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Original paper

Synthesis and biological evaluation of ATP analogues acting at putative purinergic P_{2X}-receptors (on the guinea pig bladder)

William HOWSON[1], Edwin Michael TAYLOR[2], Michael Edward PARSONS[2], Riccardo NOVELLI[1], Maria Aleksandra WILCZYNSKA[1] and Deborah Tracy HARRIS[2]

[1] *Department of Medicinal Chemistry, and*
[2] *Department of Pharmacology,*
Smith, Kline & French Research Ltd., The Frythe, Welwyn, Hertfordshire, AL6 9AR, U.K.

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Summary — This paper describes studies carried out on a series of ATP analogues on the guinea pig bladder, a tissue reported to possess purinergic P_{2X}-receptors.

Optimisation of the pharmacological experimental technique enabled reproducible responses to ATP to be obtained in the 0.2—100 μM concentration range and the potencies of ATP analogues relative to ATP to be accurately determined.

Alterations of the three main parts of the ATP molecule, *i.e.*, the triphosphate, ribose and base, suggest that the triphosphate group is responsible for the efficacy of the agonist, whereas the ribose and adenine moieties are associated with affinity.

Résumé — **Synthèse et évaluation biologique d'analogues de l'ATP agissant sur les récepteurs supposés purinérgiques P_{2X} dans la vessie de cobaye.** *Cet article décrit les études conduites avec une série d'analogues de l'ATP, dans la vessie isolée du cobaye, un tissu qui est supposé contenir des récepteurs purinérgiques P_{2X}.*

On a optimisé les techniques de pharmacologie expérimentale et cela a permis d'obtenir des réponses reproductibles après utilisation de l'ATP à des doses allant de 0,2 à 100 μM. L'efficacité des analogues par rapport à l'ATP a pu être déterminée avec précision.

Des changements ont été apportés aux trois parties principales de la molécule d'ATP, c'est-à-dire le triphosphate, le ribose et la base. Il semble que le groupe triphosphate soit responsable de l'efficacité de l'agoniste, alors que les parties ribose et adénine sont associées à son affinité.

quantitative analysis / ATP / purinergic / P_{2X}-receptors / guinea pig bladder

Introduction

There is a considerable resurgence of interest in the extracellular actions of purines which may be involved in important physiological regulatory processes, particularly on smooth muscle cells, platelets and vascular endothelial cells. Purines may also act as synaptic transmitters, co-transmitters and pre-synaptic modulators in the autonomic nervous system. The effect of purines on smooth muscle cells is mediated by two types of specific receptors, an adenosine or P_1-purinoceptor and an ATP or P_2-purinoceptor [1]. The actions of adenosine are mediated through an increase (A_2 subtype) or a decrease (A_1 subtype) in adenylate cyclase activity, and can be selectively inhibited by P_1-purinoceptor antagonists, such as 8-phenyltheophylline. In contrast, the actions of ATP do not involve adenylate cyclase and at present there are no truly selective, competitive P_2-purinoceptor antagonists, available.

Burnstock [2] has now proposed a further subdivision of P_2-purinoceptors into P_{2X} and P_{2Y} subpopulations. In essence, stimulation of P_{2X}-receptors causes the contraction of smooth muscle cells (*e.g.*, bladder, vas deferens and some vascular smooth muscle), whereas activation of P_{2Y}-purinoceptors results in relaxation of smooth muscle cells (*e.g.*, taenia coli, blood vessels *via* release of endothelial derived relaxant factor). The effect of ATP on hepatocytes also seems to involve P_{2Y}-purinoceptors [3]. The effect of ADP on platelets is unique and is generally separated from the P_2-purinoceptor classification attempts.

The subclassification of P_2-purinoceptors is based in part on the relative activities of some selected ATP analogues in tissues containing the putative P_{2X}- and P_{2Y}-receptor

Abbreviations: α,β-CH_2**ATP**: α,β-methylene adenosine-5′-triphosphate; β,γ-CH_2**ATP**: β,γ-methylene adenosine-5′-triphosphate; **L-AMPPCP**: (L)-β,γ-methylene adenosine-5′-triphosphate; **Homo ATP**: 5′-deoxy-5′[(hydroxypyrophosphoxy)phosphinylmethyl]adenosine; **Sp-ATPαS**: adenosine-5′-*O*-(1-thiotriphosphate)[*S*-isomer]; **Ara ATP**: 9-β-D-arabinofuranosyladenine-5′-triphosphate.

subpopulations. However, the data from tissues with P_{2X}-purinoceptors has been qualitative in nature because of the relative insensitivity to ATP, poor dose—response curves and no clearly defined maximal responses. We report here on optimisation of the guinea pig bladder preparation which allows quantitative data (ED_{50}'s, relative potencies) to be calculated for a series of ATP analogues. This data has allowed us to carry out a preliminary structural analysis study which is discussed below.

Chemistry

The majority of compounds **1**—**15** (Table I) were purchased from commercial sources and if necessary purified *via* ion—exchange chromatography on DEAE—Sephadex A-25 using a stepwise gradient of aq. $NHET_3HCO_3$; 2-CH_3S ATP **16**, Homo ATP **17** and L-AMPPCP **18** were prepared as previously reported by Cusack [4].

Table 1. Common nucleotides: characterisation and relative potency to ATP as P_{2X} agonists.

No.	Abbreviated name	Relative activity (ATP = 1)
1	ATP	1
2	ATP N^1-oxide	0.07
3	8-Br ATP	0.19
4	CTP	0.17
5	GTP	0.03
6	ITP	0.09
7	UTP	0.12
8	Ara ATP	0.77
9	2-deoxy ATP	0.16
10	ADP	0.21
11	AMP	inactive
12	α,β-CH_2ATP	1.2
13	β,γ-CH_2ATP	1.9
14	Sp-ATPαS	1.1
15	tripolyphosphate	0.01
16	2-CH_3S ATP	1.1
17	Homo ATP	0.89
18	L-AMPPCP ((L)-β,γ-CH_2ATP)	0.84

The ATP analogues in which the ribose ring has been replaced with an alkyl chain, compounds **19**, **20** and **21** (Table II), were synthesised by either of two methods, A [5] or B [6], (see Schemes 1 and 2). The first involves the mono protection of an alkyldiol **22** as its benzyl ether **23** and subsequent reaction of the remaining alcohol group to give the mesylate **24**. This was then reacted with the sodium salt of adenine in dimethylformamide (DMF), which after appropriate purification gave the 9-substituted adenine **25**. Deprotection to give **26** followed by reaction with phosphorous oxychloride gave the monophosphate which was isolated as the barium salt **27**. This was then converted into the triethylammonium salt to enhance its solubility, activated with 1,1-carbonyldiimidazole and reacted with the triethylammonium salt of pyrophosphate to give the required triphosphate **19**—**21** after purification.

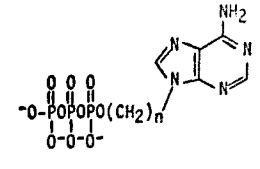
Method B (Scheme 2) starts with 5-amino-4,6-dichloropyrimidine **28** [7], this was reacted with the appropriate aminoalkylalcohol to give the diaminosubstituted pyrimidine **29**. This was cyclised with triethylorthoformate in acetic anhydride and the crude 6-chloropurine **30** obtained reacted with ammonia to give the 9-substituted adenine **26**. Subsequent steps were as described for method A.

The synthesis of compounds in which the triphosphate group of ATP has been replaced with either an amide/ester **31**, diamide **32**, amide/carboxylic acid **33**, or sulphonamide/carboxylic acid moiety **34** is outlined in Scheme 3 [8].Briefly, 5'-amino-5'-deoxyadenosine **35** was reacted with methylglutaryl chloride to give the amide **31**. This was then reacted with NH_3/MeOH to give the diamide **32** or hydrolysed with aqueous KOH to give the amide/carboxylic acid **33**. Treatment of 5'-amino-5'-deoxyadenosine **35** with 4-(chlorosulphonyl) benzoic acid gave the sulphonamide/carboxylic acid **34** after appropriate purification.

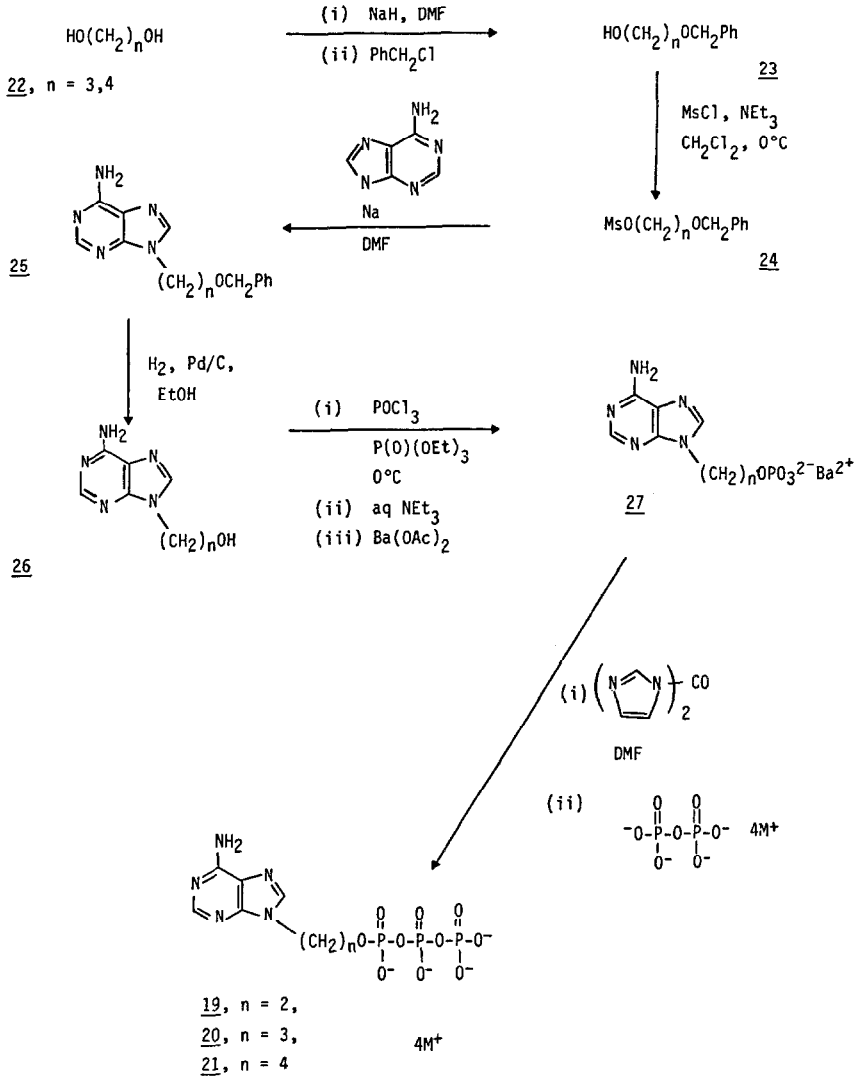
Results and Discussion

The compounds were tested as agonists on a guinea pig bladder strip preparation, where they cause a concentration-dependent contraction (see Experimental protocols for details). The results obtained are shown in Tables I, II

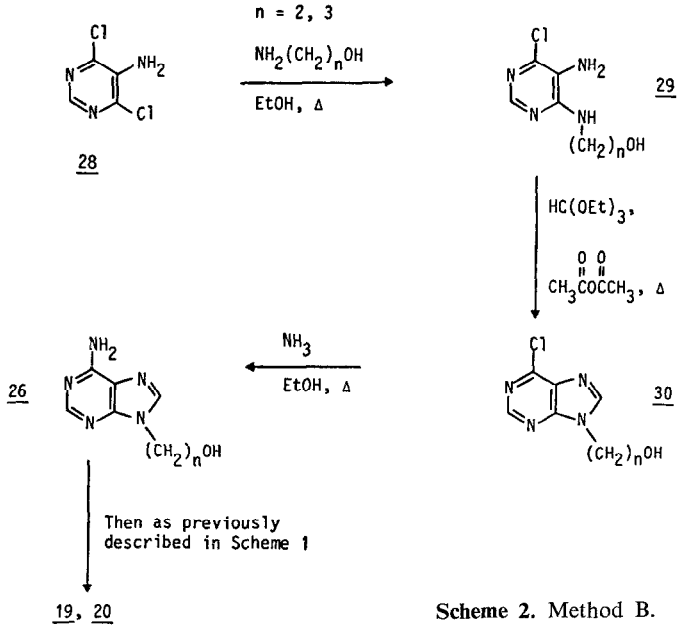
Table II. Alkyl chain analogues of ATP: characterisation and relative potency to ATP as P_{2X} agonists.



No.	*n*	Formula	Analysis	Relative activity (ATP = 1)
19	2	$C_7H_8N_5O_{10}P_3 \cdot Na_4$ 2 H_2O 0.3 C_3H_6O 0.12 NaI	C, H, N	0.04
20	3	$C_8H_{10}N_5O_{10}P_3 \cdot Na_4$ 2.5 H_2O 0.15 NaI	C, H, N	0.08
21	4	$C_9H_{12}N_5O_{10}P_3 \cdot Na_4$ 0.6 H_2O 0.16 $C_6H_{15}N$	C, H, N	0.05



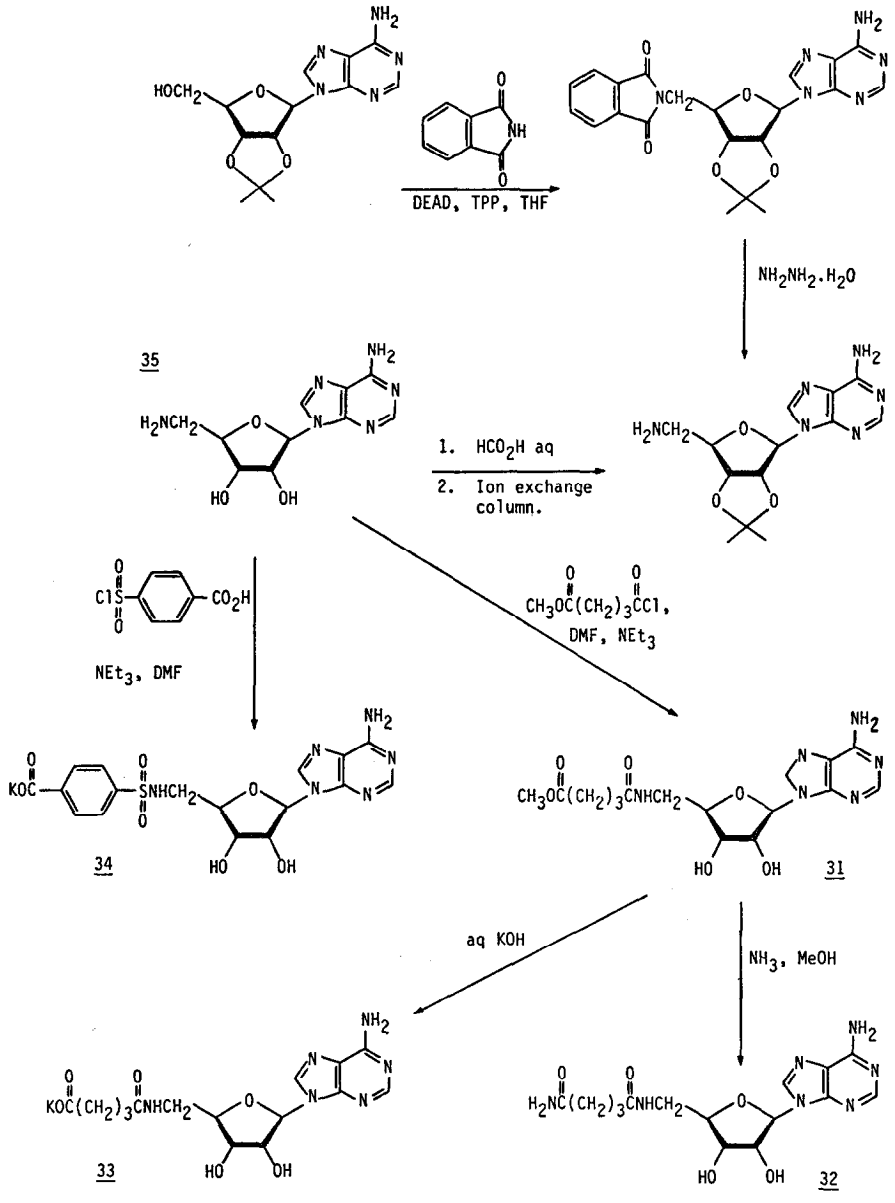
Scheme 1. Method A.



Scheme 2. Method B.

and III and activity is expressed relative to ATP **1**. The compounds were either full agonists or inactive, with none showing any antagonist activity. As the compounds are full agonists, it is reasonable to assume that the relative activity is a measure of affinity for the P_{2X}-receptor. This enabled us to carry out a preliminary structural activity study based on a series of ATP analogues, in which one of the three main parts of the ATP molecule, *i.e.*, triphosphate, ribose or base had been altered.

Alterations of the triphosphate moiety: 1) Removal of one phosphate to give ADP **10** decreases affinity 5-fold, whereas removal of two phosphates to give AMP **11** results in the complete loss of activity. In fact, AMP is a physiological antagonist to ATP in this assay, presumably acting *via* P_1 receptors (E. M. Taylor *et al.*, unpublished results). 2) Substitution of a bridging oxygen with a CH_2 group (*i.e.*, **12**, **13** and **17**), or a terminal α-P—O atom with sulphur



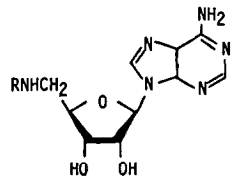
Scheme 3. Steps 1 and 2 proceed right to left to yield **35**.

(*i.e.*, **14**) has little effect. 3) Replacing the triphosphate group with more novel polar groups, such as a diamide **32**, amide/carboxylic acid **33** or sulphonamide/carboxylic acid **34**, results in the complete loss of activity. 4) Tripolyphosphate pentasodium **15** is a very weak but full agonist suggesting that this moiety is all that is necessary for agonist activity (efficacy).

Alterations of the ribose moiety: 1) Replacing this group with its mirror image while changing the β,γ-bridging oxygen for a CH_2 group to give **18** has little effect. 2) Removal (*i.e.*, **9**) or inversion (*i.e.*, **8**) of the 2′-OH group decreases affinity 5-fold or has no effect, respectively. 3) If the ribose group is replaced with a less rigid, alkyl chain of varying length (*i.e.*, **19**, **20** and **21**), affinity is decreased significantly (10—20-fold), but the compounds are still full agonists. Overall this suggests that the ribose group is not contributing to the 'agonist effect' and that it is mainly acting as a rigid spacer unit.

Alterations of the base moiety: 1) A substituent in the 2-position (*i.e.*, SCH_3, **16**) has no effect; whereas a substituent in the 8-position (*i.e.*, Br, **3**) decreases activity approximately 5-fold. This is possibly because **3** is predominantly in the *syn* conformation, whereas ATP adopts the *anti* conformation, indicating that the receptor binds ATP as the *anti* conformer. 2) The adenine nucleus can be replaced with a number of other common nucleoside heterocycles **4**, **5**, **6** and **7**. These all show reduced affinity (5—30-fold decrease) but no reduction in maximum response. This data would indicate that the adenine makes a significant contribution to affinity but not to efficacy.

Table III. Non-phosphate analogues of ATP: characterisation and relative potency to ATP as P_{2X} agonists.



No.	R	Formula	Analysis	Relative Activity (ATP=1)
31	$CO(CH_2)_3CO_2CH_3$	$C_{16}H_{22}N_6O_4$ 0.05($C_6H_{15}N$.HCl)	C,H,N	Inactive
32	$CO(CH_2)_3CONHCH_3$	$C_{15}H_{21}N_7O_5$	C,H,N	"
33	$CO(CH_2)_3CO_2K$	$C_{15}H_{19}N_6O_6$.K 0.65H_2O 0.25CH_4O 0.02CH_2Cl_2 0.02$CHCl_3$	C,H,N	"
34	SO_2-C$_6$H$_4$-CO_2K	$C_{17}H_{18}N_6O_7S$.K 1.5H_2O 0.2KOH 0.2CH_3CO_2K	C,H,N	"

Conclusion

The structural activity study described here suggests that for the P_{2X}-receptor present in guinea pig bladder, the triphosphate group is responsible for triggering an agonist effect and that the adenine and ribose just affect affinity. In fact, the ribose might just be acting as a rigid spacer unit. This analysis has enabled us to design an antagonist for P_{2X}-receptors in the guinea pig bladder (W. Howson *et al.*, in preparation).

Experimental protocols

Chemistry

Melting points were determined on a Thomas Hoover 6406-H apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Jeol PFT 100P (100 MHz) and Brucker AN 250 and 360 (250 and 360 MHz) spectrometers with $(CH_3)_4Si$ as the internal reference. The various splitting patterns were designated as follows: s: singlet; brs: broad singlet; d: doublet; t: triplet; q: quartet or quintuplet; m: multiplet. Microanalyses for elements indicated are within $\pm 0.4\%$ of the theoretical values. Purity of compounds were checked by thin—layer chromatography (TLC) analysis on silica gel 60 F_{254} plates, and components were visualised by a UV fluorescent lamp. High pressure liquid chromatography (HPLC) assays were performed using an analytical pump coupled to an HPLC column (300 × 4 mm) prepacked with 5 μm octadecyl-bonded silica and a detector set at 270 nm; elution was carried out at a flow rate of 2 ml/min with a mixture of 2% CH_3CN/98% 0.1 M KH_2PO_4, (pH = 6.8). UV spectra were recorded on Perkin—Elmer Lambda 5 spectrometer in aqueous buffered solutions.

Preparation of 21: method A

4-Benzyloxy butan-1-ol 23 (n = 4)
Butane-1,4-diol **22** (45 g, 0.5 mol) was dissolved in dry DMF (150 ml) and added dropwise over 45 min to a cooled (ice/water bath) and stirred suspension of NaH (6 g, 0.25 mol) in DMF (200 ml). The mixture was then allowed to warm to room temperature and stirred for 1 h. The mixture was then cooled to 0°C in an ice/water bath and treated with benzylchloride (28.3 ml, 0.25 mol) in DMF (150 ml). The mixture was allowed to warm to room temperature and stirred overnight. The precipitated NaCl was filtered off, the solvent removed *in vacuo*, the residue taken up in $CHCl_3$, washed with H_2O (2 ×), brine, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a brown oil. This was further purified *via* vacuum distillation to give pure **23** as an oil (34.9 g, 77%) bp: 115—118°C, 0.15 mm Hg. ^{1}H NMR ($CDCl_3$): δ 1.69 (m, 4H, CH_2CH_2); 3.52, 3.64 (t, brs, 2 × 2H, CH_2OH, CH_2O); 4.51 (s, 2H, $PhCH_2O$); 7.30 (m, 5H, ArH).

1-(Methylsulphonyloxy)-4-benzyloxybutane 24 (n = 4)
23 (34 g, 0.19 mol) and triethylamine (26.3 ml, 0.19 mol) were dissolved in CH_2Cl_2 (350 ml) and cooled to —5°C in an ice/acetone bath. Methanesulphonyl chloride (16.7 ml, 0.22 mol) in dry CH_2Cl_2 (30 ml) was then added dropwise with vigorous stirring. After 0.5 h, H_2O was added, the organic layer separated, washed with 1 M HCl, sat $NaHCO_3$ solution, brine, dried ($MgSO_4$) and the solvent removed *in vacuo* to give product **24** as an oil (48.5 g, 100%). ^{1}H NMR ($CDCl_3$): δ 1.72, 1.87 (2 × m, 2 × 2H, CH_2CH_2); 2.97 (s, 3H, CH_3), 3.51 (t, 2H, CH_2O); 4.25 (t, 2H, CH_2OSO_2); 4.50 (s, 2H, $PhCH_2O$); 7.30 (m, 5H, ArH).

9-(4'-Benzyloxybutyl) adenine 25 (n = 4)
To a stirred suspension of NaH (4.45 g, 0.185 mol) in dry DMF (300 ml), under N_2, cooled to 0°C, was added adenine (23.35 g, 0.172 mol) over 10 min. The mixture was allowed to warm to room temperature and stirred for an additional hour. The mixture was then cooled to 0°C in an ice/water bath and the mesylate **24** (48 g, 0.185 mol) in DMF (150 ml) was added over 30 min. The reaction was then allowed to warm to room temperature and stirred overnight. The solvent was then removed *in vacuo*, the residue partitioned between $CHCl_3$ and H_2O. The organic layer was washed with H_2O (4 ×), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the crude product as a yellow solid. Recrystallisation from hot EtOH gave pure product **25** (19.8 g, 38.5%) mp: 149—151°C. ^{1}H NMR ($CDCl_3$): δ 1.68, 2.02 (2 × m, 2 × 2H, CH_2CH_2); 3.51 (t, 2H, CH_2O); 4.23 (t, 2H, CH_2N); 4.49 (s, 2H, $PhCH_2O$); 6.15 (brs, 2H, NH_2); 7.30 (m, 5H, ArH); 7.78, 8.36 (2 × s, 2 × H, adenine-H). UV: λ_{max}^{pH1} 260 mm (ε = 13.8 × 10^3), $\lambda_{max}^{pH\ 7}$ 262 nm (ε = 14.1 × 10^3), λ_{max}^{pH12} 262 nm (ε = 14.1 × 10^3).

9-(4'-Hydroxybutyl) adenine 26 (n = 4)
25 (10 g, 34 mmol) was suspended in EtOH (200 ml) with 10% Pd/C (1 g) and 1 M HCl (50 ml) and hydrogenated at a pressure of 45 psi and a temperature of 50°C. When the reaction was complete, the catalyst was filtered off and the solvent removed *in vacuo* to yield the crude product. This was recrystallised from hot EtOH to give pure product **26** as the hydrochloride (7.0 g, 85%) mp: 194—195°C. ^{1}H NMR (D_2O): δ 1.57, 1.98 (2 × m, 2 × 2H, CH_2CH_2); 3.63, 4.37 (2 × t, 2 × 2H, CH_2OH, CH_2N); 8.38, 8.44 (2 × s, 2 × H, adenine H). Anal. ($C_9H_{13}N_5O$·HCl) C, H, N, Cl. UV: λ_{max}^{pH1} 259 nm (ε = 14 × 10^3), $\lambda_{max}^{pH\ 7}$ 261 nm (ε = 13.6 × 10^3), λ_{max}^{pH12} 261 nm (ε = 13.5 × 10^3). A comparison of the ^{13}C NMR spectrum for this compound with that of a compound obtained from an unambiguous synthesis showed that it was the 9-isomer. The free base of **26** was prepared by dissolving the above in 1 M NaOH, extraction with *n*-butanol (4 ×) and removal of the solvent *in vacuo* to give a white solid, mp: 198—201°C.

4-(Adenin-9-yl)-butylphosphate barium salt 27 (n = 4)
26 (1.0 g, 5 mmol) was suspended in trimethylphosphate (12 ml) and cooled to 0°C in an ice/water bath. Phosphorous oxychloride (0.9 ml, 10 mmol) was added and the mixture stirred for a further 3.5 h. The mixture was then poured onto crushed ice (50 g) containing triethylamine (7 ml, 48 mmol). The aqueous solution was then extracted with $CHCl_3$ (3 ×), and then treated with barium acetate (2.46 g, 10 mmol) and left overnight. The precipitated barium phosphate was removed from the aqueous solution by filtration and the filtrate concentrated to a small volume (10 ml). EtOH (30 ml) was added to this and the desired product **27** precipitated as the barium salt (1.8 g, 88%). ^{1}H NMR (D_2O): δ 1.71, 2.06 (2 × m, 2 × 2H, CH_2CH_2); 4.03 (q, 2H, CH_2O); 4.42 (t, 2H, CH_2N); 8.51, 8.62 (2 × s, 2 × H, adenine). UV: λ_{max}^{pH1} 260 nm (ε = 11.1 × 10^3), λ_{max}^{pH7} 262 nm (ε = 11.4 × 10^3), λ_{max}^{nH12} 262 nm (ε = 11.5 × 10^3) Anal. ($C_9H_{12}N_5O_4PBa$·2.5 H_2O·0.125 $BaPO_4$) C, H, N.

4-(Adenin-9-yl)-butyltriphosphate sodium salt **21** (n = 4)
The barium salt of the monophosphate **27** (0.5 g, 1.2 mmol) was converted into the pyridinium salt by elution from an amberlite IR-120 (pyridinium form) ion—exchange column. The aqueous solution thus obtained was treated with tributylamine (0.56 ml, 2.4 mmol) and the solvent removed *in vacuo*. Several co-evaporations with dry pyridine and then DMF gave the bis-tributyl ammonium salt of the monophosphate as a gum. This was then dissolved in dry DMF (13 ml) and 1,1′-carbonyldiimidazole (0.96 g, 6 mmol) in DMF (13 ml) was added. The mixture was stirred at room temperature under nitrogen for 5 h. The solution was then treated with dry MeOH (0.3 g, 10 mmol) to destroy excess 1,1′-carbonyldiimidazole. This gave the monophosphate activated as the imidazolate in a DMF solution. Tetrasodium pyrophosphate decahydrate (2.6 g, 6 mmol) was dissolved in H_2O (10 ml) and converted into the pyridinium salt by elution from an amberlite IR-120 (pyridinium form) ion—exchange column. The aqueous solution thus obtained was treated with tributylamine (5.6 ml, 24 mmol) and the solvent removed *in vacuo*. Several co-evaporations with dry pyridine and then DMF gave tetra-tributyl ammonium pyrophosphate as a gum. The gum was taken up in dry DMF (35 ml) and added to the DMF solution of the activated monophosphate (described above). The mixture was stirred under N_2 at room temperature for 14 h then concentrated to dryness on a rotary evaporator (bath temperature 35°C) to give crude product **21**. This was purified by ion—exchange chromatography on DEAE—Sephadex A-25 (hydrogen carbonate form) using a stepwise gradient of aq. $NHEt_3HCO_3$ (0—0.8 M). The appropriate fractions were collected, the solvent removed *in vacuo* and the residue co-evaporated with MeOH several times to give product as the triethylammonium salt. This was taken up in MeOH (5 ml) and treated with a solution of 1 M NaI in acetone, this precipitated the product **21** as the sodium salt which was collected and dried (0.13 g, 20%). ^1H NMR (D_2O): δ 1.67, 2.03 (2 × m, 2 × 2H, CH_2CH_2); 4.03 (m, 2H, CH_2O); 4.34 (t, 2H, CH_2N); 8.28, 8.30 (2 × s, 2 × H, adenine H). UV: λ_{max}^{pH1} 259 nm ($\varepsilon = 12.5 \times 10^3$), $\lambda_{max}^{pH\ 7}$ 262 nm ($\varepsilon = 12.7 \times 10^3$), λ_{max}^{pH12} 261 nm ($\varepsilon = 13 \times 10^3$).

Compound **20** was prepared similarly to compound **21** described above, starting from the appropriate diol **22** (n=3). Analytical data for **20** are as follows: ^1H NMR (D_2O): δ 2.25 (m, 2H, CH_2); 4.02 (m, 2H, CH_2O); 4.43 (t, 2H, CH_2N); 8.27, 8.29 (2 × s, 2 × H, adenine H). UV: λ_{max}^{pH1} 259 nm ($\varepsilon = 10.7 \times 10^3$), $\lambda_{max}^{pH\ 7}$ 262 nm ($\varepsilon = 10.9 \times 10^3$), λ_{max}^{pH12} 261 nm ($\varepsilon = 11.4 \times 10^3$).

Preparation of **26** (n = 2): *method B*

5-Amino-6-chloro-4-(2′-hydroxyethylamino)pyrimidine **29** (n = 2)
5-Amino-4,6-dichloropyrimidine [7] **28** (20 g, 0.12 mol) and ethanolamine (36.6 g, 0.6 mol) were dissolved in dioxane (150 ml) and treated at reflux for 18 h. The mixture was cooled, the solvent removed *in vacuo* and the residue washed with a little cold H_2O. The crude material was then recrystallised from hot H_2O to give pure product **29** (20.66 g, 91%), mp: 134—137°C. ^1H NMR (DMSO-d_6): δ 3.38, 3.48 (2 × m, 4H, 2 × CH_2); 4.80 (t, 1H, OH); 4.91 (brs, 2H, NH_2); 6.73 (brt, 1H, NH); 7.63 (s, 1H, CH). UV: $\lambda_{max}^{pH\ 7}$ 263 nm ($\varepsilon = 7.5 \times 10^3$), 290 nm ($\varepsilon = 8.0 \times 10^3$).

6-Chloro-9-(2′-hydroxyethyl) purine **30** (n = 2)
29 (10 g, 53 mmol) was dissolved in acetic anhydride (42 ml) and triethyl orthoformate (42 ml) was added. The mixture was then heated at reflux for 3 h, cooled and the solvent removed *in vacuo* to give crude product **30** as a solid. No further purification was carried out.

9-(2′-Hydroxyethyl) adenine **26** (n = 2)
30 (crude material from above) was dissolved in ammonia saturated EtOH (300 ml) and heated at 120°C for 15 h in an autoclave. The solvent was removed *in vacuo* and the residue was recrystallised from EtOH to give a fairly pure product **26** (3.2 g, 34%). HPLC (C_{18} μ-bondapak, CH_3CN/10.1 M NH_4OAc, pH = 7.0) showed this to contain a number of close running impurities which could be removed by medium pressure liquid chromatography on silica gel (15—40 μM) eluting with mixtures of 33% CH_3NH_2 in EtOH/CH_2Cl_2 to give pure product **26** (1.6 g, 17%) mp: 234—240°C. ^1H NMR (DMSO-d_6): δ 3.73 (m, 2H, CH_2OH); 4.18 (t, 2H, CH_2); 5.02 (t, 1H, OH); 7.19 (s, 2H, NH_2); 8.07, 8.13 (2 × s, 2 × H, adenine H). UV: λ_{max}^{pH1} 259 nm ($\varepsilon = 14.1 \times 10^3$), $\lambda_{max}^{pH\ 7}$ 261 nm ($\varepsilon = 14.4 \times 10^3$), λ_{max}^{pH12} 261 nm ($\varepsilon = 14.4 \times 10^3$).

Compound **19** was subsequently prepared from **26** (n = 2) *via* part of the route shown in Scheme 1 and as previously described for compound **21**, analytical data for **19** as follows: ^1H NMR (D_2O) δ 4.34 (m, 2H, CH_2O); 4.53 (m, 2H, CH_2N); 8.24, 8.28 (2 × s, 2 × H, adenine H). UV: λ_{max}^{pH1} 259 nm ($\varepsilon = 11.7 \times 10^3$), $\lambda_{max}^{pH\ 7}$ 261 nm ($\varepsilon = 11.9 \times 10^3$), λ_{max}^{pH12} 261 nm ($\varepsilon = 12.5 \times 10^3$).

Preparation of **31**, **32**, **33** *and* **34**

5′-(Methoxycarbonylpropylcarbonylamino)-5′-deoxyadenosine **31**
5′-Amino-5′-deoxyadenosine **35** [8] (3.12 g, 10 mmol) was suspended in dry DMF (250 ml) and triethylamine (3.03 g, 30 mmol) followed by the addition of methylglutaryl chloride (1.8 g, 11 mmol). The mixture was stirred at room temperature for 48 h, during this time the amine dissolved. The solvent was then removed *in vacuo* and the crude product purified by flash liquid chromatography. On silica gel (40—60 μM) eluting with mixtures of MeOH/CH_2Cl_2, crystallisation from MeOH gave pure product **31** (1.1 g, 27%), mp: 105—123°C. ^1H NMR (DMSO-d_6): δ 1.74 (m, 2H, CH_2); 2.17, 2.30 (2 × t, 2 × 2H, 2 × CH_2CO); 3.40 (m, 2H, CH_2N); 3.56 (s, 3H, CH_3O); 3.95, 4.02, 4.67 (3 × m, 3 × H, 4′, 3′, 2′-H); 5.30, 5.47 (2 × d, 2 × H, 2 × OH); 5.83 (d, 1H, 1′-H); 7.36 (brs, 2H, NH_2); 8.18, 8.34 (2 × s, 2 × H, adenine H); 8.28 (brt, 1H, NH). UV: $\lambda_{max}^{pH\ 7}$ 259 ($\varepsilon = 14.3 \times 10^3$).

5′-(Aminocarbonylpropylcarbonylamino)-5′-deoxyadenosine **32**
31 (400 mg, 1 mmol) was dissolved in an ammonia saturated methanol solution (40 ml) and stirred at room temperature for 7 days. The solvent was removed *in vacuo* and the residue crystallised from MeOH to give pure product **32** (285 mg, 75%), mp: 180—185°C. ^1H NMR (DMSO-d_6): δ 1.70 (m, 2H, CH_2); 2.05, 2.13 (2 × t, 2 × 2H, 2 × CH_2CO); 3.38 (m, 2H, CH_2N); 3.95, 4.03 (2 × m, 2 × H, 4′,3′-H's); 4.69 (m, 1H, 2′-H); 5.28, 5.48 (2 × d, 2 × H, 2 × OH); 5.83 (d, 1H, 1′-H); 6.72, 7.25 (2 × brs, 2 × H, NH_2); 7.37 (brs, 2H, NH_2); 8.19 8.32 (2 × s, 2 × H, adenine H), 8.26 (brt, 1H, NH). UV: $\lambda_{max}^{pH\ 7}$ 259 nm ($\varepsilon = 14.6 \times 10^3$).

5′-(Carboxypropylcarbonylamino)-5′-deoxyadenosine potassium salt **33**
31 (400 mg, 1 mmol) was dissolved in H_2O (1 ml) and 1 M KOH (1.1 ml) was added, the mixture was stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the residue taken up in MeOH and filtered. The MeOH was removed *in vacuo* and the residue co-evaporated with $CHCl_3$ and CH_2Cl_2 to give pure product **33** (290 mg, 70%). ^1H NMR (DMSO-d_6): δ 1.71 (m, 2H, CH_2); 1.91 (m, 2H, $CH_2CO_2^-$); 2.18 (m, 2H, CH_2CO); 3.28 (m, 2H, CH_2NH); 4.03 (m, 2H, 4′,3′-H); 4.7 (m, 1H, 2′-H); 5.84 (d, 1H, 1′-H); 7.39 (brs, 2H, NH_2); 8.22, 8.40 (2 × s, 2H, adenine H); 8.55 (brt, 1H, NHCO). UV: $\lambda_{max}^{pH\ 7}$ 259 nm ($\varepsilon = 12.8 \times 10^3$).

5′-(4-Carboxyphenylsulphonamido)-5′-deoxyadenosine potassium salt **34**
35 (1.33 g, 5 mmol) was suspended in dry DMF (50 ml), triethylamine (1.1 g, 11 mmol) was added and the mixture cooled to 0°C in an ice/water bath. To this was added 4-(chlorosulphonyl)benzoic acid (1.1 g, 5.5 mmol) and the mixture stirred and allowed to warm to room temperature, stirring was continued overnight. The triethylammonium chloride was filtered off and the solvent removed *in vacuo* to give a brown residue. The crude material was taken up in a little H_2O and the solution made acidic with 1 M HCl (pH 2). The material was then purified by flash liquid chromatography on silica gel (40—60 μM) eluting with mixtures of MeOH/CH_2Cl_2AcOH. The solid obtained was recrystallised from MeOH, treated with aq. KOH and recrystallised from MeOH to give the product as the potassium salt **34** (300 mg, 12%) mp: 247—251°C. ^1H NMR (DMSO-d_6): δ 3.05 (brs, 2H, CH_2N); 3.98 (m, 1H, 4′-H), 4.03 (m, 1H, 3′-H); 4.64 (t, 1H, 2′-H); 5.70 (m, 2H, 2 × OH); 5.82 (d, 1H, 1′-H); 7.4 (s, 2H, NH_2); 7.69, 7.98 (2 × d, 4H, aromatic H); 8.14, 8.31 (2 × s, 2H, adenine H); 8.48 (brs, 1H, $NHSO_2$). UV: $\lambda_{max}^{pH\ 7}$ 243 nm ($\varepsilon = 15.3 \times 10^3$).

Pharmacology

Isolated guinea pig bladder
Male or female Dunkin—Hartley guinea pigs (300—400 g body weight) were killed by cervical dislocation. The bladder was removed, cleared

of connective tissue and opened with transverse cuts. The mucosal layer was carefully removed with sharp scissors [9]. Four strips of detrusor muscle, 10 × 3 mm, were cut from the apical region and suspended in 10 ml organ baths containing Krebs solution at 30—31°C and continually gassed with 95% O_2 + 5% CO_2. One end of the preparation was secured to a glass holder and the other end attached to an isometric transducer. The preparations were placed under 1 g of tension and allowed to equilibrate for 30—45 min. Atropine (10^{-6} M) was added to the Krebs solution to inhibit cholinergic responses.

ATP, injected into the bath from a Hamilton syringe, caused a short-lasting increase in tension (twitch response) which was not maintained despite the continued presence of the agonist. To avoid desensitisation of the P_{2x}-purinoceptors, ATP or a related purine, was given every 15 min with frequent washing of the tissue with fresh Krebs solution between dosings. It was found that the sensitivity of the preparations to ATP increased during the first hour of dosing. After this initial increase in sensitivity was complete, a range of ATP concentrations (0.2—100 μM) was tested at 15 min intervals and a concentration—response curve constructed (Fig. 1). The bladder showed reasonable sensitivity to ATP (threshold 0.2 μM, ED_{50} 1—10 μM) and the concentration—response curve was sigmoid and showed a clear maximal response. A second and third concentration—response to ATP were similar to the first (Fig. 1) demonstrating the reproducibility of the responses.

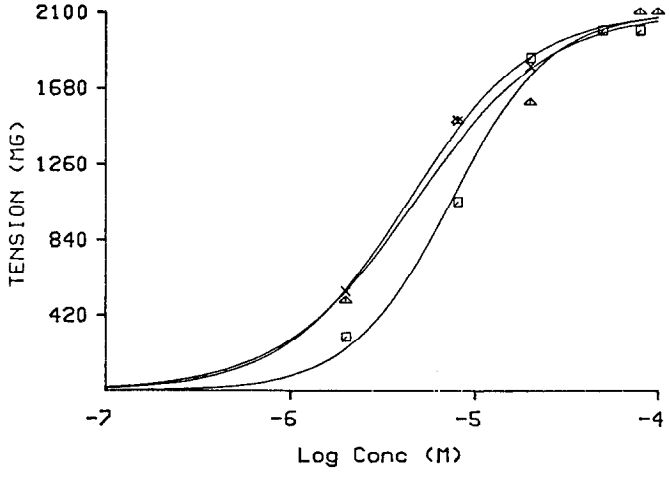


Fig. 1. Dose—response curves for ATP **1**.

Although ATP responses in a single preparation were consistent, there was some intertissue variation in the sensitivity to the agonist; because of this, test compounds were compared directly with ATP in every preparation. A concentration—response curve to ATP was obtained first, followed by the test compound and then, as a check, a second concentration—response curve to ATP was constructed. Relative potencies to the initial ATP curve were calculated using the Allfit computer programme [10]. Each compound was tested at least 4 times. All compounds were dissolved in distilled water. Composition of Krebs solution in g/l: NaCl: 7.77; KCl: 0.35; $NaH_2PO_4 \cdot 2\ H_2O$: 0.22; $NaHCO_3$: 1.37; $MgSO_4$ + H_2O: 0.35; glucose 1.41; $CaCl_2$ (1 M) 0.28 ml.

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