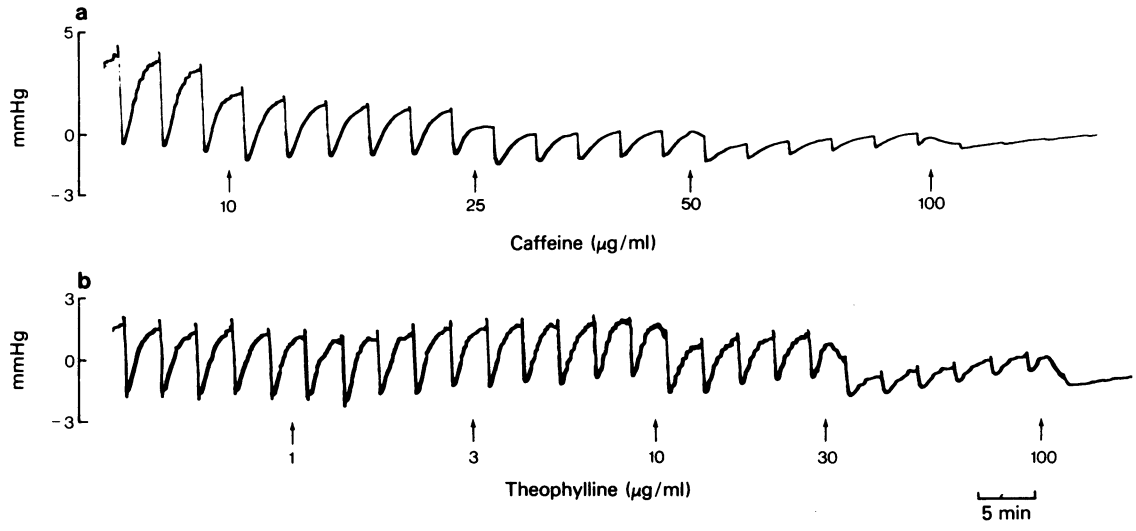

Figure 1 Guinea-pig isolated tracheal tube. Effect of (a) caffeine and (b) theophylline on non-adrenergic inhibitory response (NAIR) to nerve stimulation (20 Hz, 2 ms, 12 s)

5.1), regression slope 0.69 (0.45 to 0.94) (n = 4 to 7). The regressions were linear for both caffeine and theophylline.

Effect of other phosphodiesterase inhibitor drugs on response to adenosine

As both caffeine and theophylline inhibit the enzyme phosphodiesterase (Butcher & Sutherland, 1962), four other compounds with phosphodiesterase inhibitory activity, adenine, AH 8883, M 30966 and ICI 63197, were tested for their ability to inhibit responses to adenosine. All of the drugs relaxed the trachea in the concentration range tested. Neither adenine (up to 30 μg/ml) nor AH 8883 (up to 0.1 μg/ml) had any antagonist effect on adenosine responses, while M 30966 (0.3 to 3.0 μg/ml) and ICI 63197 (0.03 to 0.3 μg/ml) had some potentiating effect, causing 2 to 3 fold shifts to the left of adenosine concentration-effect curves. The highest concentration of each of the drugs tested caused tracheal relaxation similar in degree to that seen with caffeine (100 μg/ml) and theophylline (30 μg/ml) which produced adenosine concentration-ratios of 6.2 and 5.5 respectively (Table 1).

Effect of caffeine and theophylline on the non-adrenergic inhibitory response to nerve stimulation (NAIR)

Preliminary experiments showed that caffeine (10 to 100 μg/ml) and theophylline (3 to 30 μg/ml) reduced the amplitude of the NAIR (Figure 1). However, these responses could only be measured as absolute changes, rather than relative to the maximum relaxation of which the tissue was capable. It was, therefore, possible that the reduction in the amplitude of the NAIR was a consequence of the reduction in baseline caused by theophylline and caffeine. It has previously been shown that methacholine contracts the trachea and reduces the potency of spasmolytic drugs (Farmer & Farrar, 1976). Therefore, subsequent experiments were carried out in the presence of methacholine (1 μg/ml), atropine being omitted in these experiments. Under these conditions, caffeine (100 μg/ml) and theophylline (30 μg/ml) had little effect on the tone of the trachea, and had no consistent inhibitory effect on the NAIR at frequencies of 5 to 20 Hz.

In a second series of experiments, matched responses to nerve stimulation (20 Hz) and adenosine were obtained alternately, firstly in the absence, and then in the presence of caffeine (100 μg/ml, n = 4) or theophylline (30 μg/ml, n = 4). The experiments were carried out in the presence of methacholine (1 μg/ml). Caffeine reduced responses to adenosine by 50 to 92%, but potentiated the NAIR by 10 to 37%. Similarly, theophylline reduced responses to adenosine by 78 to 87%, but potentiated the NAIR by 5 to 37%. Typical experiments with theophylline and caffeine are illustrated in Figure 2.

Discussion

Effects of purine antagonists

In the tracheal tube preparation, quinidine, phentolamine and phenoxybenzamine were inactive against

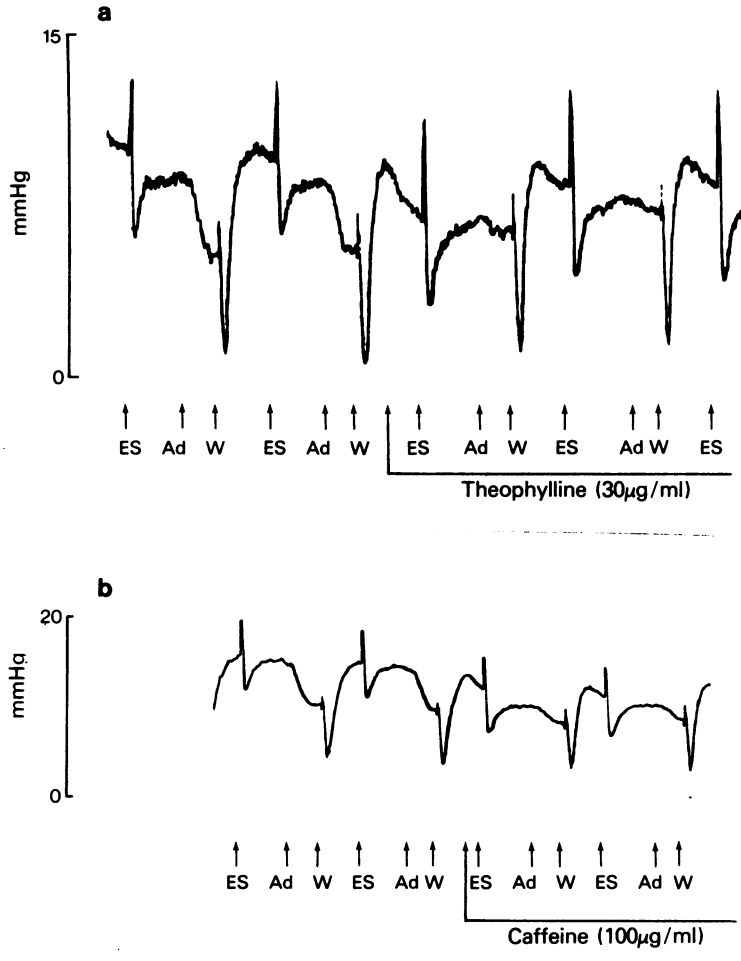

Figure 2 Guinea-pig isolated tracheal tube. Effect of (a) theophylline (30 μg/ml) and (b) caffeine (100 μg/ml) on matched responses to adenosine (10 μg/ml) (Ad) and non-adrenergic inhibitory response (NAIR) to nerve stimulation (20 Hz, 2 ms, 12 s, ES) in the presence of methacholine (1 μg/ml) and dipyridamole (1 μg/ml). (W) Wash out.

the spasmolytic responses to adenosine, ATP and adenine. PIT enhanced the spasmolytic potency of adenine, adenosine, ATP and also PGE_2. Thus PIT exerts a generalized sensitizing effect on responses to spasmolytic drugs in the trachea, but the mechanism is unknown.

Caffeine (25 μg/ml) relaxed the trachea and in the same concentration produced a small, but significant, antagonism of spasmolytic responses to adenosine and ATP. This effect is specific since the same concentration of caffeine did not antagonize spasmolytic responses to adenine, isoprenaline, aminophylline or PGE_2.

Effects of other xanthine derivatives

Of the other xanthine derivatives tested for antagonist activity against adenosine, theophylline was similar to caffeine in its inherent spasmolytic effects and its antagonism of responses to adenosine. Theobromine, relaxed the trachea but did not antagonize responses to adenosine. Hypoxanthine and xanthine had very little effect on spontaneous tone and had no adenosine antagonist activity.

Both caffeine and theophylline have previously been reported to block adenosine responses in various tissues, including heart, coronary vasculature, kidney

and gut (Ther *et al.*, 1957; Nichols & Walaszek, 1963; de Gubareff & Sleator, 1965; Afonso, 1970; Pöch & Kukovetz, 1971; Bünger, Haddy & Gerlach, 1975; Osswald, 1975; Ally & Nakatsu, 1976; McKenzie, Frew & Baer 1977) and responses to the closely related nucleoside, inosine, in tracheobronchial smooth muscle of the guinea-pig (Bertelli, Bianchi & Beani, 1973). The potencies of caffeine and theophylline quoted in these reports are similar to those found in the present study. Caffeine and theophylline both inhibit the enzyme phosphodiesterase and relax the trachea in the same concentration range that they have in their purine antagonist actions. It was, therefore, possible that these actions were linked. However, this idea is not supported by the results with theobromine, adenine, AH 8883, M 30966 and ICI 63197 which are phosphodiesterase inhibitors (Butcher & Sutherland, 1962; Peers & Davies, 1971; Davies, 1973; Hare, personal communication; Martin & Vardy, personal communication) and relax the trachea (this paper), but do not exhibit purine antagonist activity.

Mechanism of purine antagonism

Since the purine antagonism caused by caffeine and theophylline is not related to their phosphodiesterase inhibitory activity, an alternative mechanism must be sought. It is possible that caffeine and theophylline are competitive antagonists at the adenosine receptor, as has been suggested for theophylline in the rabbit ileum (Ally & Nakatsu, 1976). Although caffeine and theophylline caused parallel displacement of concentration-effect curves to adenosine and ATP, the slopes of the Schild plot regressions differed significantly from unity, the theoretical value for competitive antagonism. However, pA_2 values were calculated, and they were much lower than is usual for specific antagonists. These results, therefore shed little light on the mechanism by which caffeine and theophylline exert their purine antagonist activity.

The presence of adenosine receptors in guinea-pig trachea

The primary aim of this study was to identify a drug with specific adenosine-antagonist activity on guinea-pig trachea. Such a drug would support the hypothesis of Farmer & Farrar (1976) that there are adenosine-receptors in this tissue. The finding that caffeine and theophylline possess such activity, provides this support. Furthermore, the fact that caffeine, at least, also antagonizes responses to ATP, but not those to adenine, is in agreement with their further suggestion that, in this tissue, adenine nucleotides act either directly or indirectly at this adenosine receptor, whereas adenine itself does not.

The effect of caffeine and theophylline on the NAIR

The secondary aim of this study was to determine the effect of adenosine antagonists on the NAIR, where there is evidence that adenosine may be the neurotransmitter (Coleman & Levy, 1974; Coleman, 1976a). In experiments using alternate matched responses to exogenous adenosine and nerve stimulation, the ability of both caffeine and theophylline to antagonize adenosine responses, with no corresponding reduction of nervous responses, must cast some doubt on the theory of a neurotransmitter role for adenosine in the NAIR.

These results, therefore, need to be reconciled with the results of previous experiments where adenosine uptake blocking drugs enhanced, apparently selectively, the amplitude of the NAIR (Coleman & Levy, 1974; Coleman, 1976a). It is possible that the adenosine receptors activated by exogenous adenosine differ from the adenosine receptors activated by neuronally-released adenosine and that only the former are methylxanthine-sensitive. There is evidence that two different purine receptors exist (Burnstock, 1978), but the fact that caffeine effectively antagonizes effects of both adenosine and ATP in the trachea argues that if methylxanthine-resistant receptors exist in this tissue, either they are proportionately insignificant or they are inaccessible to exogenous purines. It is also possible that access of antagonists to the synaptic cleft and hence to the purine receptors involved in nervous transmission, is restricted. Such an explanation has been proposed to account for the resistance of excitatory nervous responses in the guinea-pig isolated vas deferens to α-adrenoceptor antagonists (Holman, 1970) and of various mammalian bladder preparations to muscarinic receptor antagonists (Huković, Rand & Vanov, 1965; Carpenter & Rand, 1965) although it has been contested by other workers (Ambache & Zar, 1971; Dumsday, 1971; Ambache, Dunk, Verney & Zar, 1972).

An alternative explanation is that a purine, although not itself the neurotransmitter, is released together with the neurotransmitter. Evidence that nerves may release more than one substance from their nerve endings has been reviewed by Burnstock (1976). If this is the case, then the potentiation of the NAIR seen with the adenosine uptake blocking drugs is not due to a potentiation of the effects of the true neurotransmitter, but of a purine released with it. Under normal conditions the NAIR would be due entirely to the effects of the true transmitter on its post-synaptic receptors, but in the presence of dipyridamole-like drugs the amplitude of the NAIR would be increased by the addition of a purine component as a result of the block of adenosine inactivation.

In conclusion, caffeine and theophylline are weak antagonists of adenosine, supporting the suggestion

that adenosine receptors exist in guinea-pig trachea. The use of these antagonists to investigate the possibility of purinergic neurotransmission in this tissue has led to equivocal results, and until more potent and specific antagonists at purine receptors become available, this question will not be resolved.

References

AFONSO, S. (1970). Inhibition of coronary vasodilating action of dipyridamole and adenosine by aminophylline in the dog. *Circulation Res.*, **26**, 743–752.

ALLY, A.I. & NAKATSU, K. (1976). Adenosine inhibition of isolated rabbit ileum and antagonism by theophylline. *J. Pharmac. exp. Ther.*, **199**, 208–215.

AMBACHE, N., DUNK, L.P., VERNEY, J. & ZAR, M.A. (1972). Inhibition of post-ganglionic motor transmission in vas deferens by indirectly acting sympathomimetic amines. *J. Physiol.*, **227**, 433–456.

AMBACHE, N. & ZAR, M.A. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 359–389.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BERTELLI, A., BIANCHI, C. & BEANI, L. (1973). Effect of inosine on guinea-pig bronchial muscle. *J. Pharm. Pharmac.*, **25**, 60–64.

BOWMAN, W.C. & HALL, M.T. (1970). Inhibition of rabbit intestine mediated by α- and β-adrenoreceptors. *Br. J. Pharmac.*, **38**, 399–415.

BÜNGER, R., HADDY, F-J. & GERLACH, E. (1975). Coronary responses to dilating substances and competitive inhibition by theophylline in isolated perfused guinea-pig heart. *Pflüg. Arch.*, **358**, 213–224.

BURNSTOCK, G. (1976). Do some nerve cells release more than one transmitter? *Neuroscience*, **1**, 239–248.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptors. *Proc. Int. Conf. on Biological Membranes*. New York: Raven Press.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40**, 668–688.

BUTCHER, R.W. & SUTHERLAND, E.W. (1962). Adenosine 3',5'-phosphate in biological materials. *J. biol. Chem.*, **237**, 1244–1250.

CARPENTER, F.G. & RAND, S.A. (1965). Relation of acetylcholine release to responses of the rat urinary bladder. *J. Physiol.*, **180**, 371–382.

COBURN, R.F. & TOMITA, T. (1973). Evidence for non-adrenergic inhibitory nerves in the guinea-pig trachealis muscle. *Am. J. Physiol.*, **224**, 1072–1080.

COLEMAN, R.A. (1976a). 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. (1976b). A non-adrenergic inhibitory nervous response in the trachea of the guinea-pig. *M.I. Biol. Thesis, June 1976.*

COLEMAN, R.A. & FARMER, J.B. (1971). The inducement of tone and its inhibition in isolated tracheal muscle. *J. Pharm. Pharmac.*, **23**, 220–222.

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

DAVIES, G.E. (1973). Antibronchoconstrictor activity of two new phosphodiesterase inhibitors, a triazolopyrazine (ICI 58301) and a triazolopyrimidine (ICI 63197). *J. Pharm. Pharmac.* **25**, 681–689.

DUMSDAY, B. (1971). Atropine-resistance of the urinary bladder innervation. *J. Pharm. Pharmac.*, **23**, 222–225.

FARMER, J.B. & COLEMAN, R.A. (1970). A new preparation of the isolated intact trachea of the guinea-pig. *J. Pharm. Pharmac.*, **22**, 46–50.

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.

GUBAREFF, T. DE & SLEATOR, W. JR. (1965). Effect of caffeine on mammalian atrial muscle and its interaction with adenosine and calcium. *J. Pharmac. exp. Ther.*, **148**, 202–214.

HOLMAN, M.E. (1970). Junction potentials in smooth muscle, In *Smooth Muscle*. ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 244–288. London: Edward Arnold.

HUKOVIĆ, S., RAND, M.J. & VANOV, S. (1965). Observations on an isolated innovated preparation of rat urinary bladder. *Br. J. Pharmac. Chemother.*, **24**, 178–188.

MADINAVEITIA, J. & RAVENTOS, J. (1949). Antimalarial compounds as antagonists of adenosine. *Br. J. Pharmac. Chemother.*, **4**, 81–92.

MCKENZIE, S.G., FREW, R. & BAER, H-P. (1977). Characteristics of the relaxant response of adenosine and its analogs in intestinal smooth muscle. *Eur. J. Pharmac.*, **41**, 183–192.

NAYLER, W.G., PRICE, J.M. & LOWE, T.E. (1967). Inhibition of adenosine induced coronary vasodilation. *Cardiovasc. Res.*, **1**, 63–66.

NICHOLS, R.E. & WALASZEK, E.S. (1963). Antagonism of the vaso-depressor effect of ATP by caffeine. *Fedn Proc.*, **22**, 308.

OSSWALD, H. (1975). Renal effects of adenosine and their inhibition by theophylline in dogs. *Naunyn Schmiedebergs Arch. Pharmac.*, **288**, 79–86.

PEERS, D.G. & DAVIES, J.I. (1971). Significance of caffeine-like effect of various purines, pyrimidines and derivatives on adipose-tissue phosphodiesterase. *Biochem. J.*, **124**, 8–9P.

PÖCH, G. & KUKOVETZ, W.R. (1971). Blockade of phosphodiesterase inhibiting properties of coronary dilator drugs by theophylline *in vitro*. *Naunyn Schmiedebergs Arch. Pharmac., Supp.*, **240**, 107.

RICHARDSON, J.B. & BOUCHARD, T. (1975). Demonstration of a non-adrenergic inhibitory nervous system in the trachea of the guinea-pig. *J. Allergy clin. Immunol.*, **56**, 473–480.

RIKIMARU, A., FUKUSHI, Y. & SUZUKI, T. (1971). Effects of imidazole and phentolamine on the relaxant responses of guinea-pig taenia coli to transmural stimulation and to adenosine triphosphate. *Tohoku J. exp. Med.*, **105**, 199–200.

SATCHELL, D., BURNSTOCK, G. & DANN, P. (1973). Antagonism of the effects of purinergic nerve stimulation and exogenously applied ATP on the guinea-pig taenia coli by 2-substituted imidazolines and related compounds. *Eur. J. Pharmac.*, **23**, 264–269.

SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1975). Antagonism of adenosine 5'-triphosphate-induced relaxation by 2-2'-pyridylisatogen in the taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **53**, 575–583.

THER, L., MUSCHAWECK, R. & HERGOTT, J. (1957). Antagonismus zwischen Adenosin und Methyl-Xanthinen am Reizleitungssystem des Herzens. *Naunyn Schmiedebergs Arch. exp. Path. Pharmak.*, **231**, 586–590.

WAYNE, E.J., GOODWIN, J.F. & STONER, H.B. (1949). Effect of adenosine triphosphate on electrocardiogram of man and animals. *Br. Heart J.*, **11**, 55–67.

(Received August 10, 1978
Revised September 27, 1979.)