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IDENTIFICATION OF SEPARATE RECEPTORS FOR ADENOSINE AND ADENOSINE 5′-TRIPHOSPHATE IN CAUSING RELAXATIONS OF THE ISOLATED TAENIA OF THE GUINEA-PIG CAECUM

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1 The mechanisms by which adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and adenosine relax the taenia caecum preparation of the guinea-pig have been studied. ATP and ADP produced similar effects which were qualitatively different from those of AMP and adenosine.

2 2-2′Pyridylisatogen tosylate (PIT: 50 μM for 30 min) blocked the effects of ATP and ADP, but exhibited weak activity against AMP and failed to antagonize the effects of adenosine. The action of PIT was unaffected by the inclusion of dipyridamole (2 μM) in the bathing fluid.

3 There was a significant correlation between the sensitivity of individual preparations to ATP or ADP and the blocking potency of PIT.

4 The presence of adenosine in the bathing fluid (2 mM for >30 min) desensitized the taenia to subsequent applications of adenosine. The effects of ATP were increased by this procedure.

5 The results indicate that ATP and adenosine relax the taenia by different mechanisms.

Introduction

Since Burnstock and his colleagues (Burnstock, Campbell, Satchell & Smythe, 1970; Burnstock, 1972) proposed that the chemical mediator of the non-adrenergic inhibitory response to intrinsic nerve stimulation in the gut was adenosine 5′-triphosphate (ATP), there has been renewed interest in the effects of purine derivatives on smooth muscle. A full examination of these effects has been hindered because of the lack of a specific ATP-receptor antagonist. Recently, however, 2-2′pyridylisatogen (PIT) has been shown to block the relaxation of guinea-pig isolated taenia caecum induced by ATP without preventing the inhibitory effects of noradrenaline or isoprenaline (Hooper, Spedding, Sweetman & Weetman, 1974; Spedding, Sweetman & Weetman, 1975). This antagonist has now been used to analyse the action of ATP, adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and adenosine on the isolated taenia caecum preparation of the guinea-pig. The results provide evidence that there are two or more receptors for the inhibitory effects of the purine bases.

Methods

Taenia caeci preparations were obtained from female guinea-pigs in the weight range 250–600 grams. The preparations were suspended in 10 ml isolated organ baths filled with McEwen's solution (McEwen, 1956) maintained at 35 ± 1°C and gassed with 95% O_2 and 5% CO_2. After an equilibration period of 30 min, responses were recorded isotonically on a smoked drum (magnification 1 : 4, load 1.5 grams).

Cumulative concentration-response curves (Van Rossum, 1963) for the purine bases and noradrenaline to relax the preparations were determined at 20 min intervals. Each dose of drug was allowed to produce its full effect (5–40 s contact) before the concentration of the drug in the bath was increased. Preparations with low tone, i.e. those that did not contract in the isolated organ bath so that they were 25% or less of their fully relaxed length, were not used.

The initial concentration-response curve for each agonist was disregarded. Subsequent concentration-response curves were measured and the EC_{50} (the concentration producing a 50% maximal relaxation) and slope (ratio of the concentrations producing 80% and 20% maximal effect; Stephenson, 1956) were determined. Dose-ratios were estimated as the ratio of the EC_{50} after and before the modifying drug.

In one series of experiments, attempts were made to desensitize the taenia to ATP and adenosine by including one of the bases in the bathing medium. After 30 min exposure to the desensitizing agent (2 mM), cumulative concentration-response curves for ATP, ADP, AMP, adenosine and noradrenaline were obtained.

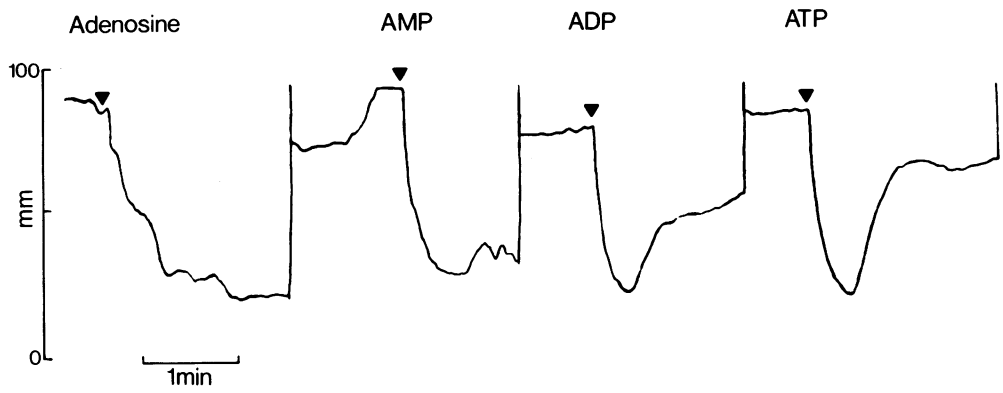

Figure 1 The effects of adenosine (400 μM), adenosine 5'-monophosphate (AMP, 120 μM), adenosine 5'-diphosphate (ADP, 40 μM) and adenosine 5'-triphosphate (ATP, 40 μM) on the taenia. Responses were obtained at 10 min intervals. Zero on the vertical calibration bar represents the maximum relaxation of the preparation to ATP.

Values in the text refer to mean ± s.e. mean. Differences in means were determined by Student's *t*-test, after checking the homogeneity of the variances (Snedecor & Cochran, 1967). Correlation coefficients were determined as described by Daniel (1974) and regression analysis by the method of Bailey (1959).

Drugs

PIT was synthesized by Dr M. Hooper of this department. The other drugs used were: adenosine 5'-triphosphate disodium salt (BDH), adenosine 5'-diphosphate disodium salt (Sigma), adenosine 5'-monophosphoric acid (BDH), adenosine (BDH); (−)-ascorbic acid and carbachol chloride (BDH); dipyridamole (Boehringer); (—)-noradrenaline bitartrate (Koch-Light). Solutions of ATP, ADP and AMP were adjusted to pH 7 with 1 M NaOH. Adenosine was dissolved in 1 M HCl and adjusted to pH 5 with 1 M NaOH. Dipyridamole was dissolved in the minimum volume of 1 M HCl. Noradrenaline was protected from oxidation by the inclusion of approximately 100 μg/ml of (−)-ascorbic acid in each dilution. The composition of the McEwen's (1956) solution was as follows (mM): NaCl 130, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 11.1 and sucrose 13.2.

Results

Responses of the taenia to the purine bases

ATP, ADP, AMP and adenosine relaxed the taenia in a concentration-dependent manner. However, there were differences in the time to peak relaxation, ATP and ADP causing a rapid relaxation of the smooth muscle, whereas the effects of adenosine and AMP developed more slowly (within 4–40 s of dosing, as opposed to 2–14 s for ATP and ADP, see Figure 1). It was also possible to subdivide the bases into two groups on the basis of the ability of the muscle partially to regain tone whilst the relaxant drug was still in contact with the preparation (Figure 1). In the presence of ATP and ADP a partial regaining of tone was consistently observed, adenosine and AMP exhibiting this effect much less frequently (Table 1), and when it occurred it was much smaller than that following ATP or ADP.

Concentration-response curves for each of the bases were determined, the tissue being dosed cumulatively. The maximum intensity of action obtained with all the purines was similar (< 10% variation, $n=5$), presumably because the smooth muscle was fully relaxed. The affinity of the drugs for the tissue (EC_{50}), the frequency of occurrence of the secondary contractions, and the slopes of the curves are shown in Table 1.

Effect of 2,2'-pyridylisatogen

PIT (50 μM for 30 min) relaxed the taenia and so carbachol (0.05–1.0 μM) was used to restore the tone of the preparation to within 20% of the control level. In the presence of this cholinomimetic agent PIT is more active (× 5) as an antagonist of ATP (Spedding *et al.*, 1975). In the present experiments, PIT (50 μM for 30 min) markedly antagonized the relaxant effects of ATP and ADP (dose-ratios 46 and 29), but the dose-ratios for AMP (5.0) and adenosine (2.0) indicated a low level of antagonism, being similar to that for noradrenaline (2.4; see Figure 2).

Although it was clear that PIT blocked the effects of ATP and ADP without greatly reducing the

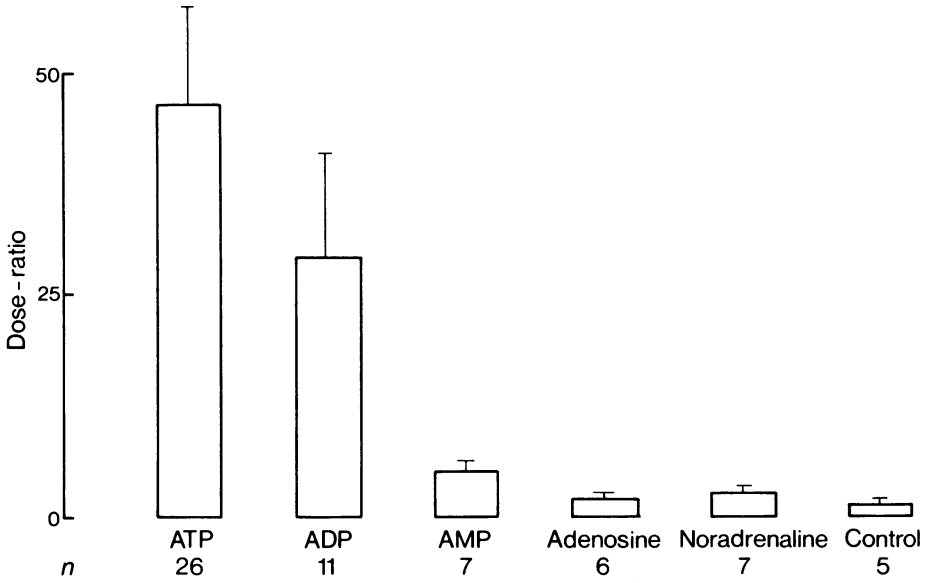

Figure 2 Effects of 2-2'pyridylisatogen tosylate (PIT; 50 μM for 30 min) on the relaxations of the taenia induced by drugs. Carbachol (0.05–1.0 μM) was used after PIT to recontract the tissue to within 20% of the original level. Control experiments in which cumulative concentration-response curves to adenosine 5'-triphosphate (ATP) were obtained when PIT was omitted are also shown. The vertical bars represent the s.e. mean. Note that only the effects of ATP and adenosine 5'-diphosphate (ADP) are antagonized, and that these dose-ratios are not significantly different ($P > 0.05$).

sensitivity of the tissue to AMP and adenosine, the magnitude of the antagonism, particularly of ADP, was not consistent. Preparations that were particularly sensitive to ADP were the most susceptible to the antagonistic action of PIT. The relationship between the effectiveness of PIT and the sensitivity of the preparations to ADP is shown in Figure 3. Analysis of the data in Figure 3 showed a significant negative correlation between the EC_{50} for ADP and the dose-ratio for PIT, the highest value for the correlation coefficient occurring when $\log_{10}$ EC_{50} was compared with the dose-ratio (Table 2). A similar correlation was found following the analysis of the results with ATP: the $\log_{10}$ EC_{50} was significantly correlated with the dose-ratio ($r = -0.67$, $P < 0.01$, $n = 19$).

Effect of dipyridamole

Although the inhibitory effects of adenosine and ATP on the taenia were dissimilar with respect to potency, onset and rate of relaxation, and susceptibility to PIT, it is known that adenosine is taken up into cells at a

Table 1 Concentration-response curve data for purines on the guinea-pig taenia

Derivative	*EC_{50} (± s.e. mean) (μM)*	*Slope (± s.e. mean)*	n	*Proportion of preparations partially regaining tone*
ATP	32 (± 9)	26 (± 5)	21	72/73
ADP	44 (± 9)	29 (± 7)	11	19/20
AMP	240 (± 70)	10 (± 2)	7	10/19
Adenosine	230 (± 79)	9 (± 2)	6	7/34

The drugs were administered cumulatively, increasing the concentration when the relaxation following the previous dose was complete. The initial curve was not used, and each tissue received only one derivative. EC_{50} is the concentration producing a 50% maximal relaxation. The slopes of the curves have been measured as the ratio of concentrations producing 80% and 20% maximal relaxations. Some additional preparations are included in the values where tone was partially regained.

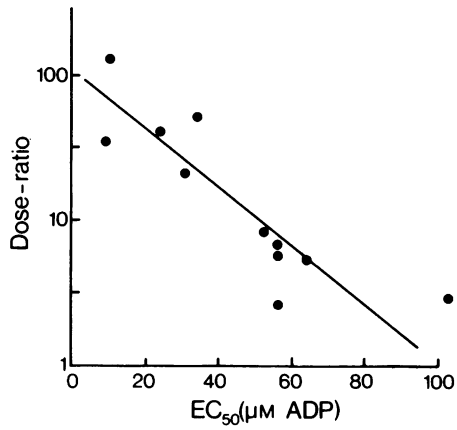

Figure 3 The relationship between the sensitivity of the taenia to adenosine 5'-diphosphate (ADP) and the dose-ratio following incubation with 2-2'pyridylisatogen tosylate (PIT). EC_{50} values were calculated from cumulative concentration-response curves to ADP before incubation with PIT (50 μM for 30 minutes). Carbachol (0.05–0.3 μM) was added after PIT to restore the tone of the preparations to within 20% of their original length. Dose-ratios were calculated from subsequent concentration-response curves. The line is the calculated regression line ($n=11$).

much faster rate than is ATP. Accordingly, the experiments were repeated in the presence of dipyridamole, a drug known to inhibit adenosine uptake into the tissues (for references see Discussion section).

The results of the experiments using dipyridamole (2 μM) are shown in Figure 4. The dose-ratio for adenosine was now less than 0.1, which indicates that the inhibitory effects of adenosine were potentiated more than tenfold. However, PIT (50 μM for 30 min) in the presence of dipyridamole failed to antagonize the relaxant effects of adenosine. In contrast, the effects of ATP were still antagonized by PIT after dipyridamole.

Desensitization experiments

In preliminary experiments, exposure of the taenia to high concentrations of ATP (2 mM) reduced the sensitivity of the preparations to subsequent additions of both ATP and adenosine, although these effects were variable. In contrast, high concentrations of adenosine caused a desensitization to adenosine but not to ATP. Consequently, a series of concentration-response curves to ATP, ADP, AMP, adenosine and noradrenaline were obtained in the presence of adenosine (2 mM) in the bathing fluid. The EC_{50} values from these experiments are compared to those obtained with normal bathing fluid in Table 3. Adenosine was the only drug whose effects were significantly reduced by the inclusion of adenosine in the medium.

Discussion

This paper contains evidence that ATP and adenosine act on different receptors, confirming the findings of Satchell, Lynch, Bourke & Burnstock (1972) that the nature of the response of the taenia to the two drugs is different. The dissimilar nature of the responses to the two bases was confirmed when PIT was found to be an effective antagonist against ATP but not adenosine-induced relaxations of the taenia. Finally, it was possible to desensitize the taenia to adenosine by including this base in the bathing medium, a procedure which increased the effectiveness of ATP.

The action of ADP resembled that of ATP in the nature of the response, in the blocking action of PIT, and in the desensitization experiments. However, the potency of PIT against ADP varied considerably. This was accounted for when the effectiveness of the

Table 2 Correlation between the sensitivity of the taenia to adenosine 5'-diphosphate (ADP) and the potency of 2-2'pyridylisatogen tosylate (PIT)

Measure of sensitivity to ADP	*Measure of potency of PIT*	r	P
EC_{50}	dose-ratio	−0.72	<0.05
$\log_{10}EC_{50}$	dose-ratio	−0.89	<0.001
EC_{50}	$\log_{10}$dose-ratio	−0.88	<0.001
$\log_{10}EC_{50}$	$\log_{10}$dose-ratio	−0.54	>0.05

ADP was administered cumulatively, increasing the concentration when the relaxation following the previous dose was complete. The initial curve was not used. EC_{50} is the concentration producing 50% maximal relaxation. The dose-ratio represents the EC_{50} after PIT (50 μM for 30 min) divided by the control EC_{50} on that preparation ($n=11$). Carbachol (0.05–0.3 μM) was used after PIT to restore the tone of the preparations to within 20% of the control level.

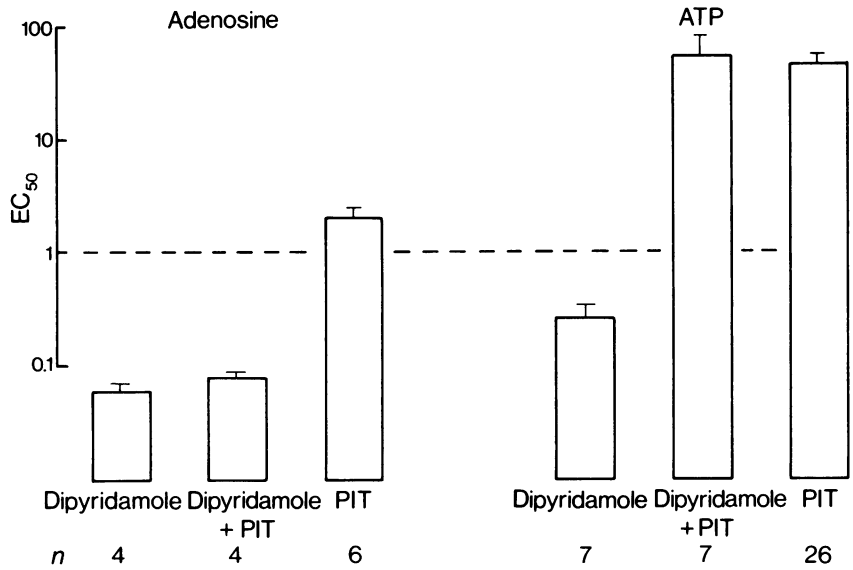

Figure 4 Effects of dipyridamole on the responses of the taenia to adenosine and adenosine 5'-triphosphate (ATP). EC_{50} values were first calculated from cumulative concentration-response curves to adenosine and ATP, then secondly after a 20 min incubation with dipyridamole (2 μM), and finally after incubation with 2-2'pyridylisatogen tosylate (PIT, 50 μM for 30 min) in the presence of dipyridamole. Carbachol (0.05–1.0 μM) was used to restore the tone of the preparations to within 20% of the original length. The results are compared with EC_{50} values obtained after PIT (50 μM for 30 min) in experiments when the dipyridamole was omitted. The vertical bars represent the s.e. mean. Note that PIT failed to block the adenosine-induced relaxations even in the presence of dipyridamole.

antagonist was considered in relation to the sensitivity of the tissue to the nucleotide: the more sensitive the preparation, the greater was the antagonism. A similar relationship existed between ATP and PIT. Such relationships may indicate that ATP, ADP and PIT act at a common site, and that the number of these sites varies from preparation to preparation. However, further studies will be necessary to establish this idea.

Table 3 Effects of including adenosine (2 mM) in the McEwen's solution on the sensitivity of the taenia to purines and noradrenaline

Drug	*EC_{50} (± s.e. mean) (μM) Control*	*In the presence of adenosine*	*Dose-ratio*	P	
ATP	32 (±9)	7 (±4)	0.2	>0.05	<0.10
ADP	44 (±9)	28 (±11)	0.6	>0.05	
AMP	240 (±70)	340 (±104)	1.4	>0.05	
Adenosine	230 (±79)	1946 (±267)	8.5	<0.001	
Noradrenaline	0.216 (±0.05)	0.197 (±0.07)	0.9	>0.05	

The drugs were administered cumulatively, increasing the concentration when the relaxation following the previous dose was complete. EC_{50} represents the concentration of drug producing 50% maximal relaxation. In this table only, the dose-ratios have been determined by dividing the mean control EC_{50} into the mean value obtained in the presence of adenosine (2 mM for >30 minutes). There were five experiments in the 'In the presence of adenosine' group, and the concentration-response curves were obtained at 20 min intervals, the order of dosing being randomized. The 'control' EC_{50} values have been taken from Table 1. Carbachol (0.05–0.2 μM) was used to restore the tone of the preparations to within 20% of the level at the start of the experiment. Note that adenosine was the only drug to which the taenia was significantly desensitized.

It was thought worthwhile to investigate whether differences in the metabolism and uptake of ATP and adenosine into cells could account for the inability of PIT to block the effects of adenosine. Consequently, the experiments were repeated with dipyridamole in the McEwen's solution. Dipyridamole reduces adenosine uptake, probably by inhibiting the intracellular phosphorylation of adenosine to ATP (Hopkins & Goldie, 1971; Hopkins, 1973; Hulme & Weston, 1974). Thus, when Hulme & Weston exposed rabbit intestine to adenosine in the presence of dipyridamole, the intracellular level of adenosine was normal or slightly elevated, but the amount of ATP was much less than in the control group. In the present experiments, PIT failed to block the inhibitory effects of adenosine even though the dipyridamole treatment increased the sensitivity of the taenia to the base 17-fold. In contrast, PIT was equally effective against ATP in the presence and absence of dipyridamole. Thus the differences between ATP and adenosine were not secondary to their disposition in the tissue.

Attempts to differentiate between the effects of ATP and adenosine by specifically desensitizing the taenia were successful when adenosine was the desensitizing agent, but not when ATP was used. The failure may have been due to metabolism of ATP to adenosine by enzymes in the taenia, and this adenosine could subsequently desensitize the tissue to adenosine as well as to ATP. When adenosine was used as the desensitizing agent, it is unlikely that ATP would be generated extracellularly because the enzymes for this biosynthesis are predominantly intracellular. Weston (1973) found that ATP desensitized rabbit duodenum to both ATP and adenosine, but not to noradrenaline and isoprenaline. However, he also found that adenosine desensitized the receptors to ATP as well as to adenosine. The difference between the rabbit duodenum and the taenia may be due to the methods of desensitization; in particular, in the present study much higher concentrations of the nucleotides were used (2 mM compared with 100 μM in Weston's experiments).

From the present evidence, which includes the nature of the responses, the blockade by PIT and the desensitization studies, it is possible to conclude that ATP and ADP act at one receptor, and that AMP and adenosine relax the taenia by some other process or processes.

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