Perspective

Purine and Pyrimidine (P2) Receptors as Drug Targets

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Introduction

Physiological evidence supporting the role of the adenine nucleotide, ATP **1** (Figure 1), as a neurotransmitter was first summarized in detail in 1972,[1] representing the basis of Burnstock's now seminal purinergic nerve hypothesis. The latter was refined in 1978 with the delineation of distinct P1 (adenosine) and P2 (ATP) receptor classes.[2] However, it is only in the past decade that definitive evidence for a family of discrete molecular targets responsive to ATP and other nucleotides, the P2 purinergic receptor family, has been obtained using molecular biological, pharmacological, and medicinal chemistry approaches.[3–11] Thus, there is now a wealth of compelling data to support a role for extracellular purine (ATP, ADP **2**) and pyrimidine (UTP **5**, UDP **6**) nucleotides acting as neurotransmitter/neuromodulators to modulate the function of a diversity of mammalian cell types and tissues under both normal and pathophysiological conditions.[3,11–15]

The concept that high-energy phosphate bond containing molecules, such as ATP, which play such a key role in all aspects of cell function, might also function as extracellular chemical messengers was initially met with considerable skepticism.[16] However, all molecules mediating cellular communication involve energy-dependent processes that involve the salvage of their breakdown products with the resynthesis of the active moiety. For ATP, this involves adenosine reuptake and the subsequent phosphorylation of the nucloside,[17] and for peptide neurotransmitters, the reassembly of the individual amino acids via energy-dependent peptide bond formation is necessary. In addition, ATP is a molecule that can be produced on demand, with the body being able to produce its own weight of the nucleotide in a single day.[18] Thus, even though extracellular ATP levels can reach millimolar concentrations in the local environment following release or cellular perturbation resulting from tissue trauma, these concentrations are relatively minor when compared to the overall steady-state nucleotide content of the cell.[16] ATP can also be constitutively released with steady-state basal extracellular concentrations in the range of 3 nM.[19] ATP and UTP can also be released by shear stress from a variety of tissues.[20,21] Intracellular ATP release occurs by vascular release, cytolysis, and activation of ABC (ATP-binding cassette) proteins.[21] The dynamic regulation of ATP production by cells may also have provided a specific selective pressure for the evolutionary progression from unicellular to multicellular organisms,[22] a role that sustains the functional importance of the purine nucleotide and its evolutionary conservation as an energy source.

ATP functions as a pluripotent signaling molecule, eliciting direct effects on cell function via its ability to activate the P2 receptor family and more indirectly via its conversion to ADP **2**, AMP **3** and adenosine **4** via a family of ectonucleotidases (E-NTPases),[23–25] resulting

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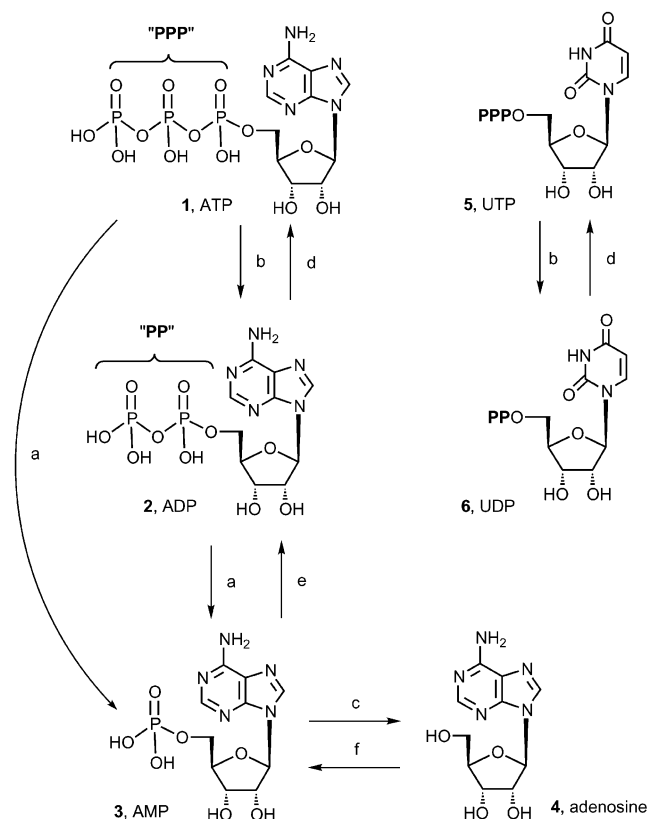
Figure 1. Metabolic interconversion of naturally occurring purine and pyrimidine nucleotides of relevance to extracellular action. Purine (adenosine derivatives, **1**–**4**) and pyrimidine (uridine derivatives, **5** and **6**) nucleosides and nucleotide are shown. "P" refers to a single phosphate group; thus, "PP" is diphosphate, etc.: (a) ectoapyrase, NTPDase1; (b) ecto-ATPase, NTPDase2; (c) 5′-nucleotidase; (d) nucleoside diphophokinase; (e) AMP kinase; (f) adenosine kinase.

in a purinergic cascade (Figure 1),[11] the functional end product of which is adenosine, the endogenous ligand for the P1 receptor family.[3,26] There is also emerging data that inorganic phosphate has the potential to function as a signaling molecule to induce gene expression.[27] ATP and its analogues can also modulate the activity of other key ATP binding proteins including the K_{ATP} ion channel family,[28,29] neuronal nicotinic acetylcholine receptors,[30,31] capsaicin-activated ion channels,[32,33] as well as E-NTPases,[34] ectoprotein kinases,[35] acetylcholinesterase, and neuromuscular neuronal nicotinic receptor expression,[36] thus making cellular responses to the nucleotide potentially complex and multifunctional. The actions of the nucleotide are therefore dependent on the enzymes and receptors modulated by ATP that are present in the extracellular milieu. The expression of these targets can change during tissue development and also as a result of disease pathophysiology.[37–40] This type of neurotransmission has been termed "domain specific",[41] with the extracellular availability of the neurotransmitter, the extent of its metabolism, and the tissue/disease specific array of its potential recognition sites determining the ultimate response of the target organ. In addition to this neurotransmitter role, ATP can also effect intercellular information transfer via adenylate charge[42] and can also modulate the set-point of signal transduction pathways affecting responses to other neurotransmitters and hormones.[43]

In the current Perspective, advances in the understanding of the molecular biology, physiology, and function of the P2 purinergic receptor family in the decade since the last Perspective on purinergic receptors[44] are reviewed together with ongoing medicinal chemistry efforts in identifying novel agonists and antagonists. In addition, the therapeutic potential for P2 receptor ligands, including ATP and UTP (Figure 1) and receptor antagonists,[13] in a variety of human disease states that include cancer, chronic obstructive pulmonary disease (COPD), chronic bronchitis, asthma, bladder and erectile dysfunction, reproduction, auditory and ocular function, pain, hemostasis (platelet aggregation, neutropenia and leukemia), neurodegeneration, and immune system function are reviewed. An alternative, complementary approach to the discovery of novel drugs interacting with the P2 receptor system is that of targeting the enzymes modulating ATP (and UTP) metabolism. However, this approach is at too early a stage to warrant coverage in the present review.

Evolution of the P2 Receptor Family

The initial characterization of P2 receptors[2] was empirically based on the rank order agonist potency of a series of ATP analogues (Figure 2) to activate functional responses in a variety of mammalian tissue preparations. While a number of distinct classes of P2 antagonists have been described (Figures 3–5), these are frequently lacking in both selectivity for, and between, P2 receptors.[45,46] The effects of both P2 receptor agonists and antagonists also show significant tissue and species dependency that further complicates pharmacological classification.[46,47] In contrast, the discovery of the alkylxanthines, caffeine, and theophylline, as adenosine receptor antagonists in the 1970s,[48,49] provided a concrete basis for an extensive effort in P1 (adenosine) receptor medicinal chemistry, resulting in the identification of several clinical drug candidates based on the xanthine pharmacophore some 20 years before the cloning of this receptor family.[50] Furthermore, synthetic chemistry efforts in the area of nucleoside triphosphates have been somewhat limited,[51] with a systematic focus on developing structure–activity relationships for the various P2 receptors and high-throughput screening approaches to identifying novel pharmacophores being relatively recent.[11,13,52–55]

Despite the inherent limitations of P2 receptor characterization in the 1980s, the concept of ATP-sensitive P2 receptors per se was expanded to encompass what were then designated as P_{2X} and P_{2Y} receptors in 1985,[56] the P_{2X} receptor being potently activated by the hydrolysis-resistant ATP bioisosteres (Figure 2A), α,β-methylene ATP (α,β-meATP, **10**) and β,γ-methylene-ATP (β,γ-meATP, **11**). Accordingly, these ATP analogues were inactive at P_{2Y} receptors, with 2-methylthioATP (2-MeSATP, **13**, Figure 2B) being the most potent agonist at this P2 receptor subtype. Pharmacological evidence for the existence of the P_{2T} platelet ADP-sensitive receptor, a pyrimidine (uracil nucleotide), P_{2U} receptor, and the pore-forming mast cell P_{2Z} receptor was summarized in the following year.[57] Unique pyrimidine-sensitive receptors, insensitive to purines, were proposed in 1989,[15] and further evidence for their existence was derived in the subsequent decade.[58,59]
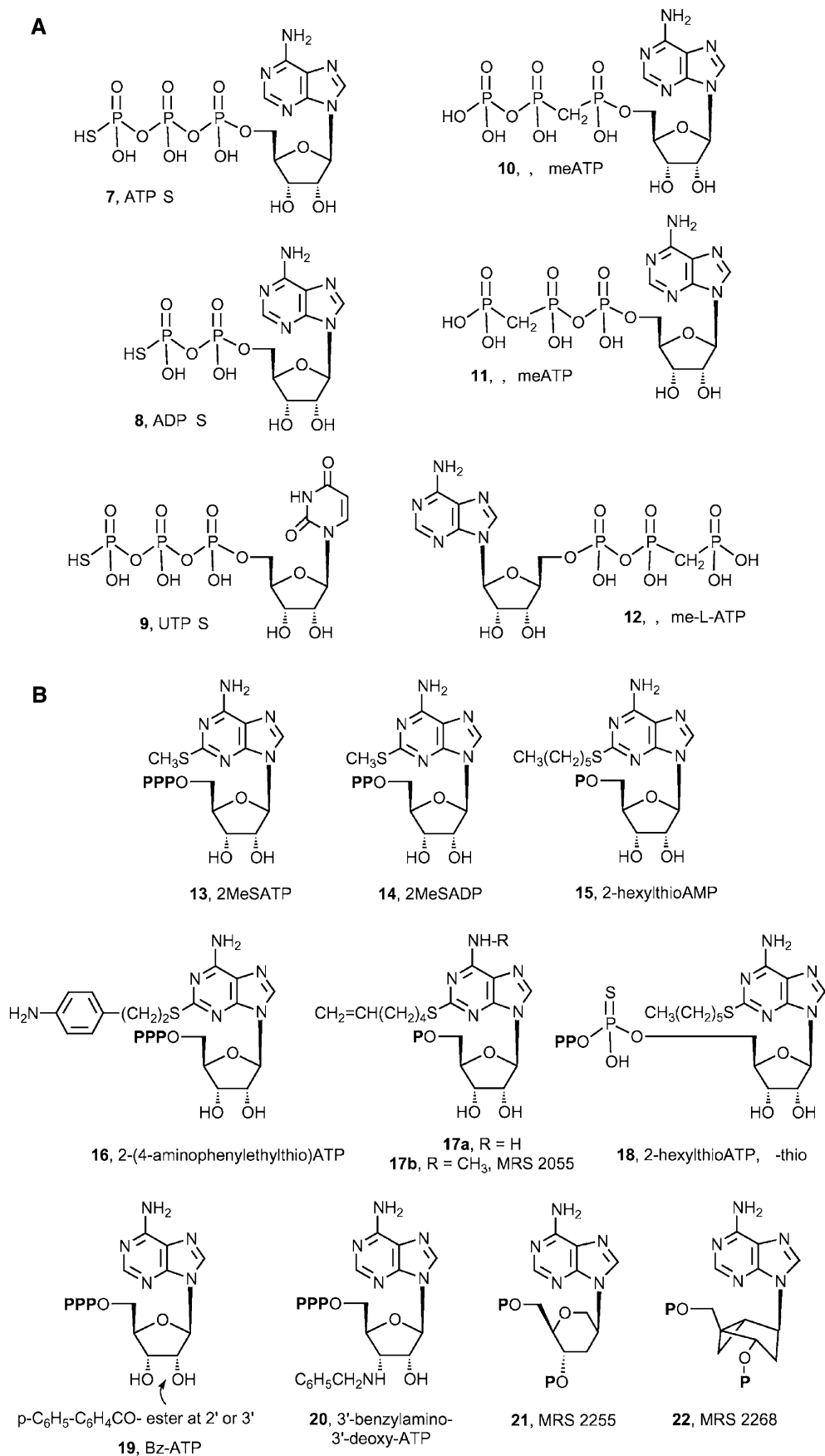
Figure 2. (A) Structures of selected adenine nucleotides modified on the phosphate moiety that have been investigated as P2 receptor agonists. (B) Structures of selected adenine nucleotides modified on the base and ribose moieties that have been investigated as P2 receptor agonists. "P" refers to a single phosphate group; thus, "PP" is diphosphate, etc.
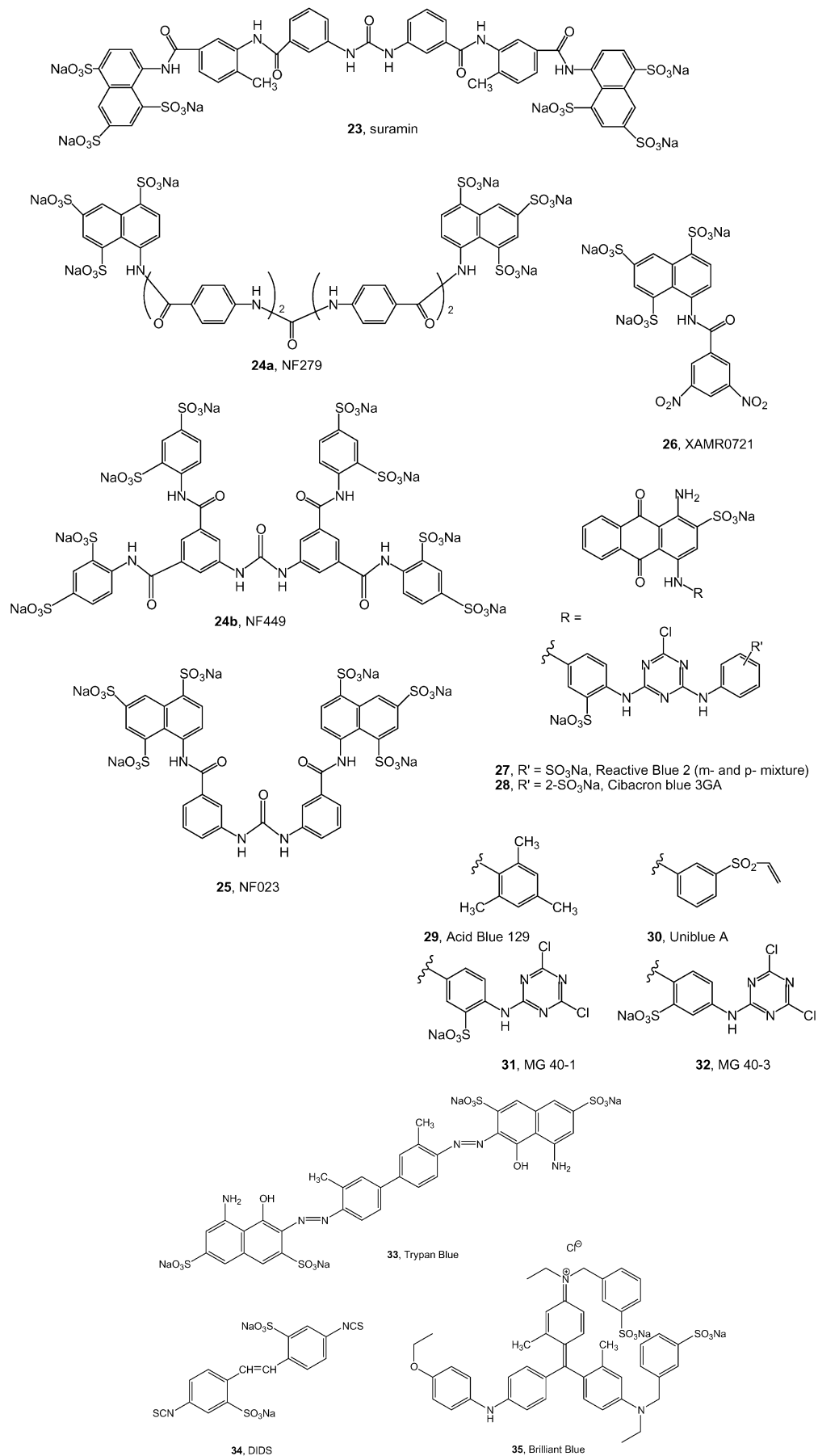
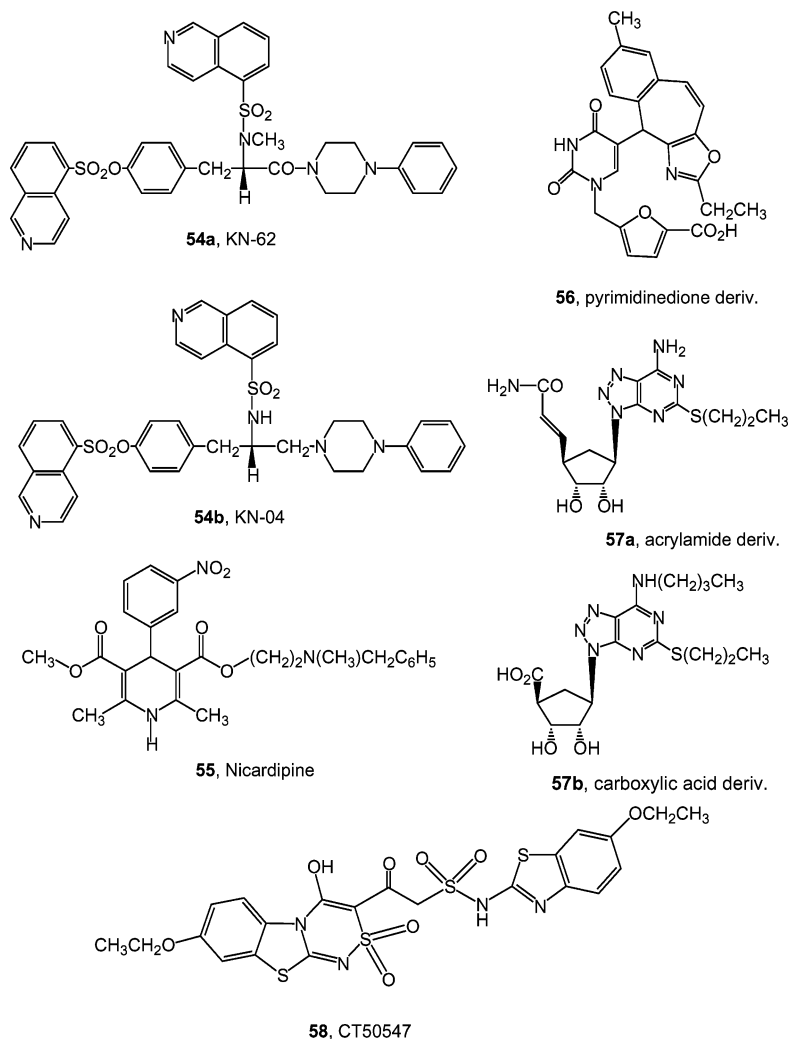**Figure 3.** Structures of selected polysulfonated aromatic molecules.
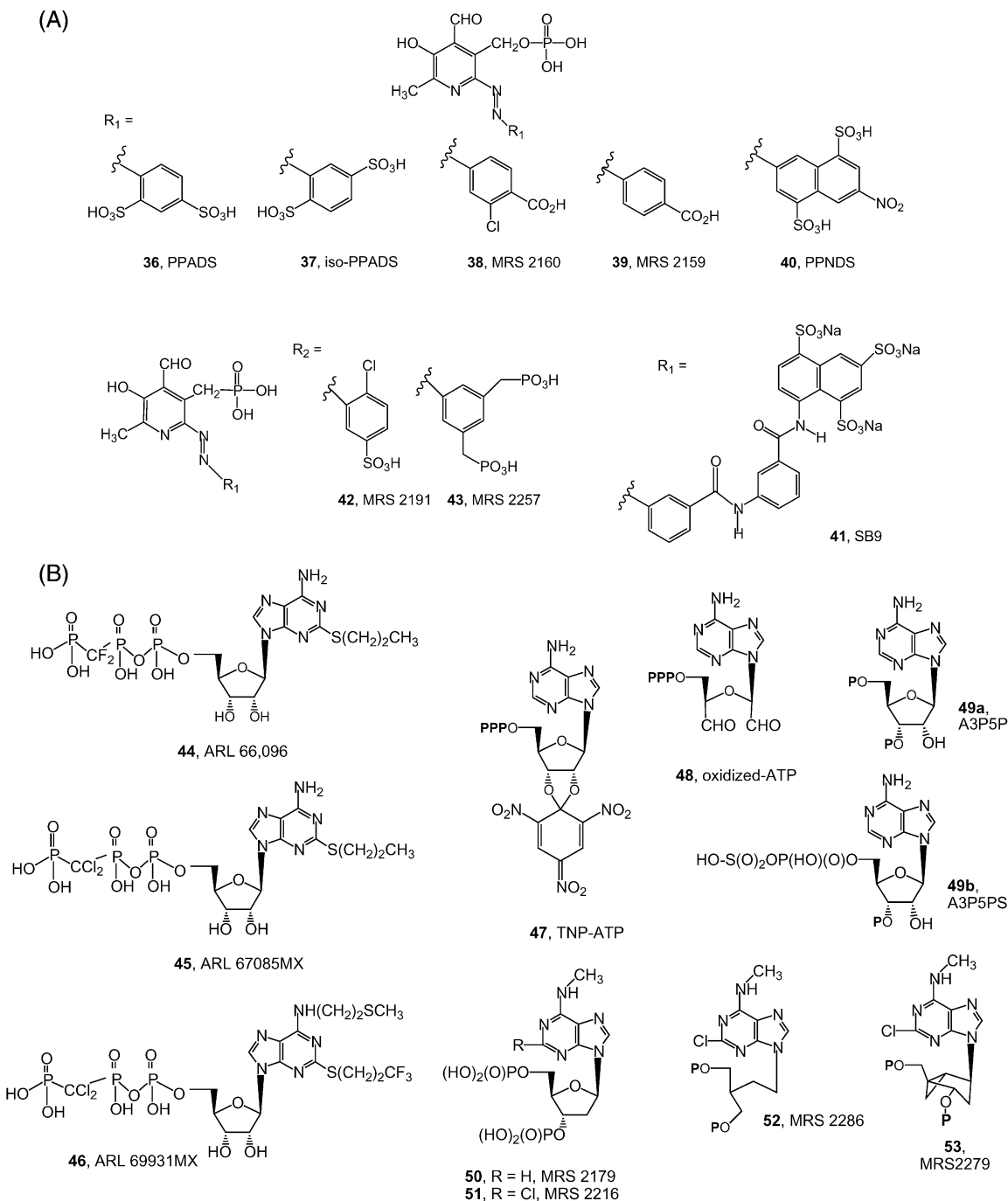
Figure 4. (A) Pyridoxal phosphate derivatives. (B) Adenine nucleotide derivatives.

In 1994, following from the seminal review of Dubyak and El Moatassim[60] and the initial successes in the cloning of P2 receptors, Abbracchio and Burnstock[61] proposed a division of P2 receptors into two separate classes based on both their structure and signal transduction properties: the P2X (Table 1), a family of ionotropic ligand gated ion channels (LGICs); the P2Y (Table 2), a metabotropic, heptahelical G-protein-coupled receptor (GPCR) family. This proposal formed the basis of the subsequent IUPHAR nomenclature subcommittee recommendations for P2 receptors.[62–64] Other putative P2 receptors including a UDP-glucose-sensitive GPCR (KIAA0001) cloned as an orphan receptor from human brain,[65] the putative P_{2D}/$P2Y_{Ap4A}$ dinucleotide receptor responsive to dinucleotide ligands (Figure 6) including Ap_4A **59a**, Ap_5A **60**, and Ap_6A **61**,[66,67] and a putative

P3 receptor responsive to both nucleosides and nucleotides[68] have been reported. The existence of these additional nucleotide-sensitive receptors has been based partly on their pharmacological characterization, again with compounds that have inherent limitations in their selectivity and potency. The dinucleotide and "P3" receptors have yet to be reported as having been cloned. A $P2X_7$ receptor paralog gene has been identified in the draft human genome sequence.[69]

It is important to note that many of the functional effects of Ap_4A and related purine and pyrimidine polynucleotides may be ascribed to the activation of other known P2 receptors,[70] and it is possible that these molecules may have the potential to act as depot sources for the generation of other nucleotides. As noted by Ralevic and Burnstock,[3] in the absence of definitive

54a, KN-62

56, pyrimidinedione deriv.

54b, KN-04

57a, acrylamide deriv.

55, Nicardipine

57b, carboxylic acid deriv.

58, CT50547

Figure 5. Non-phosphorylated and non-sulfonate derivatives that have been shown to act as P2 receptor antagonists.

molecular evidence for the existence of these other P2 receptors, "receptor subclassification based on pharmacological criteria alone is no longer tenable". Thus the nomenclature system based on receptor structure has increasingly supplanted the pharmacologically based historical system in the literature. For instance, many of the receptors described previously as "P2U/P_{2U}" are equivalent to the P2Y$_2$ receptor while the P$_{2Z}$ receptor has been cloned as the P2X$_7$ receptor[71] and the elusive P$_{2T}$ as the P2Y$_{12}$ receptor.[72]

The use of structural homology alone is, however, an insufficient basis for receptor classification. This is illustrated by the original designation of a p2y7 receptor,[73] which was, in fact, a GPCR for the leukotriene LTB$_4$.[74] The recent discovery of the orphan GPCR, SP1999,[75] which is identical to the cloned P2Y$_{12}$ receptor,[72,76] illustrates that both structural and pharmacologically relevant functions are necessary criteria for receptor classification. A novel sensory P2X receptor has also been identified in *Zebrafish*.[77]

Complexities of P2 Receptor Pharmacology

A variety of factors have tended to confound the pharmacological characterization of both native and recombinant P2 receptors. These include the already mentioned early dependence of P2 receptor classification on the rank order functional efficacy of ATP and UTP

analogues used as agonists and a lack of potent, selective, and bioavailable antagonists. In addition, the intrinsic activity and metabolic stability of the various P2 agonists can also vary as a function of the purity of the compounds used, the experimental protocol, the tissue(s) systems in which their effects are assessed, and the species used.[45–47,78] In addition to a lack of selectivity for the different P2 receptors,[45–47] many P2 receptor antagonists reported to date have the potential to interact with other biological targets. Suramin **23** (Figure 3), one of the most widely used P2 receptor antagonists, antagonizes G proteins[79] and can inhibit proteases including HIV reverse transcriptase.[80] Furthermore, ATP can function as an agonist at the rat P2Y$_4$ receptor but as an antagonist ($K_B = 700$ nM) at the human receptor homologue.[81] Receptor reserve/expression may also be a critical factor in defining pharmacophore efficacy, since ATP can function as a partial agonist[82] or antagonist[83] at human P2Y$_1$ receptors. Furthermore, many commercially available preparations of P2 receptor ligands are chemically heterogeneous or impure. For example, many of the early studies using Reactive Blue 2 **27** as a P2 antagonist relied on samples that contained only 40–50% of the active entity with the remaining components being unknown or uncharacterized. Similarly, many commercial preparations of ATP can be contaminated with UTP.
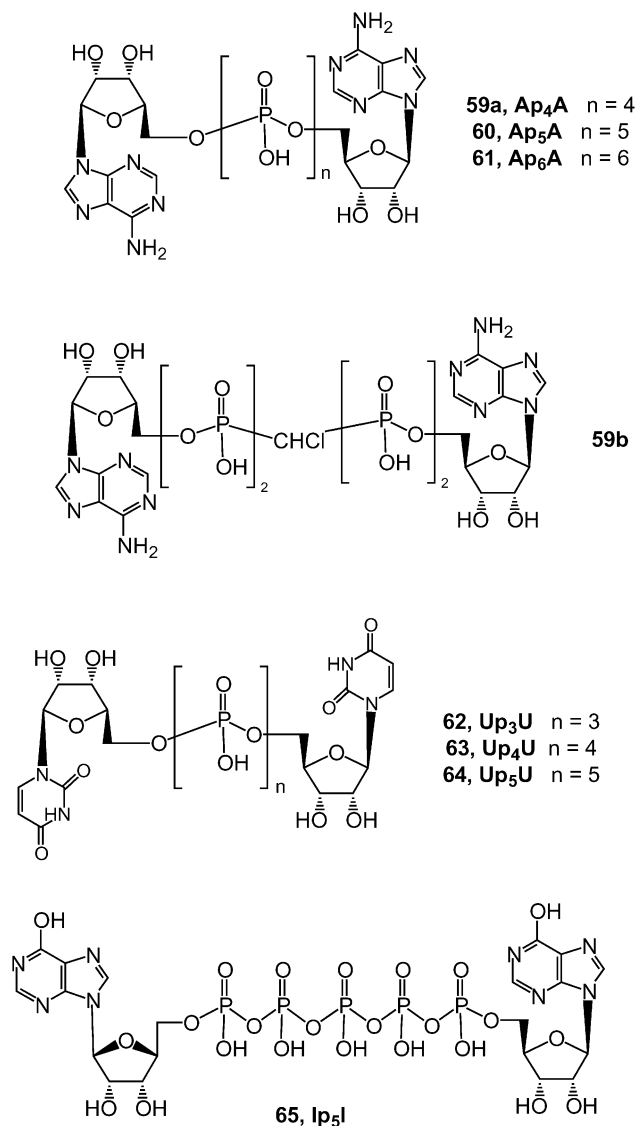
Figure 6. Structures of dinucleotide derivatives of adenine, uracil, and hypoxanthine.

There are also marked differences in the properties of native and expressed receptors both in terms of receptor expression number and in regard to the associated proteins absent (or present) in either expression systems or the natural milieu. These may provide markedly different data because of the consequent receptor density, the propensity of the expressed receptor to interact with atypical protein partners, and the nature of the reporter construct.[84] At transfected P2X$_4$ receptors, the anthelminthic ion channel blocker, avermectin noncompetitively modulates agonist responses. However, this effect does not occur in vivo.[85] Also, in native cell types, evidence exists for more than one P2 receptor subtype being expressed in a single cell. This is especially true for P2X receptors where the presence of unique, pharmacologically distinct heteromers formed from the various P2X receptor subunits in different permutations may be dynamically regulated.[86−89] This further confounds data extrapolation from one system to another. Rapid agonist-induced desensitization of P2-receptor-mediated responses would thus lead to the characterization of a compound as an antagonist.[3] In the case of the quickly desensitizing P2X$_3$ receptor

heteromer, agonist-mediated desensitization, resulting in functional receptor antagonism, can occur in the absence of detectable receptor activation.[90]

Even with bioisosteric replacements of the labile phosphate groups of ATP, e.g., **10** and **11**, these compounds can be readily hydrolyzed by members of the ectonucleotidase/E-NTPase family.[25] The activity of this family of enzymes is highly tissue-dependent and also varies according to the functional state of the tissue.[37−40,91] The net result of E-NTPase action is a reduction in the observed potency of ATP, UTP, and their respective analogues as they are broken down to nucleoside 5′-diphosphates (ADP, UDP) and to the nucleosides adenosine (from ATP) and uridine (from UTP).[25] This modification of agonist potency, which may vary from compound to compound, can then alter the rank order activity of agonists, leading to a receptor characterization that is, in part, dependent on the lability of the agonist rather than the intrinsic recognition properties of the ligand for the receptor. This represents a generic problem when selective receptor antagonists are not available.[92] Nucleotidases can also be released in soluble form under physiological conditions where they act to limit the effects of released ATP[93] with distinct species differences.[94] Since adenosine activates P1 receptors and purines can also modulate P2 receptor function,[95,96] the functional effects of these metabolic products become even more complex via the context of the purinergic cascade.[11] Finally, nucleosides also undergo extracellular rephosphorylation to regenerate both the parent nucleotides and exchange phosphates between purines and pyrimidines.[23] Several ATP analogues as well as putative P2 receptor antagonists such as ARL 66096 **44** can inhibit the enzyme ectonucleoside triphosphate diphosphohydrolyase (E-NTPDase; CD39 exists in two forms, one of which, E-NTPDase2, is specific for ATP),[97] leading to an augmentation of the effects of endogenous agonists, ATP, and/or UTP. Thus, a compound, by inhibiting CD39 activity and increasing endogenous levels of ATP, would appear to have agonist actions. The ATP derivative ARL 67156 (**66**, Figure 7) is a "nonreceptor ligand" that has been optimized to inhibit endogenous ATPase activity.[98]

A lack of reliable radioligand binding assays for the characterization of P2 receptors has led to additional confusion in the molecular characterization of P2 receptors. While various bioisosteres of ATP (e.g., [^{3}H]α,β-meATP **10**, [^{35}S]ADPβS **8**, [^{35}S]ATPγS **7**) have been used to localize P2 receptors in various tissues,[99,100] studies using transfected cells that lack functional responses to P2 agonists show high levels of specific binding of [^{35}S]ADPβS. This binding actually decreases by 25% when a functional response is introduced via transfection of the cells with cDNA for the P2X$_4$ receptor subunit.[101] [^{33}P]MRS 2179 **50** (Figure 4B), a high-affinity P2Y$_1$ receptor antagonist, has recently been developed as a receptor probe.[102]

Another critical issue in defining receptor function, not unique to P2 receptors, is that of assigning significance to changes in receptor mRNA expression versus actual receptor protein synthesis. While mRNA expression measured by Northern blot analysis may be altered as a result of tissue or cell manipulation or in diseased tissues, such message lacks function at the extracellular
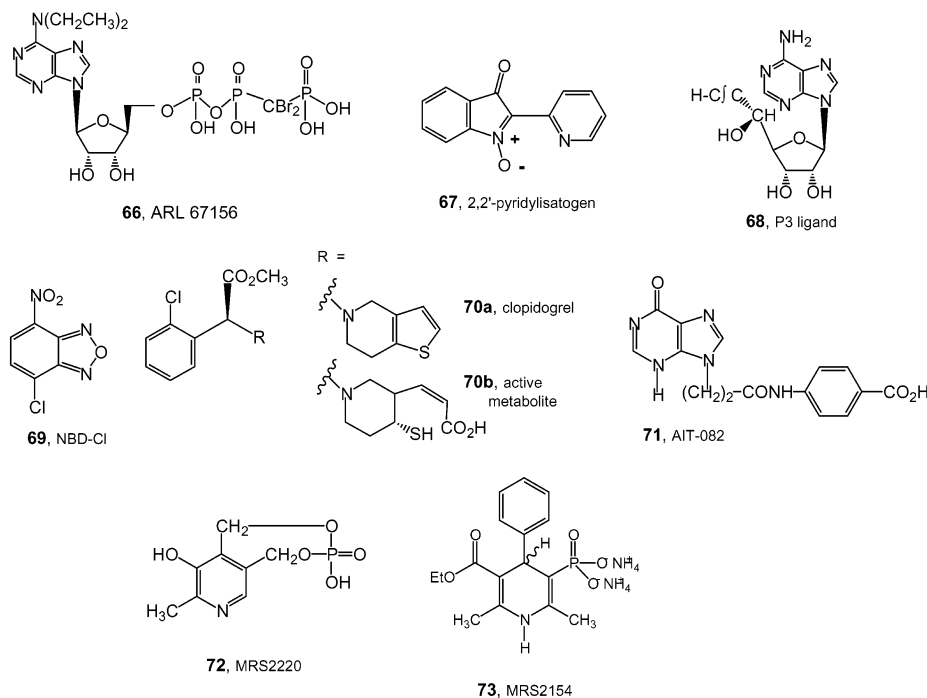
Figure 7. Structures of miscellaneous modulators of P2 receptor action, including ion channel potentiators, inhibitor of E-NTPDase, an allosteric modulator, an irreversible receptor inhibitor, and antagonist precursor for in vivo biotransformation.

level, it is only when changes in receptor protein levels are detected at the cell surface (Western blot) that definitive conclusions can be drawn regarding the physiological and pathophysiological significance of such changes.

P2 Receptors

Sixteen molecularly and functionally distinct mammalian P2 receptors have been generally accepted at the time of writing (Tables 1 and 2), the physiological function(s) of which, and their role in tissue homeostasis and pathophysiology, are currently being elucidated using a variety of pharmacological and genomic approaches including receptor antisense and receptor knockout and knockin mice.[3]

1. P2X Receptors. Functional P2X receptors are ATP-gated (ligand-gated) ion channels that mediate fast excitatory neurotransmission in excitable tissues including neurons, glia, and smooth muscle cells. ATP can elicit rapid responses (<10 ms) via these ion channels, resulting in selective permeability to Na^+, K^+, and Ca^{2+} cations.[4,9] The membrane depolarization resulting from the activation of P2X receptor multimers can lead to activation of voltage-operated ion channels, L-type Ca^{2+} channels, and Ca^{2+}-stimulated tyrosine kinases that in turn activate MAP kinases (ERK1 and ERK2) that modulate transcriptional processing.[103] MAP kinase activation is also involved in $P2Y_1$-receptor-mediated apotosis.[104]

Seven P2X receptor subunits with a two-transmembrane (2TM) motif structurally related to the amiloride-sensitive epithelial Na^+ channel of approximately 100 amino acids in length with intracellular termini and a cysteine-rich extracellular loop have been cloned and designated $P2X_{1-7}$. The degree of sequence identity between these subunits ranges from 26% for $P2X_2$ and $P2X_7$ to 47% for $P2X_1$ and $P2X_4$.[3] These subunits resemble proton-gated channels at a global but not

primary structure level[105] and form functional homomeric and heteromeric channels[86-89] that currently available evidence suggests exist as stretched trimers (Figure 2),[106,107] contrasting with the pentameric stoichiometry of other LGICs, e.g., the neuronal nicotinic receptor superfamily.[108] Unlike the latter, very little is known regarding the nature of the ATP binding site(s) on the proposed trimer, of distinct antagonist binding sites, of associated allosteric binding sites, or of ancillary proteins necessary for native receptor function.

P2X subunits, with the exception of the $P2X_6$ receptor,[4,109] can form functional homomers that are activated by ATP.[86-89] The reason for the inability of $P2X_6$ receptor subunits to form functional receptors is unclear. Functional heteromers composed of $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$, $P2X_{2/6}$, and $P2X_{1/2}$ subunits have been described.[86-89,109,110]

While there is convincing evidence for functional heteromers from in vitro studies, in situ analysis studies of P2X receptors have resulted in the identification of additional subunit combinations, e.g., a putative $P2X_{2/5}$ heteromer, that are pharmacologically distinct from those described above.[111] P2X receptors also appear to undergo activation-dependent modifications that include cellular internalization for $P2X_1$ receptors[89,112,113] or redistribution at synaptic junctions for the $P2X_2$ receptor.[114]

On the basis of their kinetic parameters, P2X receptors have been divided into three groups.[3] Group 1, which includes $P2X_1$ and $P2X_3$ homomers, is potently and rapidly activated by agonists (0.01−0.1 s) and undergoes rapid inactivation (0.1−10 s) in the presence of prolonged agonist activation. Group 2 includes $P2X_2$, $P2X_{2/3}$, $P2X_{2/6}$, and $P2X_5$ multimers that are rapidly activated (0.1−1 s) and show slow inactivation/desensitization (10−100 s) profiles. Group 3 P2X multimers include the $P2X_{1/5}$, $P2X_4$, $P2X_{4/6}$, and $P2X_7$ receptors. These show the same activation kinetics as group 2

Table 1. Mammalian P2X Receptor Genes and Functions and Localization

subunit	amino acids	GenBank accession no.			chromsomal localization	signal transduction	desensitizaiton kinetics	distribution	gene disrruption
		human	rat	mouse					
$P2X_1$	399	U45448	X80477	X84896	17p13.3	$I_{Na/K/Ca^{2+}}$	fast	smooth muscle	decreased male fertility
$P2X_2$	472	AF190822	U14414	AH009288		$I_{Na/K}$	slow pore formation	sensory neurons, brain, pancreas	
$P2X_3$	397	Y07683	X90651	undisclosed[a]	11q12	$I_{Na/K/Ca^{2+}}$	fast	nociceptive sensory neurons	antinociception bladder hyporeflexia
$P2X_4$	288	X87763	Y07684	AF089751	12q24.32	$I_{Na/K}$	slow pore formation	brain, testis, colon	
$P2X_5$	417	AF016709	X92069		17p13.3	$I_{Na/K/Ca^{2+}}$	slow	heart, adrenal medulla	
$P2X_6$	379	AB002058	X92070	AB010883		$I_{Na/K/Ca^{2+}}$	slow	brain	
$P2X_7$	595	Y09561	X95882	AJ009823	12q24.2	$I_{Na/K}$	slow pore formation	macrophages, mast cells, microglia	altered cytokine (pro-IL-1β) processing

[a] Sequence described in ref 140.

multimers but have both a fast and slow desensitization phase. In general, those P2X receptors that undergo rapid desensitization are activated by α,β-meATP, 2-MeSATP, and ATP. P2 receptors that undergo slower desensitization or show no desensitization can be subdivided into two groups, those sensitive to α,β-meATP ($P2X_{1/5}$, $P2X_4$, $P2X_{4/6}$) and those that are only weakly sensitive to this agonist ($P2X_7$).

1.1. $P2X_1$. The human, rat, and mouse $P2X_1$ subunits (Table 1) were originally cloned from vas deferens[115] and urinary bladder.[116,117] The functional $P2X_1$ receptor can be rapidly activated by ATP ($pEC_{50} = 7.3$) and its analogues (0.01−0.1 s) and undergoes rapid desensitization (0.1−10 s). The rank order potency for agonist activation of recombinant $P2X_1$ receptors was BzATP (**19**, $pEC_{50} = 8.8$) ≫ 2-Me-SATP ≥ ATP > α,β-meATP ≫ ADP. Agonists at this receptor are potently and selectively blocked by Ip_5I (**65**, $pEC_{50} = 8.0$)[70] as well as by other antagonists including TNP-ATP **47**, the suramin analogues, NF023 **25**, NF279 **24a**, NF449 **24b**, and the PPADS **36** analogues, MRS 2159 **39**, and PPNDS **40** (Figure 4A). Messenger RNA for the $P2X_1$ receptor subunit is expressed in urinary bladder, smooth muscle layers of small arteries and arterioles, vas deferens, lung and spleen, dorsal root, trigeminal and celiac ganglia, spinal cord, and brain.[115−117] A $P2X_1$ receptor has also been identified in platelets and megakaryocytes.[118] Deletion of the $P2X_1$ receptor gene in male mice[119] results in a 90% reduction in fertility, the consequence of a decrease in the amount of sperm in the ejaculate, a reflection of a 60% reduction in the contraction sensitivity of the vas deferens to sympathetic nerve stimulation and an abolition of the responsiveness to P2X receptor agonists.

1.2. $P2X_2$. The human rat $P2X_2$ subunit was initially cloned from pancreas and pheochromocytoma PC12 cells.[120−122] At the recombinant $P2X_2$ receptor, 2-Me-SATP ($pEC_{50} = 5.8$) and ATPγS are equipotent as agonists, with α,β-meATP and β,γ-meATP being inactive. PPADS (**36**, $pEC_{50} = 5.4$) blocks $P2X_2$ activation. This P2 receptor multimer is both less permeable to and has higher sensitivity to extracellular Ca^{2+}.[123] Decreases in pH enhance $P2X_2$ receptor responsiveness to ATP,[124] indicating that protons can modulate ATP affinity. $P2X_2$ message is found in pancreas, pituitary gland, vas deferens, bladder, brain, spinal cord, superior cervical ganglia, and adrenal medulla.[120−122,125] The $P2X_2$ receptor is present in rat cochlear,[126] and functional splice variants of this receptor that differ in desensitization

kinetics are present in rat cochlea and pituitary gland ($P2X_{2-1}$)[127−130] and rat cerebellum ($P2X_{2-2}$).[131] In contrast, functional splice variants of the human $P2X_2$ receptor do not differ in acute desensitization kinetics and can form functional heteromeric receptors with $P2X_3$ subunits.[132] Modeling of the expressed $P2X_2$ homomer indicates that receptor activation requires three ATP binding steps that involve binding sites that function in a positively cooperative manner.[133]

1.3. $P2X_3$. The $P2X_3$ subunit was originally cloned from rat dorsal root ganglion.[86,134] The potency order for agonist activation of the recombinant homomeric $P2X_3$ receptor is BzATP ($pEC_{50} = 7.1$) ≫ 2-MeSATP > ATP = α,β-meATP. $P2X_3$ receptor activation is potently blocked by TNP-ATP **47** ($pEC_{50} = 7.8$). The $P2X_3$ homomeric receptor undergoes rapid desensitization (<100 ms). The $P2X_{2/3}$ heteromeric receptor shares the pharmacological profile (α,β-meATP sensitivity) of the homomeric $P2X_3$ receptor and the slow desensitization kinetics of the $P2X_2$ homomer and potentially represents a naturally occurring form of the receptor that is involved in pain perception.[86,134,135] Message for the $P2X_3$ subunit was initially reported to have a relatively restricted distribution compared to other P2X receptors being expressed in a subset of sensory neurons including the trigeminal, dorsal root, and nodose ganglia. It is largely absent from smooth muscle, sympathetic, and enteric neurons.[86,134] However, $P2X_3$ receptor specific immunoreactivity in nucleus tractus solatarius and other brain regions has been recently reported.[136−138] Mouse knockouts of the $P2X_3$ receptor[139,140] show a complete absence of quickly desensitizing electrophysiological responses in dorsal root ganglia, show no apparent compensatory alterations in the expression of other P2X subunits, and show decreased nociceptive responses and bladder hyporeflexia,[139,141] highlighting the role of this receptor in sensory physiology. The incomplete reduction in nociceptive signaling following the knockout of the $P2X_3$ receptor is apparently due to a residual effect of ATP acting via a $P2Y_1$ receptor to modulate VR1 receptor function.[33] Axotomy also leads to changes in $P2Y_1$ receptor expression in dorsal root ganglion.[142] The N-type calcium channel blocker, ω-conotoxin GVIA, is also an allosteric modulator of $P2X_3$ receptor-mediated responses in rat dorsal root ganglion neurons.[143] It is more potent ($IC_{50} = 21$ nM) on $P2X_3$ homomers than on the $P2X_{2/3}$ heteromer ($IC_{50} = 3.8$ μM).

1.4. $P2X_4$. The $P2X_4$ subunit has been cloned from a variety of rat and human tissue sources.[144,145] $P2X_4$

subunit message is found in brain, spinal cord, sensory ganglia, superior cervical ganglion, lung, bronchial epithelium, bladder, thymus, salivary glands, testis, and vas deferens. Expression of $P2X_4$ subunits occurs at levels sometimes 100-fold greater than other P2X subunits,[145] particularly in regions of the cerebellum and hippocampus, where in the latter brain region there is high colocalization with AMPA-sensitive glutamatergic receptors.[146] The recombinant $P2X_4$ receptor shows greatest agonist sensitivity to BzATP and ATP (pEC_{50} = 6.3) and is insensitive to the P2 receptor antagonists suramin and PPADS.[147] Recombinant $P2X_4$ receptor functional responses are potentiated by Zn^{2+},[148] by the channel modulating macrolide anthelminthic, avermectin,[85] and by the macrolide antibiotic erythromycin, which, at concentrations that are clinically achievable (10 μM), can block the effects of ATP on calcium influx in a human lung epithelial-like carcinoma cell line.[149] Alternatively, spliced forms of the $P2X_4$ receptor subunit can form heteromers with wild-type $P2X_4$ subunits that are distinct from wild-type $P2X_4$ homomers.[147]

1.5. $P2X_5$. The $P2X_5$ subunit was initially cloned from rat celiac ganglion[109] and shows an activation profile of ATP > 2-MeSATP > ADP with α,β-meATP being inactive. It is relatively insensitive to suramin and PPADS. Message for this P2 receptor subunit is present in the ventral horn of the cervical spinal cord and in trigeminal and dorsal root ganglion neurons. The cloned chick $P2X_8$ receptor[150] has a high sequence identity (59%) with the rat $P2X_5$ receptor and may represent the avian ortholog. As previously noted above, $P2X_5$ subunits form functional heteromers with $P2X_1$ that show high sensitivity to ATP and TNP-ATP **47**.[151]

1.6. $P2X_6$. The $P2X_6$ receptor subunit was originally cloned from a rat superior cervical ganglion cDNA library.[149] Its activation profile is ATP > 2-MeSATP > ADP, with α,β-meATP being inactive. This receptor shows only partial inhibition by suramin or PPADS. Robust transfection of functional $P2X_6$ subunits has proven to be difficult. A p53-inducible P2 receptor designated P2XM, the function of which is altered in soft tissue tumors, has sequence homology similar to that of the $P2X_6$ subunit.[152]

1.7. $P2X_7$. The $P2X_7$ subunit was cloned from rat[71] and human[153] brain and macrophages.[154] It is structurally different from other P2X receptor subunits in having a longer (240 amino acid) intracellular C terminal. The $P2X_7$ receptor was previously characterized as the P_{2Z} receptor,[155] a cytolytic receptor present in cells of hematopoietic origin including mast cells, macrophages, lymphocytes, erythrocytes, and fibroblasts. Brief activation (<10 s) of the $P2X_7$ receptor results in a rapid, reversible membrane depolarization with Na^+, K^+, and Ca^{2+} influx.[155] On prolonged exposure to ATP or other P2 agonists in the presence of low levels of divalent cations, the $P2X_7$ receptor converts to a nonselective pore that is permeable to small molecules of molecular weight up to 900 Da, an event associated with cytotoxic effects, e.g., cell swelling, vacuolization, and necrotic and apoptotic cell death. Of the 240 amino acids in the intracellular C terminal, 177 are required for induction of pore formation.[71] The ability of P2 receptor agonists to induce pore formation was thought to be a unique property of the $P2X_7$ receptor, but other P2X subunit

homomers, e.g., $P2X_2$ and $P2X_4$, can also form pores upon prolonged agonist application.[156,157] The potent cytotoxin maitotoxin, derived from the dinoflagellate *Gambierdiscus toxicus*, induces a cell membrane pore that is physiologically identical to the $P2X_7$-induced pore, suggesting that this toxin may be a ligand and/or a cofactor in $P2X_7$-induced pore formation.[158] The rank order agonist potency for activation of the $P2X_7$ receptor is BzATP (pEC_{50} = 5.3) $\gg$ ATP, with 2-MeSATP, ATPγS, and ADP being inactive.[71] The human receptor has a lower sensitivity to agonists than the rat receptor.[154] Brilliant Blue G (**35**, pEC_{50} = 5.3) is a potent noncompetitive antagonist of the $P2X_7$ receptor.[159] 2',3'-Dialdehyde ATP **48** (oxidized ATP, oATP) is an irreversible inhibitor of $P2X_7$ receptors[160] and KN-62 **54a** is a noncompetitive, uncharged antagonist.[161] In macrophages and lymphocytes, $P2X_7$ receptor activation results in the activation of phospholipase D and in human macrophages elicits the release of the inflammatory cytokine IL-1β via activation of caspase-1 (IL-1β converting enzyme).[162-164] In addition to cells of hematopoietic origin, the $P2X_7$ receptor is also found on hepatocytes and acinar cells of the parotid and salivary glands. In macrophages, the $P2X_7$ receptor is also involved in the formation of multinucleated giant cells.[165] A paralog of the $P2X_7$ receptor with 80% homology to the designated $P2X_7$ receptor has been identified in the draft sequence of the human genome.[69]

Proteomic analysis of the $P2X_7$ receptor in HEK cells[166] identified a signaling complex comprised of 11 proteins that included laminin α-3, integrin β2, β-actin, supervillin, MAGuK, three heat shock proteins, phosphatidylinositol 4-kinase, and the receptor protein tyrosine phosphatase-β (RPTP-β), the last of which may modulate $P2X_7$ receptor function via control of its phosphorylation state. The C-terminal motif of the $P2X_7$ receptor contains a conserved lipopolysaccharide (LPS) binding domain (amino acids 573−590) that is structurally similar to the LPS binding site of the bactericidal/permeability increasing protein BPI.[167] Peptides derived from this latter motif bind LPS in vitro and block the ability of LPS to activate ERK1 and ERK2 and degrade IκB-α in macrophages.

A $P2X_7$ receptor knockout mouse shows a disruption in cytokine signaling cascades with perturbation of ATP-induced processing of pro-IL-1β by macrophages.[168] In some patients with B-chronic lymphocytic leukemia, the lymphocyte $P2X_7$ receptor is nonfunctional.[169]

Evaluation of single nucleotide polymorphisms (SNPs) for the $P2X_7$ receptor in patients with chronic lymphocytic leukemia[170] showed SNPs at positions 155, 348, and 496 that occurred with allele frequencies of greater than 1%. Examination of the SNP at position 496, which is present on the carboxy-terminal tail of the $P2X_7$ receptor subunit, in 45 normal subjects showed a Glu496Ala polymorphism associated with loss of function of the receptor.

2. P2Y Receptors. P2Y receptors are G-protein-coupled receptors (GPCRs) with a typical heptahelical transmembrane (7-TM) motif and are sensitive to activation by both purines (ATP, ADP) and pyrimidines (UTP, UDP).[7,10,171] Other receptors that are closest in sequence homology to P2Y receptors include angiotensin and thrombin peptide receptors, an observation that has

Table 2. Mammalian P2Y Receptor Genes and Functions and Localization

subunit	amino acids	GenBank accession no.			chromsonal localization	signal transduction	agonist	distribution	gene disruption
		human	rat	mouse					
$P2Y_1$	373	U42029	U22830	U22829	3q25	$G_{q/11}$, PLC	ADP	brain, vascular endothelia, platelets	decreased thrombosis-induced mortality
$P2Y_2$	376	U07225	U09402	L14751	11q13.5	$G_{q/11}$, PLC	UTP	epithelial cells, endothelial smooth muscle	decreased ATP-sensitive epithelial Cl$^-$ transport
$P2Y_4$	365	U40223	Y14705	NM020621	Xq13	$G_{q/11}$, PLC	UTP	placenta	
$P2X_6$	328	X97058	D63665	AF298899	11q13.3	$G_{q/11}$, PLC	UDP	spleen, smooth muscle, airway epithelia	
$P2Y_{11}$	371	AF030335				G_q, PLC, G_s, cAMP	ATP	spleen, granulocytes	
$P2Y_{12}$	342	AF313449	AF313450		3q24	G_i, cAMP	ADP	brain (glia), spinal cord, platelets	
$P2Y_{13}$	333	AF406692		AK008013		G_i, cAMP	ADP	spleen, brain, lymph nodes, bone marrow	

led to speculation that there may be an as yet unidentified endogenous peptide ligand for P2Y receptors.

The currently accepted members of this family are the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors (Table 2). The missing sequence numbers represent species homologues of other receptors, e.g., p2y3,[172] or receptors that have been misassigned to the P2Y family, e.g., p2y7,[73] which, as already noted, was subsequently cloned as an LTB$_4$ receptor.[74] $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are activated by pyrimidines.[7,10,171] Most of the P2Y receptors produce their functional effects via G protein coupling to activate phoshoplipase C (PLC), forming IP$_3$ and mobilizing intercellular calcium.[10,64] This in turn leads to activation of other signaling pathways that include protein kinase C, PLA$_2$, calcium-dependent K$^+$ channels, nitric oxide synthase (NOS), voltage-operated calcium channels, and MAP kinase pathways.[173] Some P2Y receptors are also linked to inhibition of adenylate cyclase activity.[10,64]

On the basis of their structural similarities, P2Y receptors have been divided into two distinct groups: group I consists of $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors; group II consists of $P2Y_{12}$ and $P2Y_{13}$ receptors.[174] The UDP-glucose-sensitive GPCR (KIAA0001)[65] is a potential member of this second group and has been previsionally designated as the $P2Y_{14}$ receptor.[174]

Despite emerging evidence[175] that functional dimerization of GPCRs is a rule rather than an exception, there is currently little information regarding P2Y receptor dimers, although there is evidence for $P2Y_1$/ adenosine A$_1$ receptor dimerization, the resultant oligomer showing a distinct pharmacology with A$_1$ agonists and antagonists showing reduced affinity with a concomitant 400-fold increase in affinity for the P2 agonist ADPβS.[176]

2.1. $P2Y_1$. The $P2Y_1$ receptor was the first P2 receptor to be cloned.[177−180] The rank order of agonist activation is 2-MeSATP $\geq$ ATP $\gg$ ADP, with α,β-meATP, β,γ-meATP, and UTP being inactive. MRS 2279 **53** is the most potent $P2Y_1$ antagonist yet reported.[70,181] Suramin **23** and Reactive Blue 2 (RB-2 **27**) can also block the effects of agonists at this receptor at micromolar concentrations. Activation of the $P2Y_1$ receptor results in either activation of PLC via the G$_q$ coupling protein G$_{11}$ or inhibition of adenylate cyclase via G$_i$ subunits that act independently.[10] $P2Y_1$ receptor activation can also directly modulate ion channel function, a G-protein-mediated effect that is independent of other second messenger systems.[182] In rat cerebellar neurons, $P2Y_1$

receptor activation leads to the opening of an outwardly rectifying K$^+$ current via coupling of the $\beta\gamma$ subunits of the G protein to a K$^+$ channel. $P2Y_1$ receptor gene disrupted mice show altered platelet physiology and a significantly reduced incidence of lethality from thrombosis.[183,184]

2.2. $P2Y_2$. The $P2Y_2$ receptor (originally described as the P$_{2U}$ receptor) was first cloned from the mouse neuroblastoma NG108-15 cell line[176] and subsequently from rodent[185] and human tissue.[186] ATP and UTP were equipotent in activating the receptor, with ADP, UDP, 2-MeSATP, and α,β-meATP having weak to no activity. $P2Y_2$ receptors are coupled to G$_i$ and G$_q$ proteins that mediate phospholipid breakdown, IP$_3$ formation, and calcium mobilization. In airway epithelia, biliary epithelial cell lines, and avian exocrine salt gland cells, $P2Y_2$ receptor activation leads to opening of Ca^{2+}-sensitive Cl$^-$ channels that are involved in epithelial fluid secretion.[187] $P2Y_2$ receptors negatively coupled to adenylate cyclase activation have been reported as has $P2Y_2$ activation of a member of the Kir 3.0 inward rectifier channel[188] and adenylyl cyclase via a cyclooxygenase (COX) dependent mechanism.[189] The sensitivity of $P2Y_2$ receptors to blockade by suramin **23** and PPADS **36** has provided evidence for suramin-sensitive and -insensitive responses and PPADS-sensitive and -insensitive responses suggestive of potential receptor heterogeneity,[190] although there is no molecular evidence for subtypes of the $P2Y_2$ receptor. $P2Y_2$ receptor knockout mice[191] show alterations in chloride transport function in lung and other secretory tissues.

2.3. $P2Y_4$. The $P2Y_4$ receptor is a uridine-nucleotide-specific receptor that was cloned from human placenta and rat heart.[192−195] The human $P2Y_4$ receptor has greater selectivity for UTP over ATP and is insensitive to ADP and UDP. At the rat $P2Y_4$ receptor, ATP and UTP are equipotent, with ADP, ATPγS, 2-MeSATP, and Ap4A acting as partial agonists.[194] PPADS **36** has been reported as a weak antagonist for the human $P2Y_4$ receptor, with suramin **23** being inactive.[192] The $P2Y_4$ receptor has a relatively restricted tissue expression being found only in placenta and pancreas with low levels in lung and vascular smooth muscle.[192−195] There are currently no known selective $P2Y_4$ receptor antagonists.

2.4. $P2Y_6$. The $P2Y_6$ receptor was isolated from rat aortic smooth muscle, human placenta, and human spleen.[196,197] The most potent agonist at the $P2Y_6$ receptor is UDP with weak or no effects seen with UTP, ATP,

ADP, or 2-MeSATP. The P2Y$_6$ receptor is linked to G$_q$ stimulating PLC with the formation of IP$_3$ in monocytes,[197] interleukin-8,[198] and is linked to M-type potassium channels.[199] Message for the P2Y$_6$ subunit is widely distributed including placenta, heart, lung, intestine, spleen, and airway and nasal epithelium.[196,197] There are currently no selective antagonists for the P2Y$_6$ receptor.

2.5. P2Y$_{11}$. The P2Y$_{11}$ receptor was cloned from human placenta[200] and is also found on human lymphocytes[201] and canine kidney cells.[202] The P2Y$_{11}$ receptor interacts with lymphocyte adenosine A$_{2A}$ receptors in the developmental fate of B lymphocytes[201] and is uniquely sensitive to purine nucleotides with a rank order agonist potency of ATP > 2 MeS-ATP >>> ADP, with UTP and UDP being inactive. The human receptor is also unique in that is coupled to both adenylate cyclase and phosphoinositide pathways. The canine P2Y$_{11}$ receptor in Madin−Darby kidney cells is linked to an increase in short-circuit current (I_{SC}).[202] There are currently no selective antagonists for the P2Y$_{11}$ receptor.

2.6. P2Y$_{12}$. The P2Y$_{12}$ receptor was cloned from rat and human cDNA libraries[72,75,76] and represents the elusive ADP-sensitive P2 receptor on platelets previously termed P$_{2T}$, P2Y$_{ADP}$, and P2Y$_T$. While previously thought to be a form of the P2Y$_1$ receptor,[75] the P2Y$_{12}$ receptor plays a discrete role in platelet shape change and aggregation. This confusion between the P2Y$_1$ and P2Y$_{12}$ receptors may be explained by their combined role in platelet aggregation.[203] ADP is a full agonist at the P2Y$_{12}$ receptor with ATP with its bioisosteres, including ARL 67085 **45** and AR-C 69931MX **46**, being functional antagonists[204] that, like the thienopyridines ticlopidine and clopidigrel, are active in the clinic.[205,206] Ap4A **59a**, a P^2,P^3-monochloromethylene analogue, AppCHClppA **59b**, and various phosphorothioate analogues are competitive inhibitors of platelet aggregation.[207] CT 50547 **58** is a novel, tricyclic benzothiazolo[2,3*c*]thiadiazine (IC$_{50}$ = 40 nM), a reversible P2Y$_{12}$ receptor antagonist with negligible functional activity (IC$_{50}$ > 100 μM) at the platelet P2Y$_1$ receptor.[208] The P2Y$_{12}$ receptor is present on platelets and megakaryoblastic cell lines and is coupled to a G$_{i2}$ protein that inhibits adenylate cyclase.[72] P2Y$_{12}$ knockout mice in general have a normal phenotype but exhibit prolonged bleeding time and reduced sensitivity to ADP, thrombin, and collagen.[209]

2.7. P2Y$_{13}$. The P2Y$_{13}$ receptor is the most recent member of the P2Y family to be identified.[174] Initially cloned as an orphan GPCR alternatively termed GPR86[210] and GPR94,[211] the P2Y$_{13}$ receptor has a high degree of homology compared to the P2Y$_{12}$ receptor.[174] This receptor has also been cloned as SP174.[212] On the basis of its tissue distribution, the P2Y$_{13}$ receptor has been implicated in immune system function, specifically T-cell maturation.[174,212]

3. Other Putative P2 Receptors. As an addition to the list of receptors missassigned to the P2 receptor family already documented, the p2y3 receptor is a nucleotide-sensitive avian receptor for which a mammalian homologue has not yet been described.[172] The p2y5 receptor was identified in activated chick T lymphocytes using radioligand approaches. No functional or structural support for the existence of this receptor has been reported. The P2Y$_8$ receptor was cloned from *Xenopus* neural plate and was activated to an equal degree by ATP, UTP, ITP, CTP, and GTP.[150] Mammalian homologues of this receptor have yet to be identified. The receptors cloned as P2Y$_9$ and P2Y$_{10}$ are not nucleotide receptors.[171] A novel cysteinyl leukotriene receptor 1 (CysLT1) has been identified on human cord-blood derived mast cells (hMCs) and is functionally responsive to the cysteinyl leukotrienes LTC$_4$ and LTD$_4$ and also to UDP. Interestingly, the effects of both the leukotrienes and UDP can be blocked by the CysLT1 antagonist MK 571.[213] This relationship between P2 receptors and CysLT receptors is intriguing especially in light of the misidentification of the LTB$_4$ receptor[74] as the putative P2Y$_7$ receptor,[73] suggesting perhaps that there is a close function relationship between the two receptor families. A P$_{2T}$-like receptor distinct from that on platelets that shows agonist responses to 2-MeSATP has been identified in brain capillary epithelial cells.[214]

Receptor Structure

P2X Receptors. Current evidence[106,107] indicates that functional P2X receptors have a trimeric motif. A model of the generalized P2X receptor subunit (Figure 8) shows the topography of glycosylation sites and the cytoplasmic orientation of both amino and carboxy termini. Features of the extracellular domain include 10 cysteine residues (−S), that are highly conserved among different P2X subtypes and are thought to form disulfide bridges. Four glycosylation sites have been identified on the rat P2X$_1$ receptor.[215] However, the number and location of glycosylation sites differ within the different subunits of the P2X family.[215,216] Numerous conserved positively charged residues (K and R) are present in the extracellular loop of P2X receptors, four of which at positions 68, 70, 292, and 309 are important for ATP binding to the human P2X$_1$ receptor. The neutral residue I67 of the rat P2X$_2$ receptor defines a critical region of ATP binding.[217]

By use of the "SCAM" technique, in which individual residues were replaced with cysteine and various alkylating reagents were examined for their ability to disrupt ligand recognition by reacting covalently with accessible thiols, the residues within TM2 lining the pore of the rat P2X$_2$ receptor were identified.[218] I328, N333, and T336 were at the outer vestibule of the pore, while L338 and D349 lined the cation channel. D349 was solvent-accessible only when ATP was applied. Chimeras of PX$_1$ and P2X$_2$ receptor subunits in concert with site-directed mutagenesis of the N terminal of the first transmembrane domain demonstrated a critical role for this domain in agonist effects,[219] while calcium permeability of the P2X$_2$ receptor was abolished by replacing polar amino acid residues at Thr339 and Ser340 with tyrosine.[220] A protein kinase C site on the C terminus was responsible for slowing desensitization of the P2X$_2$ receptor.[221]

P2Y Receptors. With information gathered predominately from the P2Y$_1$ receptor and the low-resolution structure of rhodopsin as a template, the structure of the chick brain P2Y$_1$ receptor was first modeled using the Quanta/Charmm software (Figure 9).[222] Ligand affinity SAR, sequence analysis of cloned P2Y receptors, and site-directed mutagenesis studies[223−225] have led to the refinement of computer-based models for ligand binding to P2Y receptors.
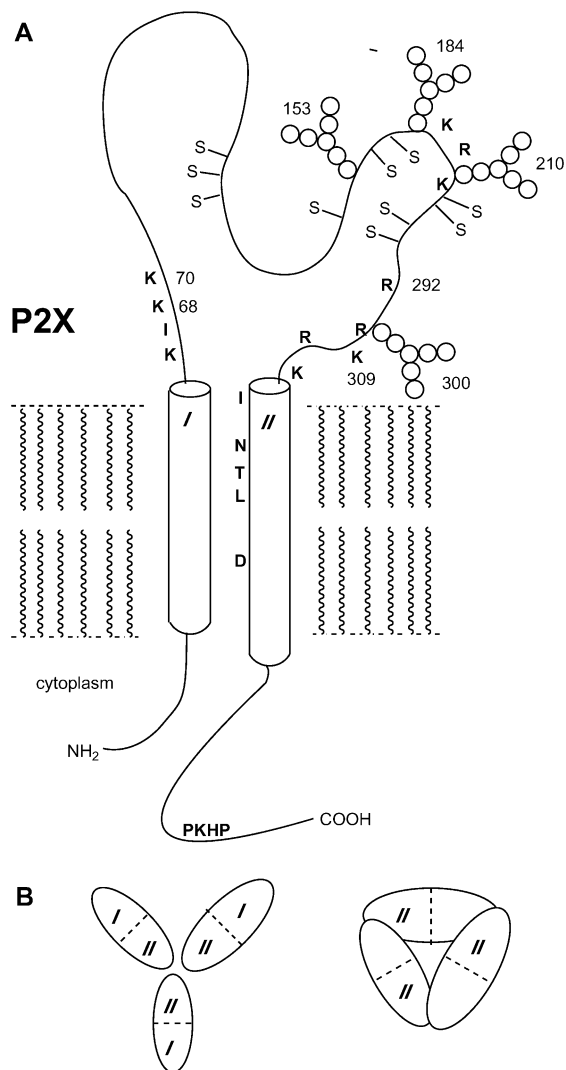
Figure 8. Schematic representation of a P2X receptor. (A) An individual subunit showing the two-transmembrane (2TM) motif and amino acid residues that are implicated in ligand binding and that maintain the conformation of the subunit. (B) Possible arrangement of subunits in a trimer, based on the finding that intersubunit recognition is dependent on residues of the second TM domain (II).

Recently a model of the human $P2Y_1$ receptor using the high-resolution structure of rhodopsin as a template resulted in a detailed model of antagonist binding to the $P2Y_1$ receptor binding.[225] Ligand docking in the $P2Y_1$ receptor model provided a hypothesis for the coordination of ATP in the TM regions, consistent with site-directed mutagenesis results (see below). The structural similarity between the potent nucleotide antagonist MRS 2179 **50** (see below) and nucleotide agonists suggests that receptor activation resulting in a specific conformational change depends on subtle differences between ligands.[226]

$P2Y_1$ receptor models have also been developed in parallel with alanine scanning mutagenic approaches.[227] Modeling of cloned $P2Y_1$ receptor sequences focused on both the seven-membrane-spanning helical domains (TMs) and the three extracellular loops (ELs). The features of the putative binding site identified within the TM region were consistent with both mutagenesis results and known ligand specificities. A new "cross docking" modeling approach,[224] which simulated the
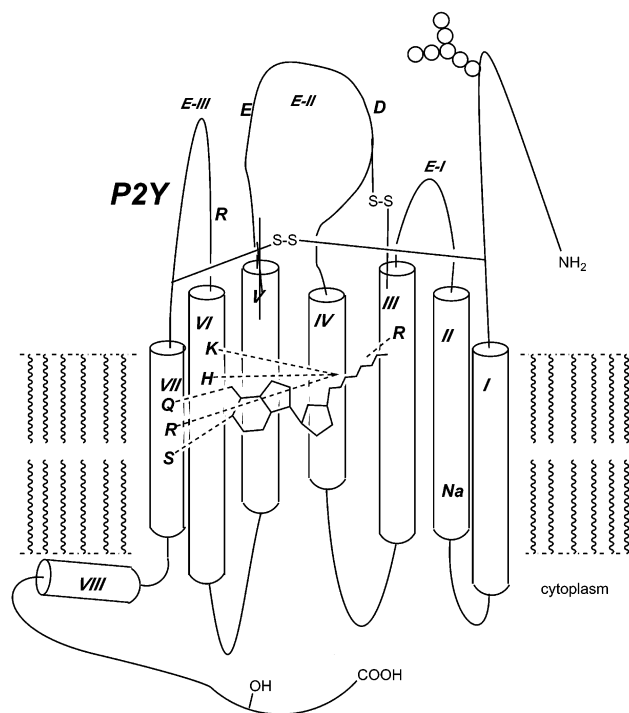
Figure 9. Schematic representation of a P2Y receptor showing the seven-transmembrane (7TM) motif and amino acid residues that are implicated in ligand binding. The helices shown are arrranged in a roughly circular bundle, which is open in this diagram for representational purposes. Model shows features of the hP2Y1 receptor important for nucleotide binding both within the TMs (3, 6, and 7) and ELs (2 and 3), including three positively charged residues (R128, K280, and R310) found to be important for ATP binding. Four Cys residues (−S), which are conserved among P2Y subtypes, form disulfide bridges. The location of the putative glycosylation site is conserved within the P2Y family. Shown also are residues within TM6 and -7, which are modulatory for the activation of the hP2Y1 receptor. Molecular modeling has predicted contact with the adenine and triphophate regions of ATP as shown. The overall structure of this GPCR representation incorporated findings from the high-resolution structure recently determined for rhodopsin. The 7TM bundle is assumed to be closed (i.e., TM1 and TM7 are in contact), and EL2 is in proximity to the TM ligand binding domain. Hydroxyl group on the C-teminal region represents potential phosphorylation sites.

reorganization of the native receptor induced by the ligand, aided in energetically refining the three-dimensional structure of the complex. To ascertain which residues of the human $P2Y_1$ receptor were involved in ligand recognition, individual residues of both the TMs (3, 5, 6, and 7) and ELs (2 and 3) were mutated to alanine and various charged residues,[227,228] and a cluster of positively charged lysine and arginine residues near the exofacial side of TMs 3 and 7 and, to a lesser extent, TM6 putatively coordinated the phosphate moieties of nucleotide agonists and antagonists. Agonists were inactive at R128A (TM3) and at R310A and S314A (TM7) mutant receptors and had a markedly reduced potency at K280A (TM6) and Q307A (TM7) mutant receptors. Positively charged residues of the human $P2Y_2$ receptor (H262, R265, and R292 in TM6 and TM7) were similarly found to be critical for activation,[224] suggesting that residues on the exofacial side of TM3 and TM7 were critical determinants of the ATP binding pocket. In contrast, there was no change in the potency

or efficacy of agonists in the S317A mutant receptor, and alanine replacement of F131, H132, Y136, F226, or H277 resulted in mutant receptors that exhibited a 7- to 18-fold reduction in potency compared to that observed with the wild-type receptor. These residues thus appear to serve a less important modulatory role in ligand binding to the $P2Y_1$ receptor.

Since changes in the potency of 2-MeSADP **14** and HT-AMP **15** paralleled the changes in potency of 2-MeSATP at the various mutant receptors, it appeared that the β- and γ-phosphates of the adenine nucleotides were less important than the α-phosphate in ligand/$P2Y_1$ receptor interactions.[227] However, T221A and T222A mutant receptors exhibited much larger reductions in triphosphate (89- and 33-fold versus wild-type receptors, respectively) versus di- or monophosphate potency, a result indicating a greater role of these TM5 residues in γ-phosphate recognition. Taken together, the results suggest that the adenosine and α-phosphate moieties of ATP bind to critical residues in TM3 and TM7 on the exofacial side of P2Y receptors.

Two essential disulfide bridges in the extracellular domains of the human $P2Y_1$ receptor were also identified: one conserved among GPCRs and another conserved between the N-terminal domain and EL3, characteristic of P2Y receptors. Several charged residues in ELs 2 (E209) and 3 (R287) were critical for receptor activation, suggesting that the role of the ELs in ligand recognition was as important as that of the TMs.[227,228] Moreover, energetically favorable "meta binding sites" in the $P2Y_1$ receptor have been defined, involving the critical residues of the ELs. At these nucleotide docking sites that are postulated to lie distal to the principal TM site, a ligand may bind en route to the principal TM binding site. Such secondary binding sites may then serve to guide the ligand in its approach to the TM binding site and reduce the energy barrier to ligand/receptor complex formation.

Development of P2 Receptor Ligands

The identification of new pharmacophores that selectively and potently interact with (a) P2 receptors and (b) individual members of the P2X and P2Y families represents a major challenge in medicinal chemistry. Much of the historical data on P2 receptor ligands have been confounded by the use of a limited repertoire of highly labile agonists, the majority related to ATP, moderately active antagonists with questionable purity, stability, and selectivity with comparisons of such compounds being made between different tissue systems using different physiological and pharmacological end points in different laboratories.[45] Evaluation of compounds in these systems is also confounded by different levels of nucleotidase activity and by species differences in receptor pharmacology. This situation has, to some extent, been simplified in recent years by the use of cell lines transfected with cDNA for the various human and rodent P2 receptors. Even so, in many instances new compounds have only been examined in limited assays, e.g., P2X or P2Y family only. This has led to the description of compounds as being receptor-selective and then being used to define new receptors/receptor systems before the compounds are found to be active at

other P2 receptors. A case in point is that of BzATP; while it is the most potent of known purine nucleotide analogues at the $P2X_7$ receptor, it is far more potent at other P2 receptors.[229] Its use as a selective ligand to delineate the involvement of $P2X_7$ receptors in a given system/tissue response can thus be misleading. Another complication is the degree to which P2 receptor ligands behave as agonists, antagonists, or partial agonists. Depending on the species, ATP can function as an agonist or an antagonist at the $P2Y_4$ receptor.[81]

Despite these caveats, there has been considerable focus on modifying the parent nucleotides and the various empirically identified antagonists, e.g., azo dyes, suramin, etc. It is only in the past 6 years, however, that molecular modeling and high-throughput screening approaches (HTS), together with the systematic development of structure−activity relationships, have been used in the identification and optimization of novel P2 receptor ligands. Anecdotally, HTS approaches have yielded disappointing results in finding either agonist pharmacophores that lack the tri- or dinucleotide motif of ATP, UTP, ADP, and UDP or antagonists that are active in vivo. Modeling approaches remain at an early stage and are obviously more promising for the better-characterized P2Y GPCR family than for members of the P2X receptor family where knowledge of subunit stoichiometry, agonist and antagonist recognition site requirements, and the role of allosteric modulators are still in their infancy.

Early studies to derive SAR relationships for a variety of adenine nucleotide analogues interacting with P2X and P2Y receptors used classical smooth muscle preparations, e.g., guinea pig *taenia coli*, rabbit aorta, and rabbit mesenteric artery, to characterize P2Y receptor interactions, while P2X receptor activity was measured in rabbit saphenous artery, guinea pig vas deferens, and urinary bladder. Receptor heterogeneity and ligand instability confounded results from these preparations. Nonetheless, they were used empirically to identify a large number of compounds that have formed the basis of emerging medicinal chemistry efforts. With the availability of cloned receptors (and sufficient resources), it is now possible to examine new compounds at all members of P2X and P2Y receptor families under comparable conditions as well as to evaluate compounds for effects on nucleotidase activity.

Development of Selective Agonists

Compounds that activate P2 receptors have distinct structural requirements from the agonists active at adenosine (P1) receptors.[45,52−55] The structure−activity relationships for a variety of adenine nucleotide analogues at P2X and P2Y purinergic receptors are shown in Tables 3 and 4. New ATP analogues containing modifications at the triphosphate, ribose 2′ or 3′, purine C2 or C8, or at the purine N^6 position have been synthesized (Figure 10).[55,230−233]

Triphosphate Modifications (Figure 2A). P2X receptors are not activated by AMP or adenosine, although ring-modified adenosine monophosphates (e.g., hexylthioAMP, **15**) can activate $P2X_1$−$P2X_4$ receptors to varying degrees.[54,232] These 2-thioether 5′-monophosphate derivatives are potent at $P2Y_1$ receptors (see below).

Table 3. Potency of Selected Agonists and Antagonists at P2X Receptors Expressed as EC50 or IC$_{50}$ Values in nM in Functional Assays (in Human or Rat)[a,b]

	P2X$_1$	P2X$_2$	P2X$_3$	P2X$_4$	P2X$_5$	P2X$_6$	P2X$_7$
Agonists							
1, ATP	56	1400	340	500, 1000	1000	1000	780,000
2, ADP	10,000	>100,000	>100,000	28,000[b]	18,000	11,000	
7, ATPγS	2300	1500	690	2300	600	1300	
8, ADPβS	2500	100,000	8800	25,000			
10, α,β–meATP	200	100,000	740	33,000	>7000	>8000	>100,000
11, β,γ–meATP	2000		9200				
13, 2-MeSATP	54	1500	350	7400, 1000	1000	600	
15, HT-AMP	840[b]	180,000	350[b]	20,000[b]			
16, PAPET-ATP	98	10,000	17	15,000[b]			
19, Bz-ATP	2	5,500	80	490	40,000	25,000	52,000
59, Ap$_4$A	182	15,000	500	1000[c]			
Antagonists							
23, suramin	1000	10,400	3000, 14,900	>100,000	4000	>100,000	92,000
25, NF023	210	>50,000	28,500	>100,000			
27, Reactive blue 2		360		128,000			
Pyridoxal-5-phosphate	3000	39,500		219,000			
36, PPADS	99	1200[e]	240, 1700	10,000, >100,000	2600	>100,000	62,000
37, isoPPADS	43	400	84				
40, PPNDS	14						
47, TNP-ATP[h]	6	2000	0.9	15,200			
50, MRS 2179	1150	>10,000	13,000	>10,000			>30,000
54a, KN-62				>100,000			34
65, Ip$_5$I	1.5	>30,000	2800	potentiator			
72, MRS 2220	10,200	>100,000	58,300	>100,000			

[a] Effects of antagonists on inward current induced by activation by ATP, at the indicated concentrations, of recombinant rat P2X$_1$ (1 μM), P2X$_2$ (10 μM), P2X$_3$ (3 μM), and P2X$_4$ (30 μM) receptors, expressed in *Xenopus* oocytes, using the twin electrode voltage clamping technique, unless otherwise noted (methods in refs 431 and 438). [b] Rat (red), human (blue). [c] Partial agonist.

Modification of the triphosphate group in the form of replacement of the bridging oxygen atoms with methylene units or of the charged oxygen atoms with sulfur has in some cases resulted in potent analogues that are resistant to degradation by nucleotidases. α,β-MeATP (α,β-methylene adenosine 5′-triphosphate, **10**) in particular is highly potent at P2X receptors,[78] being selective for group 1 subtypes (EC$_{50}$ = 1−10 μM), at which it causes rapid desensitization. α,β-MeATP also activates P2X$_{2/3}$ heteromers that are less susceptible to desensitization.[132] At P2Y receptors, α,β-MeATP is weak or inactive. Owing to the presence of the nonhydrolyzable methylene bridge, α,β-MeATP is more stable than other agonists under most assay conditions and its potency in comparison to other, hydrolyzable nucleotide agonists was therefore overestimated, being previously described as the most potent P2X receptor agonist.

Another metabolically stable analogue, β,γ-MeATP, **11**, was equipotent to ATP in eliciting vasoconstriction in the rat juxtamedullary afferent arterioles.[233] The corresponding nucleotide analogue containing the unnatural L-adenosine enantiomer β,γ-Me-L-ATP (L-adenyl 5′-(β,γ-methylene)diphosphonate, L-AMP-PCP **12**) is a more potent agonist than ATP at the P2X receptor in the guinea pig bladder[52,234] but is inactive in the guinea pig *taenia coli* P2Y receptor.

The thio substitution at the terminal phosphate also provides enzymatic stability, leading to such analogues as ATPγS (adenosine 5′-O-(3-thiotriphosphate, **7**), ADPβS (adenosine 5′-O-(2-thiodiphosphate, **8**), and UTPγS (uridine 5′-O-(3-thiotriphosphate, **9**), a potent agonist at P2Y$_2$ receptors. Compound **7** is a potent agonist at various P2Y subtypes, but not P2X subtypes, and also inhibited ecto-ATPase competitively with micromolar

Table 4. Potency of Selected Agonists and Antagonists at P2Y Receptors Expressed as EC_{50} or IC_{50} values in nM in Functional Assays (in Human, Rat, or Turkey)[a,b]

	P2Y$_1$	P2Y$_2$	P2Y$_4$	P2Y$_6$	P2Y$_{11}$ (cAMP)	P2Y$_{12}$[c]
Agonists						
1, ATP	2800	230	43,000,f / 1800	>100,000	17,000	3190f,g
2'-dATP	19,000				9000	
2, ADP	8000	>100,000				69
5, UTP	>100,000	140	2500, / 2600	6000	>100,000	
6, UDP	>100,000	>100,000		300		
7, ATPγS	1300	1720			3000	1300
8, ADPβS	96				30,000	82
10, α,β–meATP	>100,000					>100,000
11, β,γ–meATP	>100,000					>100,000
13, 2-MeSATP	8			100,000	50,000	1.1
14, 2-MeSADP	6	>100,000				
15, HT-AMP	4.8					
16, PAPET-ATP	1.5					
19, Bz-ATP					7000	
59, Ap$_4$A		720	3000			
Antagonists						
23, Suramin	>10,000d	48,000	>100,000	27%e	16,000	4000
27, Reactive blue 2	>10,000d	>100,000	33%e	87%e	>100,000	25
36, PPADS	1000, / 18,200	>10,000	>10,000	69%e		>100,000
45, AR-C67085					1500 (agonist)	1.3
50, MRS 2179	330	>100,000	>100,000	>100,000		>100,000
53, MRS 2279	52	>100,000	>100,000	>100,000	>100,000	>100,000

[a] Measured at phospholipase C coupled P2Y$_1$ receptor of turkey erythrocytes or at recombinant human P2Y$_2$ and P2Y$_4$ receptors or recombinant rat P2Y$_6$ receptors, unless otherwise noted. [b] Rat (red), turkey (green), human (blue). [c] Platelet receptor, unless otherwise noted (values for rat are from the C6 glioma cell receptor).[234,267] [d] Right shift at 30 μM and decrease in maximal effect.[267] [e] Percent inhibition at 100 μM.[193] [f] Antagonist. [g] Reference 270.

affinity.[235] UTPγS **9** is a potent P2Y$_2$ receptor agonist that is not readily degraded by nucleotidases.[236] The corresponding β-thiodiphosphate, UDPβS, selectivity activates P2Y$_6$ receptors.[236]

Adenine and Ribose Modifications (Figure 2B). a. Adenine Modifications. Pyrimidine-based nucleotides are generally weak P2X receptor agonists, although CTP is active at P2X$_3$ receptors and less so at P2X$_4$ and P2X$_{1/5}$ receptors. UTP weakly activates P2X$_3$ receptors.

Substitution of the adenine ring, particularly at the 2-position, is well tolerated. 2-MethylthioATP (2-MeSATP **13**) is one of the most potent agonists at P2Y and P2X receptors. While formerly regarded as selective for P2Y receptors, adjusting for its lability in classical smooth muscle assays in which P2X receptors have been assayed, it appears to be potent at P2X receptors.[78] It is thus typically more potent than ATP at P2X$_1$, P2X$_2$,

and P2X$_3$ receptors. At rat and human P2Y$_1$ receptors, 5'-diphosphates are generally more potent than the corresponding 5'-triphosphates. ADP and 2-MeSADP **14** are potent, full agonists, whereas ATP and 2-MeSATP **13** are less potent with reduced efficacy and at some P2 receptors act as antagonists.[81−83,237]

Attaching substituted alkylthio chains at the C2 position of **13** to create functionalized congeners as receptor probes[238] showed that chain elongation preserved potency at P2Y receptors, indicating a great tolerance for structural variation of the ligand at the C2 position. The activity of 2-thioether derivatives of ATP at P2Y receptors varied somewhat, depending on the distal structural features, and activity at P2X receptors varied to an even greater degree. The 11 ATP 2-thioethers synthesized had $K_{0.5}$ values of 1.5−770 nM in activating phospholipase C in turkey erythrocyte membranes. In P2Y-receptor-mediated smooth muscle
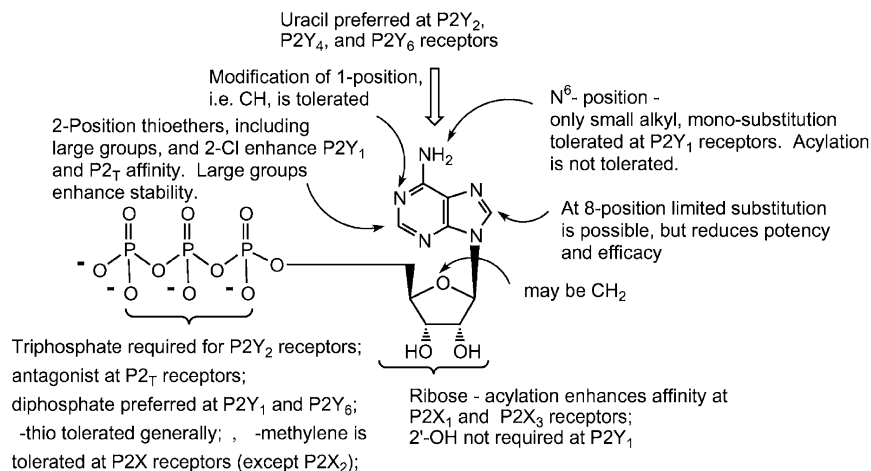
Figure 10. Summary of structure−activity relationships for P2 receptor agonists.

functional assays, 2-thioethers had EC_{50} values between 10 nM and 1 μM. A significant correlation existed for the 2-thioethers in their $pK_{0.5}$ values for inositol phosphate production and pD_2 values for P2Y-receptor-mediated relaxation in the guinea pig *taenia coli*, but not for vascular P2Y receptors or for P2X receptors. At rabbit saphenous artery P2X receptors, the thioethers were inactive but differing degrees of activity were observed in the guinea pig vas deferens and bladder depending on distal substituents in the 2-thioether moiety.

2-(7-Cyanohexylthio)ATP was more potent than 2-Me-SATP at the *taenia coli* P2Y receptor. A *p*-aminophenethylthio ether, intended as a reporter group for radioiodination and potential cross-linking to the receptor, displayed the highest affinity of all the analogues at turkey erythrocyte P2Y receptors. A *p*-nitrophenethylthio ether was relatively weak at P2Y receptors but provided selectivity for a subset (vas deferens) of the P2X receptor class. 7-Aminoheptylthio and 7-thioheptylthio ethers synthesized for their ease in further derivatization by acylation or alkylation and also to probe potential accessory binding sites on the receptor had $K_{0.5}$ values of 73−770 nM at erythrocyte P2Y receptors.[239]

The addition of a functionalized chain at the 2-position allowed for truncation of the triphosphate group with retention of affinity, thus circumventing one of the major complications in interpreting ATP pharmacological results, e.g., the impact of ectonucleotidase action. While AMP was inactive at P2Y receptors, 2-thioether analogues of AMP were full agonists at erythrocyte P2Y receptors,[232] although being generally several orders of magnitude less potent than the corresponding 2-thioether triphosphate analogue. For example, the 2-hexylthio ether of AMP, **15**, had an EC_{50} value of 59 nM in stimulating phospholipase C in turkey erythrocytes. The 2-hexenylthio ether of AMP, **17a**, was 8-fold more potent than ATP itself but was 33-fold less potent than the corresponding triphosphate. Thus, the long chain may act as a distal anchor of the ligand at an accessory binding site on the receptor. A somewhat similar approach was taken in developing the non-phosphate analogues **57a** and **57b** from AR-C 69931MX **46**.[240] Several adenosine diphosphate 2-thioether analogues were potent agonists at erythrocyte P2Y receptors.

Combinations of α-thiotriphosphate and 2-alkylthio modifications, e.g., **18**, retain activity at $P2Y_1$ receptors.[239] A further benefit of the presence of a long-chain thioether group at the 2-position was increased stability of the triphosphate group at the 5′-position. It is likely that long chains, although at a site on the molecule distal to the triphosphate group, interfere with the ATP binding site of ectonucleotidases.

Modifications of ATP other than 2-thioethers also resulted in unexpected receptor selectivity with some analogues displaying selectivity or specificity at P2X or P2Y receptors, suggesting the existence of possible subclasses. Selectivity was seen for P2Y receptor-like responses in the mesenteric artery, aorta, *taenia coli*, or erythrocytes. 8-(6-Aminohexylamino) ATP was selective for P2Y receptors in the rabbit endothelial aorta versus those in the *taenia coli*, mesenteric artery, and erythrocytes. It was inactive at P2X receptors. The potent agonist, N^6-methyl-ATP was selective for *taenia coli* P2Y receptors versus either vascular P2Y receptors or P2X receptors.[238] N^6-Ethyl-ATP was approximately equipotent to ATP at *taenia coli* P2Y receptors. N^6 Modification may prove to be a general means of increasing P2Y selectivity, since it was compatible with other modifications. A hybrid N^6-methyl and 2-thioether ATP derivative, N^6-methyl-2-(5-hexenylthio)-ATP **17b**, was a potent agonist at erythrocyte, *taenia coli*, and C6 glioma cell P2Y receptors but was inactive at P2X receptors.[238] Large groups, e.g., 2-phenylethyl, are not tolerated at the N^6-position of $P2Y_1$ receptor agonists.[222]

In summary, long-chain 2-thioethers can enhance the potency (particularly at P2Y receptors) or selectivity (particularly within the P2X class) of ATP analogues with longer-chain members tending to be stable to ectonucleotidases. The corresponding monophosphates are full P2Y receptor agonists. Modification of the ribose 2′-position and purine modifications of ATP other than 2-thioethers can result in P2Y receptor selectivity.

b. Ribose Modifications. The ribose moiety of agonists demonstrated most clearly for the $P2Y_1$ receptor is amenable to extensive modification. The weak agonist, 2′-deoxy-ATP (structure not shown) was selective for *taenia coli* P2Y receptors versus either vascular P2Y receptors or P2X receptors.[232] 3′-Deoxy-ATP is a weak, but selective, P2X agonist, while 3′-acetamido-3′-deoxy-ATP was active at both P2X receptors and

mesenteric artery P2Y receptors. 3′-Benzylamino-3′-deoxy-ATP **20** had high potency and selectivity for P2X receptors and was very potent at P2X receptors in the guinea pig vas deferens and slightly less potent in the urinary bladder and was inactive at rabbit saphenous artery P2X receptors and at all P2Y receptors. The potency of **20** at P2X receptors was approximately an order of magnitude greater than that of α,β-meATP.

Expansion of the ribose ring resulted in the *anhydro*-hexitol derivative, MRS 2255, **21**, which was monophosphorylated at two positions on the ring, e.g, a bisphosphate that was a full agonist with an EC_{50} value of 3 μM, at the turkey erythrocyte $P2Y_1$ receptor.[241]

P2 receptor nucleoside and nucleotide ligands containing conformationally rigid ribose-like rings, based on carbocyclic rings were designed using the methanocarba approach, e.g., fused cyclopropyl and cyclopentyl rings replacing the ribose moiety. The position of fusion of the cyclopropane ring determined the conformation of the ring, either Northern (N) or Southern (S). Rigid rings in the methanocarba series have defined a preference for the (N) conformation of ribose at the $P2Y_1$ receptor. MRS 2268, **22**, the (N)-methanocarba analogue of 2′-deoxyadenosine-3′,5′-bisphosphate (antagonist; see below), was a potent $P2Y_1$ agonist (EC_{50} = 155 nM), being 86-fold more potent than the corresponding (S) isomer.[241]

Dinucleotide Derivatives (Figure 4). Dinucleotides have both P2 receptor agonist and antagonist activity (see below). The activity and selectivity of diadenosine polyphosphates (Ap_nA, n = 4−6) **59**−**61** and mixed dinucleotide polyphosphates as agonists has been studied at recombinant P2X receptors and depends on the number of phosphates.[66,242−244] At the rat $P2X_1$ receptor, Ap_6A is a full agonist, while shorter homologues have increasingly diminished potency and efficacy. Uracil dinucleotides **62**−**64** function as agonists at $P2Y_2$ receptors.[245]

P2 Receptor Antagonists

Most P2 receptor antagonists have suffered from a serious lack of selectivity and potency.[4,45−47,246] The first antagonist reported was probably the alkaloid quinidine,[1] which was also active at adrenoceptors. Many early ATP antagonists were negatively charged, high molecular weight organic molecules.

Polysulfonates (Figure 3). A variety of aryl sulfonates, such as derivatives of the antiparasitic drug suramin (e.g., **23**−**26**) and derivatives of histochemical dyes, have been described as P2 receptor antagonists. These contained anthraquinone (e.g., **27**−**32**), arylazo (e.g., **33**), or triphenylmethane (e.g, **35**) moieties.

a. Suramin Class. The naphthylsulfonate derivative suramin, **23**, is a highly polypharmic ligand[247] that can act as an inhibitor of HIV reverse transcriptase[80] as a competitive antagonist at several P2 receptor subtypes[248] and vasoactive intestinal peptide receptors,[249] as an inhibitor of G protein activity,[79] and as an inhibitor of tyrosine phosphatase.[250] However, because suramin poorly penetrates cell membranes, these last actions may be relevant only in broken cell preparations.

Suramin is a large, complex, polysulfonated molecule. Thus, efforts have been made to identify the minimal pharmacophore required for P2 receptor antagonism. It is not necessary to have trisulfonate groups at both ends of the molecule.[248] A number of truncated forms of suramin, e.g., **24**−**26**, had P2 antagonist activity.[251−253] Compound **26** was selective for P2 receptors versus ectonucleotidases.[245] NF023, **25**, was approximately an order of magnitude more selective for P2X receptors in rabbit vas deferens and rat urinary bladder versus the P2Y receptors in guinea pig *taenia coli* and rat duodenum and showed 20-fold selectivity for P2X receptors in rat vas deferens versus P2Y receptors in guinea pig *taenia coli*.[254] The compound was also a selective P2X antagonist in rat, hamster, and rabbit blood vessels. NF279 **24a** had superior activity at group 1 P2X receptors compared with suramin and was more potent at $P2X_1$ than $P2X_3$ receptors.[255,256]

Many of the known P2 antagonists, e.g., suramin and its analogues, especially those acting at P2X subtypes, have a dimeric structure, suggesting a possible bifunctional interaction with P2 receptors. This seems even more likely considering the multimeric structure of P2X ion channels and the possibility that ATP recognition sites may occur between such subunits. Many of the truncated analogues of suramin also retain a symmetry suggestive of this type of bifunctional interaction. NF 449 **24b** is another suramin-derived $P2X_1$ receptor antagonist with subnanomolar activity at the receptor.[257]

b. Anthraquinone Class. Reactive Blue 2, **27**, among the most widely used P2 receptor antagonists, is a mixture of *m*- and *p*-sulfonate isomers, while Cibachron Blue 3GA, **28**, is the corresponding pure *o*-isomer.[258,259] Compound **27** was reported as a competitive P2Y antagonist at the adenylate cyclase coupled P2Y receptors of C6 glioma cells with a K_B value of 25 nM, which had at least a 50-fold higher affinity than those reported in other tissues.[260] Compound **27** blocks $P2X_1$-like receptors in rat vas deferens (IC_{50} = 30.4 μM, K_d = 11.4 μM) and $P2Y_1$-like receptors in guinea pig *taenia coli* (K_d = 3.4 μM).[261] Compound **28** is approximately 7-fold more potent than **27** at $P2X_1$-like receptors in rat vas deferens (IC_{50} = 9.6 μM, K_d = 1.6 μM) but has similar potency at $P2Y_1$-like receptors in guinea pig *taenia coli* (K_d = 2.9 μM).[259] Acid Blue 129, **29**, has modest antagonist activity at $P2Y_1$-receptors in guinea pig *taenia coli* (IC_{50} = 3.0 μM, K_d = 1.4 μM) and shows greater than 71-fold selectivity versus $P2X_1$-like receptors in rat vas deferens (K_d > 100 μM).[259] Uniblue A, **30**, is a potent antagonist at $P2X_1$-like receptors in rat vas deferens (IC_{50} = 7.5 μM, K_d = 0.8 μM) and shows greater than 125-fold selectivity versus $P2Y_1$-like receptors in guinea pig *taenia coli* (K_d > 100 μM).[259] However, **30** appeared to be an antagonist at both P2 and P1 (A_1 adenosine) receptors in the rat superior cervical ganglion.[248] MG 40-1, **31**, is a potent and highly selective antagonist at $P2X_1$-like receptors in rat vas deferens (IC_{50} = 8.8 μM) but is ineffective at $P2Y_1$-like receptors in guinea pig *taenia coli*.[258] MG 40-3, **32**, is more potent than **31** at $P2X_1$-like receptors in rat vas deferens (IC_{50} = 1.6 μM) but is far more potent and selective at $P2Y_1$-like receptors in guinea pig *taenia coli* (IC_{50} < 1 μM). **27**−**29** are reversible P2 receptor antagonists at $P2X_1$-like receptors in rat vas deferens, whereas **30**−**32** show irreversible antagonism. At the $P2Y_1$-like receptors in

guinea pig *taenia coli*, the order of potency is **32** > **29**–**27** > **31**, **30**.[261]

c. Azo Dye Class. Trypan Blue **33** had an IC$_{50}$ value of 386 μM at P2X and a value of 171 μM at P2Y receptors.[250,261] Related analogues (structures not shown), Reactive Red 2 and Acid Red 33 (P2X, K_d = 0.42 μM; P2Y, K_d = 82 nM) showed an enhanced P2 antagonist potency compared to **33**.[262]

d. Triphenylmethane Dye Class. Coumassie Brilliant Blue G **35** is a potent antagonist at P2X$_7$ receptors with an IC$_{50}$ value of approximately 400 nM,[160,263] while the potency at P2Y$_2$ receptors in rat superior cervical ganglia, at P2Y-like receptors in mouse and rat vas deferens 158,[263] and at P2X receptors in human urinary bladder was considerably lower. In the 0.1–3 μM range, **35** potentiated ATP responses at cloned human P2Y$_1$ receptors.[264] **35** is a noncompetitive antagonist at the rat P2Y$_1$ receptor at near nanomolar concentrations.[263]

e. Isothiocyanates. The ATP transport inhibitor DIDS **34** (Figure 3A) was initially used to antagonize P2X$_7$-receptor-mediated responses in rat parotid acinar cells (IC$_{50}$ = 35 μM)[265] and is particularly useful for distinguishing bladder and PC12 P2X receptors, at which it has IC$_{50}$ values of ~1 and >100 μM, respectively.[266] Other simple aromatic isothiocyanate derivatives (structures not shown) have been shown to block ATP effects of contractions of the rat vas deferens and relaxation of the carbachol-precontracted guinea pig *taenia coli* elicited by α,β-MeATP and ADPβS, respectively. The potencies of all compounds were less than that of **34** (for P2X, IC$_{50}$ = 11–54 μM; for P2Y, K_d = 10–214 μM).[267] The naphthyl derivatives showed a tendency of higher potencies at P2X receptors, but no structure–activity relationship was described at P2Y receptors.

Pyridoxal Phosphate Derivatives (Figure 4A). Other structural classes of ATP antagonists include derivatives of the coenzyme pyridoxal 5′-phosphate. A diazo derivative of pyridoxal phosphate (PPADS, **36**, Figure 4A) was an antagonist at the vas deferens P2X receptor.[263] Although shown to be 10- to 20-fold selective for P2X receptors,[268] **36** was a P2Y receptor antagonist. **36** is inactive at the adenylate cyclase coupled P2Y receptor on rat C6 glioma cells. Initially, no distinction was made between **36** and its isomer **37** because of a synthetic error. These compounds may have distinct pharmacological properties, especially with respect to the action of UTP. At P2X receptors, **37** tends to be slightly more potent than **36**.[269]

Analogues of PPADS, **37**–**41**, in which the sulfonate groups on the phenylazo ring were replaced with other functional groups, such as electron-withdrawing nitro groups, as in PPNDS, demonstrated that the sulfonate groups were not essential for antagonist activity.[270–273] Both **38** and **39** were potent antagonists of recombinant rat P2X$_1$ receptors expressed in oocytes and also completely antagonized the P2X-receptor-mediated contraction of guinea pig vas deferens and urinary bladder. At recombinant rat P2X$_2$ receptors, **38** (IC$_{50}$ = 1.1 μM) was more potent than **39**.[274] Phosphonate analogues of PPADS **42** and **43** were similar in potency to the phosphate derivatives. Compound **42** showed relatively high potency at turkey P2Y$_1$ receptors and at recombinant rat P2X$_2$ receptors, with IC$_{50}$ values of 4.4 and 1.1

μM, respectively. Replacement of the chloro substituent of **42** with a sulfonic acid eliminated binding to P2Y$_1$ receptors.[271] The most active analogue at P2X$_1$ (IC$_{50}$ = 5 nM) and P2X$_3$ (IC$_{50}$ = 22 nM) receptors was **43**, being 14-fold and 10-fold more potent than PPADS itself.[273] Compound **43** produced a nonsurmountable inhibition when tested against a range of ATP concentrations, although blockade was reversed by about 85% after 20 min of washout. The diazo linkage of PPADS was also replaced in analogues containing a carbon bridge, a modification that maintained potency at P2X receptors and enhanced chemical stability. Thus, the PPADS template can be altered at the pyridoxal and phenyl moieties to produce P2X$_1$ and P2X$_3$ receptor antagonists showing higher potency and a greater degree of reversibility than the parent compound at these group I P2X receptors.

Nucleotide Derivatives (Figure 4B). Nucleotide derivatives (Figure 4B) have long been used in various modalities to block the effects of ATP. The agonist α,β-MeATP **10** (Figure 2) can block P2X receptor responses via rapid desensitization of the receptor. Other nucleotide derivatives antagonize P2 receptor effects in a more competitive manner.[181] A number of nucleotide derivatives, e.g., **44** − **46**, have been developed as inhibitors of the platelet P2Y$_{12}$ receptor. ARL-66096 **44** is an ADP receptor antagonist that is a potent inhibitor of platelet aggregation, with the corresponding β,γ-dichloromethylene derivative ARL 67085MX **45**[275] antagonizing the ADP effects with 30000-fold selectivity for this receptor subtype. However, this compound also potently stimulates the human P2Y$_{11}$ receptor. ARL 69931MX **46** has exceptionally high potency (IC$_{50}$ = 0.4 nM) and may be more selective for the P2Y$_{12}$ subtype.[205] Compounds **57a** and **57b** were derived from ARL 69931MX, and its congeners were found to be potent P2Y$_{12}$ antagonists lacking a triphosphate group.[235]

Trinitrophenyl-ATP (TNP-ATP) **47** and the corresponding di- and monophosphate derivatives are nanomolar antagonists at P2X$_1$, P2X$_3$, and P2X$_{2/3}$ subtypes expressed in *Xenopus* oocytes,[276] while the potencies are much lower in other functional models.[273]

Oxidized ATP (oATP), **48**, proposed as an irreversible antagonist for the P2X$_7$ receptor in the J774 mouse macrophage-like cell line,[161] can antagonize the response to ATP acting via P2X$_7$ receptors without antagonizing coexpressed P2Y receptors.

Naturally occurring nucleotide bisphosphate derivatives, including **49a**,**b**, at micromolar concentrations, act as competitive antagonists or partial agonists of P2Y$_1$ receptors.[277–280] **49b** has partial agonist properties at the turkey P2Y$_1$ receptor but is a pure antagonist at human P2Y$_1$ receptors. Both 2′- and 3′-deoxy modifications of A3P5P were well tolerated at P2Y$_1$ receptors, and the removal of the free hydroxyl group decreased agonist efficacy. The introduction of a 2-chloro substituent resulted in **51**, which was a selective antagonist at P2Y$_1$ receptors, e.g., inactive at rat P2X$_1$ receptors.[280] The N^6-methyl modification resulting in the competitive antagonist MRS 2179 **50** enhanced antagonistic potency of 2′-deoxyadenosine 3′,5′-bisphosphate by 17-fold. MRS 2179 had a K_B value at turkey P2Y$_1$ receptors of 100 nM and was inactive at other P2Y receptor subtypes, including the C6 glioma cell P2Y receptor coupled to

inhibition of adenylyl cyclase (J. Boyer, unpublished results). The N^6-ethyl modification of MRS 2179 was of intermediate potency as an antagonist, while the N^6-propyl group completely abolished both agonist and antagonist properties. Thus, the N^6-binding region of the P2Y$_1$ receptor appears to contain a small hydrophobic pocket. 2-Methylthio and 2-chloro analogues were partial agonists of intermediate potency. A 2′-methoxy group provided intermediate potency as an antagonist while enhancing agonist activity. An N^1-methyl analogue was a weak antagonist with no agonist activity. An 8-bromo substitution and replacement of the N^6-amino group with methylthio, chloro, or hydroxy groups greatly reduced the ability to interact with P2Y$_1$ receptors. Benzoylation or dimethylation of the N^6-amino group also abolished the antagonist activity. MRS 2179 did antagonize electrophysiological effects of ATP at the rat P2X$_1$ receptor with an IC$_{50}$ value of 1.2 μM.[280]

By analogy to acyclic modifications of nucleosides used as antiviral agents, an acyclic modification of the ribose ring of the bisphosphate antagonists also preserved affinity at the P2Y$_1$ receptor. MRS 2286 **52** was a pure antagonist at turkey P2Y$_1$ receptors (IC$_{50}$ = 840 nM) and was inactive at P2X$_1$ receptors.[280] All acyclic analogues examined to date are inactive as agonists at P2Y$_1$ receptors.

Although introducing flexibility in the form of acyclic modifications preserved antagonistic activity, constraining the ribose ring conformationally increased the potency of P2Y$_1$ receptor antagonists. A 2-Cl-N^6-methyl-(N)-methanocarba derivative, MRS 2279 **53**, was a potent antagonist (IC$_{50}$ = 52 nM at turkey P2Y$_1$ receptors). In the (N) methanocarba analogues, as with other ribose modifications, e.g., **21**, the presence of an N^6-methyl group in these bisphosphate analogues transformed either a partial or full agonist into a pure antagonist, and the 2-chloro modification enhanced affinity.[281]

Diinosine polyphosphates (Figure 6), e.g., Ip$_n$I **65**, are highly selective antagonists for group 1[3] P2X receptors, with Ip$_5$I blocking P2X$_1$ receptors in the nanomolar range and with both Ip$_4$I and Ip$_5$I blocking P2X$_3$ receptors in the micromolar range. These polyphosphates were inactive at P2X$_2$, P2X$_4$, and P2X$_{2/3}$ receptors.[70]

Nonhighly Charged Derivatives (Figure 5). The isoquinoline derivative KN-62, **54a**, is an antagonist of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) at micromolar concentrations and a potent antagonist at P2X$_7$ receptors at even lower concentrations.[162] KN-04 **54b** has similar potency as a P2X$_7$ receptor antagonist and is inactive at CaMKII. **54a** noncompetitively antagonized P2X$_7$ receptors in HEK cells with an IC$_{50}$ value of ~15 nM. In human leukemic B lymphocytes, **54a** reduced the rate of permeability increase to larger permeant cations, like ethidium, induced by BzATP with an IC$_{50}$ value of 13 nM and complete inhibition of flux at 500 nM.[282] Compound **54a** was inactive at the neutrophil P2Y$_2$ receptor. Exploration of the SAR of KN-64 resulted in tyrosyl derivatives that were highly potent in mouse and equipotent to KN-62 at human P2X$_7$ receptors.[283,284]

The dihydropyridine nicardipine, **55**, and related derivatives can antagonize and/or potentiate P2X recep-

tor responses.[285] The pyrimidinedione derivative, **56**, is a nonnucleotide antagonist of the P2Y$_{12}$ receptor in platelets.[260,261] Thienopyridines, such as clopidogrel, have been developed as antagonists of the ADP-induced aggregation of platelets, but the actual receptor antagonist is an active metabolite of the clinically administered compound.[286]

Miscellaneous Modulators of P2 Receptors (Figure 7). Pyridylisatogen tosylate, **67** (PIT), is a weak P2 receptor antagonist that also displayed strong receptor-independent vasorelaxant effects.[287] At cloned P2Y$_1$ receptors, PIT acts as an allosteric enhancer of agonist action.[288]

NBD chloride, **69** (Figure 7), selectively blocked ADP-induced aggregation of platelets through covalent modification of aggregin, a putative (non-P2) ADP receptor, on the cell surface.[289]

The potent activity of the ω-conotoxin GVIA[142] suggests that other snail-derived peptides may function as P2X antagonists, in line with their broad effects on other ion channels.[290] The macrolides, avermectin,[85] and erythromycin[149] represent additional pharmacophores active at P2X receptors that may bind to sites distinct from those recognizing nucleotides.

Potentiators of the action of ATP at P2X receptors have also been identified. Coumassie Blue[264] and the pyridoxal phosphate derivative MRS 2220 **72** selectively enhance the effects of ATP at P2X$_1$ receptors,[291] and the charged dihydropyridine derivative MRS 2154 **73**[292] selectively enhances the effects of ATP at P2Y$_1$, P2X$_1$, and P2X$_2$ receptors.

Biological Actions and Clinical Targets. ATP is a key component of every cell in the body and is ubiquitously available in the extracellular medium as a neuromodulatory agent. Its effects on cell function are multifactoral as a distinct ligand, as part of the purinergic cascade,[11] and as a source of cellular energy. Thus, modulation of P2-receptor-mediated ATP responses may be anticipated to have profound effects on cellular and tissue function at both the cellular and intracellular levels. However, since ATP appears to be a normal constituent of the extracellular environment, it appears highly probable that functional alterations in extracellular levels of ATP and thus P2 receptor hypo- or hyperfunction, or alterations in receptor number associated with discrete disease states, will provide the opportunity for developing novel therapeutic agents that act via P2 receptors. In this context, the therapeutic areas currently of interest are pulmonary (P2Y$_2$/P2Y$_4$, Phase III), thrombosis (P2Y$_{12}$, Phase III), pain (P2X$_3$, preclinical), and bladder dysfunction (P2X$_3$, preclinical). Furthermore, as detailed below, evidence is now emerging that in some disorders, there are robust changes in P2 receptor message and/or protein that will dictate tissue responses to extracellular nucleotide concentrations.

Cardiopulmonary Function. P2X$_1$, P2X$_3$, P2X$_4$, P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors are present in human fetal heart[293] and mediate distinct effects from those of adenosine acting at P1 receptors.[294] ATP is a mediator of vagal reflexes in the heart and lung[295−299] and potently regulates vascular tone[297] causing either contraction or relaxation depending on receptor location. Nucleotide-mediated vascular contraction occurs by a

direct effect on smooth muscle cells[298] involving P2X receptor activation,[299] while relaxation involves a P2Y-receptor-mediated, endothelium-dependent mechanism.[300–303] A rat model of ischemic congestive heart failure produced by left coronary artery occlusion shows a selective down regulation of $P2X_1$ receptor mRNA in resistance vessels[304] that can be related to an attenuation of P2X-receptor-mediated pressor effects in vivo.[305] ATP may also play a role in the development of vascular disease, e.g., atherosclerosis and hypertension, via its trophic actions.[306]

In the respiratory system, the nucleotide functions as a homeostatic mediator,[307] its presence in the extracellular space modulating normal lung function. Acting via both P2X and P2Y receptors, ATP maintains the patency of the airspaces by modulating the release of phosphatidylcholine as a surfactant and by stimulating mucus secretion, facilitating mucociliary clearance, regulating ciliary beat frequency, and attenuating the inflammation associated with macrophage infiltration following respiratory tract infection, allergen inhalation, and injury. ATP and UTP, acting via $P2Y_2$ receptors, stimulate chloride secretion in airway epithelium and mucin glycoprotein release from epithelial goblet cells,[308,309] enhancing mucociliary clearance. This represents a potential treatment for cystic fibrosis (CF) and chronic bronchitis. In controlled clinical studies UTP, used in preference to ATP as a $P2Y_2$ receptor agonist because it does not form cardiovascularly active metabolites such as adenosine, dose-dependently stimulated mucociliary clearance and sputum expectoration in smokers, nonsmokers, and patients with chronic bronchitis.[310] E-NTPase-resistant analogues of UTP such as INS 365 **63**,[311] in addition to being used in CF and chronic bronchitis, may act as adjunctive agents to enhance the effectiveness of antibiotics used in the treatment of respiratory infections and thus to reduce the amounts used, potentially avoiding antibiotic resistance phenomena.

ATP may also have a direct role in asthma via its actions on bronchial innervation. P2X receptors in the ventrolateral medulla are involved in cardiorespiratory control networks.[312] The nucleotide triggers a reflex bronchconstriction via activation of P2X receptors on vagal C fibers,[313,314] and both ATP and UTP can potentiate IgE-mediated mast cell histamine release, effects involving P2Y receptors.[315] ATP acting via presynaptic P2X receptors in the nucleus tractus solitarius can facilitate glutamate release[316] as well as modulate renal and adrenal sympathetic nerve activity.[317]

Hemostasis. ADP as already discussed above is a potent platelet recruiting factor, inducing platelet aggregation.[318] This involves a complex interplay among three distinct platelet P2 receptors: a $P2Y_1$ receptor linked to phospholipase C pathways,[319] the $P2Y_{12}$ receptor linked to adenylate cyclase inhibition,[72] and a $P2X_1$ receptor.[320] In $P2Y_1$ knockout mice, which showed increased bleeding time and resistance to thromboembolism, ADP was still able to inhibit platelet adenylate cyclase activity, indicating the presence of a second ADP-responsive P2 receptor linked to adenylyl cyclase,[184,185,321] while in $P2Y_{12}$ knockout mice, there is prolonged bleeding time and reduced sensitivity to ADP, thrombin, and collagen.[210]

Hydrolysis of ADP by the E-NTPase CD39 inhibits platelet aggregation both by removing ADP and by forming adenosine that can also inhibit aggregation.[322] ATP is a competitive ADP antagonist at platelet P2Y receptors and stimulates production of PGI_2 and NO, which can also inhibit platelet aggregation and act as vasodilators. Exogenous ATP can thus act to localize thrombus formation to areas of vascular damage, controlling the relationship among hemostasis, thrombosis, and fibrinolysis. In turn, CD39 appears to act together with the extracellular nucleotides released as a result of tissue damage to modulate blood fluidity and platelet activation. Differentiation of the human promyleocytic leukemia cell line, HL-60, into neutrophils is mediated via ATP effects on the $P2Y_{11}$ receptor;[323] thus, agents acting at this receptor may be of interest in relation to the potential treatment of neutropenia and leukemia.[323,324]

AR-C 69331-MX **46** is one of a series of systemically active, synthetic $P2Y_{12}$ receptor antagonists[205] that has a safer side effect profile than aspirin and has superior antithrombotic properties compared to other modulators of platelet activity, e.g., GPIIb/IIIa antagonists, which show a narrow margin of safety.[207] An orally active $P2Y_{12}$ receptor antagonist derived from **57b** (Figure 5) is reportedly entering Phase I trials as an antithrombotic agent.[235] Both $P2Y_{11}$ and $P2Y_{12}$ receptors appear to play a role in hematopoesis.[174,324]

ATP and Neuronal Excitability. The central and peipheral nervous systems contain both P2X and P2Y receptors. ATP acts as fast transmitter in nervous tissue via activation of P2X receptors,[325,326] with other actions being mediated via P2Y receptors. ATP can produce its effects directly via actions on the postsynaptic membrane as well as via an indirect action on presynaptic P2 receptors to modulate the release of a variety of neurotransmitters[8,327] including acetylcholine,[36,328,329] norepinephrine,[330] dopamine,[331,332] serotonin,[333] and glutamate[316,334] and can enhance GABA,[335,336] vasopressin, and oxytocin release.[337] In the case of GABA, this effect of ATP appears to be mediated via $P2X_7$ receptors.[338] In nervous tissue, given the co-release of ATP with other transmitters,[3] the effects of ATP on transmission can be amplified by modulation of the effects of these other intercellular messengers. ATP also functions to transmit information between neurons and glia.[8]

Microinjection of ATP analogues into the prepiriform cortex can induce generalized motor seizures similar to those seen with NMDA and bicuculline.[339] $P2X_2$, $P2X_4$, and $P2X_6$ receptors are expressed in the prepiriform cortex,[100] suggesting that a P2X receptor antagonist may have potential as an antiepileptic.[339] These receptors are also highly expressed in cerebellum and are colocalized with AMPA-sensitive glutamatergic neurons in hippocampus, suggesting a role in the modulation of long-term potentiation.[146] $P2X_7$ immunoreactive cells are also increased in the penumbral region around the stroke focus.[154]

Auditory and Visual Function. In the auditory system, ATP, acting via P2Y receptors, depresses sound-evoked gross compound action potentials in auditory nerves and the distortion product otoacoustic emission, the latter a measure of the active process of the outer

hair cells.[340] P2X and P2Y receptors are present in the vestibular system,[126-129] and $P2X_2$ receptor splice variants are present in the cochlea. $P2X_{2-1}$ and $P2X_{2-3R}$ receptors are found in the rat, and $P2X_{2-1}$, $P2X_{2-2}$, and $P2X_{2-3}$ receptors are found in the guinea pig.[130,341] In the rat, P2X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction.[342] P2Y receptors are present in the marginal cells of the *stria vascularis*, a tissue involved in regulating the ionic and electrical gradients of the cochlea. While little is currently known regarding the pharmacology of hearing and vestibular function, ATP may regulate fluid homeostasis, cochlear blood flow, hearing sensitivity, and development. The parasensory cation flux in cochlear outer sulcus cells and vestibular transitional cells is modulated by ATP acting via a $P2X_2$ receptor that regulates endolymphatic ion homeostasis and provides protection during intense stimulation.[341] In a guinea pig model of unilateral peripheral vestibular disorder, intracochlear administration of ATP via a novel delivery system can restore normal function, an effect that preliminary data suggest is mediated by P2Y receptors.[343]

In the eye, ATP acting via both P2X and P2Y receptors modulates retinal neurotransmission, affecting retinal blood flow and intraocular pressure ATP.[344] In the ocular mucosa, $P2Y_2$ receptor activation increases salt, water, and mucus secretion and may thus represent a potential treatment for dry eye disease.[311] In the retinal pigmented layer, $P2Y_2$ receptor activation can promote fluid absorption and may be effective in treating retinal detachment.

P2X receptors are present in bipolar cells of the rat eye[345,346] where they mediate fast, direct neuronal–glial interactions[347] and appear to participate in signal transmission processes in the retina.[348] There is also emerging evidence that P2-receptor-dependent neurotransmission may play a role in the olfactory and gustatory systems.[348,349]

Pain. ATP is a cotransmitter with norepinephrine (NE) in sympathetic nerves, with acetylcholine (ACh) in parasympathetic nerves supplying the bladder, and in nonadrenergic and noncholinergic (NANC) inhibitory enteric nerves.[135] The nucleotide has both excitatory and sedative effects in the central nervous system (CNS), with both P2X and P2Y receptors being widely distributed in the central and peripheral nervous systems.[3] A specific role for ATP in pain signaling was indicated by seminal work showing that the nucleotide was released from sensory nerves,[350] that it produces fast excitatory potentials in dorsal root ganglion neurons,[351] and that it is a central mediator of primary afferent fiber conduction.[352] These actions appear to be physiologically relevant, since exogenous ATP enhances hyperalgesia in a human blister base model[353] and iontophoretic application of ATP to human skin can elicit pain.[354] The nucleotide is also a key mediator of neurogenic inflammation via its actions on P2 receptors present on neutrophils, macrophages, monocytes, and microglia, activation of which results in cytokine production and release.[60] The homomeric $P2X_3$ receptor and a heteromultimeric combination of $P2X_3$ and $P2X_2$ ($P2X_{2/3}$) receptors are expressed on a high proportion of isolectin IB4-positive neurons in dorsal root ganglion[137,355-357]

and also human dental pulp.[358] Immunohistochemical studies have shown that $P2X_3$ receptor expression is upregulated in dorsal root ganglion neurons and in ipsilateral spinal cord following chronic constriction injury (CCI) of the sciatic nerve,[359] an effect that results in a specific ectopic sensitivity to ATP, since it is not observed on contralateral (uninjured) nerves.

Many studies have shown that P2 receptor ligands modulate nociceptive behaviors in rodents.[360-363] P2 receptor agonists (e.g., ATP and the more stable analogue α,β-meATP) produce acute paw flinching following intradermal administration[364,365] and enhance the nociceptive effects of formalin[366] and carrageenan.[367] Furthermore, P2 receptor antagonists, e.g., suramin and PPADS, have antinociceptive effects in rodent models of both acute[368] and chronic nociception.[369-371] The potent P2X antagonist TNP-ATP **47** attenuates the nociceptive effects of P2 receptor agonists following intrathecal administration[372] and is antinociceptive given intradermally.[265] These data suggest that TNP-ATP can provide effective antinociception when this P2X receptor antagonist is administered directly to a relevant site of action. Consistent with these pharmacological data, $P2X_3$ knockout mice show a loss of rapidly desensitizing inward currents induced by ATP in DRG neurons[138,139] with evidence of a modest but nonsignificant increase in $P2X_2$ homomers[373] and a significantly reduced, but not elimination of, pain-related behaviors in response to intraplantar ATP or formalin.[138,139] The attenuation, but not elimination, of nociceptive responses in the $P2X_3$ knockout mice has been related[33] to a residual ATP-mediated nociceptive effect mediated via a $P2Y_1$-receptor-dependent effect on VR1 receptor function. The ability of ATP to deplete substance P from rat primary sensory neurons[374] and the blockade of the faciliatatory effects of ATP on glycine release in neurons from the trigeminal nucleus pars caudalis by Substance P[375] suggests a functional interplay between neurokinin-1 (NK-1) and P2 pain-sensing pathways.

Further strengthening the evidence for a role of $P2X_3$ receptor activation in nociception is the recent demonstration that intrathecal delivery of a $P2X_3$ receptor gene antisense oligonucleotide reduces $P2X_3$ receptor expression in dorsal root ganglion and nociception in inflammatory and neuropathic pain models.[376] The ability of systemically administered TNP-ATP to fully block acetic acid induced abdominal constrictions in mice[373] further supports a role for $P2X_3$ receptors in visceral nociception. However, other studies based on the use of P2 antagonists such as suramin and PPADS have questioned the contribution of spinal P2X receptors to pain perception.[377,378]

ATP has also been implicated in the pain associated with migraine by virtue of its effects on the neurovsaculature.[379] Coadministration of ATP with nitric oxide (NO), the nucleotide probably acting as adenosine following hydrolysis, mimics the effects of the inhalation anesthetic enflurane and also reduces the amount of inhalation anesthetic required for anesthesia.[380]

$P2X_3$ receptor mutant mice also show a marked bladder hyporeflexia, suggesting that this P2 receptor subunit plays a preferential role in visceral sensations.[141] For visceral pain, a purinergic mechanosensory transduction mechanism has evolved[135,381-384] where

distention of tubes including ureter, gut, salivary and bile ducts, and sacs such as the urinary[384] and gall bladders causes ATP release from the lining epithelial cells to act on $P2X_3$ receptors located on the subepithelial sensory nerve plexus to relay nociceptive signals to the CNS.

Trophic Actions. The viability and also the regeneration of nervous tissue are sustained by a variety of endogenous polypeptide trophic factors. Neural injury increases growth factor levels, e.g., fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor.[385,386] ATP acts in combination with these growth factors to stimulate astrocyte proliferation contributing to the process of reactive astrogliosis, a hypertrophic/hyperplastic response that is typically associated with nervous system trauma including stroke/ischemia, seizure disorders, and neurodegenerative diseases, e.g., Alzheimer's and Parkinson's diseases.

In reactive astrogliosis, astrocytes undergo process elongation and express GFAP (glial fibrillary acidic protein), an astrocyte-specific intermediate filament protein and show an up-regulation of P2X receptors.[387] ATP can increase GFAP and AP-1 complex formation in astrocytes, mimicking the actions of FGF. ATP and GTP can induce trophic factor (NGF, NT-3, FGF) synthesis in astrocytes and neurons.[388] The effects of GTP are, however, inconsistent with any known P2 receptor. A synthetic purine, Neotrofin (AIT-082 **71**, Figure 7), has been reported to up-regulate neurotrophin production and also to enhance working memory and restore age-induced memory deficits in mice.[388] While initial clinical trials of Neotrofin in Alzheimer's disease resulted in positive reports, pivotal Phase III placebo-controlled trials failed to show efficacy.

NGF can up-regulate $P2X_2$ receptor protein and induce neuritogenesis in PC12 cells. The latter effect can be blocked by a number of putative P2 receptor antagonists,[389] suggesting a potential role of P2 receptors in NGF signal transduction processes. ATP can also induce $TNF\alpha$ release from rat microglia, albeit at heroic concentrations (1 mM), an effect mediated by $P2X_7$ receptors involving sustained calcium influx and a MEK/ERK pathway.[390] This may play a key role in blunting the inflammatory response following brain trauma[387] and in the increase in $P2X_7$ receptor message in the stroke penumbra.[154] In vascular smooth muscle cells, ATP can induce cell proliferation via modulation of the cell cycle,[391] acting as a "competence" factor in combination with other growth factors to facilitate tissue repair and regeneration.

The trophic effects of purines also extend to effects on immune cell function. Activation of the macrophage $P2X_7$ receptor by ATP results in both the activation of the caspase-1 (interleukin-1β convertase; ICE),[156,163−165] leading to induction of apoptosis,[392] and nonselective ion pore formation[166] in mast cells, platelets, macrophages, and lymphocytes.[393,394]

Formation of multinucleated giant cells (MGCs) by the fusion of monocytes granulomatous inflammation can be stimulated by IFN-γ and LPS via an up-regulation of $P2X_7$ receptor expression[395] and a concomitant decrease in E-NTPase activity, increasing the susceptibility of MGCs to the cytolytic actions of extracellular ATP. The ATP-induced apoptotic cascade can involve activation of the transcription factor NF-κB[396] and is blocked by caspase inhibitors. ATP can also induce cytolysis in macrophages infected with mycobacterium via $P2X_7$-receptor-mediated apoptotic and necrotic events.[397] While the novel antimicrobial activity of ATP was initially thought to have potential utility in the treatment of tuberculosis, studies in $P2X_7$ receptor knockout mice showed that this receptor, while involved in bacterial killing, was not essential for the antimicrobial effects of ATP.[169] Activation of the $P2Y_6$ receptor stimulates IL-8 production from monocytes.[199]

The involvement of nucleotides in developmental processes,[398] where purinergic effects on tissue differentiation precede those of adrenergic signaling, indicates that in many respects, purine-related effects on development parallel the role of purines following tissue trauma, e.g., angiogenesis, cell proliferation, etc.

Bacterial Infection. The intriguing,[397] albeit non-mandatory role[169] of the $P2X_7$ receptor in macrophage killing of ingested mycobacterium together with the ability of the macrolide antibiotic erythromycin to interact with the $P2X_4$ receptor at therapeutic concentrations[149] suggests that purinergic signaling mechanisms may be involved in the regulation of bacterial growth. $P2X_4$ receptors appear to be the site at which erythromycin can block the effects of ATP on calcium influx, thus representing a potentially novel target to identify compounds that suppress fluid secretion in chronic respiratory tract infections.

Bacterial interactions with host cells involve the binding of bacteria to a membrane glycolipid asialoGM1 (ASGM1). Binding of *P. aeruginsa* flagellin to ASGM1 present on human HM3 epithelial cells promotes the autocrine release of ATP from host cells, activating a P2Y receptor, possibly the $P2Y_{11}$, on the host cell to elicit a pathogenic defensive response,[399] suggesting, despite the evidence from the $P2X_7$ receptor knockout mouse,[169] that nucleotides may play an important, perhaps "multireceptor"-mediated[297] role in the host response to bacterial infection.[397]

Lower Urinary Tract Function. Urinary bladder function is regulated by sympathetic and parasympathetic input with ATP mimicking the effects of parasympathetic nerve stimulation, resulting in bladder contraction[400−403] via activation of P2X receptors in the smooth muscle of the urinary bladder detrusor muscle that is involved in bladder emptying.[403] Detrusor dysfunction results in urge urinary incontinence (UUI), a major health problem in the aging female population. P2X receptors are also present in the bladder urothelium[404] with the $P2X_1$ receptor being the predominant subtype in adult bladder, with the rank order of other P2X receptors being $P2X_1 \gg P2X_4 > P2X_2 > P2X_7 > P2X_5 \gg P2X_3 = P2X_6$.[405] The $P2X_4$ receptor shows high levels of expression in the developing, but not adult, bladder.[406]

The urethral relaxation that leads to micturition involves ATP functioning as a cotransmitter with nitric oxide (NO). NO mediates the first stage of relaxation[403] with ATP acting via P2 receptors to mediate the second phase of the voiding response. Serosal ATP release occurs in rabbit[407] and guinea pig[384] bladder because of the hydrostatic pressure changes associated with bladder filling. Muscarinic receptors, through which ditro-

pan and tolterodine produce their clinically beneficial effects on UUI, mediate 15% of the neurogenic contraction in rat urinary bladder. An additional 50% of the response appears to be mediated via P2X receptor mechanisms,[408] suggesting that P2X receptor antagonists may have more potential in the treatment of UUI than cholinergic-based drugs. Partial bladder outlet obstruction in rabbit leads to an increase in purinergic and a decrease in cholinergic components of nerve-mediated detrusir contaction,[409] a finding that may explain the poor responses to anticholinergic therapy in patients with detrusor instability.

Bladder distension in rats via the use of a 10 μM solution of α,β-MeATP reduces afferent nerve activity by 75% compared to saline controls[410] while $P2X_3$ receptor knockout mice have a marked bladder hyporeflexia, further supporting a role for P2 receptors in the processing of sensory information in the bladder.[141] $P2X_3$ receptor immunoreactivity has been localized to the urothelium in rat and human bladder, indicating a nonneuronal localization leading to the suggestion,[411] despite the knockout data, that $P2X_3$ receptors may not play a direct role in ATP-mediated sensory responses in the urinary bladder.

Tissue from patients with idiopathic detrusor instability lack $P2X_3$ and $P2X_5$ receptor innervation,[412] while $P2X_2$ receptors in the posterior wall of the bladder were increased with other P2X receptors being decreased.[413] Furthermore, in unstable bladder tissue but not that from controls, there was a significant purinergic component in nerve-mediated contractions. In tissue from patients with symptomatic outlet obstruction, $P2X_1$ receptor expression was increased, suggestive of an enhanced purinergic role in the unstable bladder resulting from outlet obstruction.[409,414]

Detrusor strips from patients with interstitial cystitis, a chronic, symptomatic disorder of the bladder in females, showed an increased sensitivity to α,β-MeATP with decreased sensitivity to ACh and histamine[415] also indicative of altered purinergic neurotransmission. Both low- and high-threshold fibers in pelvic afferents discharged in response to P2 agonists, indicating that both nociceptive and nonnociceptive mechanasensory responses in the bladder are under purinergic control leading to the suggestion.[416] In male rat genitalia, $P2X_1$ and $P2X_2$ subunit antibodies show immunoreactivity in the membranes of the smooth muscle layer of the vas deferens, suggesting an involvement in sperm transport and ejaculation.[417] In male $P2X_1$ receptor knockout mice, fertility is reduced by approximately 90% with no effect on copulatory performance. This results from a decreased sperm count in the ejaculate due to a 60% reduction in the contraction sensitivity of the vas deferens to sympathetic nerve stimulation.[119] The remaining 10% in fertility rate suggests that a minor non-$P2X_1$-receptor-mediated component is involved in vas deferens smooth muscle tone. While a $P2X_1$ receptor antagonist may conceptually represent a novel, nonhormonal male contraceptive, some additional attributes will be necessary for it to be a reliable approach to birth control.

In the body of the penis, strong $P2X_1$ with less $P2X_2$ subunit immunoreactivity was present in the smooth muscle of blood vessels and the corpus cavernosum, suggestive of a role in erectile function.[418] In adult rat testis, all P2X receptor subtypes are present with the exception of $P2X_4$ and $P2X_6$.[418] $P2X_1$ receptors were found exclusively on blood cells, while $P2X_2$, $P2X_3$, and $P2X_5$ subunits were expressed differentially on germ cells throughout the stages of the cycle of seminiferous epithelium. P2X receptors were present on Sertoli but not Leydig cells. ATP, acting via germ cell P2X receptors, may therefore play a role in controlling germ cell maturation.

ATP has variable effects on cavernosal smooth muscle depending on the basal tone, relaxing corporal smooth muscle at high basal tension and contracting it at low tension.[419] Both adenosine and ATP show an enhanced sensitivity in penile tissue responses in diabetic males, although in diabetic rats, ATP-induced relaxation is decreased while the effects of adenosine, which are thought to be mediated via potassium channels, are enhanced.[420]

Hepatic Function, Diabetes, and Gastrointestinal Tract Function. There is a considerable body of data on the role of purines in the control of gut function.[421] Changes in liver cell volume, e.g., in epithelium following exposure to insulin, and the uptake of amino and bile acids increase ATP release,[41,422] resulting in a change in extracellular concentration from approximately 10 nM to greater than 300 nM.[423] ATP then acts as an autocrine regulator to modulate membrane chloride conductance and thus facilitates cell volume recovery, linking the cellular hydration state to the P2-mediated pathways involved in cellular homeostasis.[424] ATP is also involved in bile formation and biliary secretion via activation of cholangiocyte $P2Y_2$ receptors in the biliary tree.[425,426]

ATP acts in the gastrointestinal tract as a paracrine signaling molecule, with ATP released from hepatocytes activating P2 receptor signaling pathways in neighboring hepatocytes and biliary cells.[426] ATP may also act as a paracrine mediator in hepatobiliary coupling, a process coordinating the hepatocyte and ductular components of bile formation, thus having clinical potentialin increasing bile flow and in treating prolonged cholestasis.

Purines stimulate glycolysis in isolated perfused rat liver via mechanisms involving both P1 and P2 receptor activation and multiple signaling pathways resulting in an increase in hepatic glucose output.[427,428] ATP also stimulates pancreatic insulin release via a glucose-dependent, P2Y-receptor-mediated mechanism[429,430] and modulates insulin secretion by interactions with ATP-sensitive potassium channels in islet β-cells. ADP thus antagonizes the ATP inhibition of these channels by binding to the second nucleotide binding site on the associated sulfonylurea receptor (SUR),[431] thus activating K_{ATP} channels and inhibiting insulin secretion. $P2X_1$, $P2X_4$, $P2X_7$, $P2Y_1$, and $P2Y_2$ receptors are found in pancrease from rat and mouse.[432] $P2X_7$ receptors were localized in the outer periphery of the islet in α cells that were neither somatostatin- nor insulin-positive.

Both ATP and adenosine are potent stimulants of fluid and electrolyte (chloride) secretion in the colon and gall bladder and in the pancreatic and bile ducts,[424] effects that appear to primarily involve $P2Y_2$ receptor activation.

Bone Function. Both P2X and P2Y receptors are present on the two principle types of cell in bone tissue, macrophage-derived osteoclasts and osteoblasts, that originate from mesenchymal stem cells and are responsible for bone formation. ATP, released in response to shear stress[21,135,433] functions as a mechanotransducer in skeletal tissue acting as an osteoblast mitogen, potentiating the effects of growth factors on bone cells.[434] ATP, but not adenosine, can stimulate the formation of osteoclasts and their resorptive actions in vitro and can inhibit osteoblast-dependent bone formation.[435] The bisphosphonate clodronate, used in the treatment of Paget's disease and tumor-induced osteolysis, may interact with osteoclast P2 receptors[434] ADP, interacting with $P2Y_1$ receptors on mouse calvarial osteoclasts at nanomolar concentrations, and is a potent osteolytic agent, stimulating bone resorption. It is more potent than ATP being equivalent in potency to PGE_2 via a mechanism involving endogenous prostaglandin synthesis. P2 receptor agonists may thus have potential in the treatment of osteoporosis, rheumatoid arthritis, periodontitis, osteopenia, and inflammatory bone loss.[436−438]

Cancer. While there is a considerable clinical data on the potential use of ATP as a treatment for cancer and the cachexia associated with cancer,[439] little progress has been made in advancing the nucleotide to general use or more precisely in understanding its functional role in attenuating metastasis progression. Given the proapoptotic effects of the nucleotide acting via $P2X_7$ receptors,[388] it is likely that ATP effects on cancer growth may be cytokine-dependent because the nucleotide modulates cytokine release.[60,386,440,441] An alternative mechanism may be the ability of ATP to induce COX-2 expression,[190] inhibitors of the latter enzyme having been demonstrated to suppress colon carcinoma growth in controlled clinical trials.[442]

The initial reports on the utility of ATP as an antimetastatic agent[439] attributed the effects of the nucleotide to inhibition of gluconeogenesis, inhibition of the acute-phase response, and/or the decreased production of the proinflammatory cytokines IL-1 and IL-6.[440,441] In a nonrandomized clinical trial, ATP infusion (50 μg kg^{-1} min^{-1} for 96 h) at 28-day intervals to patients with advanced non-small-cell lung cancer (NSCLC) increased ATP pools in red blood cells, inhibited weight loss, reduced cachexia, and improved survival.[439] In a subsequent randomized trial in patients with advanced (stage IIIB or IV) NSCLC, intravenous ATP infused for 30 h at 2−4 week periods with outcome parameters assessed every month for 7 months resulted in a reduction in mean weight changes per month from a control value of −1.0 kg to a negligible change (0.2 kg) in the ATP-treated group.[441] Serum albumin, which was reduced by −1.3 g/L in the untreated group, and elbow flexor muscle strength, which was decreased by 6% in the untreated group, showed no change, e.g., remained normal, in the ATP-treated group. Negative effects on quality of life measures were also attenuated by ATP, with positive effects on body weight, muscle strength, and albumin concentration being especially marked in cachectic patients. There was no effect on tumor growth or survival.

In nude mice homozygous or heterozygous for the cystic fibrosis transmembrane conductance regulator (CFTR), a decrease in breast tumor implantability was reported that had been ascribed to elevated blood ATP levels.[443] Similarly, ATP was found to reduce breast tumor cell growth in vitro, supporting the concept of ATP as an antitumor agent. In cystic fibrosis (CF) patients, there is a reduced incidence of breast cancer and melanoma that is accompanied by an increase in gastrointestinal tract malignancies.[444]

$P2Y_2/P2Y_4$ receptors have been identified on human colorectal carcinoma cells that may be associated with ATP-mediated control of cell proliferation control.[445] In prostate carcinoma cells, ATP and BzATP, but not UTP or adenosine, inhibit cell growth, effects ascribed to P2X receptor activation.[446]

ATP has also been reported as inducing malignant tumor growth in brain,[447] nucleotide infusion eliciting in astrocyte proliferation, reactive astrogliosis, and glioma formation. These in vitro effects contrast with those seen in the clinical situation.[439,441]

In stratified epithelium, $P2X_5$ receptors are associated with proliferating and differentiating cells, while $P2X_7$ receptors label apoptotic cells.[448] From these findings, it has been suggested that selective $P2X_5$ and $P2X_7$ receptor agonists may have potential in the treatment of skin disorders such as psoriasis, scleroderma, and basal cell carcinoma and for restenosis following angioplasty.

Future Directions

In the past 5 years there has been an explosion in published studies characterizing P2 receptor function in a variety of tissue systems and disease states. This has been almost exclusively driven by advances in the cloning, expression, and characterization of the P2 receptor family[3] that have provided the molecular tools necessary to begin the process of understanding the role-(s) of ATP (and UTP) in disease pathophysiology. Many studies have now been done showing differences in P2 receptor expression in development in diseased tissues and from tissues derived from animal models of human disease. The recent flurry of publications on changes in P2X receptors in various bladder disorders[406,411,413,414] reflects the ease of access to tissue from routine surgical procedures and cannot be easily duplicated for other disease states, e.g., diabetes or neurodegenerative diseases involving apoptosis, where ATP may play a key role.

For the newcomer to the P2 area, the plethora of potential disease targets described above, the role of ATP in energy-dependent processes within the cell, the involvement of the nucleotide in the functional "ying−yang" outcomes of P2-mediated apoptotic signaling (e.g., increasing apoptosis to treat cancer may accelerate neurodegenerative processes, while retarding apoptosis as a treatment for Alzheimer's disease may lead to metastasis formation), and the lack of progress in apparently promising clinical studies for the use in ATP in cancer may be less suggestive of a viable approach to drug discovery than an "energy priming" effect more akin to homeopathic medicine.

Nonetheless, there are areas where ligands acting via P2 receptors are showing clinical promise. In the field

of antithrombotics, $P2Y_{12}$ receptor antagonists represented by the ATP bioisosteres AR-C 69931MX are superior in terms of safety to GPIIb/IIIa antagonists. Similarly, in the respiratory area, $P2Y_2/P2Y_4$ agonists such as INS 365 (Up4U), a direct UTP/ATP mimic, has advanced to Phase II clinical testing for chronic bronchitis. Two other areas of significant promise include pain and lower urinary tract dysfunction where considerable evidence exists for a potential role for $P2X_3/P2X_{2/3}$ receptor antagonists. These are both areas of major unmet medical need where the animal models and clinical trials are relatively facile and predictive. Finally, the unique $P2X_7$ receptor with a potential role in inflammation, cancer, and acute neurodegenerative diseases such as stroke may, either via a directly acting ligand or an allosteric modulator, e.g., KN-62-like, yield P2-based therapeutics.

The key issue in advancing the P2 area is the availability of improved ligands that not only are selective for the various P2 receptor subtypes but also have in vivo ADME (absorption, distribution, metabolism, excretion) properties that allow their use in animals to better characterize intact systems. Burgess and Cook[51] have lamented the medicinal chemistry focus in the area of nucleotide synthesis. This is highlighted by the fact that most of the P2 receptor ligands currently in use either are nonselective, e.g., suramin, or have emerged from the efforts of a half-dozen laboratories.

At the molecular design level, despite the ongoing efforts in modeling P2Y and P2X receptors,[217–227] little is known regarding the 3D structure and recognition characteristics of the physiologically relevant receptor in either normal or disease conditions. Added to this is limited knowledge regarding the structure and functional phenotype(s) of the homo- and heteromeric forms of the P2X receptor family, the potential interaction of the latter with subunits from other LGIC receptor families and knowledge regarding novel allosteric sites (e.g., those through which KN-62, avermectin, and ω-conotoxin GVIA act) that may represent novel P2 receptor targets. This is reflected in the P2Y family in the growing complexity of GPCR-oligomer-mediated signaling.[175] HTS approaches have led to the identification of compounds such as CT 50547 **58** but have not been especially fruitful given the dearth of publications to date, although, as noted,[449] an increased focus on natural products may prove to be useful.

In addition to these limitations, which, it might be noted, did not deter the successful efforts of medicinal chemists in many other areas where compound design was driven by a ligand-based SAR approach in the absence of intimate knowledge of the target, charcterization of the receptor profiles of new ligands has been less than optimal. Both native and transfection-based P2 receptor binding assays have been plagued with artifacts.[99–101] Access to high-throughput calcium imaging systems (e.g., FLIPR) to use with P2-receptor-transfected cell lines has also been limited in the academic sector as has access to the full family of P2 receptors.[450] In several instances, a compound, e.g., BzATP, is described as selective for a receptor subtype, e.g., $P2X_7$. The ligand is then used to define $P2X_7$ responses when in fact the compound is far more potent on other P2 receptors.[229] A better integrated approach to defining ligand properties (especially efficacy and selectivity) is critical to advancing the field as is the more extensive use of P2 receptor antisense and receptor knockouts. With an increased ability to measure partial and inverse agonist effects,[451,452] it will also be critical to understand the spectrum of potential functional activity in newer ligands. While both ADP and AMP are inactive at $P2X_7$ receptors in an oocyte expression system,[453] their addition after an initial exposure to ATP or BzATP resulted in concentration-dependent activation, indicating that sensitization of $P2X_7$ receptors results in ADP and AMP sensitivity. UTP and adensine remained ineffective.

An additional challenge in developing novel therapeutics acting via P2 receptors is that the actions of ATP on mammalian tissue(s) are complex in terms of the receptors themselves, the ability of the ligands, and the presence of additional ATP binding proteins. For a P2-based antithrombotic, evidence already exists that blockade of $P2Y_1$, $P2Y_{12}$, and $P2X_1$ receptors may be necessary for a full effect in blocking ADP-induced thrombosis[203] while the nociceptive effects of ATP appear to be mediated by both $P2X_3$ ($P2X_{2/3}$) and $P2Y_1$ receptors.[33] Thus, there may be potential redundancies in ATP signaling mechanisms that may argue against absolute selectivity in the design of P2 receptor antagonists.

ATP-binding molecular targets distinct from the P2 receptor family include members of the ATP binding cassette (ABC) protein family,[454] E-NTPases,[23,24] ATP-modulated potassium channels,[25,26] and the numerous enzymes that utilize ATP for their function, e.g., ectokinases, tyrosine kinases,[27] and the key enzymes in the mitochondrial phosphorylation chain. These are all potential targets for ligands interacting with P2 receptors, especially if the P2 receptors are able to cross the cell membrane.

Despite these challenges, many parallels can be drawn in the area of calcium channel blockers where prevailing wisdom at the time of Fleckenstein's seminal discoveries[455] and the subsequent discovery of the many clinically effective dihydropyridines was that disruption of cellular calcium homeostasis would either be fatal or, at the least, preclude the discovery of safe and effective drugs.

Medicinal chemistry remains the key to advancing efforts in P2-receptor-based drug discovery. With only few pharmacophores known that interact with the P2 receptor family, it will be imperative to identify novel pharmacophores beyond suramin and the plethora of nucleotide-based bioisosteres to identify nonnucleotide and "nonsuramin/PPADS, etc." pharmacophores with ADME properties consistent with "druggability". This may require a more systematic evaluation of compounds from natural sources,[449] which have been a rich source of novel pharmacophores for other receptor families. Together with an increased knowledge of the properties of native receptors including P2X and P2Y oligomers and associated allosteric sites that may have physiologically relevant effects on P2 receptor function, the choice of disease states where robust information on the native human tissue is available, e.g., bladder, will do much

to convince the skeptic of the utility of P2-receptor-based therapeutics.

Biographies

Kenneth A. Jacobson is Chief of the Molecular Recognition Section of NIDDK, National Institutes of Health in Bethesda, Maryland, where his research involves the exploration of the structure−activity relationships for compounds acting through P2 and other G-protein-coupled receptors. His research is currently directed toward the molecular characterization of purine receptors using mutagenesis, computer modeling, and novel ligand design and synthesis.

Michael F. Jarvis is Senior Group Leader and Associate Research Fellow in Global Neuroscience Research at Abbott Laboratories, Abbott Park, Illinois. A continuing research interest has been the study of the role of purines in CNS function, specifically modulation of nociceptive signaling. His current research is focused on the identification and characterization of novel analgesic drug targets and ligands.

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References

(1) Burnstock, G. Purinergic nerves. *Pharmacol. Rev.* **1972**, *24*, 509−581.

(2) Burnstock, G. A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones*; Bolis, L., Straub, R. W., Eds.; Raven: New York, 1978; pp 107−118.

(3) Ralevic, V.; Burnstock, G. Receptors for purines and pyrimidines. *Pharmacol. Rev.* **1998**, *60*, 413−492.

(4) North, R. A.; Surprenant, A. Pharmacology of cloned P2X receptors. *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 563−580.

(5) Illes, P.; Klotz, K.-N.; Lohse, M. J. Signaling by extracellular nucleotides and nucleosides. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 295−298.

(6) Lambrecht, G. Agonists and antagonists acting at P2X receptors: selectivity profiles and functional implications. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 340−350.

(7) Communi, D.; Janssens, R.; Suarez-Huerta, N.; Robaye, B.; Boeynaems, J.-M. Advances in signalling by extracellular nucleotides: the role and transduction mechanisms of P2Y receptors. *Cell. Signalling* **2000**, *12*, 351−360.

(8) Fields, D. R.; Stevens, B. ATP: an extracellular signaling molecule between neurons and glia. *Trends Neurosci.* **2000**, *23*, 625−633.

(9) Chessell, I.; Michel, A. D.; Humphrey, P. P. A. P2X receptors. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 47−63.

(10) Boarder, M. R.; Webb, T. P2Y receptors: structure and function. In *Purinergic and Pyrimidinergic Signalling*. Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 65−88.

(11) Williams, M.; Jarvis, M. F. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem. Pharmacol.* **2000**, *59*, 1173−1185.

(12) Burnstock, G.; Williams, M. P2 purinergic receptors: modulation of cell function and therapeutic potential. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 862−869.

(13) Williams, M. Clinical opportunities in purinergic neuromodulation. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P.; Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 2. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 407−434.

(14) Jacobson, K. A.; King, B. F.; Burnstock, G. Pharmacological characterization of P2 (nucleotide) receptors. *Celltransmissions* **2000**, *16*, 3−15.

(15) Seifert, R.; Schultz, G. Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides. *Trends Pharmacol. Sci.* **1989**, *10*, 365−369.

(16) Barnard, E. A.; Burnstock, G. ATP as a neurotransmitter. In *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*; Chadwick, D. J., Goode, J. A., Eds.; CIBA Foundation Symposium 198; Wiley: Chichester, 1996; Vol. 198, pp 262−265.

(17) Arch, J. R.; Newsholme, E. A. The control of the metabolism and the hormonal role of adenosine. *Essays Biochem.* **1978**, *14*, 82−123.

(18) Noji, H. The rotary enzyme of the cell: the rotation of F1-ATPase. *Science* **1998**, *282*, 1844−1845.

(19) Lazarowski, E. R.; Boucher, R. C.; Harden, T. K. Interplay of constitutively released nucleotides, nucleotide metabolism, and activity of P2Y receptors. *Drug. Dev. Res.* **2001**, *53*, 66−71.

(20) Grierson, J. P.; Meldolesi, J. Shear stress-induced $[Ca^{2+}]i$ transients and oscillations in mouse fibroblasts are mediated by endogenously released ATP. *J. Biol. Chem.* **1995**, *270*, 4451−4456.

(21) Bodin, P.; Burnstock, G. Purinergic signalling: ATP release. *Neurochem. Res.* **2001**, *26*, 959−969.

(22) Pfeiffer, T.; Schuster, S.; Bonhoeffer, S. Cooperation and competition in the evolution of ATP-producing pathways. *Science* **2001**, *292*, 504−507.

(23) Lazarowski, E. R.; Boucher, R. C.; Harden, T. K. Constitutive release of ATP and evidence for major contribution of ecto-nucleotide pyrophosphatase and nucleoside diphosphokinase to extracellular nucleotide concentrations. *J. Biol. Chem.* **2000**, *275*, 31061−31068.

(24) Zimmermann, H. Two novel families of ectonucleotidases: molecular structures, catalytic properties and a search for function. *Trends Pharmacol. Sci.* **1999**, *20*, 231−236.

(25) Zimmermann, H. Ectonucleotidases. In *Purinergic and Pyrimidinergic Signalling*. Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 209−250.

(26) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors. *Pharmacol. Rev.* **2001**, *53*, 527−552.

(27) Beck, G. R., Jr.; Zerler, B.; Moran, E. Phosphate is a specific signal for induction of osteopontin gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8352−8357.

(28) Edwards, G.; Weston, A. H. The pharmacology of ATP-sensitive potassium channels. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 597−637.

(29) Cooper, E. C.; Jan, L. Y. Ion channel genes and human neurological disease: Recent progress, prospects, and challenges. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4759−4761.

(30) Searl, T. J.; Redman, R. S.; Silinsky, E. M. Mutual occlusion of P2X ATP receptors and nicotinic receptors on sympathetic neurons of the guinea-pig. *J. Physiol. (London)* **1998**, *510*, 783−791.

(31) Khakh, B. S.; Zhou, X.; Sydes, J.; Galligan, J. J.; Lester, H. A. State-dependent cross-inhibition between transmitter-gated cation channels. *Nature* **2000**, *406*, 405−410.

(32) Kwak, J.; Wang, M. H.; Hwang, S. W.; Kim, T.-Y.; Lee, S.-Y.; Oh, U. Intracellular ATP increases capsaicin-activated channel activity by interacting with nucleotide-binding domains. *J. Neurosci.* **2000**, *20*, 8298−8304.

(33) Tominaga, M.; Wada, M.; Masu, M. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6951−6956.

(34) Redegeld, F. A.; Caldwell, C. C.; Sitkovsky, M. V. Ecto-protein kinases: ecto-domain phosphorylation as a novel target for pharmacological manipulation? *Trends Pharmacol. Sci.* **1999**, *20*, 453−459.

(35) Ehrlich, Y. H.; Kornecki, E. Ecto-protein kinases as mediators for the action of secreted ATP in the brain. *Prog. Brain Res.* **1999**, *120*, 411−426.

(36) Choi, R. C. Y.; Man, M. L. S.; Ling, K. K. Y.; Ip, N. Y.; Simon, J.; Barnard, E. A.; Tsim, K. W. K. Expression of the P2Y$_1$ nucleotide receptor in chick muscle: its functional role in the regulation of acetylcholinesterase and acetylcholine receptor. *J. Neurosci.* **2001**, *21*, 9224–9234.

(37) Clifford, E. E.; Martin, K. A.; Dalal, P.; Thomas, R.; Dubyak, G. R. Stage specific expression of P2Y receptors, ecto-apyrase and ecto-5′-nucleotidase in myeloid leukocytes. *Am. J. Physiol.* **2000**, *273* (*Cell Physiol. 42*), C973–C987.

(38) Adrian, K.; Bernhard, M. K.; Breitinger, H.-G.; Ogilvie, A. Expression of purinergic receptors (ionotropic P2X1–7 and metabotropic P2Y1–11) during myeloid differentiation of HL60 cells. *Biochim. Biophys. Acta* **2000** *1492*, 127–138.

(39) Communi, D.; Janssens, R.; Robaye, B.; Zeelis, N.; Boynaems, J.-M. Rapid up-regulation of P2Y messengers during granulocytic differentiation of HL-60 cells. *FEBS Lett.* **2000**, *475*, 39–42.

(40) Van Der Weyden, L.; Conigrave, A. D.; Morris, M. B. Signal transduction and white cell maturation via extracellular ATP and the P2Y$_{11}$ receptor. *Immunol. Cell Biol.* **2000**, *78*, 369–374.

(41) Feranchek, A. P.; Fitz, J. G. Regulation of bilary secretion by extracellular nucleotides. *Drug Dev. Res.* **2001**, *52*, 232–238.

(42) Atkinson, D. E. *Cellular Energy Metabolism and Its Regulation*; Academic: San Diego, CA, 1997.

(43) Ostrom, R. S.; Gregorian, C.; Insel, P. A. Cellular release of and response to ATP as key determinants of the set-point of signal transduction pathways. *J. Biol. Chem.* **2000**, *275*, 11735–11739.

(44) Jacobson, K. A.; van Galen, P. M.; Williams, M. Perspective: Adenosine receptors: pharmacology, structure activity relationships, and therapeutic potential. *J. Med. Chem.* **1992**, *35*, 407–422.

(45) Bhagwat, S. S.; Williams, M. P2 Purine and pyrimidine receptors: emerging superfamilies of G-protein coupled and ligand gated ion channel receptors. *Eur. J. Med. Chem.* **1997**, *32*, 183–193.

(46) Hibell, A. D.; Thompson, K. M.; Simon, J.; Xing, M.; Humphrey, P. P.; Michel, A. D. Species- and agonist-dependent differences in the deactivation-kinetics of P2X$_7$ receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2001**, *363*, 639–648.

(47) Hibell, A. D.; Thompson, K. M.; Xing, M.; Humphrey, P. P.; Michel, A. D. Complexities of measuring antagonist potency at P2X$_7$ receptor orthologs. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 947–957.

(48) DeGubareff, T.; Sleator, W., Jr. Effects of caffeine on mammalian atrial muscle and its interaction with adenosine and calcium. *J. Pharmacol. Exp. Ther.* **1965**, *148*, 202–214.

(49) Sattin, A.; Rall, T. W. The effects of adenosine and adenine nucleotides on the cyclic 3′-5′-phosphate content of guinea pig cerebral cortex slices. *Mol. Pharmacol.* **1970**, *6*, 13–23.

(50) Daly, J. W. Alkylxanthines as research tools. *J. Auton. Nerv. Syst.* **2000**, *81*, 44–52.

(51) Burgess, K.; Cook, D. Syntheses of nucleoside triphosphates. *Chem. Rev.* **2000**, *100*, 2047–2059.

(52) Cusack, N. J.; Hourani, S. M. O. Structure activity relationships for adenine nucleotide receptors on mast cells, human platelets, and smooth muscle. In *Purines in Cellular Signalling: Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer: New York, 1990; pp 254–259.

(53) Jacobson, K. A.; Hoffmann, C.; Kim, Y. C.; Camaioni, E.; Nandanan, E.; Jang, S. Y.; Guo, D. P.; Ji, X.-D.; von Kügelgen, I.; Moro, S.; King, B. F.; Brown, S. G.; Wildman, S. S.; Burnstock, G.; Boyer, J. L.; Mohanram, A.; Harden, T. K. Molecular recognition in P2 receptors: ligand development aided by molecular modeling and mutagenesis. *Prog. Brain Res.* **1999**, *120*, 119–134.

(54) Jacobson, K. A.; Suzuki, F. Recent developments in selective agonists and antagonists acting at purine and pyrimidine receptors. *Drug Dev. Res.* **1997**, *39*, 289–300.

(55) Jacobson, K. A.; Knutsen, L. P1 and P2 purine and pyrimidine receptor ligands. In *Purinergic and Pyrimidinergic Signalling*, Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 129–175.

(56) Burnstock, G.; Kennedy, C. Is there a basis for distinguishing two types of P$_2$-purinoceptor? *Gen. Pharmacol.* **1985**, *16*, 433–440.

(57) Gordon, J. L. Extracellular ATP: effects, sources and fate. *Biochem. J.* **1986**, *233*, 309–319.

(58) Communi, D.; Boeynaems, J. M. Receptors responsive to extracellular pyrimidine nucleotides. *Trends Pharmacol. Sci.* **1997**, *18*, 83–86.

(59) Connolly, G. P.; Duley, J. A. Uridine and its nucleotides: biological actions, therapeutic potential. *Trends Pharmacol. Sci.* **1999**, *20*, 218–226.

(60) Dubyak, G. R.; El Moatassim, C. Signal transduction via P$_2$-purinergic receptors for extracellular ATP and other nucleotides. *Am. J. Physiol.* **1993**, *265*, C577–C606.

(61) Abbracchio, M. P.; Burnstock, G. Purinoceptors: Are there families of P$_{2X}$ and P$_{2Y}$ purinoceptors? *Phamacol. Ther.* **1994**, *64*, 445–475.

(62) Fredholm, B. B.; Abbracchio, MP.; Burnstock, G.; Daly, J. W.; Harden, K. T.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and classification of purinoceptors: a report from the IUPHAR subcommittee. *Pharmacol. Rev.* **1994**, *46*, 143–156.

(63) Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, K. T.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and classification of purinoceptors: a report from the IUPHAR subcommittee. *Trends Pharmacol. Sci.* **1997**, *46*, 143–156.

(64) Khakh, B. S.; Burnstock, G.; Kennedy, C.; King, B. F.; North, R. A.; Seguela, P.; Voight, M.; Humphrey, P. P. A. International Union of Pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. *Pharmacol. Rev.* **2001**, *52*, 107–118.

(65) Chambers, J. K.; Macdonald, L. E.; Sarau, H. M.; Ames, R. S.; Freeman, K.; Foley, J. J.; Zhu, Y.; McLaughlin, M. M.; Murdock, P.; McMillan, L.; Trill, J.; Swift, A.; Aiyar, N.; Taylor, P.; Vawter, L.; Naheed, S.; Szekeres, P.; Hervieu, G.; Scott, C.; Watson, J. M.; Murphy, A. J.; Duzic, E.; Klein, C.; Bergsma, D. J.; Wilson, S.; Livi, G. P. A G protein-coupled receptor for UDP-glucose. *J. Biol. Chem.* **2000**, *275*, 10767–10771.

(66) Miras Portugal, M. T.; Gulaix, J.; Mateo, M.; Diaz-Hernadez, M.; Gomez-Villafuertes, R.; Castro, E.; Pintor, J. Diadenosine polyphosphates, extracellular function and catabolism. *Prog. Brain Res.* **1999**, *120*, 397–409.

(67) Pintor, J.; Miras Portugal, M. T. Receptors for diadenosine polyphosphates P$_{2D}$, *P2Y$_{ApnA}$*, P4 and dinucleotide receptors: are there too many? *Trends Pharmacol. Sci.* **2000**, *21*, 135.

(68) Shinozuka, K.; Bjur, R. A.; Westfall, D. P. Characterization of prejunctional purinoceptors on adrenergic nerves of the rat caudal artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *338*, 221–227.

(69) International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* **2001**, *409*, 860–921.

(70) King, B. F.; Liu, M.; Pintor, J.; Gulaix, J.; Miras-Portugal, M. T.; Burnstock, G. Diinosine pentaphosphate (IP$_5$I) is a potent antagonist at recombinant rat P2X$_1$ receptors. *Br. J. Pharmacol.* **1999**, *128*, 981–988.

(71) Surprenant, A.; Rassendren, F.; Kawashima, E.; North, RA.; Buell, G. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X$_7$). *Science* **1996**, *272*, 735–738.

(72) Hollopeter, G.; Jantzen, H. M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R. B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. B. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **2001**, *409*, 202–207.

(73) Akbar, G. K.; Dasari, V. R.; Webb, T. E.; Ayyanathan, K.; Pillarisetti, K.; Sandhu, A. K.; Athwal, R. S.; Daniel, J. L.; Ashby, B.; Barnard, E. A.; Kunapuli, S. P. Molecular cloning of a novel P2 purinoceptor from human erythroleukemia cells. *J. Biol. Chem.* **1996**, *271*, 18363–18367.

(74) Yokomizo, T.; Izumi, T.; Chang, K.; Takuwa, Y.; Shimizu, T. A. G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature* **1997**, *387*, 620–624.

(75) Zhang, F. L.; Luo, L.; Gustafson, E.; Laschowicz, J.; Smith, M.; Qiao, X.; Liu, Y.-H.; Chen, G.; Pramanik, B.; Laz, T. M.; Palmer, K.; Bayne, M.; Monsma, F. J., Jr. ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. *J. Biol. Chem.* **2001**, *276*, 8608–8615.

(76) Takasaki, J.; Kamohara, M.; Saito, T.; Matsumoto, M.; Matsumoto, S.-I.; Ohishi, T.; Soga, T.; Matsushime, H.; Furuichi, K. Molecular cloning of the platelet P2T$_{AC}$ ADP receptor: pharmacological comparison with another ADP receptor, the P2Y$_1$ receptor. *Mol. Pharmacol.* **2001**, *60*, 432–439.

(77) Boue-Grabot, E.; Akimenko, M.-A.; Seguela, A. Unique functional properties of a sensory neuronal P2X ATP-gated channel from Zebrafish. *J. Neurochem.* **2000**, *75*, 1600–1607.

(78) Kennedy, C.; Leff, P. How should P$_{2X}$ purinoceptors be classified pharmacologically? *Trends Pharmacol. Sci.* **1995**, *16*, 168–174.

(79) Freissmuth, M.; Boehm, S.; Beindl, W.; Nickel, P.; IJzerman, A. P.; Hohenegger, M.; Nanoff, C. Suramin analogues as subtype selective G protein inhibitors. *Mol. Pharmacol.* **1996**, *49*, 602–611.

(80) Jentsch, K. D.; Hunsmann, G.; Hartmann, H.; Nickel, P. Inhibition of human immunodeficiency virus type I reverse transcriptase by suramin-related compounds. *J. Gen. Virol.* **1987**, *68*, 2183–2192.

(81) Kennedy, C.; Qi, A.-D.; Herold, C. L.; Harden, T. K.; Nicolas, R. A. ATP, an agonist at the rat P2Y$_4$ receptor, is an antagonist at the human P2Y$_4$ receptor. *Mol. Pharmacol.* **2000**, *57*, 926–931.

(82) Palmer, R. K.; Boyer, J. L.; Schacter, J. B.; Nicolas, R. A.; Harden, T. K. Agonist actions of adenosine triphosphates at the human P2Y$_1$ receptor. *Mol. Pharmacol.* **1998**, *54*, 1118–1123.

(83) Sak, K.; Jarv, J. Adenosine triphosphate is a full antagonist at human P2Y₁ purinoceptors. *Neurosci. Lett.* **2000**, *284*, 179–181.

(84) Kenakin, T. P. The classification of seven transmembrane receptors in recombinant expression systems. *Pharmacol. Rev.* **1996**, *48*, 413–463.

(85) Khakh, B. S.; Proctor, W. R.; Dunwiddie, T. V.; Labarca, C.; Lester, H. A. Allosteric control and gating and kinetics at P2X₄ receptor channels. *J. Neurosci.* **1999**, *19*, 7289–7299.

(86) Lewis, C.; Neidhart, S.; Holy, C.; North, R. A.; Buell, G.; Surprenant, A. Coexpression of P2X₂ and P2X₃ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature* **1995**, *377*, 432–435.

(87) Torres, G. E.; Haines, W. R.; Egan, T. M.; Voight, M. M. Co-expression of P2X₁ and P2X₅ receptor subunits reveals a novel ATP-gated ion channel. *Mol. Pharmacol.* **1998**, *54*, 989–993.

(88) Le, K. T.; Boue-Grabot, E.; Archamabault, V.; Seguela, P. Functional and biochemical evidence for heteromeric ATP-gated channels composed of P2X₁ and P2X₅ subunits. *J. Biol. Chem.* **1999**, *274*, 15415–15419.

(89) Li, G.-H.; Lee, E. M.; Blair, D.; Holding, C.; Poronniki, P.; Cook, D. I.; Barden, J. A.; Bennett, M. R. The distribution of P2X receptor clusters on individual neurons in sympathetic ganglia and their redistribution on agonist activation. *J. Biol. Chem.* **2000**, *275*, 29017–29112.

(90) McDonald, H. A.; Bianchi, B. R.; McKenna, D. G.; Briggs, C. A.; Burgard, E. A.; Lynch, K. J.; Faltynek, C.; Jarvis, M. F.; Cartmell, J. Potent desensitization of human P2X₃ receptors by diadenosine polyphosphates, α,β-meATP and ATP. *Eur. J. Pharmacol.* **2002**, *435*, 135–142.

(91) Dubyak, G. R.; Clifford, E. E.; Humphreys, B. D.; Kertsey, S. B.; Martin, K. A. Expression of multiple ATP subtypes during the differentiation and inflammatory activation of myeloid leukocytes. *Drug. Dev. Res.* **1996**, *39*, 269–278.

(92) Black, J. W. Should we be concerned about the state of hormone receptor classification? In *Perspectives on Receptor Classification*; Black, J. W., Jenkinson, D. H.; Gerskowitch, V. P., Eds.; Liss: New York, 1987; pp 11–15.

(93) Tordorov, L. D.; Mihaylova-Todorova, S.; Westfall, T. D.; Sneddon, P.; Kennedy, C.; Bjur, R. A.; Westfall, D. P. Neuronal release of soluble nucleotidases and their role in neurotransmitter inactivation. *Nature* **1997**, *387*, 76–79.

(94) Westfall, T. D.; Menzies, J. R. W.; Liberman, R.; Waterson, S.; Ramphir, N.; Westfall, D. P.; Sneddon, P.; Kennedy, C. Release of a soluble ATPase from the rabbit isolated vas deferens during nerve stimulation. *Br. J. Pharmacol.* **2000**, *131*, 909–914.

(95) Masino, S. A.; Dunwiddie, T. V. Role of purines and pyrimidines in the central nervous system. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 251–287.

(96) Diaz-Hernandez, M.; Pereria, M. F.; Pintor, J.; Cuhna, R. A.; Ribeiro, J. A. Miras-Portugal, M. T. Modulation of rat hippocampal dinucleotide receptor by adenosine receptor activation. *J. Pharmacol. Exp. Ther.*, in press.

(97) Crack, B. E.; Beukers, M. W.; McKenchie, K. C. W.; IJzerman, A. P.; Leff, P. Pharmacological analysis of ecto-ATPase inhibition: evidence for combined enzyme inhibition and receptor antagonism in P₂ₓ-purinoceptor ligands. *Br. J. Pharmacol.* **1994**, *113*, 1432–1438.

(98) Crack, B. E.; Pollard, C. E.; Beukers, M. W.; Roberts, S. M.; Hunt, S. F.; Ingall, A. H.; McKechnie, K. C.; IJzerman, A. P.; Leff, P. Pharmacological and biochemical analysis of FPL 67156, a novel, selective inhibitor of ecto-ATPase. *Br. J. Pharmacol.* **1995**, *114*, 475–481.

(99) Bo, X. N.; Burnstock, G. High- and low-affinity binding sites for [³H]-α,β-methylene ATP in rat urinary bladder membranes. *Br. J. Pharmacol.* **1990**, *101*, 291–296.

(100) Simon, J.; Webb, T. E.; Barnard, E. A. Distribution of [³⁵S]-dATPαS binding sites in the adult rat neuraxis. *Neuropharmacology* **1997**, *36*, 1243–1251.

(101) Yu, H.; Bianchi, B.; Metzger, R.; Lynch, K. J.; Kowaluk, E. A.; Jarvis, M. F.; van Biesen, T. Lack of specificity of [³⁵S]-ATPγS and [³⁵S]-ADPγS as radioligands for inotropic and metabotropic P2 receptor binding. *Drug Dev. Res.* **1999**, *48*, 84–93.

(102) Baurand, A.; Raboisson, P.; Freund, M.; Leon, C.; Cazenave, J. P.; Bourguignon, J. J.; Gachet, C. Inhibition of platelet function by administration of MRS2179, a P2Y₁ receptor antagonist. *Eur. J. Pharmacol.* **2001**, *412*, 213–221.

(103) Swanson, K. D.; Reigh, C.; Landreth, G. E. ATP-stimulated activation of the mitogen-activated protein kinases through ionotrophic P2X₂ purinoreceptors in PC12 cells. Difference in purinoreceptor sensitivity in two PC12 cell lines. *J. Biol. Chem.* **1998**, *273*, 19965–19971.

(104) Sellers, L. A.; Simon, J.; Lundahl, T. S.; Cousens, D. J.; Humphrey, P. P.; Barnard, E. A. Adenosine nucleotides acting at the human P2Y₁ receptor stimulate mitogen-activated protein kinases and induce apoptosis. *J. Biol. Chem.* **2001**, *276*, 16379–16390.

(105) Waldmann, R.; Ladzunski, M. H⁺ gated cation channels: neuronal acid sensors in the NaC/DEG family of ion channels. *Curr. Opin. Neurobiol.* **1998**, *8*, 418–424.

(106) Nicke, A.; Baumert, H. G.; Rettinger, J.; Eichele, A.; Lambrecht, G.; Mutschler, E.; Schmalzing, G. P2X₁ and P2X₃ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J.* **1998**, *17*, 3016–3018.

(107) Torres, G. E.; Egan, T. M.; Voight, M. M. Identification of a domain involved in ATP-gated ionotropic receptor subunit assembly. *J. Biol. Chem.* **1999**, *274*, 22359–22365.

(108) Lloyd, G. K.; Williams, M. Perspective in Pharmacology. Neuronal nicotinic receptors as novel drug targets. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 461–467.

(109) Collo, G.; North, R. A.; Kawashima, E.; Merlo-Pich, E.; Neidhart, S.; Surprenant, A.; Buell, G. Cloning of P2X₅ and P2X₆ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.* **1996**, *16*, 2495–2507.

(110) Brown, S.; Townsend-Nicholson, A.; Jacobson, K. A.; Burnstock, G.; King, B. F. Heteromultimeric P2X₁/₂ receptors show a novel sensitivity to extracellular pH. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 673–680.

(111) Patel, M. K.; Khakh, B. S.; Henderson, G. Properties of native P2X receptors in rat trigeminal mesencephalic nucleus neurons: lack of correlation with known, heterologously expressed P2X receptors. *Neuropharmacology* **2001**, *40*, 96–105.

(112) Li, G. H.; Lee, E. M.; Blair, D.; Holding, C.; Poronnik, P.; Cook, D. I.; Barden, J. A.; Bennett, M. R. The distribution of P2X receptor clusters on individual neurons in sympathetic ganglia and their redistribution on agonist activation. *J. Biol. Chem.* **2000**, *275*, 29107–29112.

(113) Dutton, J. L.; Poronnik, P.; Li, G. H.; Holding, C. A.; Worthington, R. A.; Vandenberg, R. J.; Cook, D. I.; Barden, J. A.; Bennett, M. R. P2X₁ receptor membrane redistribution and down-regulation visualized by using receptor-coupled green fluorescent protein chimeras. *Neuropharmacology* **2000**, *39*, 2054–2066.

(114) Khakh, B. S.; Smith, W. B.; Chiu, C. S.; Ju, D.; Davidson, N.; Lester, H. A. Activation-dependent changes in receptor distribution and dendritic morphology in hippocampal neurons expressing P2X₂-green fluorescent protein receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5288–5293.

(115) Valera, S.; Hussy, N.; Evans, R. J.; Adams, N.; North, R. A.; Surprenant, A.; Buell, G. A new class of ligand-gated ion channel defined by P2X for extracellular ATP. *Nature* **1994**, *371*, 516–519.

(116) Valera, S.; Talbot, F.; Evans, R. J.; Gos, A.; Antonarakis, S. E.; Morris, M. A.; Buell, G. Characterization and chromosomal localization of a human P2X receptor from the urinary bladder. *Recept. Channels* **1995**, *3*, 283–289.

(117) Longhurst, P. A.; Schwegel, T.; Folander, K.; Swanson, R. The human P2X₁ receptor: molecular cloning, tissue distribution, and localization to chromosome 17. *Biochim. Biophys. Acta.* **1996**, *1308*, 185–188.

(118) MacKenzie, A. B.; Mauhaut-Smith, M. P.; Sage, S. O. Activation of receptor-operated cation channels via P₂ₓ₁ not P₂T purinoceptors in human platelets. *J. Biol. Chem.* **1996**, *271*, 2879–2881.

(119) Mulryan, K.; Gitterman, D. P.; Lewis, C. H.; Vial, C.; Leckie, B. J.; Cobb, A. L.; Brown, J. E.; Conley, E. C.; Buell, G.; Pritchard, C. A.; Evans, R. J. Reduced vas deferens contraction and male infertility in mice lacking P2X₁ receptors. *Nature* **2000**, *403*, 86–89.

(120) Brake, A. J.; Wagenbach, M. J.; Julius, D. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature* **1994**, *371*, 519–523.

(121) Valera, S.; Hussy, N.; Evans, R. J.; Adami, N.; North, R. A.; Surprenant, A.; Buell, G. A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. *Nature* **1994**, *371*, 516–519.

(122) Evans, R. J.; Lewis, C.; Virginio, C.; Lundstrom, K.; Buell, G.; Surprenant, A.; North, R. A. Ionic permeability of, and divalent cation effects on, two ATP-gated cation channels (P2X receptors) expressed in mammalian cells. *J. Physiol.* **1996**, *497*, 413–422.

(123) Stojilkovic, S. S.; Tomic, M.; Van Goor, F.; Koshimizu, T. Expression of purinergic P2X₂ receptor-channels and their role in calcium signaling in pituitary cells. *Biochem. Cell Biol.* **2000**, *78*, 393–404.

(124) King, B. F.; Wildman, S. S.; Ziganshin, L. E.; Pintor, J.; Burnstock, G. Effects of extracellular pH on agonism and antagonism at the recombinant P2X₂ receptor. *Br. J. Pharmacol.* **1997**, *121*, 1445–1453.

(125) Vulchanova, L.; Arvidsson, U.; Riedl, M.; Wang, J.; Buell, G.; Surprenant, A.; North, R. A.; Elde, R. Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8063−8067.

(126) Jarlebark, L. E.; Houslay, G. D.; Thorne, P. R. Immunohistochemical localization of the adenosine 5′-triphosphate-gated ion channel P2X$_2$ subunits in adult and developing rat cochlear. *J. Comp. Neurol.* **2000**, *421*, 389−301.

(127) Brandle, U.; Zenner, H. P.; Ruppersberg, J. P. Gene expression of P2X-receptors in the developing inner ear of the rat. *Neurosci. Lett.* **1996**, *273*, 105−108.

(128) Troyanovskaya, M.; Wackym, P. A. Evidence for three additional P2X$_2$ purinoceptor isoforms produced by alternative splicing in the adult rat vestibular end-organs. *Hear. Res.* **1998**, *126*, 210−209.

(129) Parker, M. S.; Larroque, M. L.; Campbell, J. M.; Bobbin, R. P.; Deininger, P. Novel variant of the P2X$_2$ receptor from the guinea pig organ of Corti. *Hear. Res.* **1998**, *121*, 62−70.

(130) Housley, G. D.; Kanjhan, R.; Raybould, N. P.; Greeenwood, D.; Salih, S. G.; Jarlebark, L. E.; Burton, L. D.; Setz, V. C.; Cannell, M. B.; Soeller, C.; Chritie, D. L.; Usami, S.; Matsubara, A.; Yoshie, H.; Ryan, A. F.; Thorne, P. R. Expression of the P2X$_2$ receptor subunit of the ATP-gated ion channel in the cochlear: implications for sound transduction and auditory neurotarnsmission. *J. Neurosci.* **1999**, *19*, 8377−8388.

(131) Simon, J.; Kidd, E. J.; Smith, F. M.; Chessell, I. P.; Murrell-Lagnado, R.; Humphrey, P. P.; Barnard, E. A. Localization and functional expression of splice variants of the P2X$_2$ receptor. *Mol. Pharmacol.* **1997**, *52*, 237−248.

(132) Lynch, K. J.; Touma, E.; Niforatos, W.; Kage, K. L.; Burgard, E. C.; van Biesen, T.; Kowaluk, E. A.; Jarvis, M. F. Molecular and functional characterization of human P2X$_2$ receptor. *Mol. Pharmacol.* **1999**, *56*, 1171−1181.

(133) Ding, S.; Sachs, F. Single channel properties of P2X$_2$ purinoceptors. *J. Gen. Physiol.* **1999**, *113*, 695−720.

(134) Chen, C. C.; Akopian, A. N.; Sivilotti, L.; Colquhoun, D.; Burnstock, G.; Wood, J. N. A P$_{2X}$ purinoceptor expressed by a subset of sensory neurons. *Nature* **1995**, *377*, 428−431.

(135) Burnstock, G. Purine-mediated signaling in pain and visceral perception. *Trends Pharmacol. Sci.* **2001**, *22*, 182−188.

(136) Yao, S. T.; Barden, J. A.; Lawrence, A. J. On the immunohistochemical distribution of ionotropic P2X receptors in the nucleus tractus solitarius of the rat. *Neuroscience* **2001**, *108*, 673−685.

(137) Vulchanova, L.; Riedl, M. S.; Shuster, S. J.; Stone, L. S.; Hargreaves, K. M.; Buell, G.; Surprenant, A.; North, R. A.; Elde, R. P2X$_3$ is expressed by DRG neurons that terminate in inner lamina II. *Eur. J. Neurosci.* **1998**, *10*, 3470−3478.

(138) Vulchanova, L.; Riedl, M. S.; Shuster, S. J.; Buell, G.; Surprenant, A.; North, R. A.; Elde, R. Immunohistochemical study of the P2X$_2$ and P2X$_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. *Neuropharmacology* **1997**, *36*, 1229−1242.

(139) Cockayne, D. A.; Zhu, Q.-M.; Hamilton, S.; Dunn, P. M.; Zhong, Y.; Berson, A.; Kassotakis, L.; Bardini, M.; Muraski, J.; Novakovic, S.; Lachnit, W. G.; Burnstock, G.; McMahon, S. B.; Ford, A. P. D. W. P2X$_3$-deficient mice display urinary bladder hyporeflexia and reduced nocifensive behavior. *Nature* **2000**, *407*, 1011−1015.

(140) Souslova, V.; Cesare, P.; Ding, Y.; Akopian, A. N.; Dickenson, A.; Boyce, S.; Hill, R.; Nebenius-Oosthuizen, D.; Smith, A. J. H.; Kidd, E. J.; Wood, J. N. Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X$_3$ receptors. *Nature* **2000**, *407*, 1015−1017.

(141) Vlaskovska, M.; Kasakov, L.; Rong, W.; Bodin, P.; Bardini, M.; Cockayne, D. A.; Ford, A. P.; Burnstock, G. P2X$_3$ knock-out mice reveal a major sensory role for urothelially released ATP. *J. Neurosci.* **2001**, *21*, 5670−5677.

(142) Xiao, H.-S.; Huang, Q.-H.; Zhang, F.-X.; Bao, L.; Lu, Y.-J.; Guo, C.; Yang, L.; Huang, W.-J.; Fu, G.; Xu, S.-H.; Cheng, X.-P.; Yan, Q.; Zhu, Z.-D.; Zhang, X.; Chen, Z.; Han, Z.-G.; Zhang, X. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8360−8365.

(143) Lalo, U. V.; Pankratov, Y. V.; Arndts, D.; Krishtal, O. A. ω-Conotoxin GVIA potently inhibits the currents mediated by P2X receptors in rat DRG neurons. *Brain Res. Bull.* **2001**, *54*, 507−512.

(144) Bo, X.; Zhang, Y.; Nassar, M.; Burnstock, G.; Schoepfer, R. A P2X purinoceptor cDNA conferring a novel pharmacological profile. *FEBS Lett.* **1995**, *375*, 129−133.

(145) Soto, F.; Garcia-Guzman, M.; Gomez-Hernandez, J. M.; Hollmann, M.; Karschin, C.; Stuhmer, W. P2X$_4$: an ATP-activated ionotropic receptor cloned from rat brain. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3684−3688.

(146) Rubio, M. E.; Soto, F. Distinct localization of P2X receptors at excitatory postsynaptic specializations. *J. Neurosci.* **2001**, *21*, 641−653.

(147) Jones, C. A.; Chessell, I. P.; Simon, J.; Barnard, E. A.; Miller, K. J.; Michel, A. D.; Humphrey, P. P. Functional characterization of the P2X$_4$ receptor orthologues. *Br. J. Pharmacol.* **2000**, *129*, 388−394.

(148) Townsend-Nicholson, A.; King, B. F.; Wildman, S. S.; Burnstock, G. Molecular cloning, functional characterization and possible cooperativity between the murine P2X$_4$ and P2X$_{4a}$ receptors. *Mol. Brain Res.* **1999**, *64*, 246−254.

(149) Zhao, D.-M.; Xue, H.-H.; Chida, K.; Suda, T.; Oki, Y.; Kanai, M.; Uchida, C.; Ichiyama, A.; Nakamura, H. Effect of erythromycin on ATP-induced intracellular calcium response in A549 cells. *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **2000** *278*, L276−L736.

(150) Bo, X.; Schoepfer, R.; Burnstock, G. Molecular cloning and characterization of a novel ATP P2X receptor subtype from embryonic chick skeletal muscle. *J. Biol. Chem.* **2000**, *275*, 14401−14407.

(151) Haines, W. R.; Torres, G. E.; Voigt, M. M.; Egan, T. M. Properties of the novel ATP-gated ionotropic receptor composed of the P2X$_1$ and P2X$_5$ isoforms. *Mol. Pharmacol.* **1999**, *56*, 720−727.

(152) Nawa, G.; Miyoshi, Y.; Yoshikawa, H.; Ochi, T.; Nakamura, Y. Frequent loss of expression or aberrant alternative splicing of P2XM, a p53-inducible gene, in soft tissue tumors. *Br. J. Cancer* **1999**, *80*, 1185−1189.

(153) Collo, G.; Neidhart, S.; Kawashima, E.; Kosco-Vilbois, M.; North, R. A.; Buell, G. Tissue distribution of P2X$_7$ receptors. *Neuropharmacology* **1997**, *36*, 1277−1283.

(154) Rassendren, F.; Buell, G. N.; Virginio, C.; Collo, G.; North, R. A.; Surprenant, A. The permeabilizing ATP receptor, P2X$_7$. Cloning and expression of a human cDNA. *J. Biol. Chem.* **1997**, *272*, 5482−5486.

(155) Virgilio, F. D.; Vishawanath, V.; Ferrari, D. On the role of the P2X$_7$ receptor in the immune system. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part II. Cardiovsacular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*; Springer Verlag: Heidelberg, 2001; pp 355−373.

(156) Virginio, C.; MacKenzie, A.; Rassendren, F. A.; North, R. A.; Surprenant, A. Pore dilation of neuronal P2X receptor channels. *Nat. Neurosci.* **1999**, *2*, 315−321.

(157) Khakh, B. S.; Bao, X. R.; Labarca, C.; Lester, H. A. Neuronal P2X transmitter-gated cation channels change their ion selectivity in seconds. *Nat. Neurosci.* **1999**, *2*, 322−330.

(158) Schilling, W. P.; Sinkins, W. G.; Estacion, M. Maitotoxin activates a nonselective cation channel and a P2Z/P2X$_7$-like cytolytic pore in human skin fibroblasts. *Am. J. Physiol.* **1999**, *277*, C755−C765.

(159) Jiang, L. H.; Mackenzie, A. B.; North, R. A.; Surprenant, A. Brilliant blue G selectively blocks ATP-gated rat P2X$_7$ receptors. *Mol. Pharmacol.* **2000**, *58*, 82−88.

(160) Murgia, M.; Hanau, S.; Pizzo, P.; Rippa, M.; Virgilio, F. Oxidized ATP. *J. Biol. Chem.* **1993**, *268*, 8199−8203.

(161) Gargett, C. E.; Wiley, J. S. The isoquinoline derivative KN-62 a potent antagonist of the P$_{2Z}$ receptor of human lymphocytes. *Br. J. Pharmacol.* **1997**, *120*, 1483−1490.

(162) Perregaux, D.; Gabel, C. A. Interleukin-1β maturation and release in response to ATP and nigericin. *J. Biol. Chem.* **1994**, *269*, 15195−15203.

(163) Perregaux, D.; Labasi, J.; Lalibete, R.; Stam, E.; Solle, M.; Koller, B.; Griffiths, R.; Gabel, C. A. Interleukin-1β posttranslational processing−exploration of P2X$_7$ receptor involvement. *Drug Dev. Res.* **2001**, *53*, 83−90.

(164) MacKenzie, A.; Wilson, H. L.; Kiss-Toth, E.; Dower, S. K.; North, R. A.; Surprenant, A. Rapid secretion of interleukin-1β by microvesical shedding. *Immunity* **2001**, *8*, 825−835.

(165) Di Virgilio, F.; Falzoni, S.; Chiozzi, P.; Sanz, J. M.; Ferrari, D.; Buell, G. N. ATP receptors and giant cell formation. *J. Leukocyte Biol.* **1999**, *66*, 723−726.

(166) Kim, M.; Jiang, L.-H.; Wilson, H. L.; North, R. A.; Surprenant, A. Proteomic and functional evidence for a P2X$_7$ receptor signalling complex. *EMBO J.* **2001**, *20*, 6347−6358.

(167) Denlinger, L. C.; Fisette, P. L.; Sommer, J. A.; Watters, J. J.; Prabu, U.; Dubyak, G. R.; Proctor, R. A.; Bertics, P. J. Cutting edge: the nucleutide receptor P2X$_7$ contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J. Immunol.* **2001**, *167*, 1871−1876.

(168) Solle, M.; Labasi, J.; Perregaux, D. G.; Stam, E.; Petrushova, N.; Koller, B. H.; Griffiths, R. J.; Gabel, C. A. Altered cytokine production in mice lacking P2X$_7$ receptors. *J. Biol. Chem.* **2001**, *276*, 125−132.

(169) Gu, B. J.; Zhang, W. Y.; Bendall, L. J.; Chessell, I. P.; Buell, G. N. Wiley, J. S. Expression of P2X$_7$ purinoceptors on human lymphocytes and monocytes: evidence for nonfunctional P2X$_7$ receptors. *Am. J. Physiol.: Cell Physiol.* **2000**, *279*, C1189−C1197.

(170) Wiley, J. S.; Gu, B. J.; Zhang, W.; Worthington, R. A.; Dao-Ung, P.; Shemon, A. N.; Sluyter, R.; Liang, S.; Barden, J. A. Genetic polymorphisms of the human P2X$_7$ receptor and relationship to function. *Drug Dev. Res.* **2001**, *54*, 72−76.

(171) Boynaems, J-.M.; Commini, D.; Suarez-Huerta, N.; Janssens, R. P2Y receptors. *Hematologica* **2000**, *85*, 15−21.

(172) Webb, T. E.; Henderson, F.; King, B. F. A novel G protein-coupled P2 purinoceptor (P$_{2Y3}$) activated preferentially by nucleoside diphosphates. *Mol. Pharmacol.* **1996**, *222*, 303−308.

(173) Sellers, L. A.; Simon, J.; Lundahl, T. S.; Cousens, D. J.; Humphrey, P. P.; Barnard, E. A. Adenosine nucleotides acting at the human P2Y$_1$ receptor stimulate mitogen-activated protein kinases and induce apoptosis. *J. Biol. Chem.* **2001**, *276*, 16379−16390.

(174) Communi, D.; Gonzalez, N. S.; Detheux, M.; Brezillon, S.; Lannoy, V.; Parmentier, M.; Boeynaems, J.-M. Identification of a novel human ADP receptor coupled to Gi. *J. Biol. Chem.* **2001**, *44*, 41479−41485.

(175) Dean, M. K.; Higgs, C.; Smith, R. E.; Bywater, R. P.; Snell, C. R.; Scott, P. D.; Upton, G. J. G.; Howe, T. J.; Reynolds, C. A. Dimerization of G-protein-coupled receptors. *J. Med. Chem.* **2001**, *44*, 4595−4614.

(176) Yoshioka, K.; Saitoh, O.; Nakata, H. Heteromeric association creates a P2Y-like adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7617−7622.

(177) Webb, T. E.; Simon, J.; Krishek, B. J.; Bateson, A. N.; Smart, TG.; King, B. F.; Burnstock, G.; Barnard, E. A. Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS Lett.* **1993**, *324*, 219−225.

(178) Lustig, K. D.; Shiau, A. K.; Brake, A. J.; Julius, D. Expression cloning of an ATP receptor from mouse neuroblastoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5113−5117.

(179) Filtz, T. M.; Li, Q.; Boyer, J.; Nicholas, R. A.; Harden, T. K. Expression of a cloned P2Y receptor that couples to phospholipase C. *Mol. Pharmacol.* **1994**, *46*, 8−14.

(180) Janssens, R.; Communi, D.; Pirotton, S.; Samson, M.; Parmentier, M.; Boeynaems, J. M. Cloning and tissue distribution of the human P2Y$_1$ receptor. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 588−593.

(181) Boyer, J.; Adams, M.; Ravi, R. G.; Jacobson, K. A.; Harden, T. K. 2-Chloro-N^6-methyl-(N)-methanocarba-2-deoxyadenosine-3,5-bisphosphate is a selective high affinity P2Y$_1$ receptor antagonist that blocks the ADP-activated P2Y$_1$ receptor of platelets without effect on the adenyl cyclase linked P2Y receptor. *Br. J. Pharmacol.*, in press.

(182) O'Grady, S. M.; Elmquist, E.; Filtz, T. M.; Nicholas, R. A.; Harden, T. K. A guanine nucleotide-independent inwardly rectifying cation permeability is associated with P2Y$_1$ receptor expression in *Xenopus oocytes. J. Biol. Chem.* **1996**, *271*, 29080−29087.

(183) Fabre, J. E.; Nguyen, M.; Latour, A.; Keifer, J. A.; Audoly, L. P.; Coffman, T. M.; Koller, B. H. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y$_1$-deficient mice. *Nat. Med.* **1999**, *5*, 1199−1202.

(184) Leon, C.; Hechler, B.; Freund, M.; Eckly, A.; Vial, C.; Ohlmann, P.; Dierich, A.; LeMur, M.; Cazenave, J.-P.; Gachet, C. Defective platelet aggregation and increased resistance to thombosis in purinergic P2Y$_1$ receptor-null mice. *J. Clin. Invest.* **1999**, *104*, 1731−1737.

(185) Godecke, S.; Decking, U. K. M.; Godecke, A.; Schrader, J. Cloning of the rat P2U receptor and its potential role in coronary vasoladialtion. *Am. J. Physiol.* **1996**, *270*, 570−577.

(186) Parr, CE.; Sullivan, DM.; Paradiso, AM.; Lazarowski, E. R.; Burch, LH.; Olsen, JC.; Erb, L.; Weisman, GA.; Boucher, R. C.; Turner, J. T. Cloning and expression of a human P$_{2U}$ nucleotide receptor, a target for cystic fibrosis pharmacotherapy. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3275−3279.

(187) Chinet, T.; Fouassier, L.; Dray-Charier, N.; Imam-Ghali, M.; Morel, H.; Mergey, M.; Dousset, B.; Parc, R.; Paul, A.; Housset, C. Regulation of electrogenic anion secretion in normal and cystic fibrosis gallbladder mucosa. *Hepatology* **1999**, *29*, 5−13.

(188) Mosbacher, J.; Maier, R.; Fakler, B.; Glatz, A.; Crespo, J.; Bilbe, G. P2Y receptor subtypes differentially couple to inwardly rectifying potassium channels. *FEBS Lett.* **1998**, *436*, 104−110.

(189) Brambilla, R.; Ceruti, S.; Malorni, W.; Cattabeni, F.; Abbracchio, M. P. A novel gliotic P2 receptor mediating cyclooxygenase-2 induction in rat and human astrocytes. *J. Auton. Nerv. Syst.* **2000**, *81*, 3−9.

(190) Insel, P. A.; Ostrom, R. S.; Zambon, A. C.; Hughes, R. J.; Balboa, M. A.; Shehnaz, D.; Gregorian, C.; Torres, B.; Firestein, B. L.; Xing, M.; Post, S. R. P2Y receptors of MDCK cells: epithelial cell regulation by extracellular nucleotides. *Clin. Exp. Pharmacol. Physiol.* **2001**, *28*, 351−354.

(191) Cressman, V. L.; Lazorowski, E.; Homolya, L.; Boucher, R. C.; Koller, B. H.; Grubb, B. R. Effect of loss of P2Y$_2$ receptor gene expression on nucleotide regulation of murine epithelial Cl$^-$ transport. *J. Biol. Chem.* **1999**, *274*, 26461−26468.

(192) Communi, D.; Pirotton, S.; Parmentier, M.; Boeynaems, J. M. Cloning and functional expression of a human uridine nucleotide receptor. *J. Biol. Chem.* **1995**, *270*, 30849−30852.

(193) Nguyen, T.; Erb, L.; Weisman, G. A.; Marchese, A.; Heng, H. H.; Garrad, R. C.; George, S. R.; Turner, J. T.; O'Dowd, B. F. Cloning, expression, and chromosomal-localization of the human uridine nucleotide receptor gene. *J. Biol. Chem.* **1995**, *270*, 30845−30848.

(194) Webb, T. E.; Henderson, D. J.; Roberts, J. A.; Barnard, E. A. Molecular cloning and characterization of the rat P2Y$_4$ receptor. *J. Neurochem.* **1998**, *71*, 1348−1357.

(195) Bogdanov, Y. D.; Wildman, S. S.; Clements, M. P.; King, B. F.; Burnstock, G. Molecular cloning and charcterzation of rat P2Y$_4$ receptor. *Br. J. Pharmacol.* **1998**, *124*, 428−430.

(196) Chang, K.; Hanaoka, K.; Kumada, M.; Takuwa, Y. Molecular cloning and functional analysis of a novel P2 nucleotide receptor. *J. Biol. Chem.* **1995**, *270*, 26152−26158.

(197) Communi, D.; Parmentier, M.; Boeynaems, J. M. Cloning, functional expression and tissue distribution of the human P2Y$_6$ receptor. *Biochem. Biophys. Res. Commun.* **1996**, *222*, 303−308.

(198) Warny, M.; Aboudola, S.; Robson, S. C.; Sevigny, J.; Communi, D.; Soltoff, S. P.; Kelly, C. P. P2Y$_6$ nucleotide receptor mediates monocyte interleukin-8 production in response to UDP or lipopolysaccharide. *J. Biol. Chem.* **2001**, *276*, 26051−26056.

(199) Fillipov, A. K.; Webb, T. E.; Barnard, E. A.; Brown, D. A. Dual coupling of heterologously-expressed rat P2Y$_6$ nucleotide receptors to N-type Ca^{2+} and M-type K$^+$-currents in rat sympathetic neurons. *Br. J. Pharmacol.* **1999**, *126*, 1009−1017.

(200) Communi, D.; Robaye, B.; Boeynaems, J. M. Pharmacological characterization of the human P2Y$_{11}$ receptor. *Br. J. Pharmacol.* **1999**, *128*, 1199−1206.

(201) Conigrave, A. D.; Fernado, K. C.; Gu, B.; Tasevski, V.; Zhang, W.; Luttrell, B. M.; Wiley, J. S. P2Y$_{11}$ receptor expression by human lymphocytes: evidence for two cAMP-linked purinoceptors. *Eur. J. Pharmacol.* **2001**, *426*, 157−163.

(202) Zambon A. C.; Brunton, L. L.; Barrett, K. E.; Hughes, R. J.; Torres, B.; Insel, P. A. Cloning, expression, signaling mechanisms, and membrane targeting of P2Y$_{11}$ receptors in Madin Darby canine kidney cells. *Mol. Pharmacol.* **2001**, *60*, 26−35.

(203) Turner, N. A.; Moake, J. L.; McIntire, L. V. Blockade of adenosine diphosphate receptors P2Y$_{12}$ and P2Y$_1$ is required to inhibit platelet aggregation in whole blood under flow. *Blood* **2001**, *98*, 3340−3345.

(204) Ingall, A. H.; Dixon, J.; Bailey, A.; Coombs, M. E.; Cox, D.; McInally, J. I.; Hunt, S. F.; Kindon, N. D.; Teobald, B. J.; Willis, P. A.; Humphries, R. G.; Leff, P.; Clegg, J. A.; Smith, J. A.; Tomlinson, W. Antagonists of the platelet P$_{2T}$ receptor: a novel approach to antithrombotic therapy. *J. Med. Chem.* **1999**, *42*, 213−220.

(205) Storey, R. F. Clinical experience with antithrombotic drugs acting on purine receptor pathways. *Drug Dev. Res.* **2001**, *52*, 202−212.

(206) Humphries, R. G. Pharmacology of AR-C69931MX and related compounds: from pharmacological tools to clinical trials. *Haematologica* **2000**, *85*, 66−72.

(207) Chan, S. W.; Gallo, S. J.; Kim, B. K.; Guo, M. J.; Blackburn, G. M.; Zamecnik, P. C. P^1,P^4-Dithio-P^2,P^3-monochloromethylene diadenosine 5′,5″-P^1,P^4-tetraphosphate: a novel antiplatelet agent. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4034−4039.

(208) Scarbrough, R. M.; Laibelman, A. M.; Lizbe, L. A.; Fretto, L. J.; Conley, P. B.; Reynolds, E. E.; Sedlock, D. M.; Jantzen, H.-M. Novel tricyclic benzothiazolo[2,3c]thiadiazine antagonists of the platelet ADP receptor (P2Y$_{12}$). *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1805−1808.

(209) Foster, C. J.; Prosser, D. N.; Agans, J. M.; Zhai, Y.; Smith, M. D.; Lachowicz, J. E.; Zhang, F. L.; Gusatfson, E.; Monsma, F. J., Jr.; Wiekowski, M. T.; Abbondanzo, S. J.; Cook, D. N.; Bayn, M. L.; Lira, S. A.; Chintala, M. S. Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. *J. Clin. Invest.* **2001**, *107*, 1591−1598.

(210) Wittenberger, J.; Schaller, H. C.; Hellebrand, S. An expressed sequence tag (EST) data mining strategy succeeding in the discovery of new G-protein coupled receptors. *J. Mol. Biol.* **2001**, *307*, 799−813.

(211) Lee, D. K.; Nguyen, T.; Lynch, K. R.; Cheng, R.; Vanti, W. B.; Arkhitko, O.; Lewis, T.; Evans, J. F.; George, S. R.; O'Dowd, B. F. Discovery and mapping of ten novel G protein-coupled receptor genes. *Gene* **2001**, *275*, 83−91.

(212) Zhang, F.; Luo, L.; Gustafson, E.; Palmer, K.; Qiao, X.; Fan, X.; Yang, S.; Laz, T. M.; Bayne, M.; Monsma, F., Jr. P2Y$_{13}$: identification and characterization of a novel G$_{\alpha1}$-coupled ADP receptor from human and mouse. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 705−713.

(213) Mellor, E. A.; Maekawa, A.; Austen, K. F.; Boyce, J. A. Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by human mast cells. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7964−7969.

(214) Simon, J.; Vigne, P.; Eklund, K. M.; Michel, A. D.; Carruthers, A. M.; Humphrey, P. P. A.; Frelin, C.; Barnard, E. A. Activity of adenosine diphosphates and triphosphates on a $P2Y_T$ -type receptor in brain capillary epithelial cells. *Br. J. Pharmacol.* **2001**, *132*, 173−182.

(215) Torres, G. E.; Egan, T. M.; Voigt, M. M.; N-linked glycosylation is essential for the functional expression of the recombinant $P2X_2$ receptor. *Biochemistry* **1998**, *37*, 14845−14851.

(216) Rettinger, J.; Aschrafi, A.; Schmalzing, G. Roles of individual N-glycans for ATP potency and expression of the rat P2X1 receptor. *J. Biol. Chem.* **2000**, *275*, 33542−33547.

(217) Newbolt, A.; Stoop, R.; Virginio, C.; Surprenant, A.; North, R. A.; Buell, G.; Rassendren, F. Membrane topology of an ATP-gated ion channel (P2X receptor). *J. Biol. Chem.* **1998**, *273*, 15177−15182.

(218) Rassendren, F.; Buell, G.; Newbolt, A.; North, R. A.; Surprenant, A. Identification of amino acid residues contributing to the pore of a P2X receptor. *EMBO J.* **1997**, *16*, 3446−3454.

(219) Haines, W. R.; Migita, K.; Cox, J. A.; Egan, T. M.; Voight, M. M. The first transmembrane domain of the P2X receptor subunit participates in the agonist-induced gating of the channel. *J. Biol. Chem.* **2001**, *276*, 32793−32798.

(220) Haines, W. R.; Voight, M. M.; Egan, T. M. Polar residues of the second transmembrane domain influence cation permeability of the ATP-gated $P2X_2$ receptor. *J. Biol. Chem.* **2001**, *276*, 30934−30941.

(221) Boue-Grabot, E.; Archambault, V.; Seguela, P. A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of $P2X_2$ ATP-gated channels. *J. Biol. Chem.* **2000**, *275*, 10190−10195.

(222) van Rhee, A. M.; Jacobson, K. A.; Garrad, R.; Weisman, G. A.; Erb, L. P2 receptor modeling and identification of ligand binding sites. In *The P2 Nucleotide Receptors*; Turner, J. T., Weisman, G., Fedan, J., Eds.; Humana Press: Clifton, NJ, 1998; pp 135−166.

(223) Erb, L.; Garrad, R.; Wang, Y.; Quinn, T.; Turner, J. T.; Weisman, G. A. Site-directed mutagenesis of P_{2U} purinoceptors. Positively charged amino acids in transmembrane helices 6 and 7 affect agonist potency and specificity. *J. Biol. Chem.* **1995**, *270*, 4185−4188.

(224) Moro, S.; Guo, D.; Camaioni, E.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Human $P2Y_1$ receptor: Molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. *J. Med. Chem.* **1998**, *41*, 1456−1466.

(225) Kim, H. S.; Barak, D.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Acyclic and cyclopropyl analogues of adenosine bisphosphate antagonists of the $P2Y_1$ receptor: Structure−activity relationships and receptor docking. *J. Med. Chem.* **2001**, *44*, 3092−3108.

(226) Jiang, Q.; Guo, D.; Lee, B. X.; van Rhee, A. M.; Kim, Y.-C.; Nicholas, R. A.; Schachter, J.; Harden, T. K.; Jacobson, K. A. A mutational analysis of residues essential for ligand recognition at the human $P2Y_1$ receptor. *Mol. Pharmacol.* **1997**, *52*, 499−507.

(227) Hoffmann, C.; Moro, S.; Nicholas, R. A.; Harden, T. K.; Jacobson, K. A. The role of amino acids in extracellular loops of the human $P2Y_1$ receptor in surface expression and activation processes. *J. Biol. Chem.* **1999**, *274*, 14639−14647.

(228) Moro, S.; Hoffmann, C.; Jacobson, K. A. Role of the extracellular loops of G protein-coupled receptors in ligand recognition: A molecular modeling study of the human $P2Y_1$ receptor. *Biochemistry* **1999**, *38*, 3498−3507.

(229) Bianchi, B. R.; Lynch, K. J.; Touma, E.; Niforatos, W.; Burgard, E. C.; Alexander, K. M.; Park, H. S.; Yu, H.; Metzger, R.; Kowaluk, E.; Jarvis, M. F.; van Biesen, T. Pharmacological characterization of recombinant human and rat P2X receptor subtypes. *Eur. J. Pharmacol.* **1999**, *376*, 127−138.

(230) Jacobson, K. A.; Fischer, B.; Maillard, M.; Boyer, J. L.; Hoyle, C. H. V.; Harden, T. K.; Burnstock, G. Novel ATP agonists reveal receptor heterogeneity within P_{2X} and P_{2Y} subtypes. In *Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology*; Bellardinelli, L., Pelleg, A., Eds.; Kluwer: Boston, 1995; pp 149−156.

(231) Brown, S. G.; King, B. F.; Kim, Y.-C.; Burnstock, G.; Jacobson, K. A. Activity of novel adenine nucleotide derivatives as agonists and antagonists at recombinant rat P2X receptors. *Drug Dev. Res.* **2000**, *49*, 253−259.

(232) Burnstock, G.; Fischer, B.; Maillard, M.; Ziganshin, A.; Ralevic, V.; Knight, G.; Brizzolara, A.; von Isakovics, A.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Structure activity relationships for derivatives of adenosine-5′-triphosphate as agonists at P_2 purinoceptors: heterogeneity within P_{2X}- and P_{2Y}-subtypes. *Drug Dev. Res.* **1994**, *31*, 206−219.

(233) Inscho, E. W.; Cook, A. K.; Mui, V.; Miller, J. Direct assessment of renal microvascular responses to P2-purinoceptor agonists. *Am. J. Physiol.* **1998**, *274*, F718−F727.

(234) Brownhill, V. R.; Hourani, S. M.; Kitchen, I. Ontogeny of P2-purinoceptors in the longitudinal muscle and muscularis mucosae of the rat isolated duodenum. *Br. J. Pharmacol.* **1997**, *122*, 225−232.

(235) Beukers, M. W.; von Kerkhof, C. J.; van Rhee, A. M.; Ardanuy, U.; Gurgel, C.; Widjaja, H.; Nickel, P.; IJzerman, A. P.; Soudijn, W. Suramin analogs, divalent cations and ATP-γ-S as inhibitors of ecto-ATPase. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *351*, 523−528.

(236) Lazarowski, E. R.; Watt, W. C.; Stutts, M. J.; Brown, H. A.; Boucher, R. C.; Harden, T. K. Enzymatic-synthesis of UTP-γ-S, a potent hydrolysis resistant agonist of P_{2U}-purinoceptors. *Br. J. Pharmacol.* **1996**, *117*, 203−209.

(237) Malmsjo, M.; Adner, M.; Harden, T. K.; Pendergast, W.; Edvinsson, L.; Erlinge, D. The stable pyrimidines, UDPβS and UDPγS discriminate between P2 receptors that mediate vascular contraction and relaxation of the mesenteric artery. *Br. J. Pharmacol.* **2000**, *131*, 51−56.

(238) Hechler, B.; Vigne, P.; Leon, C.; Breittmayer, J. P.; Gachet, C.; Frelin, C. ATP derivatives are antagonists of the $P2Y_1$ receptor: similarities to the platelet ADP receptor. *Mol. Pharmacol.* **1998**, *53*, 727−733.

(239) Fischer, B.; Chulkin, A.; Boyer, J. L.; Harden, K. T.; Gendron, F. P.; Beaudoin, A. R.; Chapal, J.; Hillaire-Buys, D.; Petit, P. 2-Thioether 5′-O-(1-thiotriphosphate)adenosine derivatives as new insulin secretagogues acting through P2Y-receptors. *J. Med. Chem.* **1999**, *42*, 3636−3646.

(240) Guile, S. D. P2T receptor antagonists−towards novel orally active antithrombotics. *Drug Dev. Res.* **2000**, *50*, 14 (S06-03).

(241) Nandanan, E.; Jang, S. Y.; Moro, S.; Kim, H.; Siddiqui, M. A.; Russ, P.; Marquez, V. E.; Busson, R.; Herdewijn, P.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Synthesis, biological activity, and molecular modeling of ribose-modified adenosine bisphosphate analogues as $P2Y_1$ receptor ligands. *J. Med. Chem.* **2000**, *43*, 829−842.

(242) Cinkilic, O.; King, B. F.; van der Giet, M.; Schluter, H.; Zidek, W.; Burnstock, G. Selective agonism of group I P2X receptors by dinucleotides dependent on a single adenine moiety. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 131−136.

(243) Miras-Portugal, M. T.; Gualix, J.; Mateo, M.; Diaz-Hernandez, M.; Gomez-Villafuertes, R.; Castro, E.; Pintor, J. Diadenosine polyphosphates: extracellular function and catabolism. *Prog. Brain Res.* **1999**, *120*, 397−409.

(244) Pintor, J.; Diaz-Hernadez, M.; Gualix, J.; Gomez-Villafuertes, R.; Hernando, F.; Miras-Portugal, M. T. Diadenosine polyphosphate receptors from rat and guinea-pig brain to human nervous system. *Pharmacol. Ther.* **2000**, *87*, 103−115.

(245) Pendergast, W.; Yerxa, B. R.; Douglass, J. G., 3rd; Shaver, S. R.; Dougherty, R. W.; Redick, C. C.; Sims, I. F.; Rideout, J. L. Synthesis and P2Y receptor activity of a series of uridine dinucleoside 5′-polyphosphates. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 157−160.

(246) Humphrey, P. P. A.; Buell, G.; Kennedy, I.; Khakh, B. S.; Michel, A. D.; Suprenant, A. M.; Trezise, D. J. New insights on P_{2X} purinoceptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *352*, 585−596.

(247) Baghdiguian, S.; Fantini, J. Suramin: a molecule with a broad spectrum of biological and therapeutic properties. *Cancer J.* **1997**, *10*, 31−37.

(248) van Rhee, A. M.; van der Heijden, M. P. A.; Beukers, M. W.; IJzerman, A. P.; Soudijn, W.; Nickel, P. Novel competitive antagonists for P_2 purinoceptors. *Eur. J. Pharmacol.* **1994**, *268*, 1−7.

(249) Jenkinson, K. M.; Reid, J. J. The P2-purinoceptor antagonist suramin is a competitive antagonist at vasoactive intestinal peptide receptors in the rat gastric fundus. *Br. J. Pharmacol.* **2000**, *130*, 1632−1638.

(250) Zhang, Y. L.; Keng, Y. F.; Zhao, Y.; Wu, L.; Zhang, Z. Y. Suramin is an active site-directed, reversible, and tight-binding inhibitor of protein−tyrosine phosphatases. *J. Biol. Chem.* **1998**, *273*, 12281−12287.

(251) Bültmann, R.; Pause, B.; Wittenburg, H.; Kurz, G.; Starke, K. P_2-purinoceptor antagonists. I. Blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by small aromatic isothiocyanato-sulfonates. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1996**, *354*, 481−490.

(252) Wittenburg, H.; Bültmann, R.; Pause, B.; Ganter, C.; Kurz, G.; Starke, K. P_2-purinoceptor antagonists. II. Blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by compounds related to Evans blue and trypan blue. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1996**, *354*, 491−497.

(253) Bültmann, R.; Wittenburg, H.; Pause, B.; Kurz, G.; Nickel, P.; Starke, K. P_2-purinoceptor antagonists. III. blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by compounds related to suramin. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1996**, *354*, 498−504.

(254) Ziyal, R.; Ralevic, V.; Ziganshin, A. U.; Nickel, P.; Ardanuy, U.; Mutschler, E.; Lambrecht, G.; Burnstock, G. NF023, a selective P_{2x}-purinoceptor antagonist in rat, hamster and rabbit isolated blood vessels. *Drug Dev. Res.* **1996**, *37*, 113.

(255) Damer, S.; Niebel, B.; Czeche, S.; Nickel, P.; Ardanuy, U.; Schmalzing, G.; Rettinger, J.; Mutschler, E.; Lambrecht, G. NF279: a novel potent and selective antagonist of P2X receptor-mediated responses. *Eur. J. Pharmacol.* **1998**, *350*, R5−R6.

(256) Rettinger, J.; Schmalzing, G.; Damer, S.; Muller, G.; Nickel, P.; Lambrecht, G. The suramin analogue NF279 is a novel and potent anatgonist selective for the P2X$_1$ receptor. *Neuropharmacology* **2000**, *39*, 2044−2053.

(257) Braun, K.; Rettinger, J.; Ganso, M.; Kassack, M.; Hildebrandt, C.; Ullmann, H.; Nickel, P.; Schmalzing, G.; Lambrecht, G. NF449: a subnanomolar potency antagonist at recombinant rat P2X$_1$ receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2001**, *34*, 285−290.

(258) Tuluc, F.; Bültmann, R.; Glänzel, M.; Frahm, A. W.; Starke, K. P2-receptor antagonists: IV. Blockade of P2-receptor subtypes and ecto-nucleotidases by compounds related to reactive blue 2. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 111−120.

(259) Connolly, G. P.; Harrison, P. J. Discrimination between UTP- and P2-purinoceptor-mediated depolarization of rat superior cervical ganglia by 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) and uniblue A. *Br. J. Pharmacol.* **1995**, *115*, 427−432.

(260) Glänzel, M.; Bültmann, R.; Starke, K.; Frahm, A. W. Proceedings of the XVIth International Symposium on Medicinal Chemistry, Bologna, Italy, 2000.

(261) Bültmann, R.; Trendelenburg, M.; Starke, K. Blockade of P_{2X}-purinoceptors by Trypan Blue in rat vas-deferens. *Br. J. Pharmacol.* **1994**, *113*, 349−354.

(262) Bultmann, R.; Starke, K. Reactive red 2. A P_{2Y}-selective purinoceptor antagonist and an inhibitor of ecto-nucleotidase. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *352*, 477−482.

(263) Soltoff, S. P.; McMillian, M. K.; Talamo, B. R. Coomassie brilliant blue G is a more potent antagonist of P2 purinergic responses than reactive blue 2 (cibacron blue 3GA) in rat parotid acinar cells. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1279−1285.

(264) Jarvis, M. F.; Wismer, C. T.; Schweitzer, E.; Yu, H.; van Biesen, T.; Lynch, K. J.; Burgard, E.; Kowaluk, E. A. Modulation of BzATP and formalin-induced nociception: attenuation by the P2X receptor antagonist, TNP-ATP and enhancement by the allosteric modulator, cibacron blue. *Br. J. Pharmacol.* **2001**, *132*, 259−269.

(265) Soltoff, S. P.; McMillian, M. K.; Talamo, B. R.; Cantley, L. C. Blockade of ATP binding site of P2 purinoceptors in rat parotid acinar cells by isothiocyanate compounds. *Biochem. Pharmacol.* **1993**, *45*, 1936−1940.

(266) Bültmann, R.; Starke, K. Blockade by 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) of P_{2X}-purinoceptors in rat vas deferens. *Br. J. Pharmacol.* **1994**, *112*, 690−694.

(267) Bültmann, R.; Trendelenburg, M.; Tuluc, F.; Wittenburg, H.; Starke, K. Concomitant blockade of P2X-receptors and ecto-nucleotidases by P2-receptor antagonists: functional consequences in rat vas deferens. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 339−344.

(268) Lambrecht, G.; Friebe, T.; Grimm, U.; Windscheif, U.; Bungardt, E.; Hildebrandt, C.; Baumert, H. G.; Spatz-Kumbel, G.; Mutschler, E. PPADS, a novel functionally selective antagonist of P2 purinoceptor-mediated responses. *Eur. J. Pharmacol.* **1992**, *217*, 217−219.

(269) Windscheif, U.; Pfaff, O.; Ziganshin, A. U.; Hoyle, C. H.; Baumert, H. G.; Mutschler, E.; Burnstock, G.; Lambrecht, G. Inhibitory action of PPADS on relaxant responses to adenine nucleotides or electrical field stimulation in guinea-pig taenia coli and rat duodenum. *Br. J. Pharmacol.* **1995**, *115*, 1509−1517.

(270) Kim, Y.-C.; Camaioni, E.; Ziganshin, A. U.; Ji, X.-J.; King, B. F.; Wildman, S. S.; Rychkov, A.; Yoburn, J.; Kim, H.; Mohanram, A.; Harden, T. K.; Boyer, J. L.; Burnstock, G.; Jacobson, K. A. Synthesis and structure activity relationships of pyridoxal-6-azoaryl-5′-phosphate and phosphonate derivatives as P2 receptor antagonists. *Drug Dev. Res.* **1998**, *45*, 52−66.

(271) Kim, Y.-C.; Brown, S. G.; Harden, T. K.; Boyer, J. L.; Dubyak, G.; King, B. F.; Burnstock, G.; Jacobson, K. A. Structure−activity relationships of pyridoxal phosphate derivatives as potent and selective antagonists of P2X$_1$ receptors. *J. Med. Chem.* **2001**, *44*, 340−349.

(272) Brown, S. G.; Kim, Y.-C.; Kim, S.-A.; Jacobson, K. A.; Burnstock, G.; King, B. F. Actions of a series of PPADS analogues at P2X$_1$ and P2X$_3$ receptors. *Drug Dev. Res.* **2001**, *53*, 281−291.

(273) Boyer, J. L.; Zohn, I. E.; Jacobson, K. A.; Harden, T. K. Differential-effects of P_{2Y}-purinoceptor antagonists on phospholipase C-coupled and adenylyl cyclase-coupled P_{2Y}-purinoceptors. *Br. J. Pharmacol.* **1994**, *113*, 614−620.

(274) Lambrecht, G.; Rettinger, J.; Baumert, H. G.; Czeche, S.; Damer, S.; Ganso, M.; Hildfebrandt, C.; Niebel, B.; Saptz-Kumbel, G.; Scmalzing, G.; Mutschler, E. The novel pyridoxal-5′-phosphate derivative PPNDS potently antagonizes activation of P2X$_1$ receptors. *Eur. J. Pharmacol.* **2000**, *387*, R19−R21.

(275) Humphries, R. G.; Tomlinson, W.; Clegg, J. A.; Ingall, A. H.; Kindon, N. D.; Leff, P. Pharmacological profile of the novel P2T-purinoceptor antagonist, FPL 67085 in vitro and in the anaesthetized rat in vivo. *Br. J. Pharmacol.* **1995**, *115*, 1110−1116.

(276) Virginio, C.; Roberston, G.; Suprenant, A.; North, R. A. Trinitrophenyl-substituted nucleotides are potent antagonists selective for P2X$_1$, P2X$_3$ and heteromeric P2X$_{2/3}$ receptors. *Mol. Pharmacol.* **1998**, *53*, 969−973.

(277) Kim, Y.-C.; Gallo-Rodriguez, C.; Jang, S. Y.; Nandanan, E.; Adams, M.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Acyclic analogues of adenosine bisphosphates as P2Y$_1$ receptor antagonists. *J. Med. Chem.* **2000**, *43*, 746−755.

(278) Gallo-Rodriguez, C.; Jang, S. Y.; Nandanan, E.; Adams, M.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. Acyclic analogues of deoxyadenosine bisphosphates as P2Y$_1$ receptor antagonists. *J. Med. Chem.* **2000**, *43*, 746−755.

(279) Camaioni, E.; Boyer, J. L.; Mohanram, A.; Harden, T. K.; Jacobson, K. A. Deoxyadenosine-biphosphate derivatives as potent antagonists at P2Y$_1$ receptors. *J. Med. Chem.* **1998**, *41*, 183−190.

(280) Boyer, J.; Adams, M.; Ravi, R. G.; Jacobson, K. A.; Harden, T. K. 2-Chloro-N^6-methyl-(N)-methanocarba-2-deoxyadenosine-3,5-bisphosphate is a selective high affinity P2Y$_1$ receptor antagonist that blocks the ADP-activated P2Y$_1$ receptor of platelets without effect on the adenyl cyclase lined P2Y receptor. *Br. J. Pharmacol.*, in press.

(281) Boyer, J. L.; Romero-Avila, T.; Schachter, J. B.; Harden, T. K. Identification of competitive antagonists of the P2Y$_1$-receptor. *Mol. Pharmacol.* **1996**, *50*, 1323−1329.

(282) Humphreys, B. D.; Virginio, C.; Surprenant, A.; Rice, J.; Dubyak, G. R. Isoquinolines as antagonists of the P2X$_7$ nucleotide receptor: high selectivity for the human versus rat receptor homologues. *Mol. Pharmacol.* **1998**, *54*, 22−32.

(283) Ravi, R. G.; Kertesy, S. B.; Dubyak, G. R.; Jacobson, K. A. Potent P2X$_7$ receptor antagonists: Tyrosyl derivatives synthesized using a sequential parallel synthetic approach. *Drug Dev. Res.* **2001**, *54*, 75−87.

(284) Khakh, B. S.; Michel, A.; Humphrey, P. P. A. Estimates of antagonist affinities at P_{2X} purinoceptors in rat vas-deferens. *Eur. J. Pharmacol.* **1994**, *263*, 301−309.

(285) Jacobson, K. A.; Kim, Y.-C.; King, B. F. In search of selective P2 interaction ligands: interaction of dihydropyridine derivatives at recombinant rat P2X$_2$ receptors. *J. Auton. Nerv. Syst.* **2000**, *81*, 152−157.

(286) Savi, P.; Pereillo, J. M.; Uzabiaga, M. F.; Combalbert, J.; Picard, C.; Maffrand, J. P.; Pascal, M.; Herbert, J. M. Identification and biological activity of the active metabolite of clopidogrel. *Thromb. Haemostasis* **2000**, *84*, 891−896.

(287) King, B. F.; Dacquet, C.; Ziganshin, A. U.; Weetman, D. F.; Burnstock, G.; Vanhoutte, P. M.; Spedding, M. Potentiation by 2,2′-pyridylisatogen tosylate of ATP-responses at a recombinant P2Y$_1$ purinoceptor. *Br. J. Pharmacol.* **1996**, *117*, 1111−1118.

(288) Foster, H.; Hooper, M.; Imam, S. H.; Lovett, G. S.; Nicholson, J.; Swain, C. J.; Sweetman, A. J.; Weetman, D. F. Increased inhibitory action against adenosine 5′-triphosphate in the isolated taenia of the guinea-pig caecum by substitution in the A-ring of 2-phenylisatogen. *Br. J. Pharmacol.* **1983**, *79*, 273−278.

(289) Puri, R. N.; Colman, R. W. Inhibition of ADP-induced platelet activation by 7-chloro-4-nitrobenz-2-oxa-1,3-diazole: covalent modification of aggregin, a putative ADP receptor. *J. Cell Biochem.* **1996**, *61*, 97−108.

(290) Shen, G. S.; Layer, R. T.; McCabe, R. T. Conopeptides: From deadly venoms to novel therapeutics. *Drug Discovery Today* **2000**, *5*, 98−106.

(291) Wang, M.-J.; Xiong, S.-H.; Li, Z.-W. Neurokinin B potentiates ATP-activated currents in rat DRG neurons. *Brain Res.* **2001**, *923*, 157−162.

(292) Jacobson, K. A.; Kim, Y.-C.; Wildman, S. S.; Mohanram, A.; Harden, T. K.; Boyer, J. L.; King, B. F.; Burnstock, G. A pyridoxine cyclic-phosphate and its 6-arylazo-derivative selectively potentiate and antagonize activation of P2X$_1$ receptors. *J. Med. Chem.* **1998**, *41*, 2201−2206.

(293) Bogdanov, Y. D.; Rubino, A.; Burnstock, G. Characterization of subtypes of the P2X and P2Y families of ATP receptors in the foetal human heart. *Life Sci.* **1998**, *62*, 697−703.

(294) Olssen, R. A.; Pearson, J. D. Cardiovascular purinoceptors. *Physiol. Rev.* **1990**, *70*, 761−845.

(295) Burnstock, G.; Kennedy, C. A dual function for adenosine 5′-triphosphate in the regulation of vascular tone. *Circ. Res.* **1985**, *58*, 319−330.

(296) Sunapoli, S. P.; Daniel, J. L. P2 receptor subtypes in the cardiovascular system. *Biochem. J.* **1998**, *336*, 513−523.

(297) Boarder, M. R.; Hourani, S. M. The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Trends Pharmacol. Sci.* **1998**, *19*, 99–107.

(298) Pelleg, A.; Vassort, G. P2 receptors in the cardiovascular system. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part II. Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*; Springer Verlag: Heidelberg, 2001; pp 73–99.

(299) Vassort, G. Adenosine 5′-triphosphate: a P2-purinergic agonist in the myocardium. *Physiol. Rev.* **2001**, *81*, 767–806.

(300) De May, J. G.; Vanhoutte, P. M. Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol. (London)* **1981**, *316*, 347–355.

(301) Ralevic, V.; Burnstock, G. Role of P_2-purinoceptors in the cardiovascular system. *Circulation* **1991**, *84*, 1–14.

(302) Kennedy, C.; Delbro, D.; Burnstock, G. P_2-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. *Eur. J. Pharmacol.* **1985**, *107*, 161–168.

(303) Ralevic, V. Roles of purines and pyrimidines in endothelium. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part II. Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*; Springer Verlag: Heidelberg, 2001; pp 100–120.

(304) Malmsjoa, M.; Bergdahla, A.; Mollera, S.; Zhaob, X. E.; Sunb, X. Y.; Hednerb, T.; Edvinssona, L.; Erlinge, D. Congestive heart failure induces downregulation of $P2X_1$-receptors in restance arteries. *Cardiovasc. Res.* **1999**, *43*, 219–227.

(305) Zhao, X.-H.; Sun, X.-Y.; Erlinge, D.; Edvinsson, L.; Hedner, T. Downregulation of adenosine and P2X receptor-mediated cardiovascular responses in heart failure rats. *Blood Pressure* **2000**, *9*, 52–161.

(306) Burnstock, G. Purinergic signaling and vascular cell proliferation and death. *Arterioscler., Thromb., Vasc. Biol.* **2002**, *22*, 364–373.

(307) Wegner, C. D. P2 receptors in the respiratory system. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part II. Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*; Springer Verlag: Heidelberg, 2001; pp 281–300.

(308) Bennett, W. D.; Olivier, K. N.; Zeman, K. L.; Hohneker, K. W.; Boucher, R. C.; Knowles, M. R. Effect of uridine 5′triphosphate plus amiloride on mucociliary clearance in adult cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 1796–1801.

(309) Stutts, M. J.; Boucher, R. C. Cystic fibrosis gene and functions of CFTR implications of dysfunctional ion transport for pulmonary pathogenesis. In *Cystic Fibrosis in Adults*; Yankaskas, J. R., Knowles, M. R., Eds.; Lippincott-Raven: Philadelphia, PA, 1999; pp 3–25.

(310) Bennett, D.; Zeman, K. L.; Foy, C.; Shaffer, C.; Johnson, F.; Sannuti, A.; Johnson, J. Aerosolized Prolucin (uridine 5′-triphosphate, UTP) acutely enhances mucociliary clearence in mild chronic bronchitis (CB). *Am. J. Respir. Crit. Care Med.* **1999**, *159*, A805.

(311) Yerxa, B. R. Therapeutic use of nucleotides in respiratory and opthalamic diseases. *Drug Dev. Res.* **2001**, *52*, 196–201.

(312) Thomas, T.; Ralevic, V.; Bardini, M.; Burnstock, G.; Spyer, K. M. Evidence for the involvement of purinergic signaling in the control of respiration. *Neuroscience* **2001**, *107*, 481–490.

(313) Pelleg, A.; Hurt, C. M. Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals. *J. Physiol.* **1996**, *490*, 265–275.

(314) McQueen, D. S.; Bond, S. M.; Moores, C.; Chessell, I.; Humphrey, P. P. A.; Dowd, E. Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anesthetized rats. *J. Physiol.* **1998**, *507*, 843–855.

(315) Schulman, E. S.; Glaum, M. C.; Post, T.; Wang, Y.; Raible, D. G.; Mohnaty, J.; Butterfield, J. H.; Pelleg, A. ATP modulates anti-IgE-induced release of histamine from human lung mast cells. *Am. J. Respir. Cell. Mol. Biol.* **1999**, *20*, 530–537.

(316) Kato, F.; Shigetomi, E. Distinct modulation of evoked and spontaneous EPSCs by purinoceptors in nucleus tractus solitarii of the rat. *J. Physiol.* **2001**, *530*, 469–486.

(317) Scisio, T. J.; Kitchen, A. M.; Augustyniak, R. A.; O'Leary, D. S. Differential patterns of sympathic responses to selective stimulation of nucleus tractus solitarius purinergic receptor subtypes. *Clin. Exp. Pharmacol. Physiol.* **2001**, *28*, 120–124.

(318) Gachet, C. ADP receptors of platelets and their inhibition. *Thromb. Haemostasis* **2001**, *86*, 222–232.

(319) Leon, C.; Hechler, B.; Vial, C.; Leray, C.; Cazenave, J.-P.; Gatchet, C. The $P2Y_1$ receptor is an ADP receptor antagonized by ATP and expressed in megakaryoblastic cells. *FEBS Lett.* **1997**, *403*, 26–30.

(320) Vial, C.; Hechler, B.; Leon, C.; Cazenave, J.-P.; Gatchet, C. Presence of $P2X_1$ purinoceptors on human platelets and megakaryoblastic cell lines. *Thromb. Haemostasis* **1997**, *78*, 1500–1504.

(321) Mahaut-Smith, M. P.; Ennion, S. J.; Rolf, M. G.; Evans, R. J. ADP is not an agonist at $P2X_1$ receptors: evidence for separate receptors stimulated by ATP and ADP on human platelets. *Br. J. Pharmacol.* **2000**, *131*, 108–114.

(322) Marcus, A. J.; Broekman, M. J.; Drosopoulos, J. H. F.; Islam, N.; Gayle, R. B., III; Pinsky, D. J.; Maliszewski, C. R. Human ectoADPase/CD 39: thromboregulation via a novel pathway. *Hematologica* **2000**, *85*, 53–57.

(323) Conigrave, A. D.; van der Weyden, L.; Holt, L.; Jiang, L.; Wilson, P.; Christopherson, R. I.; Morris, M. B. Extracellular ATP-dependent suppression of proliferation and induction of differentiation of human HL-60 leukemia cells by distinct mechanisms. *Biochem. Pharmacol.* **2000**, *60*, 1585–1591.

(324) Communi, D.; Janssens, R.; Robaye, B.; Zeelis; Boeynaems, J.-M. Role of $P2Y_{11}$ receptors in hematopoiesis. *Drug Dev. Res.* **2001**, *52*, 156–163.

(325) Khakh, B. S. Molecular physiology of P2X receptors and ATP signaling at synapses. *Nat. Rev. Neurosci.* **2001**, *2*, 165–174.

(326) Robertson, S. J.; Burnashev, N.; Edwards, F. A. Ca^{2+} permeability and kinetics of glutamate receptors in rat medial habenula neurones: implications for purinergic transmission in this nucleus. *J. Physiol.* **1999**, *518*, 539–549.

(327) Cuhna, R. A.; Riberio, J. A. ATP as a presynaptic modulator. *Life Sci.* **2000**, *68*, 119–137.

(328) Cuhna, R. A.; Ribeiro, J. A.; Sebastiao, A. M. Purinergic modulation of the evoked release of [^{3}H] acetylcholine from the hippocampus and cerebral cortex of the rat: role of the ecto-nucleotidases. *Eur. J. Neurosci.* **1994**, *6*, 33–42.

(329) Salgado, A. I.; Cuhna, R. A.; Ribeiro, J. A. Facilitation by P_2 receptor activation of acetylcholine release from rat motor nerve terminals: interaction with presynaptic nicotinic receptors. *Brain Res.* **2000**, *877*, 245–250.

(330) Koch, H.; von Kugelgen, I.; Starke, K. P_2-receptor-mediated inhibition of noradrenaline release in the rat hippocampus. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1997**, *355*, 707–715.

(331) Krugel, U.; Kittner, H.; Illes, P. Adenosine 5′-triphosphate-induced dopamine release in the rat nucleus accumbens in vivo. *Neurosci. Lett.* **1999**, *265*, 49–52.

(332) Trendelenburg, A. U.; Bultmann, R. P_2 receptor-mediated inhibition of dopamine release in rat neostriatum. *Neuroscience* **2000**, *96*, 249–252.

(333) von Kugelgen, I.; Koch, H.; Starke, K. P_2 receptor-mediated inhibition of serotonin release in rat brain cortex. *Neuropharmacology* **1997**, *36*, 1221–1227.

(334) Gu, J. G.; MacDermott, A. B. Activation of ATP P_{2X} receptors elicits glutamate release from sensory neuron synapses. *Nature* **1997**, *389*, 749–753.

(335) Inoue, K.; Koizumi, S.; Ueno, S.; Kita, A.; Tsuda, M. The functions of ATP receptors in the synaptic transmission in the hippocampus. *Prog. Brain Res.* **1999**, *120*, 193–221.

(336) Gomez-Villafuertes, R.; Gualix, J.; Miras-Portugal, M. T. Single GABAergic synaptic terminals from rat midbrain exhibit functional P2X and dinucleotide receptors, able to induce GABA secretion. *J. Neurochem.* **2001**, *77*, 84–93.

(337) Kapoor, J. R.; Sladek, C. D. Substance P and NPY differentially potentiate ATP and adrenergic stimulated vasopressin and oxytocin release. *Am. J. Physiol.* **2001**, *280*, R69–R78.

(338) Wang, C.-M.; Chang, T.-Y.; Kuo, J. S.; Sun, S. H. Activation of $P2X_7$ receptors induced [^{3}H]GABA release from the RBA-2 type-2 astrocyte cell line through a Cl^-/HCO_3-dependent mechanism. *Glia* **2001**, *37*, 8–18.

(339) Knutsen, L. J. S.; Murray, T. F. Adenosine and ATP in epilepsy. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley: New York, 1997; pp 423–447.

(340) Kujawa, S. G.; Erosteguli, C.; Fallon, M.; Christ, J.; Bobin, R. P. Effects of adenosine 5′-triphosphate and related agonists on cochlear function. *Hear. Res.* **1984**, *76*, 87–100.

(341) Lee, J. H.; Chiba, T.; Marcus, D. C. $P2X_2$ receptor mediates stimulation of parasensory cation absorption by cochlear outer sulcus cells and vestibular transitional cells. *J. Neurosci.* **2001**, *21*, 9168–9174.

(342) Housley, G. D. Physiological effects of extracellular nucleotides in the inner ear. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 575–800.

(343) Shimogori, H.; Yamashita, H. Rapid correction of vestibular imbalance by intracochlear administration of ATP in a guinea pig model of unilateral peripheral vestibular disorder. *Neurosci. Lett.* **2001**, *315*, 69–72.

(344) Pintor, J.; Peral, A.; Hoyle, C. H. V.; Redick, C.; Douglass, J.; Sims, I.; Yerxa, B. Effects of diadenosine polyphosphates on tear secretion in New Zealand White rabbits. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 291–297.

(345) Jabs, R.; Guenther, E.; Marquordt, K.; Wheeler-Schilling, T. H. Evidence for P2X$_3$, P2X$_4$, P2X$_5$ but not for P2X$_7$ receptors in Mueller cells of the rat retina. *Mol. Brain Res*. **2000**, *76*, 205–210.

(346) Wheeler-Schilling, T. H.; Marquordt, K.; Kohler, K.; Jabs, R.; Guenther, E. Expression of purinergic receptors in bipolar cells of the rat retina. *Mol. Brain Res*. **2000**, *76*, 415–418.

(347) Newman, E. A.; Zahs, K. R. Modulation of neuronal activity by glial cells in the retina. *J. Neurosci*. **1998**, *18*, 4022–4028.

(348) Housley, G. D. Nucleoside and nucleotide transmission in sensory systems. In *Purinergic and Pyrimidinergic Signalling*, Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 339–369.

(349) Bo, X.; Alavi, A.; Xiang, Z.; Oglesby, I.; Ford, A.; Burnstock, G. Localization of ATP-gated P2X$_2$ and P2X$_3$ receptor immuoreactive nerves in rat taste buds. *NeuroReport* **1999**, *10*, 1107–1111.

(350) Houlton, P. The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. *J. Physiol*. **1959**, *145*, 494–504.

(351) Jahr, C. E.; Jessell, T. M. ATP excites a subpopulation of rat dorsal horn neurones. *Nature* **1983**, *304*, 730–733.

(352) Fyffe, R. E. W.; Perl, E. R. Is ATP a central synaptic mediator for certain primary afferent fibersfrom mammalian skin? *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *81*, 6890–6893.

(353) Bleehen, T.; Keele, C. A. Observation on the algogenic actions of adenosine compounds on the human blister base preparation. *Pain* **1977**, *3*, 367–377.

(354) Hamilton, S. G.; Warburton, J.; Bhattacharjee, A.; Ward, J.; McMahon, S. B. ATP in human skin elicits a dose-related pain response under conditions of hyperalgesia. *Brain Res*. **2000**, *123*, 1238–1246.

(355) Collo, G.; North, R. A.; Kawashima, R.; Merlo-Pich, E.; Neidhart, S.; Surprenant, A. Cloning of P2X$_5$ and P2X$_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci*. **1996**, *16*, 2495–2507.

(356) Guo, A.; Vulchanova, L.; Wang, J.; Li, X.; Elde, R. Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X$_3$ purinoceptor and IB4 binding sites. *Eur. J. Neurosci*. **1999**, *11*, 946–958.

(357) Burgard, E. C.; Niforatos, W.; van Biesen, T.; Lynch, K. J.; Touma, E.; Metzger, R. E.; Kowaluk, E. A.; Jarvis, M. F. P2X receptor-mediated ionic currents in dorsal root ganglion neurons. *J. Neurophysiol*. **1999**, *82*, 1590–1598.

(358) Alavi, A. M.; Dubyak, G. R.; Burnstock, G. Immunohistochemical evidence for ATP receptors in human dental pulp. *J. Dent. Res*. **2001**, *80*, 476–483.

(359) Novakovic, S. D.; Kassotakis, L. C.; Oglesby, I. B.; Smith, J. A. M.; Eglen, R. M.; Ford, A. P. D. W.; Hunter, J. C. Immunocytochemical localization of P$_{2X3}$ purinoceptors in sensory neurons in naïve rats and following neuropathic injury. *Pain* **1999**, *80*, 273–282.

(360) Chen, Y.; Shu, Y.; Zhao, Z. Ectopic purinergic sensitivity develops at sites of chronic nerve constriction injury in rat. *NeuroReport* **1999**, *10*, 2779–2782.

(361) Jarvis, M. F.; Jovaluk, E. A. Pharmacological characterization of P2X$_3$ homomeric and heteromeric ion channels in nociceptive signaling and behavior. *Drug Dev. Res*. **2000**, *52*, 220–231.

(362) Chizu, B.; Illes, P. P2X receptors and nociception. *Pharmacol. Rev*. **2000**, *53*, 553–568.

(363) Salter, M. W.; Sollevi, A. Roles of purines in nociception and pain. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 371–401.

(364) Bland-Ward, P. A.; Humphrey, P. P. A. Acute nociception mediated by hindpaw P2X receptor activation in the rat. *Br. J. Pharmacol*. **1997**, *122*, 365–371.

(365) Hamilton, S. G.; Wade, A.; McMahon, S. B. The effects of inflammation and inflammatory mediators on nociceptive behavior induced by ATP analogues in the rat. *Br. J. Pharmacol*. **1999**, *126*, 326–332.

(366) Hamilton, S. G.; McMahon, S. B.; Lewin, G. R. Selective activation of nociceptors by P2X receptor agonists in normal and inflamed rat skin. *J. Physiol*. **2001**, *534*, 437–445.

(367) Sawynok, J.; Reid, A. Peripheral adenosine 5′-triphosphate enhances nociception in the formalin test via activation of a purinergic P2X receptor. *Eur. J. Pharmacol*. **1997**, *330*, 115–121.

(368) Driessen, B.; Reimann, W.; Selve, N.; Friderichs, E.; Bultmann, R. Antinociceptive effect of intrathecally administered P2-purinoceptor antagonists in rats. *Brain Res*. **1994**, *666*, 182–188.

(369) Tsuda, M.; Koizumi, S.; Kita, A.; Shigemoto, Y.; Ueno, S.; Inoue, K. Mechanical allodynia caused by intraplantar injection of P2X receptor agonist in rats: Involvement of heteromeric P2X$_{2/3}$ receptor signaling in capsaicin-insensitive primary afferent neurons. *J. Neurosci*. **2000**, *20*, RC90 (1–5).

(370) Tsuda, M.; Ueno, S.; Inoue, K. In vivo pathway of thermal hyperalgesia by intrathecal administration of α,β-methyleneATP in mouse spinal cord: Involvement of glutamate−NMDA receptor system. *Br. J. Pharmacol*. **1999**, *127*, 449–456.

(371) Tsuda, M.; Ueno, S.; Inoue, K. Evidence for the involvement of spinal endogenous ATP and P2X receptors in nociceptive responses caused by formalin and capsaicin in mice. *Br. J. Pharmacol*. **1999**, *128*, 1497–1504.

(372) Honore, M. P.; Mikusa, J.; Bianchi, B.; McDonald, H.; Cartmell, J.; Faltynek, C.; Jarvis, M. F. TNP-ATP, a potent P2X$_3$ receptor antagonist blocks acetic acid-induced abdominal constriction in mice: comparison reference analgesics. *Pain* **2002**, *96*, 99–105.

(373) Zhong, Y.; Dunn, P. M.; Bardini, M.; Ford, A. P. D.W.; Cockayne, D. A.; Burnstock, G. Changes in P2X responses of sensory neurons from P2X$_3$-deficient mice. *Eur. J. Neurosci*. **2001**, *14*, 1784–1792.

(374) Nakatsuka, T.; Mena, N.; Ling, J.; Gu, J. G. Depletion of substance P from rat primary sensory neurons by ATP, an implication of P2X receptor-mediated release of substance P. *Neuroscience* **2001**, *107*, 293–300.

(375) Wang, Z. M.; Katsurabayashi, S.; Rhee, J. S.; Brodwick, M.; Akakie, N. Substance P abolishes the faciliatatory effects of ATP on spontaneous glycine release in neurons of the trigeminal nucleus pars caudalis. *J. Neurosci*. **2001**, *21*, 2983–2991.

(376) Honore, P.; Kage, K.; Mikusa, J.; Watt, A. T.; Johnston, J. F.; Wyatt, J. R.; Faltynek, C.; Jarvis, M. F.; Lynch, K. Analgesic profile of intrathecal P2X$_3$ antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. *Pain*, in press.

(377) Stanfa, L. C.; Kontinen, V. K.; Dickenson, A. H. Effects of spinally administered P2X receptor agonists and antagonists on the responses of dorsal horn neurons recorded in normal, carrageenan-inflamed and neuropathic rats. *Br. J. Pharmacol*. **2000**, *129*, 351–359.

(378) Liu, T.; Tracey, D. J. ATP P$_{2X}$ receptors play little role in the maintinence of neuropathic hyperalgesia. *NeuroReport* **2000**, *11*, 1669–1672.

(379) Grafstein, B.; Liu, S.; Cotrina, M. L.; Goldman, S. A.; Nedergaard, M. Meningeal cells can communicate with astrocytes by calcium signaling. *Ann. Neurol*. **2000**, *47*, 18–25.

(380) Fukunaga, A. F. Purines in anesthesia. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1987; pp 471–493.

(381) Burnstock, G. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechano-sensory transduction. *J. Anat*. **1999**, *194*, 335–342.

(382) Burnstock, G. P2X receptors in sensory neurons. *Br. J. Anesthesiol*. **2000**, *84*, 476–488.

(383) Burnstock, G. Expanding field of purinergic signaling. *Drug Dev. Res*. **2001**, *52*, 1–10.

(384) Knight, G. E.; Bodin, P.; De Groat, W. C.; Burnstock, G. ATP is released from guinea pig ureter epithelium on distension. *Am. J. Physiol. Renal Physiol*. **2002**, *283*, F281–F288.

(385) Neary, J. T.; Rathbone, M. P.; Cattabeni, F.; Abbracchio, M. P.; Burnstock, G. Trophic actions of extracellular nucleotides and nucleosides on glial and neuronal cells. *Trends Neurosci*. **1996**, *19*, 13–18.

(386) Neary, J. T.; Abbracchio, M. P. Trophic actions of purines and pyrimidines. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 305–338.

(387) Franke, H.; Grosche, J.; Schadlich, H.; Krugel, U.; Allgaier, C.; Illes, P. P2X receptor expression on astrocytes in the nucleus accumbens of rats. *Neuroscience* **2001**, *108*, 421–429.

(388) Rathbone, M. P.; Middlemiss, P. J.; Gysbers, J. W.; Andrew, C.; Herman, M. A. R.; Redd, J. K.; Ciccarelli, R.; Di Iorio, P.; Caciagli, R. Trophic effects of purines in neurons and glial cells. *Prog. Neurobiol*. **2000**, *59*, 663–690.

(389) D'Ambrosi, N.; Cavaliere, F.; Merlo, D.; Milazzo, L.; Mercanti, D. Volonte, C. Antagonists of P2 receptor prevent NGF-dependent neuritogenesis in PC12 cells. *Neuropharmacology* **2000**, *39*, 1083–1094.

(390) Hide, I.; Tanaka, M.; Inoue, A.; Nakajima, K.; Kohsaka, S.; Inoue, K.; Nakata, Y. Extracellular ATP triggers tumor necrosis factor-α release from rat microglia. *J. Neurochem*. **2000**, *75*, 965–972.

(391) Malam-Souley, R.; Campan, M.; Gadeau, A. P.; Desgranges, C. Exogenous ATP induces limited cell cycle progression of arterial smooth muscle cells. *Am. J. Physiol*. **1993**, *265*, C783–C788.

(392) Chow, S. C.; Kass, G. E. N.; Orrenius, S. Purines and their role in apoptosis. *Neuropharmacology* **1997**, *36*, 1149–1156.

(393) Di Virgilio, F.; Sanz, J. M.; Chiozzi, P.; Falzoni, S. The P2Z/P2X$_7$ receptor of microglial cells: a novel immunomodulatory receptor. *Prog. Brain Res.* **1999**, *120*, 55−78.

(394) Ferrari, D.; La Sala, A.; Chiozzi, P.; Morelli, A.; Falzoni, S.; Girolomoni, G.; Idzko, M.; Dichmann, S.; Norgauer, J.; Di Virgilio, F. The P2 purinergic receptors of human dendritic cells: identification and coupling to cytokine release. *FASEB J.* **2000**, *14*, 2466−2476.

(395) Humphreys, B. D.; Dubyak, G. R. Induction of the P2Z/P2X$_7$ nucleotide receptor and associated phospholipase C activity by lipopolysaccharide and IFN-γ in the human THP-1 monocytic cell line. *J. Immunol.* **1996**, *157*, 5627−5637.

(396) Ferrari, D.; Wesselborg, S.; Bauer, M. K. A.; Schulze-Osthoff, K. Extracellular ATP activates transcription factor NF-κB through the P2Z purinoceptor by selectively targeting NF-κB p65 (RelA). *J. Cell. Biol.* **1997**, *139*, 1635−1643.

(397) Lammas, D. A.; Stober, C.; Harvey, C. J.; Kendrick, N.; Panchalingam, S.; Kumararatne, D. S. ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z (P2X$_7$) receptors. *Immunity* **1997**, *7*, 433−444.

(398) Burnstock, G. Purinoceptors: ontogeny and phylogeny. *Drug Dev. Res.* **1996**, *39*, 204−242.

(399) McNamara, N.; Khong, A.; McKemy, D.; Caterina, M.; Boyer, J.; Julius, D.; Basbaum, C. ATP transduces signals from ASGM1, a glycolipid that functions as a bacterial receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9086−9091.

(400) Burnstock, G.; Cocks, T.; Crowe, R.; Kasakov, I. Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmacol.* **1978**, *63*, 125−138.

(401) Burnstock, G. Purinergic signaling in the lower urinary tract. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 1. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 424−515.

(402) Dean, D. M.; Downie, J. W. Contribution of adrenergic and "purinergic" neurotransmission to contraction in rabbit detrusor. *J. Pharmacol. Exp. Ther.* **1978**, *207*, 431−445.

(403) Yoshimura, N.; de Groat, W. C. Neural control of the lower urinary tract. *Int. J. Urol.* **1997**, *4*, 111−125.

(404) Pinna, C.; Puglis, C.; Burnstock, G. ATP and vasoactive intestinal polypeptide relaxant responses in hamster isolated proximal urethra. *Br. J. Pharmacol.* **1998**, *124*, 1069−1074.

(405) Lee, H. Y.; Bardini, M.; Burnstock, G. Distribution of P2X receptors in the urinary bladder and the ureter of the rat. *J. Urol.* **2000**, *163*, 2002−2007.

(406) O'Reilly, B. A.; Kosaka, A. H.; Chang, T. K.; Ford, A. P. D. W.; Popert, R.; Rymer, J. M.; McMahon, S. B. A quantitative analysis of purinoceptor expression in human fetal and adult bladders. *J. Urol.* **2001**, *165*, 1730−1734.

(407) Chancellor, M. B.; Kaplan, S. A.; Blaivas, J. G. The cholinergic and purinergic components of detrusor contracility in a whole rabbit bladder model. *J. Urol.* **1992**, *148*, 906−909.

(408) Hashimoto, M.; Kokubun, S. Contribution of P$_2$-purinoceptors to neurogenic contraction of rat urinary bladder smooth muscle. *Br. J. Pharmacol.* **1995**, *115*, 636−640.

(409) Calvert, R. C.; Thompson, C. S.; Khan, M. A.; Mikhailidis, D. P.; Morgan, R. J.; Burnstock, G. Alterations of cholinergic and purinergic signaling in a model of the obstructed bladder. *J. Urol.* **2001**, *166*, 1530−1533.

(410) Namasivayam, S.; Eardley, I.; Morrison, J. F. Purinergic sensory neurotransmission in the urinary bladder: an in vitro study in the rat. *BJU Int.* **1999**, *84*, 854−860.

(411) Elneil, S.; Skepper, J. N.; Kidd, E. J.; Williamson, J. G.; Ferguson, D, R. Distribution of P2X$_1$ and P2X$_3$ receptors in the rat and human urinary bladder. *Pharmacology* **2001**, *63*, 120−128

(412) Moore, K. H.; Ray, F. R.; Barden, J. A. Loss of purinergic P2X$_3$ and P2X$_5$ receptor innervation in human detrusor from adults with urge incontinence. *J. Neursoci.* **2001**, *21*, RC166/1−RC166/6.

(413) O'Reilly, B. A.; Kosaka, A. H.; Knight, G. F.; Chang, T. K.; Ford, A. P. D. W.; Rymer, J. M.; Popert, R.; Burnstock, G.; McMahon, S. B. P2X receptors and their role in female detrusor instability. *J. Urol.* **2002**, *167*, 157−164.

(414) O'Reilly, B. A.; Kosaka, A. H.; Chang, T. K.; Ford, A. P. D. W.; Popert, R.; McMahon, S. B. A quantitative analysis of purinoceptor expression in the bladders of patients with symptomatic outlet obstruction. *BJU Int.* **2001**, *87*, 617−622.

(415) Palea, S.; Artibani, W.; Ostardo, E.; Trist, D. G.; Pietra, C. Evidence for purinergic neurotransmission in human urinary bladder affected by interstistial cystitis. *J. Urol.* **1993**, *150*, 2007−2012.

(416) Rong, W.; Spyer, K. M.; Burnstock, G. Activation and sensitization of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. *J. Physiol.*, in press.

(417) Lee, H. Y.; Bardini, M.; Burnstock, G. P2X receptor immunoreactivity in the male genital organs of the rat. *Cell Tissue Res.* **2000**, *300*, 321−330.

(418) Glass, R.; Bardini, M.; Robson, T.; Burnstock, G. Expression of nucleotide P2X receptor subtypes during spermatogenesis in the adult rat testis. *Cells Tissues Organs* **2001**, *169*, 377−387.

(419) Wu, H. Y.; Broderick, G. A.; Suh, J. K.; Hypolite, J. A.; Levin, R. M. Effects of purines on rabbit corpus cavernosum contractile activity. *Int. J. Impot. Res.* **1993**, *5*, 161−167.

(420) Gür, S.; Öztürk, B. Altered relaxant responses to adenosine and adenosine 5′-triphosphate in the corpus cavernosum from men and rats with diabetes. *Pharmacology* **2000**, *60*, 105−112.

(421) Burnstock, G. Purinergic signaling in the gut. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; Springer Verlag: Heidelberg, 2001; pp 141−238.

(422) Schlosser, S. F.; Burgstahler, A. D.; Nathanson, M. H Isolated rat hepatocytes can signal to other hepatocytes and bile duct epithelial cells by release of nucleotides. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9948−9953.

(423) Feranchak, A. P.; Fitz, J. G.; Roman, R. M. Volume-sensitive purinergic signaling in human hepatocytes. *J. Hepatol.* **2000**, *33*, 174−182.

(424) Roman, R. M.; Fitz, J. G. Emerging roles of purinergic signaling in gastrointestinal epithelial secretion and hepatobiliary function. *Gastroenterology* **1999**, *116*, 964−979.

(425) Chari, R. S.; Shutz, M.; Haebig, J. E.; Shimokura, G. H.; Cotton, P. B.; Fitz, J. G.; Myers, W. C. Adenosine nucleotides in the bile. *Am. J. Physiol.* **1996**, *270*, G246−G252.

(426) Schenkler, T.; Romac, J. M.-J.; Sharara, A.; Roman, R. M.; Kim, S.; LaRusso, N.; Liddle, R.; Fitz, J. G. Regulation of biliary secretion through apical purinergic receptors in cultured rat cholangiocytes. *Am. J. Physiol.* **1996**, *273*, G1108−G1117.

(427) Keppens, S.; De Wulf, H. Characterization of the biological effects of 2-methylthio-ATP on rat hepatocytes: clear-cut differences from ATP. *Br. J. Pharmacol.* **1991**, *104*, 301−304.

(428) Haussinger, D.; Stehle, T.; Gerok, W. Actions of extracellular ATP and UTP in perfused rat liver. *Eur. J. Biochem.* **1987**, *167*, 65−71.

(429) Loubatrieres-Mariani, M.-M.; Hillaire-Buys, D.; Chapal, J.; Betrand, G.; Petit, P. P2 purinoceptor agonists: new insulin secretogogues potentially useful in the treatment of non-insulin-dependent diabetes mellitus. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997; pp 253−260.

(430) Petit, P.; Hillaire-Buys, D.; Loubatrieres-Mariani, M.-M.; Chapal, J. Purinergic receptors and the pharmacology of type 2 diabetes. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 2. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 377−391.

(431) Nichols, C. G.; Shyng, S.-L.; Nestorowicz, A.; Glaser, B.; Clement, J. P., IV; Gonzalez, G.; Aguilar-Bryan, L.; Permutt, M. A.; Bryan, J. Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* **1996**, *272*, 1785−1787.

(432) Coutinho-Silva, R.; Parsons, M.; Robson, T.; Burnstock, G. Changes in the expression of P2 receptors in rat and mouse pancreas during development and aging. *Cell Tissue Res.* **2001**, *306*, 373−383.

(433) Morrison, M. S.; Turin, L.; King, B. F.; Burnstock, G.; Arnett, T. R. ATP is a potent stimulator of the activation and formation of rodent osteoclasts. *J. Physiol. (London)* **1998**, *511*, 495−500.

(434) Dixon, S. J.; Sims, S. M. P2 purinergic receptors on osteoblasts and osteoclasts: potential targets for drug development. *Drug Dev. Res.* **2000**, *49*, 187−200.

(435) Naemsch, L. N.; Du, X.; Sims, S. M.; Dixon, J. P2 nucleotide receptors in osteoclasts. *Drug. Dev. Res.* **2001**, *53*, 130−139.

(436) Hoebertz, A.; Meghi, S.; Burnstock, G.; Arnett, T. R. Extracellular ADP is a powerful osteolytic agent: evidence for signaling through the P2Y$_1$ receptor on bone cells. *FASEB J.* **2001**, *15*, 1139−1148.

(437) Ferrier, J. Purinergic and pyrimidinergic receptor signaling in bone cells. In *Purinergic and Pyrimidinergic Signalling*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Vol. 151, Part 2. Molecular Aspects, Central and Peripheral Nervous System Function*; Springer Verlag: Heidelberg, 2001; pp 393−406.

(438) Steinberg, T. H.; Jørgensen, N. R.; Bong, J. S.; Henriksen, Z.; Atal, N.; Lin, G. C.; Bennett, B. D.; Eriksen, E. F.; Sørensen, O. H.; Civitelli, R. P2-mediated responses in osteoclasts and osteoclast-like cells. *Drug Dev. Res.* **2001**, *53*, 126−129.

(439) Rapaport, E. ATP in the treatment of cancer. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997; pp 545−553.

(440) Wang, P.; Ba, Z. F.; Morrison, M. H.; Ayala, A.; Dean, R. E.; Chaudry, I. H. Mechanism of the beneficial effects of ATP-MgCl$_2$ following trauma-hemorrhage and resuscitation: down regulation of inflammatory cytokine (TNF, IL-6) release. *J. Surg. Res.* **1992**, *52*, 364−371.

(441) Agteresch, H. J.; Dagnelie, P. C.; van der Gaast, A.; Stijnen, T.; Wilson, J. H. P. Randomized clinical trial of adenosine 5′-triphosphate in patients with advanced non-small-cell lung cancer. *J. Natl. Cancer Inst.* **2000**, *92*, 321−328.

(442) Kawamori, T.; Rao, C. V.; Seibert, K.; Reddy, B. S. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res.* **1998**, *58*, 409−412.

(443) Abraham, E. H.; Vos, P.; Kahn, J.; Grubman, S. A.; Jefferson, D. M.; Ding, I.; Okunieff, P. Cystic fibrosis hetero- and homozygosity is associated with inhibition of breast cancer growth. *Nat. Med.* **1996**, *2*, 593−596.

(444) Neglia, J. P.; Fitzsimmons, S. C.; Maisonneuve, P.; Schoni, M. H.; Schoni-Affolter, F.; Corey, M.; Lowenfels, A. B. The Cystic Fibrosis and Cancer Study Group. The risk of cancer among patients with cystic fibrosis. *N. Eng. J. Med.* **1995**, *332*, 494−499.

(445) Hopfner, M.; Lemmer, K.; Jansen, A.; Hanski, C.; Riecken, E. O.; Gavish, M.; Mann, B.; Buhr, H.; Glassmeier, G.; Scherubl, H. Expression of functional P2-purinergic receptors in primary cultures of human colorectal carcinoma cells. *Biochem. Biophys. Res. Commun.* **1998**, *251*, 811−817.

(446) Janssens, R.; Boynaems, J.-M. Effects of extracellular nucleotides and nucleosides on prostate carcinoma cells. *Br. J. Pharmacol.* **2001**, *132*, 536−546.

(447) Franke, H.; Kruegel, U.; Illes, P. P2 receptor-mediated proliferative effects on astrocytes in vivo. *Glia* **1999**, *28*, 190−200.

(448) Groschel-Stewart, U.; Bardini, M.; Robson, T. A.; Burnstock, G. Localization of P2X$_5$ and P2X$_7$ receptors by immunohistochemistry in rat stratified squamous epithelium. *Cell Tissue Res.* **1999**, *29,* 599−605.

(449) Demain, A. L. Prescription for an ailing pharmaceutical industry. *Nat. Biotechnol.* **2002**, *20*, 331.

(450) Williams, M. Challenges in developing P2 purinoceptor-based therapeutics. In *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*; Chadwick, D. J., Goode, J. A., Eds.; CIBA Foundation Symposium 198; Wiley: Chichester, 1996; Vol. 198; pp 309−321.

(451) Kenakin, T. P. Efficacy at G-protein-coupled receptors. *Nat. Rev. Drug Discovery* **2002**, *1*, 103−110.

(452) Christopoulos, A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nat. Rev. Drug Discovery* **2002**, *1*, 198−210.

(453) Chafke, Y.; Seguin, R.; Antel, J. P.; Morisette, C.; Malo, D.; Henderson, D.; Seguela, P. ADP and AMP induce interleukin-1β release from microglial cells through activation of ATP-primed P2X$_7$ receptor channels. *J. Neurosci.* **2002**, *22*, 3061−3069.

(454) Delombe, S.; Escande, D. ATP-binding proteins as targets for drugs discovery. *Trends Pharmacol. Sci.* **1996**, *17*, 273−275.

(455) Fleckenstein-Grun, G.; Fleckenstein, A. Calcium—a neglected key factor in arteriosclerosis. The pathogenic role of arterial calcium overload and its prevention by calcium antagonists. *Ann. Med.* **1991**, *23*, 589−599.