Br. J. Pharmac. (1979), **67**, 153–158

RELAXATION OF ISOLATED TAENIA COLI OF GUINEA-PIG BY ENANTIOMERS OF 2-AZIDO ANALOGUES OF ADENOSINE AND ADENINE NUCLEOTIDES

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1 2-Azido photoaffinity analogues of adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and adenosine have been synthesized and tested on guinea-pig taenia coli.

2 2-Azido-ATP and 2-azido-ADP were approximately 20 times more potent than ATP as relaxants of taenia coli, and required prolonged washout times before recovery of the muscle.

3 2-Azido-AMP and 2-azidoadenosine were 2 to 12 times more potent than ATP, but took much longer (up to 100 s) to reach maximal relaxation. This behaviour is different from that of AMP and adenosine which were much less potent than ATP.

4 L-Enantiomers of adenosine and adenine nucleotides were also tested. L-ATP and L-ADP were 3 to 6 times less potent than ATP and ADP, and L-AMP and L-adenosine were inactive. 2-Azido-L-ATP and 2-azido-L-ADP were approximately 120 times less potent than 2-Azido-ATP and 6 times less potent than ATP as relaxants of taenia coli. 2-Azido-L-AMP and 2-azido-L-adenosine were almost inactive.

5 2-Azido derivatives are photolysed by u.v. irradiation to reactive intermediates. 2-Azido-ATP and 2-azidoadenosine might be suitable photoaffinity ligands for labelling putative P2 and P1 purine receptors respectively. 2-Azido-L-ATP and 2-azido-L-adenosine could be useful controls for non-specific labelling.

Introduction

Adenosine and adenine nucleotides have an inhibitory and relaxant effect on mammalian gut, and evidence suggests that adenosine 5'-triphosphate (ATP) might act as a transmitter released from non-cholinergic, non-adrenergic inhibitory nerves which have been termed purinergic (Burnstock, 1971). Recently, purine receptors have been divided into two groups, P2 receptors having a greater response to ATP and adenosine 5'-diphosphate (ADP), and P1 receptors having a greater response to adenosine and adenosine 5'-monophosphate (AMP) (Burnstock, 1978).

Some 2- substituted analogues of adenosine and adenine nucleotides applied exogenously to guinea-pig taenia coli are potent relaxants, with higher relative activity than the unsubstituted compounds (Satchell & Maguire, 1975). 2-Azidoadenosine, a photolysable analogue of adenosine, is a good inhibitor of ADP-induced aggregation of human platelets, and acts on adenylate cyclase. 2-Azido-ADP on the other hand is a more potent aggregator of human platelets than ADP itself (Cusack & Born, 1977). It appeared that 2-azido analogues of adenosine and adenine nucleotides could be active on guinea-pig taenia coli, and since they are photolysable (Cusack & Born, 1976) might enable purine receptors to be labelled by binding to them irreversibly after u.v. irradiation.

Methods

Taenia coli preparations were obtained from male guinea-pigs (350 to 600 g). Isolated muscle strips were suspended in 10 ml of modified Krebs solution (Bülbring, 1953), gassed with a mixture of O_2 (95%) and CO_2 (5%) at 35°C in a conventional organ bath. After an equilibration period of 30 min, responses were recorded isotonically with a photoelectric transducer coupled to a pen recorder (load 1.5 g). Each dose of drug was allowed to produce its maximal effect (exposure time 20 s to 3 min). Log dose-response curves, maximal responses, pD_2, and relative

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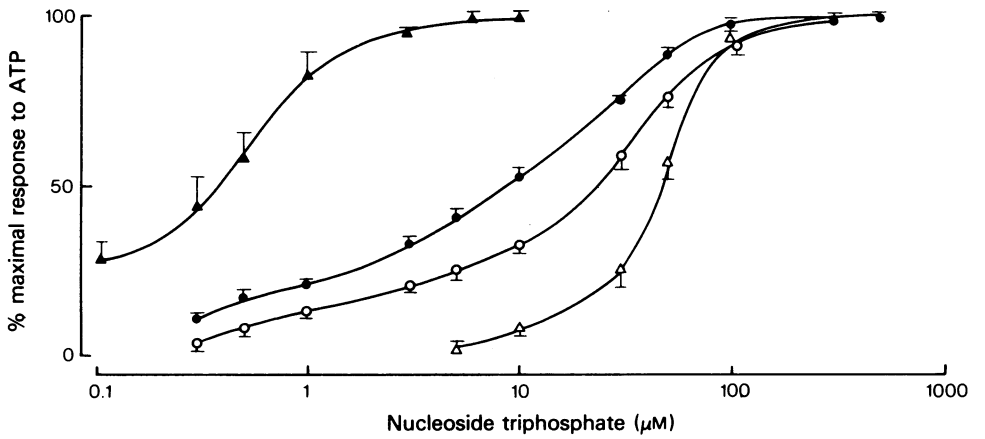

Figure 1 Relaxation of guinea-pig isolated taenia coli preparations by analogues of ATP. (●) ATP; (○) L-ATP; (▲) 2-azido-ATP; (△) 2-azido-L-ATP. Each point is the mean of at least 3 observations from at least 3 different animals. Vertical bars show s.e. mean.

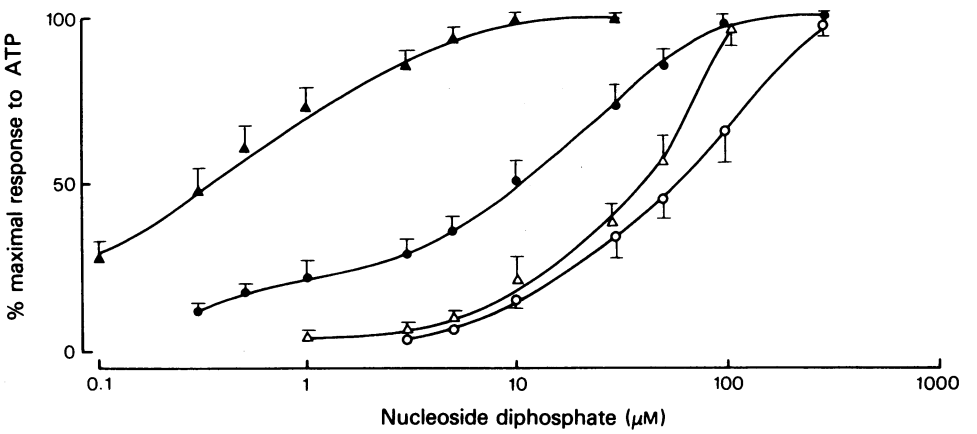

Figure 2 Relaxation of guinea-pig isolated taenia coli preparations by analogues of ADP. (●) ADP; (○) L-ADP; (▲) 2-azido-ADP; (△) 2-azido-L-ADP. Each point is the mean of at least 3 observations from at least 3 different animals. Vertical bars show s.e. mean.

activities were calculated as described by Satchell & Maguire (1975).

Drugs

Adenosine, adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), 2,6-dichloropurine, and 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-*O*-acetate were obtained from Sigma, London. 2,3,5-Tri-*O*-benzoyl-β-L-ribofuranosyl-1-*O*-acetate and 9-β-L-ribofuranosyladenine (L-adenosine) were prepared by the method of Acton, Ryan & Goodman (1964). 2-Chloroadenosine, m.p. 142-5°C, $[\alpha]_D^{20}$ −40.5° (c 0.3 water) was prepared by fusion of 2,6-dichloropurine with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-*O*-acetate at 155°C (Gough & Maguire, 1967) followed by treatment with methanolic ammonia; and 2-azidoadenosine, m.p. 157–160°C, $-N_3$ ν_{max}^{DMSO} 2155 cm^{-1}, was obtained by further treatment with hydrazine followed by nitrous acid (Schaeffer & Thomas, 1958). Similarly 2-chloro-9-β-L-ribofuranosyladenine (2-chloro-L-adenosine) m.p. 143-6°C, $[\alpha]_D^{20}$ + 39° (c 0.3, water) was synthesized by fusion of 2,3,5-tri-*O*-benzoyl-β-L-ribofuranosyl-1-*O*-acetate with 2,6-dichloropurine followed by treatment with ammonia; and further treatment with hy-

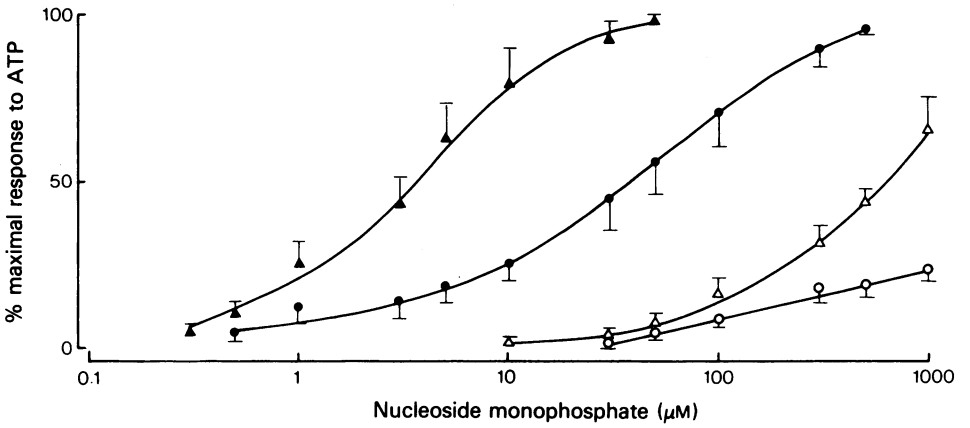

Figure 3 Relaxation of guinea-pig isolated taenia coli preparations by analogues of AMP. (●) AMP; (○) L-AMP; (▲) 2-azido-AMP; (△) 2-azido-L-AMP. Each point is the mean of at least 3 observations from at least 3 different animals. Vertical bars show s.e. mean.

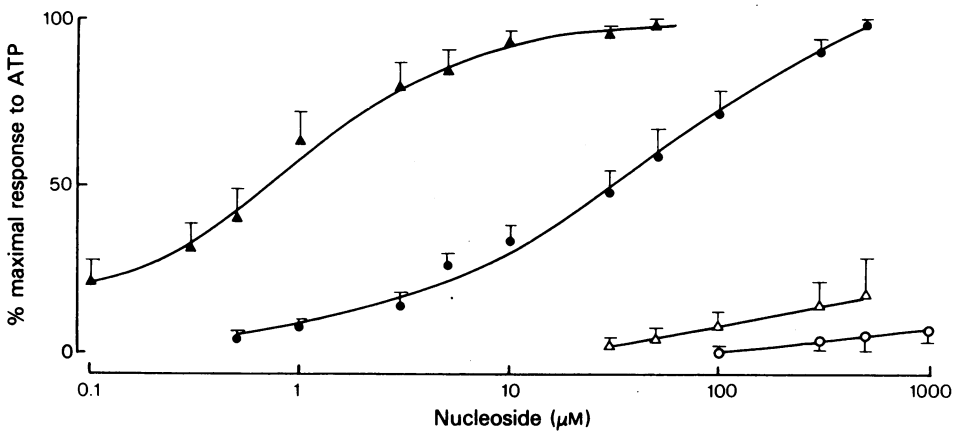

Figure 4 Relaxation of guinea-pig isolated taenia coli preparations by analogues of adenosine. (●) Adenosine; (○) L-adenosine; (▲) 2-azidoadenosine; (△) 2-azido-L-adenosine. Each point is the mean of at least 3 observations from at least 3 different animals. Vertical bars shown s.e. mean.

drazine and nitrous acid afforded 2-azido-L-adenosine, m.p. 156–9°C, $-N_3$ ν_{max}^{DMSO} 2155 cm^{-1}.

β-L-Adenosine 5′-monophosphate (L-AMP) was obtained by phosphorylation of L-adenosine with phosphoryl chloride ($POCl_3$) in triethyl phosphate (TEP) (Holý & Šorm, 1971). β-L-Adenosine 5′-diphosphate (L-ADP) and β-L-adenosine 5′-triphosphate (L-ATP) (Holý & Šorm, 1971) were synthesized by further phosphorylation in hexamethyl phosphoramide (HMPT) with carbonyl diimidazole (CDI) and tri-*n*-butylammonium ortho- or pyrophosphate respectively (Ott, Kerr, Hansbury & Hayes, 1967).

2-Azidoadenosine 5′-monophosphate (2-azido-AMP) and 2-azidoadenosine 5′-diphosphate (2-azido-ADP) were prepared from 2-azidoadenosine as reported (Cusack & Born, 1977), and 2-azidoadenosine 5′-triphosphate (2-azido-ATP) was synthesized by phosphorylation of 2-azido-AMP with tri-*n*-butylammonium pyrophosphate and CDI in HMPT, and purified by chromatography on DEAE-cellulose hydrogen carbonate. $\lambda_{max}^{pH\,7}$ 273, 309 nm, purine: total P: acid labile P: inorganic P, 1:3.0:2.0:0. 2-Azido-β-L-adenosine 5′-monophosphate (2-azido-L-AMP), 2-azido-β-L-adenosine 5′-diphosphate (2-azido-L-

Table 1 Effect of enantiomers of adenosine and adenine nucleotides, and enantiomers of 2-azidoadenosine and 2-azidoadenine nucleotides on isolated taenia coli

Compound	*No. of muscle strips (No. of animals)*	*Time to reach maximal relaxations ± s.e. mean*	*pD_2 ± s.e. mean*	*Maximal response ± s.e. mean*	*Relative affinity*	*Ratio activity of enantiomers*
ATP	26 (18)	13.6 ± 1.2	5.08 ± 0.09	1.00 ± 0.01	1.0	2.8
L-ATP	6 (3)	20.0 ± 2.2	4.64 ± 0.09	1.00 ± 0.01	0.36	
2-Azido-ATP	9 (6)	29.9 ± 3.6	6.43 ± 0.11	0.99 ± 0.01	22.4	124.4
2-Azido-L-ATP	4 (3)	35.8 ± 2.7	4.33 ± 0.05	1.01 ± 0.02	0.18	
ADP	15 (10)	15.9 ± 2.3	5.00 ± 0.13	1.00 ± 0.02	0.83	5.9
L-ADP	9 (8)	17.8 ± 1.5	4.24 ± 0.09	0.98 ± 0.04	0.14	
2-Azido-ADP	14 (11)	50.5 ± 5.1	6.48 ± 0.14	1.01 ± 0.01	25.1	125.5
2-Azido-L-ADP	6 (6)	28.6 ± 2.2	4.39 ± 0.09	0.97 ± 0.05	0.20	
AMP	7 (4)	53.2 ± 2.9	4.43 ± 0.17	0.97 ± 0.01	0.22	Not calculated
L-AMP	6 (4)	Inactive		0.99 ± 0.02		
2-Azido-AMP	8 (6)	75.6 ± 4.2	5.46 ± 0.11		2.4	240
2-Azido-L-AMP	8 (6)	89.0 ± 11.7	3.19 ± 0.10	Not calculated	0.01	
Adenosine	12 (8)	70.3 ± 3.6	4.52 ± 0.20	0.99 ± 0.01	0.27	Not calculated
L-Adenosine	3 (3)	Inactive				
2-Azidoadenosine	8 (6)	95.0 ± 4.8	6.18 ± 0.15	0.98 ± 0.02	12.6	Not calculated
2-Azido-L-adenosine	8 (6)	Inactive				

ADP) and 2-azido-β-L-adenosine 5'-triphosphate (2-azido-L-ATP) were synthesized by identical procedures from 2-azido-L-adenosine.

All nucleosides and nucleotides were chromatographically homogeneous in at least two solvent systems. Each pair of enantiomers had identical physical constants except for their equal but opposite rotations.

Results

Responses of guinea-pig taenia coli to adenosine and adenine nucleotides

ATP, ADP, AMP and adenosine relaxed guinea-pig taenia coli in a dose-dependent manner, the order of potency being ATP > ADP > AMP = adenosine. ATP and ADP caused a rapid relaxation of the muscle (13 to 16 s) which partially regained tone before washout, while AMP and adenosine took a longer time (50 to 70 s) to effect relaxations without regaining tone before washout. Washout of higher doses of ATP and ADP caused a rebound excitation (4 to 8 min), but this effect was not observed with AMP or adenosine. Log dose-response curves for adenosine and adenine nucleotides, and L-adenosine and adenine L-nucleotides were determined (Figures 1 to 4). The maximal relaxations induced by adenosine and adenine nucleotides and L-ATP and L-ADP were similar, but L-ATP and L-ADP were 3 to 6 times less active than ATP and had comparably shaped log dose-response curves. L-AMP and L-adenosine were almost inactive (Table 1).

Responses of guinea-pig taenia coli to 2-azido analogues of adenosine and adenine nucleotides

2-Azido analogues of ATP, ADP, AMP, and adenosine relaxed guinea-pig taenia coli in a dose-dependent manner and were all more potent (2 to 25 times) than their respective unsubstituted compounds, the order of potency being 2-azido-ATP = 2-azido-ADP > 2-azido-AMP < 2-azidoadenosine. 2-Azido-ATP and 2-azido-ADP caused less rapid relaxation (30 to 50 s) than ATP, while 2-azido-AMP and 2-azidoadenosine took considerably longer (75 to 95 s). Unlike ATP, with all 2-azido analogues a prolonged washout time was required before recovery of the muscle preparation (accompanied by increased spontaneous activity), and with higher concentrations of 2-azido analogues complete recovery could not be achieved. Log dose-response curves for 2-azido analogues were all steeper than those obtained for ATP.

2-Azido-L-ATP and 2-azido-L-ADP were about 5 times less active than ATP and about 125 times less active than 2-azido-ATP. 2-Azido-L-AMP and 2-azido-L-adenosine were almost inactive except at high concentrations of 2-azido-L-adenosine.

Discussion

The higher potencies of the 2-azido derivatives compared to unsubstituted adenosine and adenine nucleotides, and the steeper log dose-response curves, are in agreement with results reported for other 2-substitutions, including 2-chloro (Satchell & Maguire, 1975), and confirmed by us (unpublished results). We have found that 2-azido derivatives fall into two groups based on their relative potencies. In addition, the responses of taenia coli to L-adenosine and adenine-L-nucleotides, and 2-azido-L-adenosine and 2-azidoadenine-L-nucleotides, also fall into two groups, in as far as that L-ATP and L-ADP and 2-azido-L-ATP and 2-Azido-L-ADP are nearly as active as ATP itself, while L-adenosine and L-AMP, and 2-azido-L-adenosine and 2-azido-L-AMP are almost without activity.

These two groups may arise from an action on two different purine receptors, a P1 receptor acting on adenylate cyclase and a P2 receptor maximally stimulated by ATP. If the P1 receptor is linked to adenylate cyclase, then the inactivity of L-adenosine and 2-azido-L-adenosine is consistent with our findings for adenosine-stimulated adenylate cyclase in human platelets where L-adenosine and 2-azido-L-adenosine are inactive (Cusack & Born, unpublished observations).

The postulated P2 receptor appears to have lower stereospecificity, possibly because binding of the 5'-triphosphate moiety is of greater importance. L-ATP might be useful for studies on purinergic transmitter mechanisms since, for example, although complexing of divalent cations is identical for L-ATP and ATP, the steric presentation to ATP receptors must be different.

2-Azidoadenosine and 2-azidoadenine nucleotides are converted to reactive intermediates by u.v. irradiation (Cusack & Born, 1977). It might be possible therefore to label P1 and P2 receptors by u.v. irradiation of purine receptor preparations in the presence of 2-azidoadenosine and 2-azido-ATP respectively.

2-Azido-L-adenosine and 2-azido-L-adenine nucleotides, because of their identical chemistry but smaller potency, should be useful controls for the nonspecific labelling which often occurs in photolysis experiments (Ruoho, Kiefer, Roeder & Singer, 1973).

We thank the Medical Research Council for financial support (G 978/38/SA), Dr J.M. Young for the loan of an organ bath and transducer, Mr G. Baker & Mr G. Oakes for preparation of the diagrams, Mrs T. Milner for technical assistance at the beginning of this work, and Professor G.V.R. Born, F.R.S. for encouragement. Correspondence to N.J.C. please.

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(Received January 12, 1979.)