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VI. Nomenclature and Classification of Purinoceptors*

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I. Introduction

"Receptors recognize a distinct chemical entity and translate information from that entity into a form that the cell can read to alter its state" (Kenakin et al., 1992). Even though the receptors are often pharmacologically defined on the basis of synthetic compounds, they are assumed to have developed to respond to endogenous molecules. Therefore, receptors are generally named on the basis of their natural ligands. Hence, it is appropriate to very briefly summarize the evidence that purine nucleotides and nucleosides are natural ligands for a wide class of receptors.

In a seminal paper, Drury and Szent-Györgyi (1929) showed that adenosine exerted a large number of biological effects, including bradycardia and vasodilation. A wider interest in the role of adenosine followed from the demonstration in 1963 that adenosine can be produced by the hypoxic heart. Two groups independently formulated the hypothesis that adenosine may be involved in the metabolic regulation of coronary blood flow (Berne, 1963; Gerlach et al., 1963). The observation by de Gubareff and Sleator (1965) that the actions of adenosine in heart tissue could be blocked by caffeine suggested the existence of an adenosine receptor. The potent cardiovascular effects of adenosine led to an interest in the synthesis of new adenosine analogs, and careful dose-response studies with a number of these drugs (Cobbin et al., 1974) strongly suggested the presence of a receptor for adenosine-like compounds. Sattin and Rall (1970) reported that adenosine increased cyclic AMP accumulation in slices of rodent brain and that this adenosine-induced second-messenger response was blocked by methylxanthines. Their findings suggested that adenosine receptors exist in the central nervous system. The essentially simultaneous findings by McIlwain (1972), that such brain slices actually elaborate adenosine in concentrations that would be sufficient to elevate cyclic AMP, provided support that these putative receptors were physiologically occupied by adenosine. Thus, in the 1970s there was good evidence that there were receptors for adenosine at which methylxanthines acted as antagonists. Biochemical evidence for the existence of multiple adenosine receptors was subsequently provided by the demonstration that adenosine analogs increased cyclic AMP production in some preparations and decreased it in others. Because the relative agonist potency for a variety of adenosine analogs was different for these two types of effects, the presence of two classes of receptors, called A_1 and A_2 (van Calker et al., 1979) or R_i and R_a (Londos et al., 1980), was proposed. The A_1/A_2 nomenclature is now generally used.

The presence of receptors for ADP, particularly on

* The classification of purinoceptors described in this review has been sanctioned by the IUPHAR Committee on Receptor Nomenclature and Drug Classification. As stated in the review, the nomenclature may have to be revised when more information becomes available. IUPHAR Purinioceptor Classification Subcommittee: Prof. Bertil B. Fredholm (Chairman), Section of Molecular Neuropharmacology, Department of Physiology and Pharmacology, Karolinska Institutet, S.-171 77 Stockholm, Sweden (phone: Int + 46-8-728 79 39, fax: Int + 46-8-33 16 53); Dr. Alison Abbott, Nature, Macmillan Magazines Ltd., Sandstraße 41, D.-8000 München 2, Germany (phone: Int + 49-89-52 70 36, fax: Int + 49-89-523 22 22); Prof. Geoffrey Burnstock, University College London, Gower Street, London WC1E 6BT, United Kingdom (phone: Int + 44-71-387 70 50, fax: Int + 44-71-380 73 49); Dr. John W. Daly, Ph.D., Chief, Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Building 8, Room 1A-15, Bethesda, Maryland 20892, USA (phone: Int + 1-301-496 40 24, fax: Int + 1-301-402 00 08); Dr. Paul Leff, Manager of Pharmacology, Fisons Pharmaceuticals, Research and Development Labs, Pharmaceutical Division, Bakewell Road, Loughborough, Leistershire LE11 0RH, United Kingdom (phone: Int + 44-509-61 10 11, fax: Int + 44-509-21 04 50); Prof. T. Kendall Harden, Department of Pharmacology, University of North Carolina, School of Medicine, Chapel Hill, North Carolina 24999, USA (phone: Int + 1-919-966-3744, fax: Int + 1-919-966-5640); Prof. Dr. med. Ulrich Schwabe, Pharmakologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 366, D.-6900 Heidelberg 1, Germany (phone: Int + 49-6221-56 39 02, fax: Int + 49-6221-56 39 44); Dr. Michael Williams, Divisional Vice President, Neuroscience Research, Abbott Laboratories, D.-464, AP10, Abbott Park, Illinois 60064-3500, USA (phone: Int + 1-708-937 81 86, fax: Int + 1-708-937-9195).

TABLE 1
Original criteria for distinguishing two types of purinoceptors

Antagonists	Agonist preferences	Changes in cyclic AMP	Induction of prostaglandin synthesis
P_1 receptors			
Methylxanthines*	ADO > AMP > ADP > ATP†	Yes‡	No§
P_2 receptors			
Quinidine*			
Imidazolines		No‡	Yes§
2,2′-Pyridylisatogen	ATP > ADP > AMP > ADO†		
Apamin			

* We still lack good antagonists at P_2 receptors (see section III.B and Table 4). There are apparently adenosine receptors (A_3 receptors, see below) where the classical methylxanthines are very poor antagonists.

† Adenosine (ADO) and AMP do not activate P_2 receptors. Adenine nucleotides may or may not be agonists at adenosine receptors.

‡ Not all adenosine receptors (P_1 purinoceptors) affect cyclic AMP formation. Conversely, adenine nucleotides may affect cyclic AMP formation.

§ Not all effects of ATP or ADP are mediated through changes in prostaglandin formation. Adenosine effects on Ca^{2+} may be associated with prostaglandin formation.

TABLE 2
Subdivision of purinoceptors into P_1 and P_2 types. Current recommendations

Receptor class	P_1 purinoceptors	P_2 purinoceptors
Effector system	G-protein coupled	G-protein coupled Intrinsic ion channel Nonselective pore
Natural ligand(s) so far identified	Adenosine	ATP, ADP, diadenosinetetraphosphate, (UTP?)*

* Whereas UTP is an endogenous compound and a ligand at some P_2 purinoceptors, it remains to be shown that endogenous UTP acts via such receptors in vivo.

blood platelets, was also recognized several decades ago. Studies of the factors in blood that induce platelet aggregation led to the identification of ADP as an active component present in red blood cell extracts (Gaarder et al., 1961). The evidence that ADP and adenosine (presumably A_2) receptors exist on platelets was summarized by Haslam and Cusack (1981).

Four decades ago, ATP was shown to produce important cardiovascular effects (Green and Stoner, 1950) and to be released from sensory nerves (Holton and Holton, 1954; Holton, 1959), hinting at a role in neural transmission. In his landmark review of purinergic nerves, Burnstock (1972) postulated the existence of specific ATP receptors. Although evidence in support of this idea was not overwhelming at the time, many subsequent studies have supported the existence of receptors for extracellular ATP (Burnstock and Brown, 1981; Gordon, 1986; O'Connor et al., 1991). Similarly, the evidence is now compelling that ATP plays important physiological and/or pathophysiological roles in a variety of biological systems, including that of a neurotransmitter in peripheral and central neurons. Finally, diadenosinetetraphosphate is a dinucleotide stored in synaptic vesicles and chromaffin granules (Flodgaard and Klenow, 1982; Rodriguez del Castillo et al., 1988) and released therefrom (Pintor et al., 1991a, 1992). The purine dinucleotide also binds with subnanomolar affinity to receptors (Pintor et al., 1991b, 1993) and exerts biological effects (Pintor et al., 1993), indicating that it is an endogenous purinoceptor ligand.

Thus, strong evidence for the presence of receptors for the endogenous ligands adenosine, ADP, ATP, and diadenosinetetraphosphate had accumulated. This group of receptors is called the *purinoceptors*. If at some future time there is compelling evidence that UTP, or another pyrimidine nucleotide, is an *endogenous ligand* at receptors that respond poorly or not at all to ATP, then this terminology may need revision.

II. General Considerations concerning the Classification of Purinoceptors

Classification of receptors should preferably be based on combined structural and pharmacological information (Kenakin et al., 1992). We are not yet in this ideal situation in the area of purinoceptors, and any proposed classification scheme by necessity must be tentative. In 1978 Burnstock made the important suggestion that there exists a family of receptors called purinergic receptors that can be subgrouped into two subclasses, P_1 and P_2 (Burnstock, 1978). A somewhat extended version of this classification scheme (Burnstock, 1980) is shown in table 1. The scheme has been extremely influential and was adopted by numerous authors in the field. It should, however, be borne in mind that the original criteria have been continuously updated and modified with the availability of new information (see footnotes to table 1). The current criteria for the subclassification are summarized table 2.

Structural information is now available for several subtypes of receptors for adenosine (Maenhaut et al., 1990; Libert et al., 1991; Mahan et al., 1991; Fink et al., 1992; Stehle et al., 1992; Salvatore et al., 1992; Zhou et al., 1992). The same is true for some P_2 receptors (Lustig et al., 1993; Webb et al., 1993; for comparisons between

TABLE 3
*Classification of adenosine receptors (P_1 purinoceptors)**

Name	A_1	A_{2a}	A_{2b}	A_3
Structure	(Name of clone)			
Deduced molecular mass†				
Amino acids	326	410–412	332	320
kDa	37	45	36	36
G-protein coupling	$G_{i(1-3)}$	G_s	G_s	Yes
Effectors‡	↓ cyclic AMP ↑IP_3 ↑ K^+ ↓ Ca^{2+}	↑ cyclic AMP	↑ cyclic AMP	↓ cyclic AMP
Agonists§	*High:* (0.3–3 nM) CPA, CHA, R-PIA, ADAC	*High:* (1–20 nM) NECA, CGS 21680, APEC, Ado	*High:* (0.5–5 μM) NECA	*High:* (<10 nM) APNEA, N^6-benzyl-NECA
	Intermediate: (3–30 nM) NECA, 2-Cl-Ado, Ado	*Intermediate:* (20–200 nM) 2-Cl-Ado, CV 1808, R-PIA, ADAC	*Intermediate:* (5–20 μM) 2-Cl-Ado, Ado, R-PIA	*Intermediate:* (10–30 nM) NECA, R-PIA
	Low: (30–350 nM) S-PIA, DPMA	*Low:* (200–500 nM) CPA, CHA, S-PIA	*Low:* (20–100 μM) S-PIA	*Low:* (100–1000 nM) CGS 21680
	Very low: (>350 nM) CV 1808, CGS 21680, APEC		*Very low:* (>100 μM) CGS 21680, CV 1808	*Very low:* (>1 μM) Ado
Antagonists§	*High:* (0.5–2 nM) CPX,‖ XAC	*High:* (20–100 nM) XAC, CSC, KF 17837, CGS 15943	*High:* (20–100 nM) XAC, CPX,‖ 8-PT, CGS 15943	*High:* (1–20 nM) BW-A 522¶
	Intermediate: (2–200 nM) CPT, 8-PT, CGS 15943	*Intermediate:* (0.2–2 μM) CPT, CPX,‖ 8-PT	*Intermediate:* (0.5–10 μM) 8-pSPT	
	Low: (1–20 μM) Theophylline., 8-pST, IBMX, KF 17387	*Low:* (2–20 μM) DMPX, 8-pSPT, IBMX, theophylline	*Low:* (10–20 μM) Theophylline DMPX, IBMX	
	Very low: (>20 μM) Caffeine, DMPX, CSC	*Very low:* (>30 μM) Caffeine	*Very low:* (>30 μM) Caffeine, KF 17837	*Very low:* (>100 μM) 8-PT, XAC, IBMX
Radioligands	CHA (agonist) CPX‖ (antagonist)	CGS 21680 (agonist) XAC (antagonist)#	None available	^{125}I-APNEA (agonist) ^{125}I-AB-MECA**
Distribution††	Brain (highest in cortex, hippocampus, cerebellum), testis, adipose tissue, heart, kidney	Brain (highest in striatum, nucleus accumbens, tuberculum olfactorium)	Wide. High in gastrointestinal tract	Testis. Wide in some species‡‡

* Abbreviations: BW-A 522, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)-1-propylxanthine; CPA, N^6-cyclopentyladenosine; CHA, N^6-cyclohexyladenosine; R-PIA, N^6-(*R*-phenylisopropyl)-adenosine; S-PIA, N^6-(*S*-phenylisopropyl)-adenosine; ADAC, adenosine amine congener; NECA, 5′-*N*-ethyl-carboxamidoadenosine; CPA, N^6-cyclopentyladenosine; CV 1808, 2-phenylaminoadenosine; CGS 21680, 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; APEC, 2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CPX (DPCPX), 1,3-dipropyl-8-cyclopentylxanthine; XAC, xanthine amine congener; CPT, 8-cyclopentyltheophylline; 8-PT, 8-phenyltheophylline; CGS 15943, 9-chloro-2-(2-furanyl)-5,6-dihydro-[1,2,4]-triazolo[1,5]quinazolin-5-imine monomethanesulfonate; 8-pSPT, 8-*p*-sulfophenyltheophylline; IBMX, 3-isobutyl-1-methylxanthine; KF 17387, 1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine; DMPX, 1,3-dimethyl-7-propylxanthine; CSC, 8-(3-chlorostyryl)caffeine; APNEA, N^6-2-(4-aminophenyl)ethyladenosine; AB-MECA, N^6-(3-iodo-4-aminobenzyl(-5′-*N*-methyl-carboxamidoadenosine; 2-Cl-ado, 2-chloroadenosine; Ado, adenosine.

Additional binding sites for adenosine analogs have been reported: (*a*) Certain adenine derivatives (e.g., 2′,5′-dideoxyadenosine) are inhibitors of adenylyl cyclase via a so called P-site (Londos and Wolff, 1977). (*b*) A ubiquitous nonreceptor-binding site, adenotin, for NECA, has been well characterized from human placenta (Hutchinson and Fox, 1989) and human platelets (Lohse et al., 1988). (*c*) A high-affinity binding site for NECA in the brain with a pharmacological profile that differs from reported adenosine receptors and from adenotin has been described (Lorenzen et al., 1992, 1993). (*d*) A binding site for CGS 21680 with properties that differ from those of A_{2a} receptors has been reported (Johansson et al., 1993). (*e*) A binding site for CV 1808 with pharmacological properties different from known adenosine receptors has been reported and denoted "A_4" by the authors (Cornfield et al., 1992). (*f*) Two further adenosine analogs which, when available, would be useful are 2-hexynyl-NECA (Cristalli et al., 1992) and 2-(*p*-methylphenyl)ethyl-Ado (Daly et al., 1993). The former has intermediate potency at A_1 receptors and high potency at A_{2a} receptors, whereas the latter has low potency at A_1 receptors and high potency at A_{2a} receptors (as defined above). Both presumably will have very low potency at A_{2b} receptors.

several recombinant P_2 receptors, see Barnard et al., 1994). Often, but not always, the information from the cloned receptors has supported earlier attempts at classification based on pharmacological criteria. Only limited structural information is presently available for receptors for adenine nucleotides. Thus, the current recommendations about the classification of receptors for adenosine are firmer than those regarding the adenine nucleotides. The pharmacological evidence is also more substantial in the case of the adenosine receptors than in the case of receptors for adenine nucleotides.

It has been strongly emphasized that antagonists, rather than agonists, are the preferred tools for pharmacological classification (Kenakin et al., 1992). The reason for this is that apparent agonist potency depends strongly not only on agonist binding to the receptor but also on the entire signal transduction machinery. Unfortunately, much of the pharmacological classification in the purinoceptor field rests on relative agonist potencies. Antagonists at adenosine receptors in many instances do not show the degree of selectivity that is ideal. Binding assays can be used for some of the receptors, but ligands are lacking for others. Thus, comparisons between receptors often have been based on different types of assays. In the case of receptors for ADP and ATP, classification is even more problematic in that currently available antagonists have not been unequivocally shown to be specific and selective.

One final consideration is of particular importance in the area of purinoceptors. It has been emphasized that extreme care must be taken to ensure that assays of biological activity are carried out under equilibrium conditions and that complications due to sites of loss or the influence of endogenous ligands are avoided (Kenakin et al., 1992). Because the purine nucleosides and nucleotides are extremely important metabolically, cells have elaborated very efficient systems for their degradation and/or uptake into cells. Such removal mechanisms are present on virtually all cells; this is in contrast to the situation for many neurotransmitters, for which sites of loss are particularly abundant in the nerves that use them but virtually absent elsewhere. Adenosine and several adenosine analogs are rapidly taken up and/or metabolized by transporters and enzymes. The efficiency of this process is extraordinary; the half-life of adenosine injected into the blood stream is on the order of 1 second (Möser et al., 1989). Nucleotides are also very rapidly degraded, and the degradation products are taken up by cells. Some of the nucleotide analogs and their breakdown products interfere not only with the receptors but also with the removal systems. Conversely, the available agents that may reduce the breakdown of the nucleotides have the possibility of interacting with the receptors. Finally, it is often difficult to achieve a temporal equilibrium in the case of at least some adenine nucleotide receptors, which desensitize very rapidly. Consequently, the evidence for subtypes of adenine nucleotide receptors that is based on relative agonist potency in complex biological systems must be regarded as tentative rather than definitive.

The IUPHAR receptor nomenclature committee also has suggested that classification-neutral labels such as 1–2–3 are to be preferred to other labels. In the case of purinoceptors, Burnstock (1978, 1980) proposed a P_1 and P_2 nomenclature for adenosine and adenine nucleotide (ATP, ADP) receptors, respectively. A classification into A_1 and A_2 adenosine receptors was proposed in 1979 (van Calker et al., 1979) and is now generally accepted, along with the A_{2a} and A_{2b} nomenclature. The basis for the latter is that the two cloned receptors show considerable sequence homology and essentially similar signal transduction mechanisms and can be readily distinguished based on pharmacological criteria. The P_1 receptor designation is particularly used to contrast it with P_2 receptors. A series of letters has been allocated in a rather random manner (P_{2X}, P_{2Y}, P_{2T}, P_{2S}, P_{2N}, P_{2U}, P_{2Z}, P_{2D}) for the adenine nucleotide receptors.

TABLE 3—*continued*

† For further details, see Linden et al. (1993b).

‡ This list is not complete. An attempt has been made to limit the presentation to effects that are probably a direct consequence of G-protein interaction. In addition, there are a number of consequences of phosphorylation events.

§ The agonists have been grouped into four categories according to potency: high, intermediate, low, and very low. In the case of A_1 receptors, binding data are from rat brain, rat fat cells, and DDT_1 MF-2 cells. Corresponding functional data exist for rat fat cells and DDT_1 MF-2 cells. The potency figures are from the binding assays. In the case of A_{2a} receptors, the binding data were obtained from rat striatum or PC12 cells. Corresponding functional assays are available in the same preparations (Hide et al., 1992). In the case of A_{2b} receptors, no binding data are available. Most data were obtained from cyclic AMP accumulation in cells or brain slices. The data listed are based on adenylyl cyclase measurements (Bruns, 1981; Brackett and Daly, 1993). The data concerning A_3 receptors is based on adenylyl cyclase in transfected Chinese hamster ovary cells (Zhou et al., 1992).

‖ CPX is also commonly referred to as DPCPX.

¶ Whereas classical xanthines are poor ligands (at least in the rat), some 8-substituted compounds are quite active, at least on human and sheep A_3 receptors (Linden et al., 1993a; Salvatore et al., 1993; Fozard and Hannon, 1993).

XAC has been used as an A_{2a} antagonist ligand, e.g., in human platelets (Ukena et al., 1986).

** Linden et al. (1993), Jacobson et al. (1993c).

†† Distribution information is based on Northern blots (all receptors), in situ hybridization (A_1, A_{2a}), and receptor autoradiography (A_1, A_{2a}).

‡‡ The distribution is reported to be quite restricted in the rat (Zhou et al., 1992) but wide in, e.g., sheep (Linden et al., 1993a).

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                                              TM1
A1                              MPPYISAFQAAYIGIEVLIALVSYPGNVL
A2a                                 MGSSVYITVELAIAVLAILGNVL
A2b                              MQLETQDALVVALELVIAALAVAGNVL
A3                           MKANNTTTSALWLQITYITMEAAIGLCAVVGNML
P2yl   MTEALISAALNGTQPELLAGGWAAGNATTKCSLTKTGFQFYYLPTVYILVFITGFLGNSV
P2u         MAADLEPWNSTINGTWEGDELGYKCRFNEDFKYVLLPVSYGVVCVLGLCLNVV

             TM2                                           TM3
A1     VIWAVKVNQALRDATTFCFIVSLAVADVAVGALVIPLAILLINIGPQTYFHT CLMVACP
A2a    VCWAVWINSNLQNVTNF FVVSLAAADIAVGVLAIPFAITTISTGFCAACHG CLFFACF
A2b    VCAAVGASSALQTPTNY FLVSLAIADVAVGLFAIPFAITTISLGFCTDFHS CLFLACF
A3     VIWVVKLNRTLRTTT FYFIVSLALADIAVGVLVIPLAIAASAWRSRCTSMA CLFMSCV
P2yl   AIWMFVFHMRP WSGISVYMFNLALADFLYVLTLPALIFYYFNKTDWIFGDVMCKLQR F
P2u    ALYIFLCRLKT WNASTTYMFHLAVSDSLYAASLPLLVYYYARGDHWPFSTVLCKLVR F

                                              TM4
A1     VLILTQSSILALLAIA VDRYLRVKIPLRYKTVVTQRRAVAITG CWILSLVVGLTPLFG
A2a    VLVLTQSSIFSLLAIA IDRYIAIRIPLRYNGLVTGVRAKGIIAICWVLSFAIGLTPMLG
A2b    VLVLTQSSIFSLLAVA VDRYLAIRYPLRYKGLVTGTRARGIIAVLWYLAFGIGLTPFLG
A3     LLVFTHASIMSLLAIAAVDRYLRVKLTVRYRTVTTQRRIWLFLGLCWLVSFLVGLTPMFG
P2yl   IFHVNLYGSILFLTCISVHRYTGVVHPLKSLGRLKKKNAVYVSSLVWALVVAVIAPILF
P2u    LFYTNLYCSILFLTCISVHRCLGVLRPLHSLRWGRARYARRVAAVVWVLVLACQAPVLY

                                                      TM5
A1      NNRLSVVEQDWAANGSVGEPV     IKCEFEKVISMEYMVYFNFFVWVLPPLL  LMV
A2a     WNNCSQKDGNSTKTCGEGRVT       CLFEDVVPMNYMVYYNFFAFVLTPLL  LML
A2b    WNSKDRATSNCTEPGNDGITTNKSCCPYKCLFENVVPMSYMVYFNFFGCVLPPLL  IMM
A3      WNRKVTLE             LSQNSSTLSCHFRSVVGLDYMVFFSFITWILIPLV  VMC
P2yl                    YSGTGVRRNKTITCY  DTTADEYLRSYFVYSMCTTVFMFCIPF
P2u                     FVTTSVRGTR ITCH  DTSARELFSHFVAYSSVMLGLLFAVPF

                                                  TM6
A1      LIYLEVFYLIRKQLNKK  VSASS GDPQKYYGKDVKIAKSLALILFLFALSWLPLHILN
A2a     AIYLRIFLAARRQLKQMESQPLPG ERTRSTLQKEVHAAKSLAIIVGLFALCWLPLHIIN
A2b     VIYIKIFMVACKQLQHMELM     EHSHTTLQREIHAAKSLAMIYGIFALCWLPYHAIN
A3      IIYLDIFYIIRNKLSQ    NLTGFRETRAFYGREFKTAKSLFLVLFLFALCWLPLSIIN
P2yl    IVILGCYGLIVKALI   YKDLDNSP LRR       KSIYLVIIVLTVFAVSYLPFHVMK
P2u     SVILVCYVLMARRLLKPAYGTTGGLPRAKR       KSVRTIALVLAVFALCFLPFHVTR

                                  TM7
A1      CITLF          CPT  CQKPSILIYIAIFLTHGNSAMNPIVYAFRIQKFRVTFLK
A2a     CFTFF          CST CRHAPPWLMYLTIILSHSNSVVNPFIYAYRIREFRQTFRK
A2b     CITIF          HPALAKDKPKWVMNVAILLSHANSYYNPIVYAYRNRDFRYSFHR
A3      FVSYF          NVKI    PEIAMCLGILLSHANSMMNPIVYACKIKKFKETYFV
P2yl    TLNLRARLDFQTPQM CAF  NDKVYATYQVTRGLASLNSCVDPILYFLAGDTF
P2u     TLYYSFR   SLDLSCH  TLNAINMAYKITRPLASANSCLDPVLYFLAGQRLVRFARD

A1      IWNDHFRCQPKPPIDEDLPEEKEA
A2a     IIRTHVLRRQEPFQAGGSSAWALAAHSTEGEQVSLRLNGHPLGVWANGSATH
A2b     IISRYYLCQTDTKGGSGQAGGQSTFSLSL
A3      ILRACRLCQTSDSLDSNLEQTTE
P2yl       RRRLSRATRKSSRRSEPNVQSKSEEMTLNILTEYKQNGDTSL
P2u    AKPPTEPTPSPQARRKLGLHRPNRTVRKDLSVSSDDSRRTESTPAGSETKDIRL

A2a    SGRRPNGYTLGLGGGGSAQGSPRDVELPTQERQEGQEHPGLRGHLVQARVGASSWSSEFA

A2a     PS
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FIG. 1. Amino acid sequences of purinoceptors deduced from cloned DNAs, aligned for maximum homology. Note that the P_2 receptor sequences belong to a completely different family than do the adenosine (P_1) receptors. The adenosine receptors listed here are all from the rat: A_1 (Mahan et al., 1991), 326 amino acids; A_{2a} (Fink et al., 1992; Furlong et al., 1992), 410 amino acids; A_{2b} (Stehle et al., 1992), 332 amino acids; A_3 (Meyerhof et al., 1991; Zhou et al., 1992), 319 amino acids. Other such recombinant sequences known are for the human (Salvatore et al., 1992; Libert et al., 1992; Townsend-Nicholson and Shine, 1992), canine (Libert et al., 1991), and bovine (Olah et al., 1992; Tucker et al., 1992) A_1 receptors; the human (Salvatore et al., 1992) and canine (Maenhaut et al., 1990) A_{2a} receptors; and the human (Salvatore et al., 1992) A_{2b} receptor; each of these is extremely homologous to the corresponding rat receptor. The rabbit A_2 receptor was also found to be highly homologous, and genomic cloning revealed that the receptor gene has an intron (Bhattacharya et al., 1993). The chicken P2y1 receptor has 362 amino acids (Webb et al., 1993), and the mouse P2u receptor (Lustig et al., 1993) has 373 amino acids. The approximate start positions of the transmembrane helices, as designated on the basis of hydropathy plots, are shown by the symbols TM1 to TM7.

III. Proposed Receptor Classification

A. Adenosine (P_1) Receptors

The terms adenosine receptor or P_1 purinoceptor are used to designate this family of receptors. The term P_1 is useful in situations in which comparison is made between P_1 and P_2 purinoceptors. The subtypes of adenosine/P_1 purinoceptors are designated as A_1, A_2, A_3, ... receptors and can be further divided, e.g., into A_{2a}, A_{2b} (table 3); this is in agreement with the above-mentioned general recommendation for subtype numbering of the IUPHAR committee on receptor nomenclature. Additions of receptors into this scheme will be made if, and only if, both structural and pharmacological evidence indicate a specific subtype. Such criteria involve evidence that the structure is different from that of already established members of the adenosine family *in the same species*. This information must be supplemented by the demonstration that either the receptor has a unique distribution among cells and tissues or the cloned receptor exhibits a unique pharmacology. Because all adenosine receptors as yet characterized are G-protein coupled (see below), unique pharmacology should be demonstrated in an expression system in which the receptor couples to relevant G-proteins.

The predicted amino acid sequences of some of the recombinant adenosine receptors are shown in figure 1. There appear to be four major classes of these receptors. The A_{2a} receptors are similar to the A_{2b} receptors in the transmembrane parts but differ from the A_{2b} (and other adenosine receptor types) in having a considerably larger COOH-terminal domain. There are differences in the primary structure of adenosine receptors of a single subtype cloned from different species, which may help to explain the differences that have been shown in binding studies (Ferkany et al., 1986; Stone et al., 1988).

G-protein-coupled receptors show some structural similarities with bacteriorhodopsin, the structure of which has been determined with high-resolution electron cryomicroscopy (Henderson et al., 1990). Three-dimensional models of G-protein-coupled receptors may be constructed using this as a template (Hibert et al., 1991; Dudley et al., 1993), and attempts to model the ligand-receptor interaction have been made (van Galen et al., 1990; van der Wenden et al., 1992). Functional models of the adenosine receptors may serve as an aid in the synthesis of novel ligands (Ijzerman et al., 1992; van Galen et al., 1992; Jacobson et al., 1993b). A schematic representation of some aspects of these models is shown as figure 2.

All of the recombinant adenosine receptors have the general structure that would place them in the rhodopsin-like group of the superfamily of G-protein-coupled receptors. The A_2 receptors have been defined on the basis of their ability to stimulate adenylyl cyclase. Thus, they probably interact with the G-protein, G_s. It is not known whether there are other G-proteins that can interact with A_2 receptors. Similarly, it is not known whether G_s activated by adenosine receptors can interact with effectors other than adenylyl cyclase (cf. the β-adrenoceptor in the heart which activates a Ca^{2+} channel

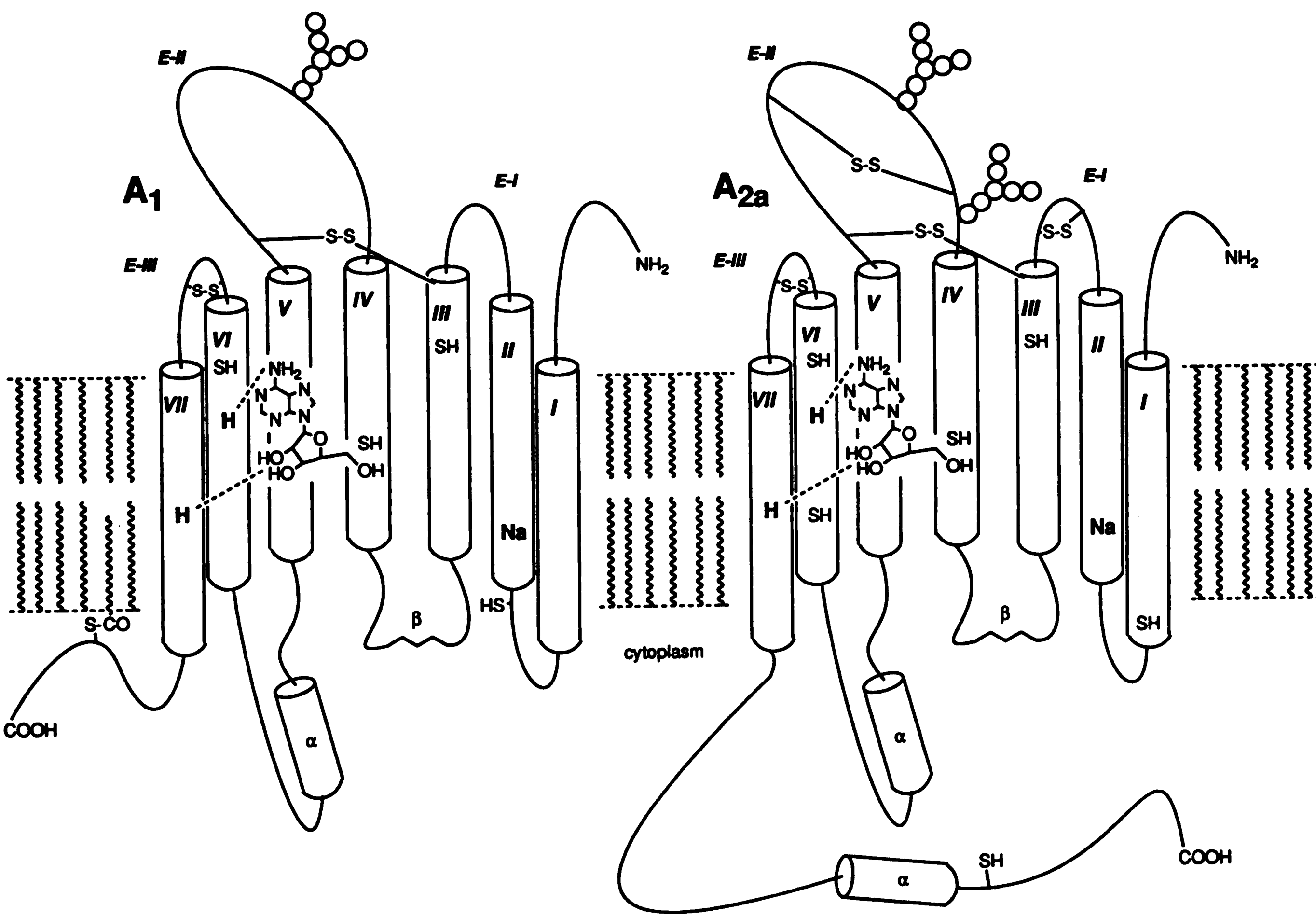
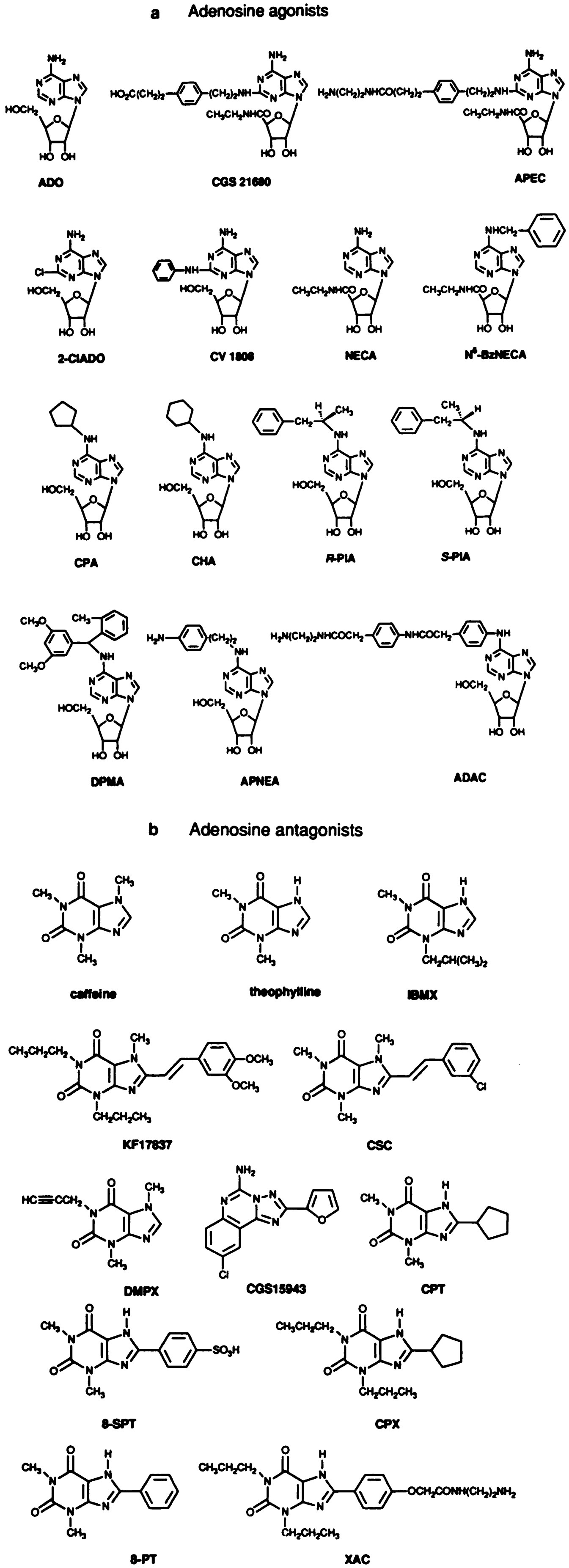


FIG. 2. Proposed models of A_1 and A_{2A} adenosine receptor proteins based on sequence analysis (van Galen et al., 1992) and computer-assisted molecular modeling (Ijzerman et al., 1992). The seven transmembrane helices (I through VII) are arranged in a counterclockwise orientation (looking from the extracellular side) according to the experimentally determined structure of bacteriorhodopsin. Actually, I and VII are in proximity, forming a barrel shape, which surrounds the ligand-binding site. Histidyl residues (H) in the sixth and seventh helices are proposed to hydrogen bond to adenosine, through the purine N^6- and ribose 2′,3′ positions, respectively. The locations of cysteinyl residues (SH) and hypothetical disulfide bridges (S-S, Jacobson et al., 1993d) are indicated. Glycosylation occurs on the second extracellular loop (E-II) in both receptors. In the A_1 receptor, a potential palmitoylation site (S-CO) is shown as forming an additional anchor of the carboxy-terminal segment in the phospholipid bilayer. Cytoplasmic segments show a hypothetical secondary structure (α and β), predicted using computational algorithms (van Galen et al., 1992).

via G_s), but experience from other signal transduction cascades suggests that this is a distinct possibility.

The A_1 receptor has been shown to couple with $G_{i\text{-}1}$, $G_{i\text{-}2}$, $G_{i\text{-}3}$, and G_o but not with G_s or G_z (Freissmuth et al., 1991; Munshi et al., 1991). In practically all instances (but see Fredholm et al., 1989; Thompson et al., 1992), responses to adenosine A_1 receptor activation are blocked by pertussis toxin, which is compatible with an involvement of the G_i/G_o family of G-proteins. In agreement with this, adenosine A_1 receptors can induce a variety of different cellular responses, including inhibition of adenylyl cyclase (van Calker et al., 1978; Londos et al., 1980), stimulation of K^+ conductance (Trussell and Jackson, 1985), inhibition of a Ca^{2+} conductance, probably through an N-type channel (Scholz and Miller, 1991), stimulation of phospholipase C, and generation of a Ca^{2+} and protein kinase C signal (Gerwins and Fredholm, 1992, 1994). Other reported effects include inhibition of inositol phospholipid hydrolysis (Kendall and Hill, 1988; Delahunty and Linden, 1988) and inhibition of transmitter release by a mechanism that does not involve a change in membrane K^+ and Ca^{2+} conductances (Scholz and Miller, 1991). Based on evidence from other G-protein-coupled receptors it seems likely that some of these responses are mediated by the α-subunits of the G-proteins, whereas other effects may be due to the β,γ-subunits (Birnbaumer, 1992). Probably the degree of activation of the G-protein, and hence of the receptor, may differ by orders of magnitude depending on which subunit(s) mediates the response (Birnbaumer, 1992). The broad range of signaling responses emphasizes that it is not fruitful to attempt to subclassify adenosine receptors solely on the basis of whether the effects are mediated via cyclic AMP or not (Ribeiro and Sebastiao, 1986; Fredholm and Dunwiddie, 1988). Furthermore, the absolute potency of agonists in producing an effect can-



not be used to classify receptors. Regarding the newly identified A_3 receptor (Zhou et al., 1992; Meyerhof et al., 1991), little is so far known about its G-protein coupling. Because A_3 receptors mediate inhibition of adenylyl cyclase, a G_i-like protein is a probable partner.

A list of drugs that appear to be useful to classify adenosine receptors is given in figure 3. The battery of pharmacological tools currently available for the classification of adenosine receptors is far from optimal, especially for use in a functional context, and some points need to be emphasized.

First, sensitivity to methylxanthines cannot be taken as a universal sign of adenosine receptor involvement. Methylxanthine-induced blockade of a response is still highly suggestive of an involvement of adenosine receptors, but a lack of inhibition can no longer be taken as conclusive evidence against an adenosine receptor being involved. Because the methylxanthine-insensitive A_3 receptor (table 3) may be present in significant quantities outside the testis, several older reports of methylxanthine-insensitive adenosine effects may have to be reinterpreted. Indeed, in a recent report it was shown that N^6-2-(4-aminophenyl)ethyladenosine which shows a high affinity for the A_3 receptors (Zhou et al., 1992) produces xanthine-insensitive hypotension in pithed rats (Fozard and Carruthers, 1993). The A_3 receptor also appears to mediate xanthine-resistant adenosine actions on mast cells (Ramkumar et al., 1993). It was recently found that N^6-benzyl-*5′-N-ethyl-carboxamidoadenosine* may be an A_3-selective agonist (14-fold vs. A_1 and A_{2a} receptors; van Galen et al., 1993). Some 8-phenyl-substituted xanthines are potent antagonists, BW-A 522 being the most potent, with nanomolar affinities at least at ovine and human A_3 receptors (Linden et al., 1993a; Salvatore et al., 1993; Fozard and Hannon, 1993).

Second, antagonists that are both selective and easily soluble are established for the A_1 receptor type. However, both absolute and relative potencies for these selective antagonists at A_1 receptors may differ among species. The more selective antagonists at A_2 receptors (Sarges

FIG. 3. Ligands used to classify adenosine receptors. Adenosine agonists (a) include those that are selective for A_1 receptors [N^6-cyclopentyladenosine (CPA) > N^6-cyclohexyladenosine (CHA) > N^6-(*R*-phenylisopropyl)-adenosine (R-PIA)], A_{2a} receptors {2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CG21680)>2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (APEC) > 2-phenylaminoadenosine (CV1808)}, and A_3 receptors [N^6-benzyl-5′-*N*-ethyl-carboxamidoadenosine (NECA)]. NECA and 2-chloroadenosine (2-ClADO) are essentially nonselective. Adenosine antagonists (b) include such that are A_1 selective {1,3-dipropyl-8-cyclopentylxanthine (CPX, or sometimes DPCPX); 8-cyclopentyltheophylline (CPT) [and xanthine amine congener (XAC)]}, those that are A_{2a} selective [8-(3-chlorostyryl)caffeine (CSC); 1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF 17837)], moderately A_2 selective [1,3-dimethyl-7-propylxanthine (DMPX) and 9-chloro-2-(2-furanyl)-5,6-dihydro-[1,2,4]-triazolo[1,5]quinazolin-5-imine monomethanesulfonate (CGS 15943)}. Caffeine, theophylline, 3-isobutyl-1-methylxanthine (IBMX), and 8-*p*-sulfophenyl theophylline are essentially nonselective. The latter compound penetrates the blood-brain barrier poorly. (For other abbreviations, see footnote to table 3.)

TABLE 4
*Reported P_2 purinoceptors**

Name	P_{2X}	P_{2Y}	P_{2U}	P_{2T}	P_{2Z}	P_{2D}
Structure (known)	No	Yes†	Yes‡	No	No	No
Molecular mass						
(aa)			373‡			
(kDa)		42†	42	43§		
Type	Intrinsic ion channel‖	G-protein-coupled¶	G-protein-coupled#	G-protein-coupled	Nonselective pore	G-protein coupled?
Effectors	Na^+, K^+, Ca^{2+}	↑IP_3/Ca^{2+}/DAG; ↓cAMP, ↑phospholipase A_2(↓K^+-conductance††	IP_3/Ca^{2+}/DAG; Ca^{2+}, Cl^-, and K^+ currents	IP_3/Ca^{2+}/DAG; cAMP	Na^+, K^+, Ca^{2+}	↑Ca^{2+}**
Agonist	αβ-MeATP ≥ βγ-MeATP > ATP ≥ ADP > 2MeSATP >> UTP‡‡	2-MeSATP > ATP = ADP >> αβ-MeATP >> UTP§§	UTP ≥ ATP = ATPγS >> 2-MeSATP = αβ-MeATP‖‖	2-substituted ADP > ADP¶¶	ATP^{4-}	Ap_4A > ADPβS > AMP-PNP > Ap_5A > αβ-MeATP >> 2-MeSATP
Antagonist	Desensitization## by αβ-MeATP ANAPP$_3$ Suramin*** (pK_B = 5.0) PPADS††	Suramin (pA_2-5.0)‡‡‡	None known§§§	ATP (pA_2 = 4.6)‖‖‖ Suramin (pA_2 = 4.6)¶¶¶·### FPL 66096 (pK_B = 8.7)###		None known
Radioligand	[^{3}H]D-αβ-MeATP (pK_H = 9.0; pK_L = 7.0****	[35S]ADPβS (affinity: pK_d = 8.0)††††		β[^{32}P]2-MeSADP (pK_d = 8.0)‡‡‡‡ [^{35}S]ATPαS (pK_d = 8.5)§§§§		[^{3}H]Ap_4A (1 × 10^{-10} M; 0.6 μM)‖‖‖‖
Distribution	Smooth muscles, brain, heart, spleen¶¶¶¶	Wide distribution####	Wide. Found in many cultured cells and in vascular muscle	Platelets	Mast cells,***** macrophages, vas deferens†††††	Chromaffin cells, rat brain synaptosomes

* Abbreviations: IP_3, inositol triphosphate; DAG, diacylglycerol; Me, methyl; cAMP, cyclic AMP; PNP, Ap_4A, diadenosine tetraphosphate; Ap_5A, diadenosine pentaphosphate; ANAPP$_3$, arylazido aminopropionyl ATP.

† Webb et al. (1993).

‡ Lustig et al. (1993).

§ Determined by photoaffinity labeling using 2-(*p*-axidophenyl)-ethylthioadenosine 5′-diphosphate (Cristalli and Mills, 1993).

‖ Benham and Tsien (1987), Dubyak (1991), Bean (1992).

¶ Responses to P_{2Y} receptors are often, but not always, blocked by pertussis toxin indicating involvement of G_i/G_0 proteins. Often (Bruner and Murphy, 1993) pertussis toxin affords a partial blockade indicating involvement of also G_q/G_{11} proteins. In some instances (e.g., C6-2B glioma cells) the response (↓ cyclic AMP) is fully pertussis toxin sensitive.

P_{2U} receptor-mediated responses are often only partially blocked by pertussis toxin, suggesting that they are mediated by both G_i/G_0 and by G_q/G_{11} proteins (Gerwins and Fredholm, 1992).

** The increase in $[Ca^{2+}]_i$ appears to be via mobilization of intracellular stores (Castro et al., 1992).

†† Shen and North (1993); Dubyak, 1991; Boyer et al., 1993.

‡‡ There are some minor differences between the potency order as determined in functional assays (e.g., rabbit ear artery; O'Connor et al., 1990) and in binding assays (Bo and Burnstock, 1992). The channel activity studied by patch-clamp is somewhat different in that ATP is equipotent with αβ-MeATP and βγ-MeATP (Bean, 1992).

§§ Important differences have been noted. For relaxation of rat pulmonary vessels the potency order (relative to that of the most potent compound in the series) was 2-MeSATP (1) > ATP (0.02) = ADP (0.02) > βγ-MeATP (0.01) > αβ-MeATP (0.006) (Liu et al., 1989); responses to activation of the cloned P_{2Y1} receptor showed the order: 2-MeSATP (1) = ATP (1) > ADP (0.05) >> αβ-MeATP, whereas binding to turkey erythrocytes shows the order: 2-MeSATP (1) > ATP (0.1) ≥ ADP (0.07) > αβ-MeATP (0.002) > βγ-MeATP (0.0006) (Cooper et al., 1989).

‖‖ UTP is sometimes considerably more potent than ATP (van Rhee et al., 1993).

¶¶ Cusack and Hourani (1981; 1982a), MacFarlane (1983), Greco et al. (1991).

Desensitization is often rapid and may be irreversible, especially with photoactivated ANAPP$_3$ (Hogaboom et al., 1980; Kasakov and Burnstock, 1983).

*** Leff et al. (1990).

††† Ziganshin et al. (1993).

‡‡‡ Hoyle et al. (1990).

§§§ There are reports that suramin is (van der Zee et al., 1992) and is not (Wilkinson et al., 1993) an antagonist.

‖‖‖ Cusack and Hourani (1982b).

¶¶¶ Hourani et al. (1992).

2-Propylthio-D-β,γ-difluoromethylene ATP (Humphries et al., 1993).

**** Bo and Burnstock (1990).

†††† Cooper et al. (1989).

‡‡‡‡ MacFarlane et al. (1983).

§§§§ Greco et al. (1991).

et al., 1990; Shimada et al., 1992) have usually not been examined in well-characterized functional systems, and information derives mainly from binding assays. Recent data indicate that 1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine is a selective A_{2a} antagonist in functional assays (Shimada et al., 1992; Fredholm et al., unpublished observations). A related xanthine, 8-(3-chlorostyryl)caffeine, was recently found to be a very selective A_{2a} antagonist (vs. A_1), also in vivo (Jacobson et al., 1993b). A whole family of 8-styrylxanthines with A_{2a} selectivity and varying physicochemical properties has been described (Jacobson et al., 1993a). These compounds are not yet readily available, which limits their usefulness in defining criteria for receptor classification.

Third, CGS 21680 and other 2-substituted adenosine analogs play an important role in pharmacological subclassification of A_2 receptors. These compounds discriminate well between A_{2a} and A_{2b} receptors. Cellular responses to CGS 21680 (and related compounds) may in addition depend critically on factors, such as receptor density and amounts and types of G-proteins and adenylyl cyclases, and not solely on the presence or absence of a specific adenosine A_{2a} receptor subtype. However, recent data suggest that CGS 21680 may also bind to a structure, which may be a functional receptor, that is different from hitherto recognized adenosine receptors (Johansson et al., 1993; Cunha, Johansson, and Fredholm, unpublished data).

B. P_2/ATP Purinoceptors

The first overview of the functional effects of various ATP analogs was given by Burnstock and Kennedy (1985). By analyzing the nature of the responses to ATP and related compounds in a number of different biological systems, they discriminated between two major classes of receptors that were named P_{2X} and P_{2Y} purinoceptors, respectively. The two postulated P_2 receptors were discriminated on the basis of response profiles to a number of ATP analogs: $\alpha\beta$-MeATP > $\beta\gamma$-MeATP > ATP = 2-MeSATP = ADP for the P_{2X} subtype; 2-MeSATP > ATP ≫ $\alpha\beta$-MeATP = $\beta\gamma$-MeATP for the P_{2Y} subtype. P_{2X} receptors were also proposed to be quickly desensitized by $\alpha\beta$-MeATP. The agonist response profiles in the guinea pig isolated bladder and taenia coli were considered to represent prototypical P_{2X} and P_{2Y} receptors (Burnstock, 1991; Cusack, 1993). These studies were extended to other smooth muscle preparations, and P_{2X} and P_{2Y} purinoceptor activation was correlated with contraction and relaxation, respectively, but exceptions have been noted (Bailey and Hourani, 1992). The classification (table 4) rests very much on the potency of phosphothioate ATP derivatives, which were originally thought to be "nonhydrolyzable," but many have more recently been shown to be degraded by ectonucleotidases (Cusack, 1993). Among the analogs 2-MeSATP, $\alpha\beta$-MeATP, L-$\beta\gamma$-MeATP may be particularly useful in discriminating between P_{2X} and P_{2Y} receptors (Hourani et al., 1985). Desensitization by $\alpha\beta$-MeATP and $ANAPP_3$ (table 4) has so far not been reported to occur except at P_{2X} receptors. Novel selective agonists for P_{2X} and P_{2Y} receptors have been introduced (Fischer et al., 1993; Burnstock et al., 1994), and studies with these ligands suggest that the receptors may exist in several subtypes.

Most recent evidence suggests that the P_{2X} purinoceptor family represents an intrinsic ion channel permeable to Na^+, K^+, and Ca^{2+} (Bean, 1992). P_{2Y} purinoceptors constitute G-protein-linked receptors, often coupled to stimulation of phospholipase C activity and, hence, to inositol trisphosphate formation (O'Connor et al., 1991), but additional transduction mechanisms, including modulation of cyclic AMP generation (Okajima et al., 1989; Yamada et al., 1992; Boyer et al., 1993) and arachidonic acid mobilization (Bruner and Murphy, 1990, 1993) have also been demonstrated.

After the 1985 Burnstock and Kennedy proposal, Gordon (1986) further subdivided the P_2 purinoceptors by assigning the name P_{2T} to the receptor for ADP on blood platelets (Humphries et al., 1993) and P_{2Z} for the "receptor" that mediates responses to ATP^{4-} in mast cells (Dahlqvist and Diamant, 1974) and macrophages (Steinberg and Silverstein, 1987), which appears to represent the opening of a fairly nonselective type of pore. There is now good evidence that there are receptors that respond to UTP, ATP, and ATPγS, but not to 2-MeSATP or $\alpha\beta$-MeATP, which has led to the definition of the so-called "P_{2U}" or "nucleotide" or "pyrimidine" receptor (table 4; O'Connor et al., 1991; Dubyak, 1991). There

TABLE 4—*continued*

‖‖ High-affinity sites represent less than half the total binding sites (Pintor et al., 1993). The second set of figures within parentheses are K_i values for a low affinity site.

¶¶¶¶ Contractile responses in smooth muscle have been well characterized, e.g., in rabbit mesenteric artery (Burnstock and Warland, 1987) and rabbit ear artery (O'Connor et al., 1990). Binding has been studied, e.g., in rat bladder, brain, heart, vas deferens, and spleen (Bo and Burnstock, 1990; Michel et al., 1993). ATP-induced channel activity has been studied, e.g., in PC12 cells, but this probably is a secondary event (Nakazawa et al., 1991), sensory neurons (Krishtal et al., 1983; Bean et al., 1990) and ear artery muscle (Benham and Tsien, 1987).

Functional responses have been studied, e.g., in guinea pig aorta (Dainty et al., 1992) and in turkey erythrocytes (Berrie et al., 1989). Binding has been studied in turkey erythrocytes (Cooper et al., 1989).

***** Dahlqvist and Diamant (1974), Steinberg and Silverstein (1987).

††††† Fedan et al. (1990).

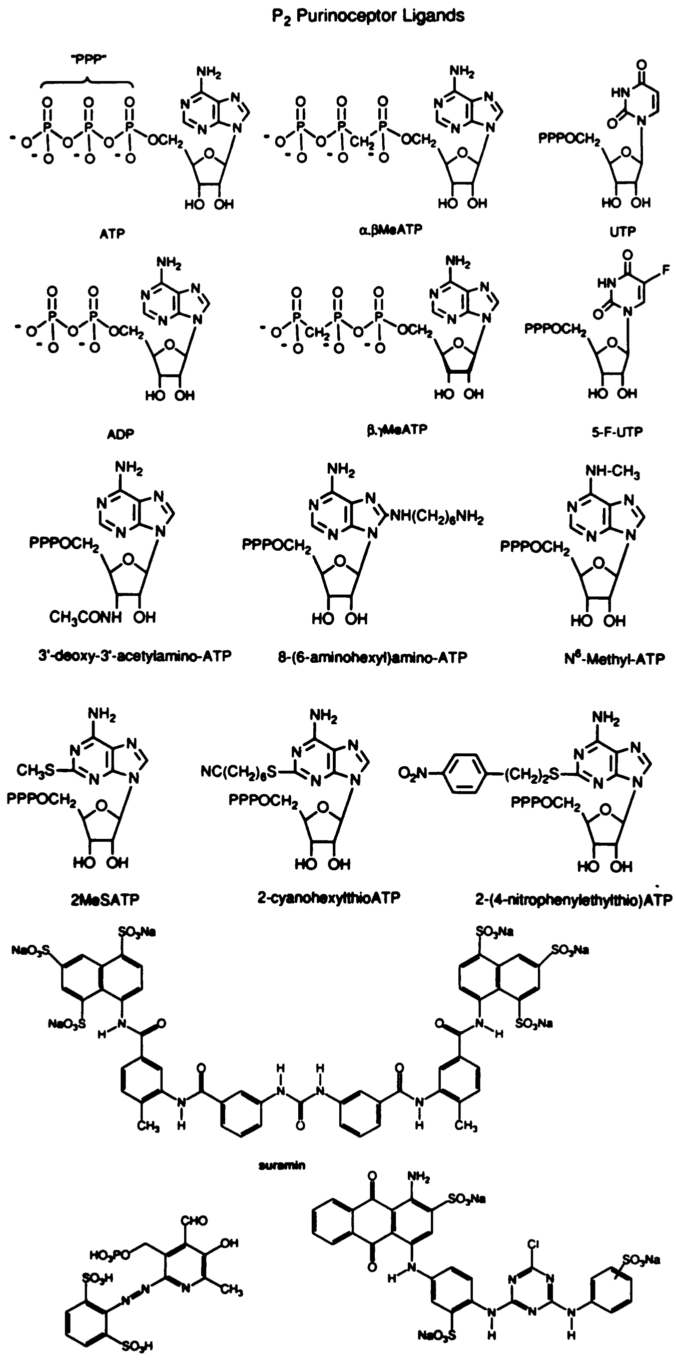


FIG. 4. Ligands used to characterize P_2 purinoceptors. The structures shown are of the D-isomers. Some studies have also been done on L-isomers. α,β-methylene (Me) ATP is a potent and selective P_{2X} agonist. 8-(6-aminohexylamino)ATP is selective for P_{2Y} vs. P_{2X} receptors and may discriminate between subforms (Burnstock et al., 1994). UTP and 5-F-UTP are active at P_{2U} but not at P_{2Y} receptors. 2-MethylthioATP is active at P_{2Y} but not at P_{2U} receptors. None of these are very active at P_{2X} receptors. The long-chain functionalized congeners (Fischer et al., 1993) 2-(*p*-nitrophenylthio)ATP and 2-(6-cyanhexylthio)ATP maintain or increase potency at P_{2Y} receptors. The nitro derivative may discriminate between forms of P_{2X} receptors. N^6-methyl ATP may discriminate between forms of P_{2Y} receptors. The structures of some compounds—suramin, pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), and Reactive Blue—that have antagonistic properties are also shown.

also appears to be a receptor for diadenosinetetraphosphate, which was called a P_{2D} subtype (Hilderman et al., 1991; Castro et al., 1992). Some characteristics of the P_2 purinoceptors are summarized in table 4.

A problem that has always hampered research in the P_2 purinoceptor field is the lack of selective antagonists. The trypanoside suramin (Dunn and Blakeley, 1988; Voogd et al., 1993) sometimes behaves as a competitive antagonist but does not appear to distinguish between the P_{2X} and P_{2Y} subtypes (Hoyle et al., 1990). Suramin is also very effective in inhibiting the actions of certain growth factors (Betsholtz et al., 1986; Peng et al., 1991), presumably secondarily to interactions with the corresponding receptors (Eriksson et al., 1991). The potency of suramin against basic fibroblast growth factor (Peng et al., 1991; IC_{50} is in the low micromolar range) is at least as high as against P_2 receptors. Suramin is also an inhibitor of several enzymes, including 5'-nucleotidase (Hourani and Chown, 1989), something that is of particular concern when the compound is used to discriminate between P_1 and P_2 actions. 2-2'-Pyridylisatogen was reported to be a weakly selective antagonist of the relaxant effects of ATP in smooth muscle (Spedding et al., 1975) but did not antagonize the effects of adenosine (Spedding and Weetman, 1976). Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid, synthesized by Lambrecht and co-workers (1992), is a novel type of P_2 antagonist with a potency at P_{2X} receptors in the nanomolar range (Ziganshin et al., 1993). The displacement of [^{3}H]$\alpha\beta$MeATP was biphasic, suggesting multiple affinity sites or multiple receptor subtypes (Ziganshin et al., 1993). Reactive Blue 2 has been reported to selectively antagonize ATP actions at the P_{2Y} subtype, although concentration and time of exposure are critical. The structures of a number of compounds useful for the study of P_2 purinoceptors are shown in figure 4.

Molecular information concerning P_2 purinoceptors is becoming available. *Xenopus* oocytes injected with mRNA from embryonic guinea pig brain (Fournier et al., 1990; Honoré et al., 1991), promyelocytic leukemia cells (HL60; Murphy and Tiffany, 1990), J774 murine macrophage-like cells (Hickman et al., 1993; Nuttle et al., 1993), or guinea pig vas deferens (Russell et al., 1993) were conferred with the ability to respond to ATP. The pharmacology corresponded to that of several of the proposed P_2 receptor subforms.

P_{2Y} (Webb et al., 1993) and P_{2U} purinoceptors (Lustig et al., 1993) have recently been cloned. As shown from figure 1, these receptors are more similar to each other than they are to adenosine receptors. Interestingly, they are not closer to the adenosine receptor than to other G-protein-coupled receptors. Based on such a comparison of these sequences with a more recently cloned ADP receptor, it has been proposed that the G-protein-coupled P_2 purinoceptors will constitute a distinct family within the superfamily of G-protein-coupled receptors (Barnard et al., 1994).

P_{2Y} purinoceptor-mediated responses show different agonist pharmacology in a variety of tissues and preparations (Burnstock, 1991; Fischer et al., 1993), suggesting a subclassification of the "classic" P_{2Y} purinoceptor. Other data suggest that the currently designated "P_{2U}," "P_{2T}," and "P_{2D}" purinoceptor subtypes may have to be reclassified.

On these grounds it must be emphasized that the current classification of the P_2 series must be considered unsatisfactory for the long term. When additional structural information is obtained and truly selective antagonists become available, a revised nomenclature will be established. Even now it is clear that there is a basis for distinguishing two major families of P_2 purinoceptors, one coupled to intrinsic ion channels and the other coupled to G-proteins. For the transition period the IUPHAR Committee on Receptor Nomenclature and Drug Classification, in keeping with the proposal by Abbracchio and Burnstock (1994), recommends that any new subtypes of G-protein-coupled receptor be termed $P2Y_1$, $P2Y_2$, $P2Y_3$,... purinoceptors and any new subtypes of intrinsic ion channel be termed $P2X_1$, $P2X_2$, $P2X_3$,... purinoceptors. The 2X and 2Y are not subscripted, to avoid confusion with previous usage and to facilitate the use of lower case, as in $p2y_1$, $p2y_2$,... to refer to cloned receptors whose correspondence to a pharmacologically defined subtype has not been firmly established. Lower case is being used in this way in other IUPHAR nomenclatures, e.g., adrenoceptors, muscarinic cholinoceptors, and 5-hydroxytryptamine receptors. The term P2Z purinoceptor should be reserved for novel receptor structures that do not correspond to the P2X and P2Y purinoceptor structure. A possible example could be the mast cell P_2 purinoceptor, if this is established to be a nonselective ion pore opened by ATP. Although not an ideal system of classification, it does allow consecutive numbering and obviates the need for arbitrary designation of letters when new subtypes are identified.

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