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Activation of P_1- and P_{2Y}-purinoceptors by ADP-ribose in the guinea-pig taenia coli, but not of P_{2X}-purinoceptors in the vas deferens

[1]Charles H.V. Hoyle & Gareth A. Edwards

Department of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT

1 The activity of adenosine 5'-diphosphoribose (ADP-ribose), a ribosylated purine nucleotide, was investigated on the carbachol-contracted taenia coli, a tissue possessing P_1- (A_2) and P_{2Y}-purinoceptors and on the guinea-pig vas deferens which possesses P_{2X}-purinoceptors.

2 In the vas deferens, where ATP (1 μM–1 mM) produced concentration-dependent contractions, ADP-ribose was without effect at concentrations up to 1 mM.

3 In the taenia coli, ADP-ribose (0.1 μM–1 mM) produced concentration-dependent relaxations with a potency similar to that of adenosine, but less than that of ATP. The pD_2 values for ADP-ribose, adenosine and ATP were 4.5 ± 0.07 (27), 4.4 ± 0.10 (9) and 5.5 ± 0.14 (21), respectively. The time-course of the relaxations elicited by ADP-ribose was found to be significantly longer than that for ATP and significantly shorter than that for adenosine.

4 The P_1-purinoceptor antagonist, 8-phenyltheophylline (5 μM), produced parallel rightward shifts in the concentration-response curves of the relaxations of the taenia coli elicited by ADP-ribose and adenosine but not ATP.

5 Dipyridamole (0.3 μM), a purine nucleoside uptake inhibitor, potentiated the responses to adenosine and ADP-ribose in the taenia coli. These potentiations were sensitive to 8-phenyltheophylline (5 μM).

6 Reactive blue 2, a P_{2Y}-purinoceptor antagonist, antagonized the inhibitory responses of ADP-ribose and ATP in the taenia coli, without significantly altering the inhibitory responses of either adenosine or noradrenaline.

7 In the presence of the potassium channel blocker, apamin (0.3 μM), the inhibitory responses of ADP-ribose were severely attenuated, and the inhibitory responses of ATP in the taenia coli were converted to transient contractions. Further addition of 8-PT blocked the residual responses of ADP-ribose.

8 The P_2-purinoceptor antagonist, suramin (500 μM), antagonized responses to ATP and ADP-ribose, but not adenosine. Further addition of 8-PT antagonized the residual responses to ADP-ribose, but not to ATP.

9 It is concluded that ADP-ribose has a mixed pharmacological profile, evoking both P_1 (A_2)-purinoceptor-mediated responses and P_{2Y}-purinoceptor-mediated responses, while being inert at P_{2X}-purinoceptors. It is suggested that ADP-ribose may provide a useful starting point for the generation of structural analogues which have specific activity at the P_{2Y}-purinoceptor.

Keywords: ADP-ribose; apamin; ATP; guinea-pig intestine; purinoceptors; reactive blue 2; smooth muscle; suramin; guinea-pig vas deferens

Introduction

Purine nucleosides and nucleotides have long been known to have potent extracellular actions on a variety of tissues. As early as 1929, Drury & Szent-György published findings of cardiac and vascular actions of adenine compounds. Since then, purine derivatives have also been shown to have potent pharmacological activity in many peripheral organs and the central nervous system. ATP has been proposed as the principle neurotransmitter from some non-adrenergic, non-cholinergic nerves and to be a co-transmitter with noradrenaline, acetylcholine and other substances (see Burnstock, 1972; 1976; 1990; Hoyle & Burnstock, 1991a).

Based on criteria of differences in relative agonist potencies, selective antagonism and differences in transduction mechanisms, Burnstock (1978) proposed that two types of purinoceptors could be distinguished: P_1- and P_2-purinoceptors. Both these classes of purinoceptors have subsequently been found not to be homogeneous groups and have been further subdivided (see Hoyle & Burnstock, 1991b). The P_1-purinoceptors, which have a relative agonist potency order of adenosine ≥ adenosine monophosphate (AMP) > adenosine diphosphate (ADP) ≥ adenosine 5'-triphosphate (ATP), have been divided into A_1 and A_2 subtypes (see Burnstock & Buckley, 1985), and a third subclass of P_1-purinoceptors, A_3 (Ribeiro & Sebastião, 1986; Sebestião & Ribeiro, 1988) has more recently been proposed.

P_2-purinoceptors, which have an agonist potency order of ATP ≥ ADP > AMP ≥ adenosine, were subdivided by Burnstock & Kennedy (1985), into P_{2X}- and P_{2Y}-purinoceptors, based largely on the rank order of agonist potency of structural analogues of ATP and also on the activity of antagonists at the P_{2X}-purinoceptor. The P_2-purinoceptor has been further subclassified (Gordon, 1986), with P_{2T}-purinoceptors, activated by ADP, suggested as being present only on thrombocytes and megakaryocytes, and P_{2Z}-receptors, activated by ATP^{4-}, mediating permeabilisation of mast cells and other blood cells.

It has been suggested that adenine dinucleotides, including

[1] Author for correspondence.

nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP), might act on a receptor class distinct from P_1- and P_2-purinoceptors (Hoyle, 1990). Adenosine 5'-diphosphoribose (ADP-ribose) is chemically related to NAD and NADP, and NAD can be regarded as ADP-ribose covalently attached to the vitamin nicotinamide by a high energy β-N-glycosidic bond. ADP-ribose itself is an ADP moiety with a second ribose sugar attached to the β-phosphate via an esteric linkage. An increasing number of studies have provided evidence suggesting that mono/poly (ADP-ribose) may regulate the activities of a number of enzymes, either by covalent attachment to, or by physical association with, these enzymes (Hussain *et al.*, 1989). ADP-ribosylation of specific proteins is thought to occur in almost all forms of life and almost all compartments of the cell, and has been implicated in the control of a number of biological events (Hayaishi & Ueda, 1977; Ueda & Hayaishi, 1985). Although its intracellular roles have been widely researched, the pharmacological profile of ADP-ribose on extracellular receptors has not to our knowledge been evaluated.

Since ADP-ribose is an adenine nucleotide it was thought that it would be more likely to act upon P_2- than P_1-purinoceptors and therefore the tissues chosen for investigation were the guinea-pig vas deferens, which has P_{2X}-purinoceptors on the smooth muscle, and the taenia coli which possesses P_{2Y}-purinoceptors (Burnstock & Kennedy, 1985). The taenia coli also possesses P_1-purinoceptors of the A_2 subtype (Burnstock *et al.*, 1984).

Methods

Guinea-pig taenia coli

Male guinea-pigs, weighing between 300 and 600 g, were killed by a blow to the head and exsanguination. The ventral and medial taenia coli were dissected free. Segments, approximately 2 cm long, were suspended in 5 or 10 ml organ-baths containing modified Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.4, $NaHCO_3$ 16.3, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.7 (Bülbring, 1953). The organ-baths were constantly gassed with 95% O_2/5% CO_2 and the organ-baths and reservoirs of Krebs solution were maintained at 36–37°C.

The strips of taenia coli were initially loaded with a tension of 1 g and were then allowed to equilibrate for approximately 45 min, with the bathing solution being changed every 15 min. Contractions were recorded by either a Dynamometer UF1 or Grass FTOC3 force-displacement transducer, and displayed on a Grass 79D polygraph.

Carbachol (100 nM) was used to induce a sustained submaximal (approximately 80%) contraction upon which the relaxant effects of the test drugs were demonstrable. When the maximal relaxation due to the applied drug had been observed, the organ-bath was washed several times with fresh Krebs solution. After 7–10 min, carbachol was re-added and the next concentration of the drug tested. The drugs were usually tested in multiples of 1 and 3 decades of concentration units; intermediate concentrations were tested in those cases where there was a steep concentration-response relationship. This was carried out in a range of concentrations from below the threshold for observable response to maximal response or a maximal organ-bath concentration of 1 mM (or 0.5 mM in the case of adenosine).

When dipyridamole, 8-phenyltheophylline (8-PT) or reactive blue 2 (RB2) were used, the agent was added to the stock of Krebs solution and the preparations were allowed to equilibrate for 30 min. Apamin was added directly to the organ-bath and allowed to equilibrate for minimum of 20 min.

Responses to ADP-ribose, ATP and noradrenaline were tested first in the absence of RB2 and then compared with responses on the same preparations in the presence of RB2. Since RB2 tends to have a general desensitizing action on all drugs responses beyond a period of about 2 h (Burnstock & Warland, 1987), only one non-cumulative concentration-response curve was performed on each preparation once RB2 had been added to the organ-bath.

Because of the limited availability of suramin, and the relatively high concentrations that are needed, full concentration-response relationships were not determined in its presence. Instead, concentrations of agonists that produced approximately a 50% relaxation under control conditions were tested following incubation of the preparation in suramin for 30 min, and again in the additional presence of 8-PT. Also, because full concentration-relaxation relationships for ADP-ribose could not be obtained in the presence of apamin, in one series of experiments concentrations of ADP-ribose that produced approximately 50% relaxations in the control situation were tested in the presence of apamin, and again in the additional presence of 8-PT.

Guinea-pig vas deferens

Male guinea-pigs, weighing between 300 and 600 g were killed by a blow to the head and exsanguination, their abdomens were opened and the vasa deferentia removed. Segments, 2 cm long, were suspended in 5 or 10 ml organ-baths containing modified Krebs solution (as described above). The organ-baths were constantly gassed with 95% O_2/5% CO_2 and were maintained at 36–37°C.

The preparations were allowed to equilibrate for at least 30 min under a resting tension of approximately 1 g, with the bathing solution being changed every 15 min. Contractile responses were recorded as described above. Concentration-response curves were obtained following the non-cumulative addition of ATP in a range from below the threshold of observable response to 1 mM. The concentrations tested were in multiples of 1 and 3 decades of concentration units. For ADP-ribose a range of concentrations between 1 μM and 1 mM were tested in a non-cumulative manner. After the maximal response to the applied drug had been observed, the organ bath was washed several times with fresh Krebs solution and 7–10 min were allowed before the next application of a drug.

A series of experiments was carried out in order to assess possible effects of ADP-ribose on the response to ATP. Having established a concentration-response relationship of ATP on a preparation, ADP-ribose (1 μM–1 mM) was added to the organ-bath and after an interval ranging from 2 to 22 min ATP was again tested.

Drugs used

Adenosine 5'-diphosphoribose, adenosine 5'-triphosphate (sodium salt), adenosine hemisulphate, noradrenaline ((±)-L-arterenol bitartrate), apamin, 8-phenyltheophylline and reactive blue 2 (Cibracron blue 3GA) were all obtained from Sigma. Dipyridamole (Persantin) was supplied by Boehringer Ingelheim. 8-Phenyltheophylline was dissolved in 80% v/v methanol/20% molar NaOH to produce a stock solution of 10 mM; noradrenaline was dissolved in 0.1 mM ascorbic acid to produce a stock solution of 10 mM and adenosine was dissolved in Krebs solution to give a stock of 10 mM; subsequent dilutions of all three drugs were in distilled water. In order to obtain an organ-bath concentration of 0.5 mM, 500 μl of the adenosine stock was added to the 10 ml organ-bath, while other additions were of 30 μl or 100 μl. All other drugs were made up in distilled water to produce stock solutions so that additions to the 10 ml organ-bath were 30 μl or 100 μl, and those to the 5 ml organ-baths were 15 μl or 50 μl.

Analysis of results

In the experiments on the taenia coli, responses were calculated as the percentage reduction of the carbachol-induced contraction. The mean response of the preparations from each animal was calculated at each concentration of the test-drug used. These mean responses for each animal were then subjected to a probit transformation (Bliss, 1935; Finney, 1971) which converts the sigmoidal log-concentration curve to a straight line. Linear regression of the probit values against log concentration was then carried out in order to interpolate the log-concentrations yielding 1, 5, 10, 20, 35, 50, 65, 80, 90, 95 and 99% responses (Hoyle & Greenberg, 1988). The means and standard errors of the log concentrations for each of these percentage points were calculated and used to plot the summed concentration-response curve. This method averages the concentration-response curve horizontally and therefore avoids a bias towards a lower slope (see Waud, 1975). Since the range of response from 1–99% is used, this method is also more informative than taking the mean of linear regression over the 20–80% portion of the concentration-response curve.

In the experiments where dipyridamole, 8-PT or RB2 were used, the means and the standard errors of the pD_2 values (negative logarithm of EC_{50}) were calculated from the concentration-response relationships and were then used in Student's paired t tests. The slopes of the concentration-response curves in the presence of an agent were also compared with the control curves by paired t tests.

In the experiments with apamin, maximal relaxation of the carbachol-contraction in response to ADP-ribose was not attained at the concentrations tested. Analysis by the method outlined above was therefore inappropriate; the results were analysed by Students paired t test of the mean relaxant responses produced by ADP-ribose at concentrations of 10 μM, 30 μM and 100 μM.

Measurements of the time to reach maximum response were made for responses to ATP, adenosine and ADP-ribose at approximately their EC_{50} concentrations in the control experiments. Such measurements were also made in the presence of dipyridamole (0.3 μM), which is an inhibitor of nucleoside uptake (Kolassa *et al.*, 1970).

In experiments on the vas deferens the responses to the drugs were expressed relative to the response elicited by the administration of ATP (1 mM). The means and standard errors of the average response of the preparations from each animal for each concentration applied were calculated

In the text, values are presented as mean ± s.e.mean with the number of replicates given in parentheses. When mulitiple comparisons were made, analysis of variance followed by Tukey's procedure was used. For all tests a probability level of 0.05 or less was considered significant.

Table 1 Carbachol-contracted guinea-pig taenia coli: pD_2 values of the inhibitory responses of ADP-ribose, ATP, noradrenaline (NA) and adenosine, and the effects of 8-phenyltheophylline (8-PT), dipyridamole (Dip), and reactive blue 2 (RB2)

Agents	$pD_2 \pm$ s.e. (n)
ADP-ribose	4.50 ± 0.07 (27)
ADP-ribose + 5 μM 8-PT	4.12 ± 0.07 (7)*
ADP-ribose + 0.3 μM Dip	5.54 ± 0.24 (4)*
ADP-ribose + 0.3 μM + 5 μM 8-PT	4.31 ± 0.10 (4)*
ADP-ribose + 30 μM RB2	3.96 ± 0.17 (7)*
ATP	5.50 ± 0.14 (21)
ATP + 5 μM 8-PT	5.39 ± 0.15 (7)
ATP + 30 μM RB2	4.59 ± 0.16 (9)*
Adenosine	4.40 ± 0.10 (9)*
Adenosine + 5 μM 8-PT	3.69 ± 0.08 (5)
Adenosine + 0.3 μM Dip	5.84 ± 0.22 (4)*
Adenosine + 0.3 μM Dip + 5 μM 8-PT	4.67 ± 0.16 (4)*
NA	6.50 ± 0.34 (7)
NA + 30 μM RB2	6.20 ± 0.12 (7)

*$P<0.05$, paired t test against control

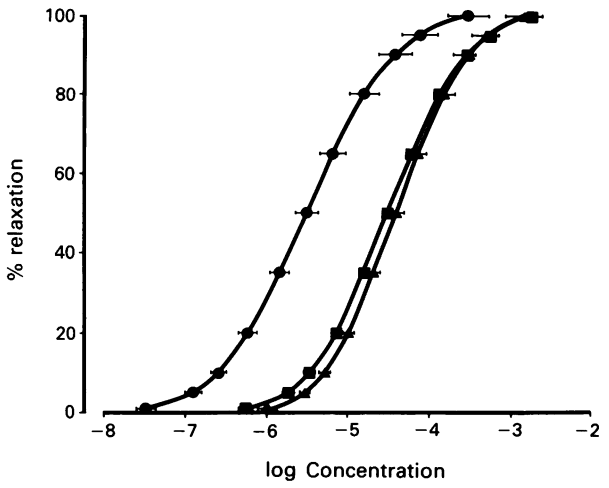

Figure 1 Concentration-response curves for ATP (●, $n = 21$), ADP-ribose (■, $n = 27$) and adenosine (▲, $n = 9$) in causing relaxation of carbachol-contracted guinea-pig taenia coli. The points represent the mean and the horizontal lines the s.e.mean.

Results

Guinea-pig taenia coli

The results of the experiments carried out on the taenia coli are summarised in Tables 1 and 2.

ADP-ribose, in a concentration range of 0.1 μM to 1 mM, produced concentration-dependent relaxations of the carbachol-contracted taenia coli with a pD_2 value of 4.50 ± 0.07 (27). ATP (0.03 μM–1 mM) produced concentration-dependent relaxations with a pD_2 value of 5.50 ± 0.14 (21). Adenosine (0.3 μM–500 μM) produced concentration-dependent relaxations with a pD_2 value of 4.40 ± 0.10 (9). The concentration-response curves for ADP-ribose, ATP and adenosine are illustrated in Figure 1.

The time taken for the peak response to half-maximal concentrations of ATP, adenosine and ADP-ribose to develop were all significantly different from one another, with mean times (s) to reach maximum response being 9 ± 1 (21) for ATP, 14 ± 1 (27) for ADP-ribose, and 21 ± 3 (9) for adenosine.

Effects of 8-phenyltheophylline

The effects of 8-PT (5 μM) on the concentration-response relationships for adenosine, ADP-ribose and ATP are shown in Figure 2.

8-PT (5 μM) produced significant rightward shifts in the concentration-response curves of both adenosine and ADP-ribose, with the pD_2 value of adenosine being reduced from 4.34 ± 0.15 to 3.69 ± 0.08 (5) ($P<0.01$) and the pD_2 value of ADP-ribose being reduced from 4.47 ± 0.05 to 4.12 ± 0.07 (7) ($P<0.01$). The shift in the pD_2 value for adenosine was significantly greater than that for ADP-ribose ($P<0.01$). No significant change was found in the slope of the concentration-response curves for either drug in the presence of 8-PT.

For ATP no significant changes were found in either the

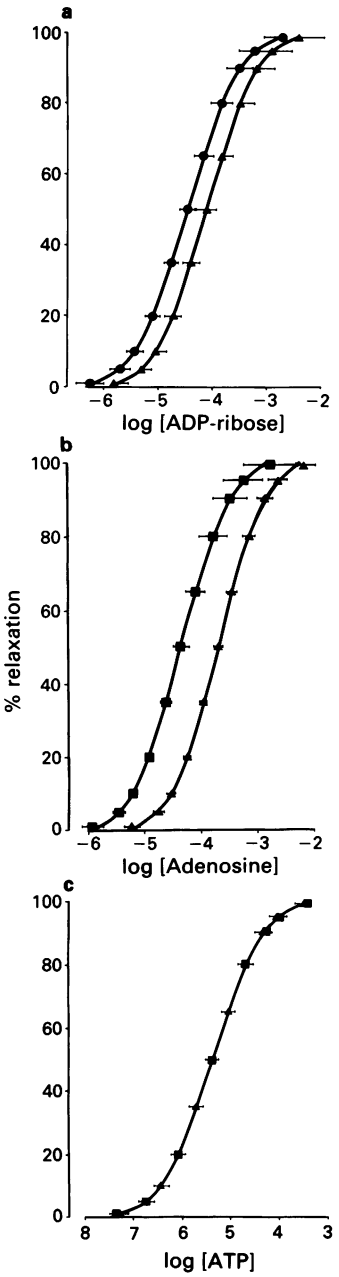

Figure 2 Effect of 8-phenyltheothylline (8-PT, 5 μM) on the inhibitory responses of carbachol-contracted guinea-pig taenia coli to ADP-ribose, adenosine and ATP. Concentration-response curves are shown for (a) ADP-ribose prior to administration of 8-PT (●) and in the presence of 8-PT (▲) ($n = 7$); (b) adenosine prior to administration of 8-PT (■) and in the presence of 5 μM 8-PT (▲) ($n = 5$); and (c) ATP prior to administration of 8-PT (■) and in the presence of 8-PT (▲) ($n = 7$). The points represent the mean and the horizontal lines the s.e.mean.

pD_2 values, which altered from 5.39 ± 0.15 to 5.39 ± 0.17 (7), or the slope of the concentration-response curve, in the presence of 8-PT.

Effects of dipyridamole

In the presence of dipyridamole (0.3 μM) no observable changes in the levels of spontaneous activity or the sensitivity of the taenia preparations to carbachol were noted. The effects of dipyridamole (0.3 μM), and 8-PT (5 μM) in the presence of dipyridamole (0.3 μM), on the concentration-response relationships for ADP-ribose and adenosine are shown in Figure 3. Relaxant responses evoked by ATP were

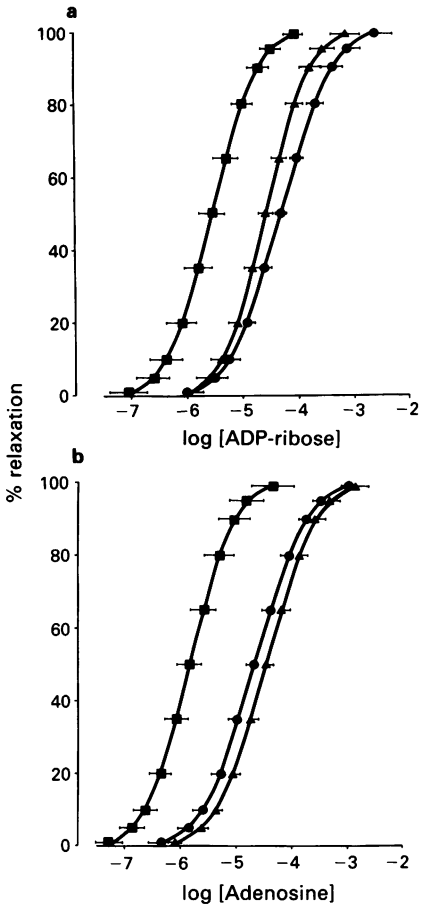

Figure 3 Effects of dipyridamole (0.3 μM) and 8-phenyltheophylline (8-PT, 5 μM) in the presence of dipyridamole on the inhibitory responses of carbachol-contracted guinea-pig taenia coli to ADP-ribose and adenosine. Concentration-response curves are shown for (a) ADP-ribose in the absence of dipyridamole and 8-PT (▲), in the presence of dipyridamole (■), and in the presence of dipyridamole plus 8-PT (●); and (b) adenosine in the absence of dipyridamole and 8-PT (▲), in the presence of dipyridamole (■), and in the presence of dipyridamole plus 8-PT (●). The points represent the mean and the horizontal lines the s.e.mean.

unaffected by dipyridamole (0.3 μM). In the absence and presence of dipyridamole the pD_2 values for ATP were 5.44 ± 0.10 (4) and 5.44 ± 0.15 respectively. There was no significant difference between the slopes of the concentration-response relationships.

The concentration-response curves of both adenosine and ADP-ribose were significantly shifted to the left in the presence of dipyridamole (0.3 μM). The pD_2 value of adenosine increased from 4.48 ± 0.15 to 5.84 ± 0.22 (4) ($P < 0.001$), while that of ADP-ribose shifted from 4.59 ± 0.13 to 5.54 ± 0.24 (4) ($P < 0.02$). The shift in the pD_2 value for adenosine was significantly greater than that for ADP-ribose ($P < 0.01$). No significant changes were found in the slopes of the concentration-response curves for either drug in the presence of dipyridamole (0.3 μM).

In the presence of dipyridamole (0.3 μM), 8-PT (5 μM) produced rightward shifts in the concentration-response curves of adenosine and ADP-ribose. The pD_2 values of adenosine was significantly reduced from 5.84 ± 0.22 to 4.67 ± 0.16 (4) ($P < 0.01$) and the pD_2 value of ADP-ribose was reduced from 5.54 ± 0.24 to 4.31 ± 0.10 (4) ($P < 0.01$). The shift in the pD_2 value for adenosine in the presence of dipyridamole, produced by 8-PT, was not significantly different from that for ADP-ribose. No significant changes were found in the slopes of the concentration-response curves for either agonist.

Analysis of the responses to EC_{50} concentrations of adenosine showed a significant lengthening of the time to reach maximal response in the presence of dipyridamole (0.3 μM). Prior to the addition of dipyridamole the time taken to reach maximal response for adenosine was 23 ± 6 s (4), while in the presence of dipyridamole, adenosine (EC_{50} value) brought about a response taking a mean of 57 ± 10 s (4) ($P<0.05$). A significant change was also found in the time to reach maximal response to ADP-ribose (EC_{50} value), taking a mean of 13 ± 2 s to reach maximal response in the control experiments, and 24 ± 6 s (4) ($P<0.05$) to reach a maximal response in the presence of dipyridamole.

Effects of apamin

In the presence of apamin (0.3 μM) a considerable increase in the frequency and magnitude of spontaneous activity of the taenia preparations was seen, although the magnitude of the contractions elicited by carbachol (0.1 μM) was not significantly affected. At low concentrations of ATP (1–30 μM) the normal inhibitory responses were greatly reduced or abolished. At higher concentrations of ATP (100 μM–1 mM), the normal inhibitory responses were converted to transient contractions.

The inhibitory responses induced by ADP-ribose at 10 μM, 30 μM and 100 μM were significantly reduced in the presence of apamin (0.3 μM), but contractile responses were not unmasked. The mean percentage relaxation induced by ADP-ribose at 10 μM was significantly reduced from 22.8 ± 5.1% to 4.7 ± 2.0% (9) ($P<0.01$), while relaxations to ADP-ribose at 30 μM were reduced from 44.4 ± 9.2% to 13.1 ± 3.0% (9) ($P<0.01$) and relaxations to ADP-ribose at 100 μM were reduced from 70.9 ± 8.0% to 19.7 ± 5.0% (9) ($P<0.001$). In a further series of experiments, when the concentration of ADP-ribose was adjusted to produce a near 50% relaxation (log [ADP-ribose] = 4.5 ± 0.20 (4), which produced a relaxation of 50.4 ± 2.47%), apamin (0.3 μM) reduced this response to 15.7 ± 4.3%, and further incubation with 8-PT (5 μM) caused the relaxant response to be reduced to 1.0 ± 4.0%. The effects of both apamin and 8-PT were significant ($P<0.01$ and $P<0.05$, respectively).

Effects of reactive blue 2

In the presence of RB2 (30 μM) no effect was seen on the carbachol-induced contractions of the strips of taenia coli. The effects of RB2 (30 μM) on the concentration-response relationships for ADP-ribose, ATP and noradrenaline are illustrated in Figure 4.

At a concentration of 30 μM, RB2 produced a significant rightward shift in the ADP-ribose concentration-response curve, reducing the pD_2 value from 4.41 ± 0.19 to 3.96 ± 0.17 (7) ($P<0.02$). The slope of the ADP-ribose concentration-response curve was not significantly altered.

The concentration-response curve of ATP was also subjected to a rightward parallel shift in the presence of RB2 (30 μM), with the pD_2 value being reduced from 5.52 ± 0.25 to 4.59 ± 0.16 (9) ($P<0.01$) in the presence of RB2 (30 μM). No significant differences were found in the slopes of these concentration-response curves.

No significant change was seen in the slope or the pD_2 values of the concentration-response curves for either adenosine or noradrenaline in the presence of RB2 (30 μM). For adenosine the pD_2 values in the absence and presence of RB2 were 4.56 ± 0.05 (6) and 4.60 ± 0.07, respectively. For noradrenaline, in the absence of RB2 the pD_2 was 6.49 ± 0.12 (7), compared with a value of 6.20 ± 0.12 in presence of RB2.

Effects of suramin

The effects of suramin (500 μM) on responses to adenosine, ATP and ADP-ribose are summarised in Table 2. Suramin had no significant effect on the relaxant responses to adenosine, but significantly reduced the responses evoked by ATP and ADP-ribose. Further addition of 8-PT (5 μM) antagonized the responses to adenosine, did not significantly affect the residual responses to ATP, but significantly inhibited the residual responses to ADP-ribose (Table 2).

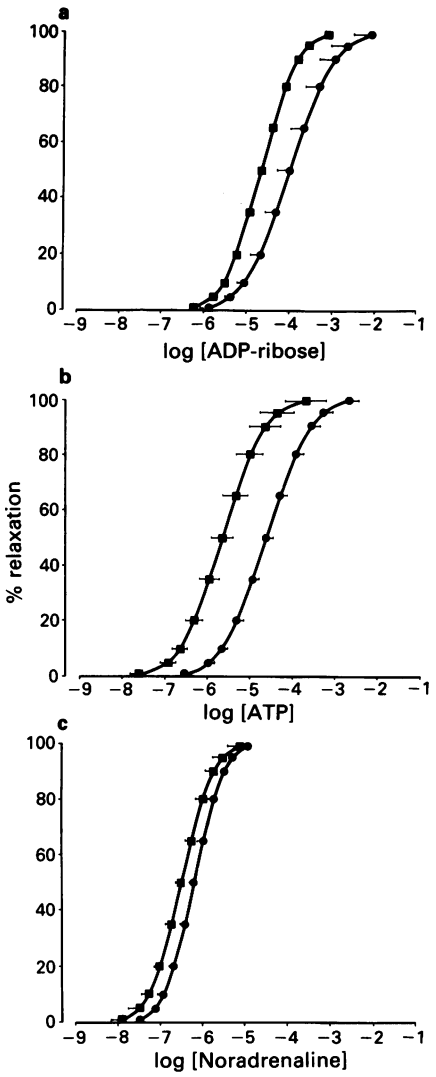

Figure 4 Effect of reactive blue 2 (RB2, 30 μM) on the inhibitory responses of carbachol-contracted guinea-pig taenia coli to ADP-ribose. Concentration-response curves are shown for (a) ADP-ribose in the absence of RB2 (■) and in the presence of RB2 (●) ($n = 7$); (b) ATP in the absence of RB2 (■), and in the presence of RB2 (●) ($n = 9$); and (c) noradrenaline in the absence of RB2 (■), and in the presence of RB2 (●) ($n = 7$). The points represent the mean and the horizontal lines the s.e.mean.

Guinea-pig vas deferens

ATP, tested in a range of concentrations of 1 μM to 1 mM, produced concentration-dependent transient contractions in the guinea-pig vas deferens. The mean increase in tension elicited by ATP at 1 mM was 1.50 ± 0.49 g (7). ADP-ribose in a range of concentrations from 1 μM to 1 mM failed to produce any observable change in the tension of the vas deferens preparations.

There were no changes in the response of the vas deferens preparations to ATP when ADP-ribose, at various concentrations from 1 μM to 1 mM, was allowed to incubate in the organ-baths for various times from 2–22 min prior to administration of ATP.

Table 2 Effects of suramin (500 μM), alone and in combination with 8-phenyltheophylline (8-PT, 5 μM) on the relaxant responses to adenosine, ATP and ADP-ribose in the carbachol-contracted guinea-pig taenia coli

		Relaxation (%)		
Agonist	*−log(conc)*	*Control*	*+Suramin*	*+Suramin and 8-PT*
Adenosine	4.4 ± 0.13	55.3 ± 5.78	56.2 ± 12.01	6.8 ± 1.39[a]
ATP	5.6 ± 0.24	55.9 ± 7.10	15.5 ± 9.24[b]	8.6 ± 3.35
ADP-ribose	4.4 ± 0.06	58.2 ± 7.57	35.7 ± 8.25[b]	1.7 ± 1.73[a]

Data are given as mean ± s.e.mean, from at least four animals. The concentration of the agonists (first column) that produced approximately a 50% relaxation of the carbachol-contracted taenia coli were subsequently tested in the presence of suramin (500 μM), and in the presence of suramin plus 8-PT (5 μM). Statistical significances were (paired Student's *t* tests): [a]$P<0.05$ versus suramin; [b]$P<0.05$ versus control.

Discussion

The results from the experiments on the taenia coli show that ADP-ribose is pharmacologically active in this tissue, producing concentration-dependent relaxations with a potency similar to that of adenosine. The antagonism of the inhibitory responses to ADP-ribose by 8-PT (5 μM) indicates that these responses, at least in part, are mediated through P_1-purinoceptors.

The potentiation of the action of adenosine on the guinea-pig taenia coli by dipyridamole (0.3 μM) and the antagonism by 8-PT, found in this study is consistent with previous studies (Satchell *et al.*, 1972; Satchell & Burnstock, 1975; Burnstock *et al.*, 1984). The potentiation of the inhibitory actions of ADP-ribose by dipyridamole indicates that ADP-ribose might have been degraded to adenosine which contributed to the overall response. The potentiation of ADP-ribose by dipyridamole and the sensitivity to 8-PT strongly indicate that adenosine, from some source or other, was involved in the overall response.

The extracellular catabolism of adenine nucleotides is thought to occur by sequential stepwise degradation such that ATP is catabolized to ADP then to AMP, and then to adenosine with at least three enzymes being involved (Gordon, 1986; Fleetwood *et al.*, 1989; Culic *et al.*, 1990). The final common ectoenzyme in the conversion of adenine 5'-nucleotides to adenosine is 5'-nucleotidase, which is thought to be exclusively localized on the extracellular face of the plasma membrane (De Pierre & Karnovsky, 1974). All of the naturally occurring ribo- and deoxyribo-nucleotide 5'-monophosphates are substrates of 5'-nucleotidase, while nucleotide 5'-diphosphates and -triphosphates are not substrates of the enzyme, but do inhibit it (Burger & Lowenstein, 1970).

If the degradation of ADP-ribose to adenosine occurs as a result of the actions of the same ectoenzymes that are thought to act on ATP to produce adenosine, resulting in the ability of dipyridamole to potentiate ADP-ribose, it is strange that in the absence of dipyridamole the actions of ADP-ribose were antagonized by 8-PT while those of ATP were not, and that dipyridamole did not potentiate ATP.

Alternatively, indirect activation of P_1-purinoceptors by ADP-ribose could be brought about by the evoked release of purine compounds from the muscle itself. Nucleotides and dinucleotides have been shown to be able to induce release of labelled adenosine from rodent vas deferens (Stone, 1981), and it is possible that a similar mechanism could occur in the taenia coli. Further experiments are needed to determine whether or not ADP-ribose evokes release of adenosine from the guinea-pig taenia coli.

The findings of increased spontaneous activity, the reduction of inhibitory responses to ATP and the appearance of contractile responses to ATP in the presence of apamin (0.3 μM) are consistent with the findings of other studies (Banks *et al.*, 1979; Maas & Den Hertog, 1979; Maas *et al.*, 1980; Brown & Burnstock, 1981; Fedan *et al.*, 1984). That the inhibitory responses to ADP-ribose were strongly attenuated by apamin indicates that for at least part of its action ADP-ribose shares characteristics associated with activation of P_2-purinoceptors rather than P_1-purinoceptors. Although apamin antagonizes P_2-purinoceptor-mediated activity, it is not specific for this receptor type since its mechanism involves the blocking of calcium-dependent potassium channels. Thus, an action of ADP-ribose on P_2-purinoceptors cannot be definitely concluded from the results with apamin alone. However, it is known that apamin does not antagonize the activity of adenosine on the guinea-pig taenia coli (Brown & Burnstock, 1981); thus it can be said that the activity of ADP-ribose cannot be solely limited to activation of adenosine receptors.

Further evidence for the involvement of P_2-purinoceptors in the action of ADP-ribose on the taenia coli comes from the experiments carried out with RB2 and suramin. The antagonism of the inhibitory responses to ATP but not to adenosine or noradrenaline in the presence of RB2 is consistent with the findings of Burnstock *et al.* (1986), and with the view that in this tissue, at this concentration RB2 is a selective antagonist at P_{2Y}-purinoceptors. Thus, the antagonism of the inhibitory responses to ADP-ribose and ATP by RB2, but not to adenosine, indicates that the inhibitory responses to ADP-ribose were mediated, at least in part, by P_{2Y}-purinoceptors. Suramin is selective antagonist of P_2-purinoceptors (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990), but it does not discriminate between the P_{2X} and P_{2Y} subtypes (Hoyle *et al.*, 1990). Nevertheless, the antagonism by suramin of responses to ATP and ADP-ribose, but not adenosine, further supports the case that ADP-ribose is acting on P_2-purinoceptors in the taenia coli.

It is also interesting to note that both apamin and suramin, at the concentrations tested, blocked the responses to ATP to a greater extent than they did ADP-ribose, and that the residual responses to ADP-ribose (but not ATP) were inhibited by 8-PT. The fact that a combination of apamin and 8-PT, or suramin and 8-PT, was needed almost to abolish the responses to ADP-ribose, rather than any one of these antagonists alone, is further evidence that both P_1- and P_2-purinoceptors are involved in the responses to ADP-ribose.

The contractile responses of the guinea-pig vas deferens to ATP have been noted in several studies, and the P_2-purinoceptors mediating such contractions were defined as P_{2X}-purinoceptors (Burnstock & Kennedy, 1985). The results of the present study, showing that ADP-ribose over a concentration range of 1 μM to 1 mM had no effect on the tension of the preparations of the vas deferens, are in agreement with the results of Fedan *et al.* (1986), who found ADP-ribose to be without activity in this tissue, even at a concentration of 10 mM. ADP-ribose was found to be similarly inert in producing any changes in the response of the vas deferens to ATP. These results imply that ADP-ribose is neither an agonist nor an antagonist of P_{2X}-purinoceptors.

The mixed pharmacology of ADP-ribose can be said to be remarkable considering its structural similarity to ATP, from

which it varies only by the replacement of the terminal phosphate by a ribose moiety. This replacement of a phosphate by a ribose group somehow conferred an additional P_1-purinoceptor activity on ADP-ribose that is lacked by ATP, while also removing its ability to act as an agonist of P_{2X}-purinoceptors.

The compound adenosine 5'-(2-fluorodiphosphate) (ADPβF) has been shown to be a specific agonist for P_{2Y}-purinoceptors (Hourani *et al.*, 1988). Although the relaxation of the guinea-pig taenia coli evoked by ADPβF is unaffected by 8-PT (Hourani *et al.*, 1988), it has been suggested that ADPβF can act on P_1-purinoceptors mediating relaxation of the rabbit jugular vein (Wood *et al.*, 1989). Nevertheless it is interesting to note that substitution on the β-phosphate of ADP by either F^- or a ribose moiety can confer P_{2Y} specificity over P_{2X}. Furthermore, if this ribose moiety is also linked to a nicotinamide group, forming NAD, all P_2-purinoceptor activity is lost (Burnstock & Hoyle, 1985).

In conclusion, it can be said that ADP-ribose has a mixed pharmacological profile. The inhibitory responses to ADP-ribose in the guinea-pig taenia coli appear to involve adenosine acting on a P_1-purinoceptor, probably via an indirect mechanism involving either partial degradation of ADP-ribose to adenosine or the induction of release of adenosine from the muscle itself. It would seem appropriate that future experiments might investigate whether adenosine is formed or released during the course of ADP-ribose action. ADP-ribose also appears to act on P_{2Y}-purinoceptors in the taenia coli while having no observable action on the P_{2X}-purinoceptors of the guinea-pig vas deferens. This selectivity between the two P_2-purinoceptor subtypes indicates that ADP-ribose could be a useful parent compound in the development of a specific P_{2Y}-purinoceptor agonist if a modification could be made which eliminates its P_1-purinoceptor activity. Also, the activity of ADP-ribose might indicate that in addition to its ubiquitous distribution as an intracellular regulatory compound, during evolution it may also have developed a role as a messenger involved in cell to cell communication.

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