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Evidence that the P_1-purinoceptor in the guinea-pig taenia coli is an A_2-subtype

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1 The effects of 5′-N-ethylcarboxamidoadenosine (NECA), L-NECA, 2-chloroadenosine, N^6-phenylisopropyladenosine (L-PIA and D-PIA), cyclohexyladenosine (CHA), and adenosine were examined on the guinea-pig taenia coli.

2 All the analogues except L-NECA caused relaxations; the order of potency for the series was: NECA > 2-chloroadenosine > L-PIA > CHA > D-PIA > adenosine.

3 L-PIA was twice as potent as D-PIA in inducing relaxations of the guinea-pig taenia coli.

4 Adenosine and its analogues that induce relaxation all caused a slow membrane hyperpolarization; differences in the rates of hyperpolarization and latencies were apparent, although not statistically significant.

5 The duration of the response to adenosine was significantly less than that for any adenosine analogue.

6 Ion studies, using the sucrose gap, revealed that responses to the analogues were attenuated in elevated extracellular potassium or reduced extracellular chloride.

7 8-Phenyltheophylline, a potent P_1-purinoceptor antagonist, caused a rightward shift of all the adenosine and analogue concentration-response curves.

8 Dipyridamole, an adenosine uptake inhibitor, potentiated the relaxations to adenosine but had no significant effect on the relaxations induced by the analogues.

9 It is concluded that NECA, 2-chloroadenosine, L-PIA, CHA, D-PIA and adenosine mediate their relaxant effects via an extracellular P_1-purinoceptor which displays characteristics of the A_2-subtype as determined by the rank order of agonist potency. Electrophysiological analysis of the responses to each of the analogues did not reveal any marked differences in the modes of action even between NECA and L-PIA (preferential A_2-and A_1-receptor agonists, respectively).

Introduction

The longitudinal smooth muscle strip on the guinea-pig caecum has been shown to contain separate purinoceptors for adenosine (P_1) and ATP (P_2), both of which mediate relaxation (Brown & Burnstock, 1981; Satchell & Maguire, 1982). In many other systems, where adenosine mediates various responses, it seems likely that extracellular adenosine receptors consist of subtypes. Van Calker *et al.* (1979) proposed the subtype A_1 for a high affinity adenosine receptor associated with inhibition of adenylate cyclase and the subtype A_2 for a low affinity adenosine receptor associated with stimulation of adenylate cyclase in cultured brain cells. Londos *et al.* (1980) on the other hand, used the term R_i-receptor corresponding to A_1- and R_a-receptor corresponding to A_2. It has been claimed that the A_1- and A_2-receptors may be differentiated by the use of N^6 and 5′ substituted adenosine analogues (Londos *et al.*, 1980). The A_1- site prefers N^6 substituted compounds such as N^6-phenylisopropyladenosine (L-PIA), while at the A_2-site 5′ substituted analogues such as 5′-N-ethylcarboxamideadenosine (NECA) are more potent. In addition, at the A_1-site, a high degree of stereoselectivity for L-PIA over its isomer (D-PIA) is displayed. However, as pointed out by Daly (1982), very few adenosine actions can be categorically linked to the adenylate cyclase system and some receptor subtypes examined appear to display both A_1 and A_2 characteristics (see Paton, 1981).

In the present study we have compared the effects of NECA, 2-chloroadenosine, L-PIA, D-PIA, cyc-

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lohexyladenosine (CHA), and adenosine on the guinea-pig taenia coli by investigating mechanical and electrical changes induced by these compounds.

Methods

Guinea-pigs of either sex (300–650 g) were stunned and bled. The longitudinal muscle of the caecum (taenia coli) together with its underlying myenteric plexus, was dissected out and placed in Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 16.3, $MgSO_4$ 0.6, $CaCl_2$ 2.5 and glucose 7.7.

Organ bath studies

Strips of taenia coli approximately 1.5 cm in length were attached by thread to a rigid support and then transferred to 10 ml organ baths, where they were continually gassed with 95% O_2 plus 5% CO_2 and maintained at 36°C. The preparations were initially placed under a resting tension of 1 g and allowed to equilibriate for 1 h. Mechanical activity was measured under isometric conditions with a Dynamometer UFl force transducer and recorded on a Grass polygraph. The tone of the preparation was standardized by the addition of carbachol (50 nM), to allow quantification of the magnitude of inhibitory responses. In some experiments this concentration was increased (up to 100 nM) in order to maintain a standard level of tone throughout. Concentration-response curves for one analogue with or without 8-phenyltheophylline (8-PT) or dipyridamole were obtained from each preparation. A half hour equilibration period was allowed for 8-phenyltheophylline or dipyridamole.

Sucrose gap studies

Strips of taenia coli, approximately 1.5 cm in length were mounted in a single sucrose gap apparatus as described by Bülbring & Burnstock (1960). The tissue was placed under 2 g tension and allowed to equilibrate for 1 h. The temperature of the bathing solution was maintained at 36°C, and the solution was continuously bubbled with 95% O_2 plus 5% CO_2.

Tension changes were recorded via a Dynamometer UF1 isometric transducer and displayed on a Grass Polygraph. Membrane potential changes were recorded via two silver/silver chloride electrodes across an agar/3 M KCl bridge. The electrodes were connected through a high impedance amplifier, and potential and tension changes were displayed simultaneously on a Grass polygraph and Tektronix oscilloscope. The oscilloscope display was stored by means of a moving film Grass camera.

Adenosine and its analogues were applied to the preparation in the Krebs flow from a constant pressure infusion pump. At least 10 min washout was allowed between subsequent drug applications. In

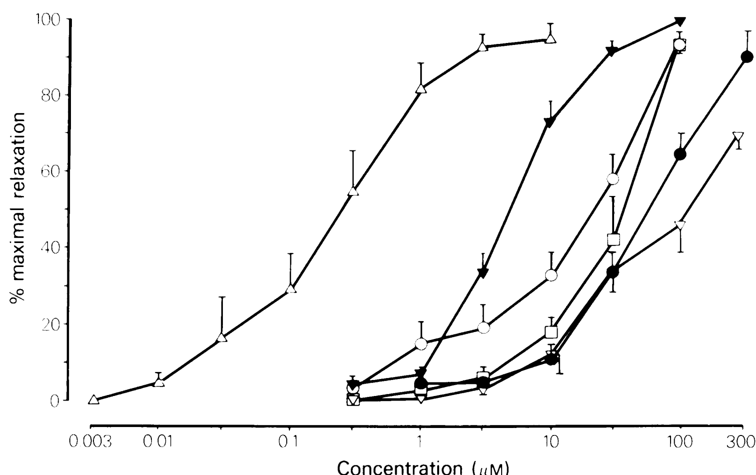

Figure 1 Log concentration-response curves for adenosine and adenosine analogues for the relaxation of the isolated carbachol-contracted guinea-pig taenia coli by 5′-N-ethylcarboxamideadenosine (NECA) (△), 2-chloroadenosine (▼), L-N^6-phenylisopropyl adenosine (L-PIA) (o), cyclohexyladenosine (CHA) (□), D-N^6-phenylisopropyladenosine (D-PIA) (●) and adenosine (▽). Each point is the mean of at least 13 experiments except for D-PIA where the mean is at least 4. Vertical bars show s.e.mean. Each agonist is significantly more or less potent than any other at EC_{50} concentrations ($P < 0.05$; Student's *t* test) except that there is no significant difference between CHA (□) and L-PIA (○) and between adenosine (▽) and D-PIA (●).

Table 1 EC_{50}'s for adenosine and analogues on the taenia coli of the guinea-pig in the presence of 8-phenyltheophylline (8-PT) (10 μM) or dipyridamole (0.5 μM)

	NECA	*2-Chloro-adenosine*	*L-PIA*	*CHA*	*D-PIA*	*Adenosine*
EC_{50} (μM)	0.47 ± 0.12 (13)	6.28 ± 1.36 (15)	28.6 ± 4.7 (13)	33.5 ± 6.8 (15)	56.8 ± 8.2 (6)	109.0 ± 27.4 (13)
pD_2	6.33	5.20	4.54	4.48	4.25	3.96
Relative activity	234	17	4	3	2	1
In the presence of 8-phenyltheophylline (10 μM)						
EC_{50} (μM)	20.7 ± 10.9 (6)	43.5 ± 13.8 (7)	39.0 ± 6.5 (6)	80.8 ± 4.6 (6)		176.0 ± 52.9 (6)
pD_2	4.68	4.36	4.41	4.09		3.75
Relative antagonism	45	6.9	1.4	2.4		1.6
In the presence of dipyridamole (0.5 μM)						
Control EC_{50} (μM)	0.22 ± 0.07 (3)	5.05 ± 1.34 (4)	36.7 ± 8.81 (4)	34.0 ± 8.65 (4)		106.0 ± 66.0 (3)
Control pD_2	6.65	5.30	4.44	4.47		3.98
EC_{50} (μM)	0.18 ± 0.02 (3)	2.77 ± 0.80 (4)	28.7 ± 7.73 (4)	46.6 ± 16.5 (3)		6.92 ± 4.10 (3)
pD_2	6.74	5.56	4.54	4.35		5.16
Relative potentiation	1.2	1.8	1.3	0.8		15.1

Values ± s.e. mean (*n*).
pD_2 = negative log of EC_{50}
Relative activity = ratio of analogue EC_{50} to adenosine EC_{50}, relative antagonism = ratio of analogue EC_{50} in presence and absence of 8-PT and relative potentiation = ratio of analogue EC_{50} in absence and presence of dipyridamole.

some experiments extracellular potassium was raised by equimolar substitution of KCl with NaCl. Where a low chloride solution was required the concentration of sodium isethionate was 133.3 mM and that of potassium metabisulphite 4.7 mM. The other salts were as indicated above.

Stock solutions of drugs were made up in the following: CHA-50% ethanol/50% distilled water; PIA-dimethylsulphoxide; 8-phenyltheophylline-80% v/v methanol containing 0.2 M NaOH; adenosine, 2-chloroadenosine, NECA were made up in distilled water. All subsequent dilutions were made in distilled water, or for sucrose-gap experiments were diluted in Krebs solution.

Drugs used

Adenosine (Sigma), 2-chloroadenosine (Sigma), dipyridamole (Persantin, Boehringer Ingelheim), (−)-N^6-phenylisopropyladenosine (Boehringer Mannheim), (+)-N^6-phenylisopropyladenosine (Research Biochemicals Inc.), 8-phenyltheophylline (Calbiochem), cyclohexyladenosine (Calbiochem), carbamylcholine (Sigma), D- and L-5′-N-ethylcarboxamidoadenosine was kindly synthesized and donated by Dr N.J. Cusack.

Statistical analyses of the results were carried out using Student's unpaired *t* test.

Results

Organ bath experiments

Potency series Adenosine, 2-chloroadenosine, NECA, L-PIA, D-PIA and CHA all caused a concentration-dependent relaxation of the carbachol-contracted taenia. L-NECA, however, was all but ineffective in concentrations up to 30 μM. NECA was the most potent of the analogues studied, with an EC_{50} of 0.47 ± 0.12 μM. Adenosine was the least potent with an EC_{50} of 109.0 ± 27.4 μM. The rank order of potency based on the EC_{50} values was NECA > 2-chloroadenosine > L-PIA > CHA > D-PIA > adenosine (Figure 1, Table 1).

Antagonism by 8-phenyltheophylline 8-Phenyltheophylline (10 μM), a potent P_1-purinoceptor antagonist, shifted each concentration-response curve to the right. The degree of antagonism varied, being most effective against NECA and least effective against L-PIA (Table 1, Figure 2).

Effect of dipyridamole Dipyridamole (0.5 μM), an adenosine uptake inhibitor, significantly potentiated the relaxations induced by adenosine, thus causing a leftward shift in the concentration-response curve (Figure 2e). Dipyridamole (0.5 μM) was without sig-

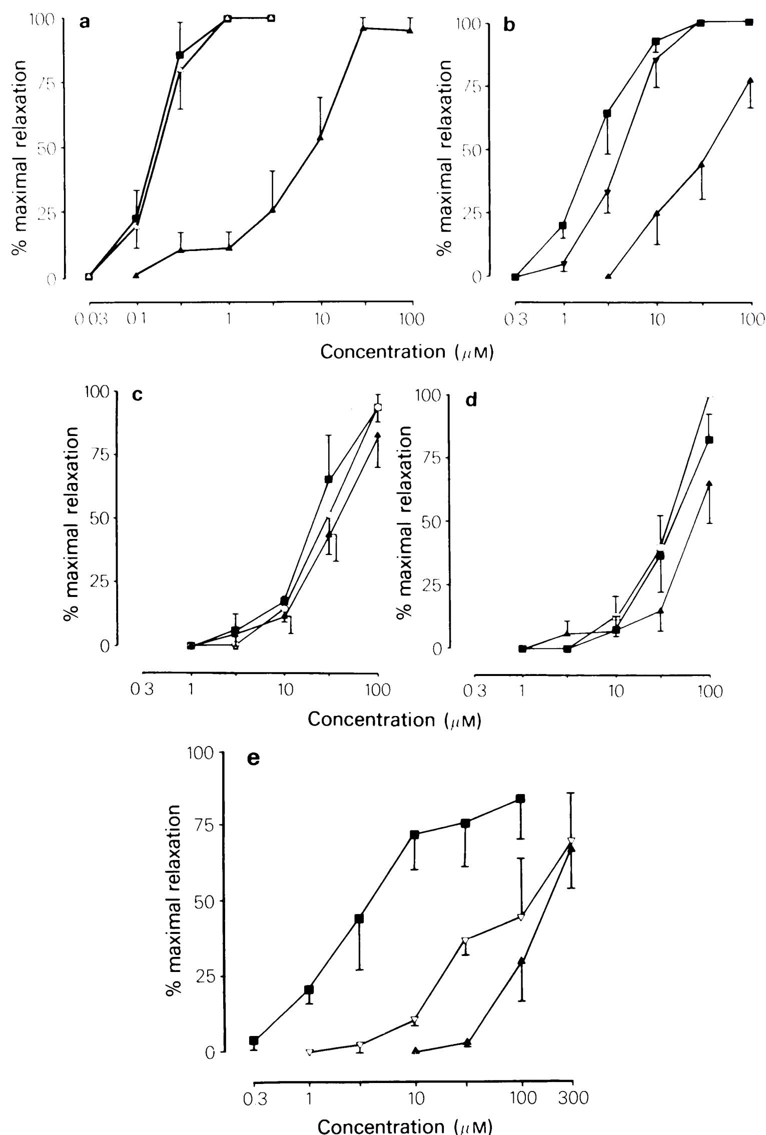

Figure 2 Effects of 8-phenyltheophylline (8-PT) and dipyridamole on the log concentration-response curves of adenosine and its analogues in the carbachol-contracted guinea-pig taenia coli. (a) 5′-N-ethylcarboxamido-adenosine (NECA) (■), in the presence of 8-PT (10 μM, ▲) or dipyridamole (0.5 μM, △). At concentrations below 10 μM, NECA is antagonized by 8-PT (10 μM) ($P<0.05$). Dipyridamole (0.5 μM) does not significantly affect NECA responses. (b) 2-Chloroadenosine (▼), in the presence of 8-PT (10 μM, ▲) or dipyridamole (0.05 μM, ■). 2-Chloroadenosine is antagonized at concentrations below 100 μM by 8-PT (10 μM) ($P<0.05$) and is not significantly affected by dipyridamole (0.5 μM). (c) L-N^6-phenylisopropyl adenosine (L-PIA) (o), in the presence of 8-PT (10 μM, ▲) or dipyridamole (0.5 μM, ■). At 10 μM L-PIA is antagonized by 8-PT (10 μM) ($P<0.05$) but is otherwise not significantly affected 8-PT or dipyridamole. (d) Cyclohexyladenosine (CHA) (□), in the presence of 8-PT (10 μM, ▲) or dipyridamole (0.5 μM, ■). Although some shift in the presence of 8-PT occurs CHA is not significantly affected by either 8-PT (10 μM) or dipyridamole (0.5 μM). (e) Adenosine (▽), in the presence of 8-PT (10 μM, ▲) or dipyridamole (0.5 μM, ■). At concentrations below 100 μM, adenosine was potentiated by dipyridamole (0.5 μM), ($P<0.05$) and antagonized by 8-PT (10 μM), ($P<0.05$). In all figures points represent the mean of at least 6 for controls, 5 for 8-PT and 4 for dipyridamole. Vertical bars show s.e.mean if not occluded by symbol.

nificant effect on concentration-response curves for NECA, 2-chloroadenosine, L-PIA and CHA (Figure 2a,b,c,d,).

Sucrose gap studies

Adenosine, NECA, 2-chloroadenosine, L-PIA, and CHA each caused relaxations associated with membrane hyperpolarizations or the slowing of spontaneous action potentials. The effect was concentration-dependent, marked membrane hyperpolarization occurring at the higher concertrations studied (See Figures 3, 4a, and 5a). Equipotent concentrations of analogues, as determined from mechanical effects in the organ bath, produced similar degrees of membrane hyperpolarization recorded in the sucrose gap. These results therefore show that a close correlation between membrane hyperpolarization and muscle tone exists for the action of these analogues on the P_1-purinoceptor. Although NECA is about 80 times more potent than CHA (Table 1, Figure 1), both compounds produce similar degrees of hyperpolarization at equipotent concentrations. Although there was no significant difference betwcen rates of hyperpolarization for equipotent concentrations of analogues, NECA, the most selective A_2-receptor agonist, hyperpolarized the membrane at a rate which was 30% faster than the most selective A_1-receptor agonists, L-PIA and CHA (Table 2).

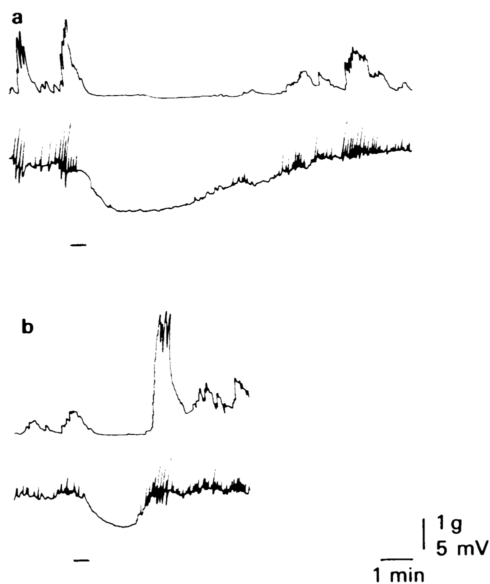

Figure 3 Upper traces represent tension, lower traces represent membrane potential (sucrose gap recording). (a) 5'-N-ethylcarboxamidoadenosine (NECA) EC_{50} (0.47 μM) 30 s bolus injected into Krebs flow induces relaxation and hyperpolarization. (b) Adenosine equipotent concentration EC_{50} (110 μM) induces similar relaxation and hyperpolarization but with shorter duration of action. Horizontal bars show period of drug application.

The mean latency for onset of response did not differ significantly for any of the analogues tested (Table 2). The duration of action of adenosine was significantly shorter than that of any of the other analogues when measured at EC_{90} concentrations (Table 2, Figure 3). This probably reflects differences in breakdown and uptake of adenosine compared with the deactivation processes for the synthetic analogues.

Ion studies

The effects of raising the extracellular potassium concentration from 4.7 mM to 8.0 mM on the responses to the adenosine analogues were investigated. Hyperpolarizations and rates of hyperpolarization were consistently depressed (Table 3), although no consistent changes in latencies were apparent. Antagonism by high K^+ was further demonstrated by raising the extracellular K^+ concentration to 32 mM when all responses to the analogues were completely abolished (Figure 4). Reducing the extracellular chloride concentration from 43 mM to 5 mM initiated membrane depolarization and increased spike discharge. After several minutes in the

Table 2 Membrane potential changes measured using the sucrose gap technique

EC_{90}	*Latency* (*s*) (n)	*Hyperpolarization* (mV)	*Duration* (s)	*Rate of Hyperpolarization* ($\mu V s^{-1}$)
NECA	31 ± 4	8.6 ± 1.1	343 ± 39	270 ± 63
7.9 μM	(10)	(9)	(10)	(9)
2-Chloro-	27 ± 4	6.6 ± 1.4	498 ± 144	239 ± 54
adenosine	(8)	(8)	(9)	(8)
34.0 μM				
L-PIA	40 ± 8	6.4 ± 1.3	319 ± 64	203 ± 49
45.0 μM	(11)	(11)	(10)	(11)
CHA	29 ± 4	8.5 ± 1.6	292 ± 56	202 ± 27
55.0 μM	(9)	(10)	(10)	(10)
Adenosine	27 ± 8	6.1 ± 0.8	*143 ± 33	217 ± 43
210 μM	(8)	(7)	(8)	(7)

Values ± s.e. mean (n)
All drugs were applied at EC_{90} concentrations for 15 or 30 s. Latency was measured from the beginning of drug application until the onset of the decline in spontaneous activity. Duration was taken as the period until control membrane potential had returned to the original level before drug application. Rate of hyperpolarization was taken as (half hyperpolarization level) ÷ (time to attain this level from onset of hyperpolarization). $^*P < 0.05$, value is significantly less than the values for any other duration.

Table 3 Effects of raised extracellular potassium (8 mM) on membrane potential changes measured using the sucrose gap technique

	Latency (s)		*Hyperpolarization* (mV)		*Rate of Hyperpolarization* ($\mu V s^{-1}$)	
	Control	*High K^+ (8 mM)*	*Control*	*High K^+ (8mM)*	*Control*	*High K^+ (8mM)*
NECA 7.9 μM	31 ± 4 (10)	38 ± 12 (6)	8.6 ± 1.1 (9)	5.6 ± 2.3 (6)	270 ± 63 (9)	108 ± 37 (5)
2-Chloro-adenosine 34 μM	27 ± 4 (8)	39 ± 5 (4)	8.6 ± 1.8 (8)	3.6 ± 1.5 (5)	239 ± 54 (8)	170 ± 13 (4)
L-PIA 45 μM	40 ± 8 (11)	37 ± 3 (4)	6.4 ± 1.3 (11)	2.2 ± 1.6 (6)	203 ± 48 (11)	104 ± 23 (3)
CHA 55 μM	29 ± 4 (9)	33 ± 4 (7)	8.5 ± 1.6 (10)	5.2 ± 1.5 (8)	204 ± 30 (9)	170 ± 31 (5)

Values ± s.e.mean (*n*).
Each analogue was applied at EC_{90} concentration for 30 s. The measurements are as defined for Table 2.

low Cl^- solution, the membrane became depolarized to such an extent that spiking no longer occurred. The responses to each of the adenosine analogues was completely abolished by the low chloride solution (Figure 5).

Discussion

The results presented show that adenosine-induced relaxation of the guinea-pig taenia coli is mediated

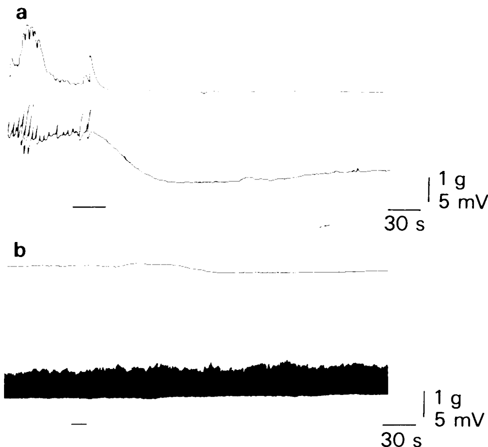

Figure 4 Upper traces represent tension, lower traces represent membrane potential (sucrose gap recording). (a) 2-Chloroadenosine EC_{90} (34 μM) abolishes spontaneous activity and causes hyperpolarization. (b) High potassium Krebs solution (32 mM) depolarized the muscle membrane and markedly increased spontaneous activity and tension. The response to 2-chloroadenosine EC_{90} (34 μM) is abolished. Horizontal bars show period of drug application.

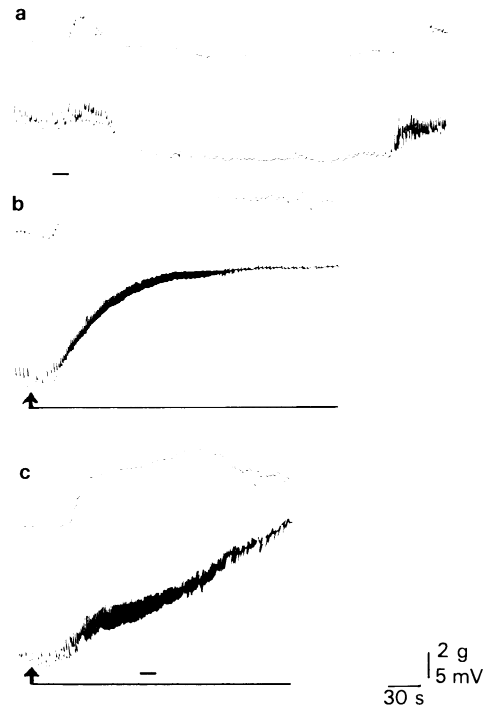

Figure 5 Upper traces represent tension, lower traces represent membrane potential (sucrose gap recording). (a) L-N^6-phenylisopropyladenosine (L-PIA) EC_{90} (45 μM) in normal Krebs solution causes hyperpolarization and cessation of spontaneous activity. (b) Low chloride Krebs solution (Cl^- 5 mM) causes 25 mV depolarization and increased tension. (c) L-PIA EC_{90} (45 μM) during low chloride perfusion. The response to L-PIA (45 μM) was antagonized. Arrows indicate beginning and duration of low chloride Krebs perfusion. Horizontal bars show L-PIA application.

via a receptor which closely resembles the A_2-receptor as defined in other systems (for review, seeDaly 1983). Compounds which are substituted at the C^5 position of the ribose ring (e.g. NECA) are more potent at relaxing the guinea-pig taenia coli than compounds which are substituted at the N^6 amino position on the purine ring (e.g. L-PIA, D-PIA and CHA).

Such a rank order resembles adenosine-induced relaxation of the guinea-pig trachea (Brown & Collis, 1982), adenosine-induced inhibition of platelet aggregation (Cusack & Hourani, 1981) and adenosine-induced stimulation of steroidogenesis in cultured adrenal and Leydig I-10 tumour cells (Londos *et al.*, 1980).

In addition, our experiments revealed that the adenosine receptor in the guinea-pig taenia coli did not show marked stereoselectivity for PIA. This lack of stereoselectivity has been claimed to indicate the presence of an A_2-rather than an A_1-receptor, since, when L-PIA acts on an A_1-receptor it is 100 times more potent than D-PIA (Smellie *et al.*, 1979; Bruns *et al.*, 1980).

However, the A_1/A_2-adenosine receptor classification requires careful interpretation. The original definitions were drawn from diverse isolated tissues, cell lines in culture, primary cell cultures and membrane fractions, and were based largely on the ability of the analogues to stimulate or inhibit adenylate cyclases (Van Calker *et al.*, 1979; Londos *et al.*, 1980). More recently, such classifications have been based entirely on rank orders or on biochemical assay (Paton, 1981, Brown & Collis, 1982; Shütz *et al.*, 1982; Collis, 1983), and as pointed out by Daly (1982), only a limited number of adenosine actions can be linked categorically to the adenylate cyclase system.

Methylxanthines have been shown to antagonize competitively both A_1-and A_2-adenosine receptors (Londos *et al.*, 1978; Bruns *et al.*, 1980). Such findings are in agreement with the ability of 8-phenyltheophylline, a potent P_1-purinoceptor antagonist (Griffith *et al.*, 1981), to antagonize the responses to adenosine and to all of its analogues, in the guinea-pig taenia coli. In addition, the adenosine analogues can be said to be acting via an extracellular adenosine receptor since methylxanthines do not antagonize the intracellular 'P' site (Bruns, 1980).

Dipyridamole, which enhances the adenosine-induced relaxations of the taenia by preventing adenosine uptake (Satchell & Burnstock, 1975), did not significantly alter the relaxations to any of the adenosine analogues. This is further evidence for an action on an extracellular receptor since intracellular actions of adenosine would be prevented by an uptake blocker. In addition, since the adenosine analogues do not share the adenosine uptake mechanism sensitive to dipyridamole, (Mah & Daly, 1976), it is not surprising that this drug does not alter the concentration-response curves of the analogues. In addition, we have shown that the termination of the action of the analogues is not dependent on an uptake process in the smooth muscle system studied here.

It is interesting to speculate as to where adenosine would appear in the potency series in the absence of uptake and breakdown mechanisms since, unlike adenosine, claims have also been made that the adenosine analogues are resistant to deamination (Nair & Wiechert, 1980). One way of testing this is to consider the potency of 2-chloroadenosine, since substitution at the C^2 position on the purine ring favours neither the A_1- nor the A_2-receptor selectively (Daly, 1982). Alternatively, it can be seen from our results that adenosine in the presence of dipyridamole has an EC_{50} value which lies very close to that of 2-chloroadenosine. The potency series should then perhaps be rewritten as NECA $>$ 2-chloroadenosine = adenosine $>$ L-PIA $>$ CHA $>$ D-PIA, and such a series would be in closer agreement with other A_2 systems where adenosine appears as intermediate between NECA and L-PIA (Londos *et al.*, 1980; Cusack & Hourani, 1981).

The electrophysiological studies did not reveal evidence for more than one subtype of P_1-purinoceptor since no significant differences were seen between the extents of hyperpolarization, rates of hyperpolarization or latencies for the responses to any of the analogues. Such information cannot be gleaned from organ bath studies which could only be said to reveal a dominant A_2-sub-class of P_1-purinoceptor. In addition, responses to preferential A_1-receptor agonists (L-PIA, CHA) and the preferential A_2-receptor agonist (NECA) were antagonized to similar extents by high potassium and low chloride conditions. Further, all adenosine analogues appeared to involve changes in both potassium and chloride permeabilities, as has been claimed for the action of adenosine (Axelsson & Holmberg, 1969; Ferrero & Frischknecht, 1983).

The only significant differences in the characteristics of the membrane hyperpolarization induced by EC_{90} concentrations of each analogue were in the duration of the adenosine response compared with that of the analogues. This shorter duration is readily explained by the uptake and metabolism of adenosine, but not of the other analogues (Mah & Daly, 1976).

Although the physiological response to adenosine and the involvement of an action on an adenylate cyclase system has only been categorically linked in a few instances, it is generally accepted that adenosine receptor activation induces changes in cyclic adenosine monophosphate (cyclic AMP) levels

(Daly, 1982). Adenosine causes membrane hyperpolarization of smooth muscle (Axelsson & Holmberg, 1969; Ferrero & Frischknecht, 1983), and of hippocampal neurones (Siggins & Schubert, 1981; Reddington *et al.*, 1982). Similarly, in the present study adenosine and its analogues caused smooth muscle membrane hyperpolarization. It is not clear whether the membrane hyperpolarization occurs as a result of increased levels of intracellular cyclic AMP. Contrary to expectation for an A_2-receptor, Baer & Muller (1983) have claimed that in the guinea-pig taenia coli the adenosine analogues do not stimulate adenylate cyclase. It is also noteworthy that some actions of adenosine can be overcome by raising extracellular calcium (Silinsky, 1981; Stone, 1981; Schrader *et al.*, 1975).

It is proposed that the P_1-purinoceptor in the guinea-pig taenia coli is of the A_2-subtype. Such a proposal is made on the rank order of agonist potency of adenosine analogues in inducing relaxation and hyperpolarization and the lack of stereoselectivity displayed for PIA. The changes which occur as a result of adenosine receptor activation with adenylate cyclase remain to be investigated, in order to confirm our proposed classification.

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