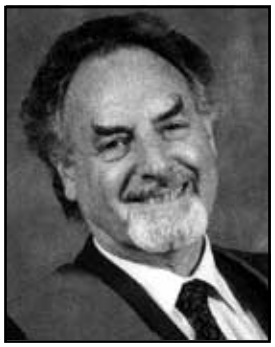
Introduction: P2 Receptors

Geoffrey Burnstock*

Autonomic Neuroscience Institute, Royal Free and University College, London NW3 2PF, U.K.



Abstract: The current status of ligand gated ion channel P2X and G protein-coupled P2Y receptor subtypes is described. This is followed by a summary of what is known of the distribution and roles of these receptor subtypes. Potential therapeutic targets of purinoceptors are considered, including those involved in cardiovascular, nervous, respiratory, urinogenital, gastrointestinal, musculo-skeletal and special sensory diseases, as well as inflammation, cancer and diabetes. Lastly, there are some speculations about future developments in the purinergic signalling field.

HISTORICAL BACKGROUND

The first paper describing the potent actions of adenine compounds was published by Drury & Szent-Györgyi in 1929 [1]. Many years later, ATP was proposed as the transmitter responsible for non-adrenergic, non-cholinergic transmission in the gut and bladder and the term 'purinergic' introduced by Burnstock [2]. Early resistance to this concept appeared to stem from the fact that ATP was recognized first for its important intracellular roles and the intuitive feeling was that such a ubiquitous and simple compound was unlikely to be utilized as an extracellular messenger.

Purinergic receptors were first defined in 1976 [3] and two years later a basis for distinguishing two types of purinoceptor, identified as P1 and P2 (for adenosine and ATP/ADP, respectively), was defined [4]. At about the same time, two subtypes of the P1 (adenosine) receptor were recognised [5,6], but it was not until 1985 that a pharmacological basis for distingushing two types of P2 receptors (P2X and P2Y) was proposed [7]. A year later, two further P2 purinoceptor subtypes were named, a P2T receptor selective for ADP on platelets and a P2Z receptor on macrophages [8]. Further subtypes followed, perhaps the most important of which being the P2U receptor which could recognise pyrimidines such as UTP as well as ATP [9]. However, to provide a more manageable framework for newly identified nucleotide receptors, Abbracchio and Burnstock [10] proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G protein-coupled purinoceptors, based on studies of transduction mechanisms [11] and the cloning of nucleotide receptors [12-15]. This nomenclature has been widely adopted and currently seven P2X subtypes and eight P2Y receptor subtypes are recognised, including receptors that are sensitive to pyrimidines as well as purines [16-18].

*Address correspondence to this author at Autonomic Neuroscience Institute, Royal Free and University College, Rowland Hill Street, London NW3 2PF, U.K.; Tel: 020 7830 2948; Fax: 020 7830 2949; E-mail: g.burnstock@ucl.ac.uk

1568-0266/04 $45.00+.00

It is widely recognised that purinergic signalling is a primitive system [19] involved in many non-neuronal as well as neuronal mechanisms and in both short-term and long-term (trophic) events [20], including exocrine and endocrine secretion, immune responses, inflammation, pain, platelet aggregation, endothelial-mediated vasodilatation, cell proliferation and death [8, 21-23].

P2X Receptors

Members of the existing family of ionotropic $P2X_{1-7}$ receptors show a subunit topology of: intracellular N- and C-termini possessing consensus binding motifs for protein kinases; two transmembrane spanning regions (TM1 and TM2), the first involved with channel gating and second lining the ion pore; a large extracellular loop, with 10 conserved cysteine residues forming a series of disulphide bridges; a hydrophobic H5 region close to the pore vestibule, for possible receptor/channel modulation by cations (magnesium, calcium, zinc, copper and proton ions); and an ATP-binding site, which may involve regions of the extracellular loop adjacent to TM1 and TM2. The $P2X_{1-7}$ receptors show 30-50% sequence identity at the peptide level. The stoichiometry of $P2X_{1-7}$ receptors is now thought to involve three subunits which form a stretched trimer [17].

The pharmacology of the recombinant P2X receptor subtypes expressed in oocytes or other cell types is often different from the pharmacology of P2Y-mediated responses in naturally occurring sites. Several contributing factors may account for these differences. First, heteromultimers as well as homomultimers are involved in forming the trimer ion pore. For example, heteromultimers are clearly established for $P2X_{2/3}$ in nodose ganglia [24-29]. $P2X_7$ does not form heteromultimers, and $P2X_6$ will not form a functional homomultimer [30, 31]. Second, spliced variants of P2X receptor subtypes might play a part. For example, a splice variant of $P2X_4$ receptor, while it is nonfunctional on its own, can potentiate the actions of ATP through the full-length $P2X_4$ receptors (e.g. [32]). Third, the presence of powerful ectoenzymes in tissues that rapidly break down purines and pyrimidines is not a factor when examining recombinant receptors [33].

Many pharmacological and operational differences are exhibited by the P2X receptor family. The kinetics of activation, inactivation and deactivation also vary considerably amongst P2X receptors. Calcium permeability is high for some P2X subtypes, a property that may be functionally important.

P2Y Receptors

Metabotropic $P2Y_{1-14}$ receptors are characterised by a subunit topology of: extracellular N-terminus and intracellular C-terminus, the latter possessing consensus binding motifs for protein kinases; seven transmembrane spanning regions which help to form the ligand docking pocket; a high level of sequence homology between some transmembrane spanning regions, particlarly TM3, 6 and 7; structural diversity of intracellular loops and C-terminus amongst P2Y subtypes, so influencing the degree of coupling with $G_{q/11}$, G_s and G_i proteins. Each P2Y receptor binds to a single heterotrimeric G protein (typically $G_{q/11}$), although $P2Y_{11}$ can couple to both $G_{q/11}$ and G_s whereas $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ couple to G_i. P2Y receptors may form homo- and heteromultimeric assemblies under some conditions, and many tissues express multiple P2Y subtypes [18]. P2Y receptors show a low level of sequency homology at the peptide level (19-55% identical) and, consequently, show significant differences in their pharmacological and operational profiles. In response to nucleotide activation, recombinant P2Y receptors either activate phospholipase C and release intracellular calcium or affect adenylyl cyclase and alter cAMP levels. There is little evidence to indicate $P2Y_{5,9,10}$ sequences are nucleotide receptors or affect intracellular signalling cascades. Endogenous P2Y receptors show a greater diversity in intracellular signalling and can activate phospholipases A_2, C and D, MEP/MAP kinase, Rho-dependent kinase and tyrosine kinase, as well as coupling both positively and negatively to adenylyl cyclase.

2-MethylthioADP is a potent agonist of mammalian $P2Y_1$ receptors [34], and MRS 2179 is a potent antagonist [35]; MRS 2269 and MRS 2286 have been identified as selective antagonists [36]. At $P2Y_2$ and $P2Y_4$ receptors in the rat, ATP and UTP are equipotent, but the two receptors can be distinguished with antagonists, i.e. suramin blocks $P2Y_2$, while Reactive blue 2 blocks $P2Y_4$ receptors [37,38]. $P2Y_6$ is UDP-selective, while $P2Y_7$ turned out to be a leukotriene receptor. $P2Y_8$ is a receptor cloned from frog embryos, where all the nucleotides are equipotent [39], but no mammalian homologue has been identified to date, apart from a recent report of $P2Y_8$ mRNA in undifferentiated HL60 cells [40]. $P2Y_{11}$ is unusual in that there are two transduction pathways, adenylate cyclase as well as inositol triphosphate, which is the second messenger system used by the majority of the P2Y receptors. The $P2Y_{12}$ receptor found on platelets was not cloned until recently [41], although it only has 19% homology with the other P2Y receptor subtypes. It seems likely to represent one of a subgroup of P2Y receptors for which transduction is entirely through adenylate cyclase. A further receptor, $P2Y_{13}$, has recently been identified [42], which is related to the $P2Y_{12}$ receptor, and the UDP-glucose receptor which has been recently renamed as the $P2Y_{14}$ receptor [18,43,44].

There are suggestions that P2Y receptors can be divided into two subgroups on the basis of structural similarities: (i) $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ and (ii) $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. In addition, the non-mammalian $p2y_8$ and tp2y receptors are activated by all triphosphate nucleotides. Table 1 summarises the status of the P2 receptor subtype agonists and antagonists currently available [18].

DISTRIBUTION AND ROLES OF PURINOCEPTOR SUBTYPES

P2X Receptors

The distribution of P2X receptor subtypes based on Northern blot and *in situ* hybridization studies [45] has been extended, after antibodies to these receptors became available, by immunohistochemical localization at both light microscopic [46-58] and electron microscopic levels [59-63]. For example, while it was originally thought that smooth muscle contained only $P2X_1$ receptors, there is now evidence for the presence of $P2X_2$, $P2X_4$ and probably $P2X_5$ receptors as both homomultimers and heteromultimers [64, 66]. $P2X_1$ receptors, which, in earlier studies, were not considered to be present in the brain, now have been found at postjunctional sites in synapses in the cerebellum [60].

The $P2X_1$ receptor is characterised by rapid desensitization and potent actions of αβ-methylene ATP (αβ-meATP), and there are now very potent selective antagonists for this receptor, such as trinitrophenyl ATP [67], diinosine pentaphosphate [68], NF449 [69] and MRS 2257 [70] widespread in the central nervous system and have been found at both pre- and postsynaptic sites in the hypothalamus [62,63]). A feature of the $P2X_2$ receptor is lack of fast desensitisation and high sensitivity to acidity and Zn^{2+} [71,72]. $P2X_3$ receptors are interesting in that they are predominantly localised on sensory nerves, particularly the small nociceptive neurons in the dorsal root, trigeminal and nodose ganglia [73]. The central projections are located in inner lamina II of the dorsal horn of the spinal cord, and peripheral extensions have been noted in the skin, tongue [74] and most recently in tooth pulp [75] and bladder [76]. There is recent evidence for $P2X_2$ and $P2X_3$ labelling of endothelial cells of microvessels in brain, thymus, thyroid, and gut and also in epithelial cells in the thyroid [54,61,77,78]. $P2X_4$ and $P2X_6$ receptors are prominent in the central nervous system, but are unusual in that responses to purines mediated by these receptors are not blocked by agents commonly used as P2 antagonists [79]. $P2X_5$ receptors have been shown to be associated with proliferating and differentiating epithelial cells in the skin and hair follicles [53], in the bladder, ureter, and vagina [55,57,58]. $P2X_5$ receptors are also prominent in a number of cell types in embryonic development [80, 81]. $P2X_7$ receptors are unique in that, as well as a cation pore, a large, 4-nm pore can be formed, which appears to be linked with apoptosis, perhaps associated with the elongated C-terminus of this receptor [82]. The $P2X_7$ receptor is often internalized in cells, although under pathological conditions such as ischemia and cancer, it becomes externalized, leading to apoptosis. Fluorescent green protein coupled to $P2X_7$ receptors provide a valuable technique for observing the movement of receptors in living cells [83].

Table. 1. Mammalian P2 Receptors and Assessment of Activities of Agonists and Antagonists (* Selective).

	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$	$P2X_{2/3}$	$P2X_{1/5}$	$P2X_{4/6}$	$P2Y_1$	$P2Y_2$	$P2Y_4$	$P2Y_6$	$P2Y_{11}$	$P2Y_{12}$	$P2Y_{13}$	$P2Y_{14}$
Agonists																		
ATP	✓✓✓	✓✓	✓✓✓	✓✓	✓✓	✓	✓	✓✓✓	✓✓✓	✓✓	✓	✓✓✓	✓✓	-	✓			
ADP	✓	-	✓	-	-	-	-		✓✓		✓✓		✓			✓✓	✓✓	
2-MeSATP	✓✓✓	✓✓	✓✓✓	✓✓	✓✓	✓	✓				✓✓		✓	✓	✓	✓	✓✓	
PAPET-ATP	✓✓	✓	✓✓✓	✓							✓✓✓*							
2-MeSADP				✓							✓✓✓*			✓✓		✓✓✓	✓✓✓	
HT-AMP	✓✓	-	✓✓✓	✓✓							✓✓							
α,β-meATP	✓✓✓*	-	✓✓✓*	✓	✓	✓	-	✓✓✓	✓✓	✓			-					
β,γ-meATP	✓✓	-	✓✓	-	✓		-						-					
ATPγS	✓✓	✓✓	✓✓	✓	✓✓	✓	-				✓	✓	✓		✓✓✓	✓		
ATPβS											✓✓				✓	✓✓		
Bz-ATP	✓✓✓	✓✓	✓	✓✓✓	✓✓		✓✓						✓ [antag]		✓✓✓			
UDP-glucose																		✓✓✓*
UDPβS														✓✓✓*				
2-dATP															✓✓			
Ap_4A	✓✓✓	✓	✓✓✓	✓	✓							✓✓	✓✓					
UTP	-	-	-	-	✓							✓✓✓	✓✓✓	✓				
UTPγS												✓✓✓*						
UDP					-							✓	✓✓	✓✓✓				
CTP	✓	✓	✓	-	✓								✓✓					
Antagonists																		
PPADS	✓✓	✓✓	✓✓	-	✓✓	✓	✓					✓	✓	✓✓				
isoPPADS	✓✓	✓✓	✓✓															
PPNDS	✓✓*																	
Suramin	✓✓	✓	✓	-	✓✓	-	-				✓	✓	-	-	✓✓	✓		
NF023	✓✓*	✓	✓															
Reactive blue 2	✓	✓✓	✓	-	✓						✓		✓	✓✓	✓	✓✓		
MRS 2179	✓	-	✓	-							✓✓✓	-	-	-				
MRS 2279	-		-								✓✓✓*							
TNP-ATP	✓✓✓*	✓	✓✓✓*	✓	✓		✓											
KN-62							✓✓✓ (h)											

((Table 1. Contd….)

	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$	$P2X_{2/3}$	$P2X_{1/5}$	$P2X_{4/6}$	$P2Y_1$	$P2Y_2$	$P2Y_4$	$P2Y_6$	$P2Y_{11}$	$P2Y_{12}$	$P2Y_{13}$	$P2Y_{14}$
AR-C67085 MX															✓✓* [ag]	✓✓✓*		
2-MeSAMP																✓✓		
Brilliant Blue G	✓	✓	-				✓✓✓ (r)											
Ip_5I	✓✓✓*	-	✓	-														
MRS 2257	✓✓✓*		✓✓*															
NF279	✓✓✓*	✓	✓	-			✓											

Number of ticks (✓) indicates relative potency with respect to agonist/antagonist concentration. Agonists: ✓✓✓<1μM, ✓✓ 1-10μM, ✓>10μM, - virtually inactive. Antagonists: ✓✓✓<10nM, ✓✓10nM-300nM, ✓>300nM, - virtually inactive. *, selective agonist or antagonist. *h human, r rat. ATP, Adenine-5'-triphosphate; ADP, adenosine-5'-diphosphate; 2-MeSATP, 2-methylthioadenosine 5'-triphosphate; PAPET, 2-[2-(4-aminophenyl)ethyl-thio]adenosine 5'-triphosphate; 2-MeSADP, 2-methylthio ADP; HT-AMP, 2-(hexylthio) adenosine 5'-monophosphate;* α, β-*meATP,* α, β *methylyne ATP;* β, γ-*meATP,* β, γ *methylene ATP; BzATP, benzoyl benzoyl ATP; 2-dATP, deoxyATP; AP_4A, P^1, P^4-Di-(adenosine-5')tetraphosphate ammonium; UTP, uridine triphosphate, UDP, uridine diphosphate; CTP, cytidine triphosphate; PPADS, pyridoxal-phosphate-6-azophenyl-2', 4'-disulphonic acid; iso-PPADS, pyridoxal-phosphate-6-azophenyl-2', 5'-disulphonic acid; PPNDS, pyridoxal-5'-phosphate-6-(2'-napthylazo-6'-nitro-4', 8' disulfonate; NF023, 8, 8'-[carbonylbis (imino-3, 1-phenylenecarbonyl-imino)] bis-(1, 3, 5-naphthalene trisulphonate; MRS 2179, N^6-methyl-2'deoxyadenosine 3', 5'-bisphosphate; MRS 2269, N^6-methyl-1, 5-anhydro-2-(adenin-9-yl)-2, 3-dideoxy-D-arabino-hexitol-4, 6-bis (diammonium phosphate); TNP-ATP, trinitrophenol-ATP, KN-62, 1-[N, O-bis (5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine; AR-C67085 MX, 2-propylthio-D-*β-γ-*dichloromethylene ATP; IP_5I; diinosine pentaphosphate, MRS 2257, pyridoxal-5'-phosphonate-6-azophenyl-3', 5'-bismethyl phosphonate; NF279, (8, 8'-(carbonylbis(imino-4, 1-phenylene carbonylimino-4, 1-phenylene carbonyl-imino))bis (1, 3, 5-naphthalenetrisulphonic acid).* Table reproduced with permission from [18].

The proposal in 1976 that more than one transmitter can be released from nerve terminals (the cotransmitter hypothesis [3]) is now widely accepted, and the focus has been on defining the "chemical coding" of various nerve types, i.e. to describe the combination of neurotransmitters in these nerves and their projections to various sites. ATP appears to be a cotransmitter in many nerve types, perhaps reflecting the primitive nature of purinergic signalling [19, 84]. ATP is a cotransmitter with noradrenaline and neuropeptide Y in sympathetic nerves, with acetylcholine and vasoactive intestinal peptide in some parasympathetic nerves, with nitric oxide and vasoactive intestinal peptide in enteric NANC inhibitory nerves, and with calcitonin gene-related peptide and substance P in sensory-motor nerves. There is also evidence for ATP with γ-aminobutyric acid in retinal nerves and for ATP with glutamate or with dopamine in nerves in the brain. ATP provokes a fast, short-lasting twitch response via P2X receptors, while the slower component is mediated by G protein-coupled adrenoceptors and muscarinic receptors, respectively, in sympathetic nerves supplying visceral and vascular smooth muscle and in parasympathetic nerves supplying the urinary bladder [85,86]. In the gut, ATP released from NANC inhibitory nerves produces the fastest response, nitric oxide gives a less rapid response, and vasoactive intestinal peptide produces slow tonic relaxations. In all cases of cotransmission, there are considerable differences in the proportion of the cotransmitters in nerves supplying different regions of the gut or vasculature, between species and in different pathophysiological conditions [87].

The first clear evidence for nerve-nerve purinergic synaptic transmission was published in 1992. Excitatory postsynaptic potentials in the coeliac ganglion were reversibly antagonized by suramin [88, 89] and similar experiments were carried out in the medial habenula in the brain, showing reversible block of excitatory postsynaptic potentials by suramin [90]. Since then, other examples of purinergic synaptic transmission have been identified [91, 92] and there have been many articles describing the distribution of various P2 receptor subtypes in the brain and spinal cord and electrophysiological studies of the effects of purines in brain slices, isolated nerves, and glial cells [93, 94]. Synaptic transmission also has been found in the myenteric plexus [87] and in various sensory and sympathetic and pelvic ganglia [95].

In addition to the examples of short-term signalling, there are now many examples of purinergic signalling concerned with long-term (trophic) events, such as development and regeneration, proliferation, and cell death [20,96,97]. For example, αβmeATP produces proliferation of glial cells, whereas adenosine inhibits proliferation. $P2X_5$ and $P2X_6$ receptors have been implicated in the development of chick skeletal muscle [80]. In recent studies of purinoceptor expression in the mouse myotubes, we have shown progressive expression of $P2X_5$ (from E14 to E18), $P2X_6$ (from E16 to E18) and $P2X_2$ (from E18 to postnatal day 7) [81].

P2Y Receptors

P2Y purinoceptor-mediated responses occur in non-neuronal and non-muscular cell types, as well as in the nervous system. Examples include endothelial cells, which express $P2Y_1$, $P2Y_2$ and probably $P2Y_4$ receptors that, when occupied, release nitric oxide leading to vasodilatation. $P2Y_1$ receptors in pancreatic β-cells are involved in insulin secretion [98]. $P2Y_2$ receptors have been identified in hepatocytes [99]; $P2Y_{12}$, $P2X_1$, and $P2Y_1$ receptors in platelets [100], $P2Y_2$ receptors on myelinating Schwann cells and $P2Y_1$ receptors on non-myelinating Schwann cells [101]. P2Y receptors also are involved in signalling to endocrine cells, leading to hormone secretion [77,102-106]. There is a widespread presence of P2Y receptor subtypes in different regions of kidney glomeruli, tubules and collecting ducts

[107-113]. P2Y receptors are strongly represented in the brain, both at presynaptic sites and on glial cells [114-119].

There are also examples of P2Y purinergic signalling concerned with long-term events such as development and regeneration, and proliferation, particularly in glial, vascular smooth muscle and endothelial cells. A $P2Y_8$ receptor was cloned from the frog embryo, which appears to be involved in the development of the neural plate [39]. $P2Y_1$ receptors seem to have a role in cartilage development in limb buds and in development of the mesonephros [120].

POTENTIAL THERAPEUTIC POTENTIAL OF PURINOCEPTORS

There is increasing interest in the therapeutic potential of purinergic compounds in a wide range of disease conditions both in relation to P1 receptors [121-124] and P2 receptors [20,125,127]. A number of purine-related compounds have been patented [128].

The autonomic nervous system shows marked plasticity, i.e. the expression of cotransmitters and receptors show dramatic changes during development and ageing in nerves that remain after trauma,or surgery and in disease conditions. There are a number of examples where the purinergic component of cotransmission is increased in pathological conditions [20].

Cardiovascular System

Adenosine and ATP are very much involved in the mechanisms underlying local control of vessel tone [129] as well as cell migration, proliferation and death during angiogenesis, atherosclerosis and restenosis following angioplasty [130]. ATP, released as a cotransmitter from sympathetic nerves, constricts vascular smooth muscle via P2X receptors, while ATP released from sensory-motor nerves during 'axon reflex' activity dilates vessels via P2Y receptors. Further, ATP released from endothelial cells during changes in flow (shear stress) or hypoxia acts on P2Y receptors in endothelial cells to release nitric oxide (NO) resulting in relaxation. Adenosine, following breakdown of extracellular ATP, produces vasodilatation via P1 receptors. P2X receptors are also present on endothelial cells and appear to be associated with cell adhesion and permeability.

There have been very promising recent developments concerning purinergic antithrombotic drugs. Platelets are known to express both P2Y and P2X purinoceptors [41,131]. Recent 'mega' clinical trials (CAPRIE [132] and CURE [133]) provided clear evidence that clopidogrel and ticlopidine, which are antagonists to the $P2Y_{12}$ receptor, reduce the risks of recurrent strokes and heart attacks, especially when combined with aspirin. Patents have been lodged for the application of P1 receptor subtype agonists and antagonists for myocardial ischaemia and reperfusion injury, cerebral ischaemia and stroke [122].

ATP plays a significantly greater cotransmitter role in sympathetic nerves supplying hypertensive blood vessels [134]. Upregulation of $P2X_1$ and $P2Y_2$ receptor mRNA in hearts of rats with congestive heart failure has been reported [135].

Neurology

Agonists and antagonists of ATP are being explored as therapeutic agents for a number of neurological conditions. For example, microinjection of ATP analogs into the prepiriform cortex induces generalized motor seizures [136]. $P2X_2$, $P2X_4$ and $P2X_6$ receptors are expressed in the prepiriform cortex, suggesting that a P2X receptor antagonist may have potential as a neuroleptic agent**.**

ATP, given systemically, elicits pain responses, and endogenous ATP may contribute to the pain associated with causalgia, reflex sympathetic dystrophy, angina, migraine, lumbar, pelvic and cancer pain [137]. $P2X_3$ receptors are selectively localized on sensory neurones in trigeminal, nodose, and dorsal root ganglia (DRG) and the terminals of these nociceptive neurones in skin and visceral organs represent unique targets for novel analgesic agents that function as $P2X_3$ receptor antagonists. Nonspecific P2 receptor antagonists, e.g. suramin, are antinociceptive and $P2X_3$ receptor knockout mice have reduced nociceptive inflammatory responses [138].

Trophic factors ensure neuronal viability and regeneration. Neuronal injury releases fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor [97]. In combination with these growth factors, ATP can act to stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis, a hypertrophic/ hyperplastic response that is associated with brain trauma, stroke/ischemia, seizures and neurodegenerative disorders.

Respiratory System

The use of theophylline, an adenosine receptor antagonist, as an antiasthmatic agent has focussed attention on the development of novel P1 receptor antagonists as asthmatic medication [139]. ATP may also have a direct role in asthma via its actions on bronchial innervation. Nucleotides trigger a reflex bronchoconstriction via activation of a P2X receptor on vagal C fibres, and both ATP and UTP can potentiate IgE-mediated mast cell histamine release, effects involving P2Y receptors [140].

In Type II alveolar epithelial cells, ATP and UTP stimulate P2Y receptor-mediated surfactant secretion and transepithelial chloride secretion; there are abnormalities in this mechanism in cystic fibrosis [141]. Nucleotides also increase mucous secretion from goblet cells and increase the ciliary beat frequency of airway epithelial cells. Purinergic compounds are being explored for the treatment of cystic fibrosis and to improve clearance of secretions from bronchi in chronic obstructive pulmonary disease (COPD), and for sputum expectoration in smokers [141,142]. Pulmonary hypertension can be a problem in patients with COPD; it is a life threatening condition and intravenous ATP infusion in these patients produces a significant decrease in mean pulmonary arterial pressure and pulmonary vascular resistance, without any changes in mean systemic arterial pressure [143].

Urinogenital System

In the normal human bladder, atropine will block at least 95% of parasympathetic nerve-mediated contraction,

indicating that its innervation is predominantly cholinergic; purinergic signalling is responsible for the atropine-resistant component of contraction [86]. There are now a number of examples where the purinergic component of cotransmission is increased in pathological conditions [20,86]. One example is that purinergic nerve-mediated contraction of the human bladder is increased to 40% in pathophysiological conditions such as interstitial cystitis, outflow obstruction, idiopathic instability and possibly also neurogenic bladder.

Purinergic signalling also appears to play a role in afferent sensation from the bladder. ATP is released from urothelial cells when the bladder is distended. Sensory nerve recording has indicated that $P2X_3$ receptors are involved in mediating the nerve-responses to bladder distension, providing mechanosensory feedback involving both the micturition reflex and pain [76,144]. This, too, might be a potential target for pharmacological manipulation in the treatment of detrusor instability.

Penile erectile function is dependent upon a delicate balance between contracting and relaxing factors in the corpus cavernosum smooth muscle, which are modulated by signalling from both nerves and endothelial cells. Evidence has accumulated to support a pivotal role for non-adrenergic, non-cholinergic neurotransmitters. NO plays a central role in mediating cavernosal smooth muscle relaxation, but other neurotransmitters can modulate this action and may play a role in erectile dysfunction. ATP potently relaxes cavernosal smooth muscle strips *in vitro*, an action pharmacologically consistent with P2Y receptors. Indeed, P2Y receptors are present on both cavernosal smooth muscle cells and endothelial cells, and ATP is released from a subpopulation of the cavernosal nerves. It appears that smooth muscle relaxation is caused both by ATP acting directly on the cavernosal smooth muscle cells and indirectly, mediated by NO released from the endothelial cells. ATP-mediated cavernosal relaxation is impaired in diabetes mellitus (independent of NO) implying that purinergic signalling may be involved in the pathophysiology of erectile dysfunction [145].

The potential role of $P2X_3$ receptors in mechanosensory transduction has already been mentioned in relation to the bladder. However, there is increasing evidence that this is not an isolated phenomenon and that ATP released from the epithelial lining of other organs such as the ureter, gut or bile duct following distension may act on $P2X_3$ receptors on afferent nerves in the subepithelial plexuses to provide sensory feedback and, in the case of the ureter, renal colic pain. $P2X_3$ receptors have been found on the suburothelial nerve plexus and both the human and guinea-pig ureter have been shown to release ATP in a pressure-dependent fashion when distended [146]. This ATP release is abolished when the urothelium is removed, and sensory nerve-recording studies during ureteral distension demonstrate purinergic involvement, suggesting that specific $P2X_3$ antagonists may have efficacy in alleviating renal colic [144].

There is a substantial presence of purinoceptors in the kidney, including subtypes involved in the regulation of renin secretion, glomerular filtration and transport of water, ions, nutrients and toxins. ATP and adenosine have been used to protect kidneys from renal ischaemic/reperfusion injury and are being explored for the treatment of chronic renal failure and transplantation-induced erythrocytosis [147].

Gastroenterology

Purinergic signalling plays a major role in different activities of the gut [87]. ATP is a cotransmitter in non-adrenergic, non-cholinergic nerves that are responsible for the inhibitory phase in peristalsis; it participates in synaptic transmission in the myenteric and submucosal ganglia; it is involved in vascular control of the gastrointestinal tract and in control of mucosal secretion.

There are a limited number of studies to date on changes in purinergic signalling in diseased gut, but for example, ATP and adenosine have been implicated in the development of gastric ulcers, Hirschsprung's and Chagas' diseases, ischaemia and colonic tumours [87]. Intrinsic, as well as extrinsic, sensory neurones in both myenteric and submucous plexuses of the gut, show positive immunoreactivity for $P2X_3$. It is proposed that during moderate distension, low threshold intrinsic enteric sensory fibres are activated via $P2X_3$ receptors by ATP released from mucosal epithelial cells, leading to reflexes concerned with propulsion of material down the gut. In contrast, it is proposed that with substantial (colic) distension, which is often associated with pain, higher threshold extrinsic sensory fibres are activated by ATP released from the mucosal epithelia, which pass messages through the dorsal root ganglia to pain centres in the central nervous system [144]. $P2X_3$ receptor expression is increased in human inflammatory bowel disease, suggesting a potential new therapeutic target for dysmotility and pain [148].

Musculo-skeletal System

Purinergic signalling is implicated in bone development and remodelling. P2X and P2Y receptors are present on osteoclasts with P2Y receptors only being present on osteoblasts. ATP, but not adenosine, stimulates the formation of osteoclasts and their resorptive actions *in vitro* and can inhibit osteoblast-dependent bone formation. The bisphosphonate, clodronate, which is used in the treatment of Paget's disease and tumour-induced osteolysis, may act via osteoclast P2 receptors. A recent study has shown that very low (nM) concentrations of ADP acting through $P2Y_1$ receptors turn on osteoclast activity [149]. Modulation of P2 receptor function may have potential in the treatment of osteoporosis [150].

Immune System and Inflammation

ATP and adenosine are released at sites of inflammation. ATP is involved in the development of inflammation by a combination of actions: release of histamine from mast cells, provoking production of prostaglandins and production and release of cytokines from immune cells [151]. In addition to the roles of purines in inflammation, they have a broad range of functions via purinergic receptors on immune cells, including killing of intracellular pathogens by inducing apoptosis of host macrophages, chemoattraction and cell adhesion [152]. Purinergic compounds may turn out to be

useful for the treatment of neurogenic inflammation, rheumatoid arthritis, and periodontitis [22].

Oncology

The anticancer activity of adenine nucleotides was first described by Rapaport in 1983. Intraperitoneal injection of ATP into tumour-bearing mice resulted in significant anticancer activity against several fast-growing aggressive carcinomas [125]. ATP inhibits the growth of murine colonic adenocarcinoma and human pancreatic carcinoma in mice and inhibited the weight loss in these mice. Growth of prostate cancer cells *in vitro* is inhibited by up to 90% by ATP via P2-receptors, although it is not yet clear which subtype mediates this effect and whether it is a directly antiproliferative effect or a pro-apoptotic effect [153]. Phase I clinical trials showed that infusion of ATP into patients with advanced cancer was feasible, but was limited by chest tightness and dyspnoea, probably due to conversion to adenosine [125]. A phase II trial has been carried out in patients with non-small cell lung cancer and intravenous ATP for 96 hours at 4 week intervals reduced weight loss [125]. A combination of interferon-γ and ATP is being explored for the treatment of acute myeloid leukaemia. Further clarification of the mechanisms mediating the dramatic anti-neoplastic effects of ATP for breast, ovarian and colorectal carcinoma [154] may yield new purinergic agents which are more specific, better tolerated and more appropriate for human trials.

Diabetes

P2Y receptors are present on pancreatic β-cells and are involved in insulin secretion. ATP stimulates pancreatic insulin release via a glucose-dependent, P2Y receptor-mediated mechanism [155] and also modulates insulin secretion by interactions with ATP-sensitive potassium channels in islet β-cells. The potential role of purinergic compounds as novel treatment for diabetes, especially Type II, has yet to be fully explored.

Special Senses

In the ear, ATP, acting via P2Y receptors, depresses sound-evoked gross compound action potentials in the auditory nerve and the distortion product otoacoustic emission, the latter being a measure of the active process of the outer hair cells [156]. P2X splice variants are found on the endolymphatic surface of the cochlear endothelium, an area associated with sound transduction. Both P2X and P2Y receptors have been identified in the vestibular system. It has been suggested that ATP may regulate fluid homeostasis, cochlear blood flow, hearing sensitivity and development, and thus may be useful in the treatment of Ménières disease, tinnitus, and sensorineural deafness.

In the eye, ATP, acting via both P2X and P2Y receptors, modulates retinal neurotransmission affecting retinal blood flow and intraocular pressure. The ATP analogue β,γ-methyleneATP has greater efficacy in reducing intraocular pressure (40%) than muscarinic agonists like pilocarpine (25%) or β-adrenoceptor blockers (30%), raising the potential for purinergic agents in glaucoma. $P2Y_2$ receptor activation increases salt, water, and mucus secretion and thus represents a potential treatment for dry eye conditions [141]. In the retinal pigmented layer, $P2Y_2$ receptor activation promotes fluid absorption and may be involved in retinal detachment.

FUTURE DIRECTIONS

Several clinically relevant pharmacological interventions are already being explored. However, one of the main reasons that most purinergic therapies are still to be established is the current sparcity of P2 receptor subtype-specific agonists and antagonists which are effective *in vivo*. In addition to the development of selective agonists and antagonists, for the different P2 receptor subtypes, therapeutic strategies are likely to include agents that control the expression of P2 receptors, inhibitors of extracellular breakdown of ATP and enhancers or inhibitors of ATP transport. The interactions of purinergic signalling with other established signalling systems will also be an important way forward. The development of new investigative tools and recent advances in the understanding of cell biology of purinergic signalling, as well as new compounds, hopefully will lead to significant progress in this field.

REFERENCES

[1] Drury, A.N.; Szent-Györgyi, A. The physiological activity of adenine compounds with special reference to their action upon the mammalian heart. *J. Physiol.* (*Lond*) **1929**, *68***,** 213-237.

[2] Burnstock, G. Purinergic nerves. *Pharmacol. Rev*. **1972**, *24***,** 509-581.

[3] Burnstock, G. Do some nerve cells release more than one transmitter? *Neuroscience* **1976**, *1***,** 239-248.

[4] Burnstock, G. A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach* (Straub, R.W. & Bolis, L., eds), Raven Press, New York **1978**, *pp. 107-118.* .

[5] Van Calker, D.; Müller, M.; Hamprecht, B. Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem*. **1979**, *33***,** 999-1005.

[6] Londos, C.; Cooper, D.M.; Wolff, J. Subclasses of external adenosine receptors. Proc. *Natl. Acad. Sci*. USA **1980**, *77***,** 2551-2554.

[7] Burnstock, G.; Kennedy, C. Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol*. **1985**, *16***,** 433-440.

[8] Gordon, J.L. Extracellular ATP: effects, sources and fate. *Biochem. J*. **1986**, *233***,** 309-319.

[9] O'Connor, S.E.; Dainty, I.A.; Leff, P. Further subclassification of ATP receptors based on agonist studies. *Trends Pharmacol. Sci*. **1991**, *12***,** 137-141.

[10] Abbracchio, M.P.; Burnstock, G. Purinoceptors: are there families of P_{2X} and P_{2Y} purinoceptors? *Pharmacol. Ther*. **1994**, *64***,** 445-475.

[11] Dubyak, G.R. Signal transduction by P_2-purinergic receptors for extracellular ATP. Am. J. Respir. *Cell Mol. Biol*. **1991**, *4***,** 295-300.

[12] 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.* USA **1993**, *90***,** 5113-5117.

[13] Webb, T.E.; Simon, J.; Krishek, B.J.; Bateson, A.N.; Smart, T.G.; 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.

[14] 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.

[15] Valera, S.; Hussy, N.; Evans, R.J.; Adani, N.; North, R.A.; Surprenant, A.; Buell, G. A new class of ligand-gated ion channel defined by P_{2X} receptor for extra-cellular ATP. *Nature* **1994**, *371***,** 516-519.

[16] Ralevic, V.; Burnstock, G. Receptors for purines and pyrimidines. *Pharmacol. Rev*. **1998**, *50***,** 413-492.

[17] Khakh, B.S.; Burnstock, G.; Kennedy, C.; King, B.F.; North, R.A.; Séguéla, P.; Voigt, 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**, *53***,** 107-118.

[18] Burnstock, G. Introduction: ATP and its metabolites as potent extracellular agonists.In: *Current Topics in Membranes, Purinergic Receptors and Signalling* Ed E. M. Schwiebert. Academic Press, San Diego. **2003,** *Vol 54,* pp 1-27.

[19] Burnstock, G. Purinoceptors: Ontogeny and Phylogeny. *Drug Dev. Res.* **1996**, *39***,** 204-242.

[20] Abbracchio, M.P.; Burnstock, G. Purinergic signalling: pathophysiological roles. Jpn. *J. Pharmacol.* **1998**, *78***,** 113-145.

[21] Olsson, R.A.; Pearson, J.D. Cardiovascular purinoceptors. *Physiol. Rev*. **1990**, *70***,** 761-845.

[22] Dubyak, G.R.; El Moatassim, C. Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am. *J. Physiol*. **1993**, *265***,** C577-C606.

[23] Burnstock, G. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology* **1997**, *36***,** 1127-1139.

[24] Lewis, C.; Neidhart, S.; Holy, C.; North, R.A.; Buell, G.; Surprenant, A. Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature* **1995**, *377***,** 432-435.

[25] Radford, K.M.; Virginio, C.; Surprenant, A.; North, R.A.; Kawashima, E. Baculovirus expression provides direct evidence for heteromeric assembly of $P2X_2$ and $P2X_3$ receptors. *J. Neurosci*. **1997**, *17***,** 6529-6533.

[26] Le, K.T.; Babinski, K.; Seguela, P. Central $P2X_4$ and $P2X_6$ channel subunits coassemble into a novel heteromeric ATP receptor. *J. Neurosci*. **1998**, *18***,** 7152-7159.

[27] Torres, G.E.; Haines, W.R.; Egan, T.M.; Voigt, M.M. Co-expression of $P2X_1$ and $P2X_5$ receptor subunits reveals a novel ATP-gated ion channel. *Mol. Pharmacol*. **1998**, *54***,** 989-993.

[28] 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.

[29] King, B.F.; Townsend-Nicholson, A.; Wildman, S.S.; Thomas, T.; Spyer, K.M.; Burnstock, G. Coexpression of rat $P2X_2$ and $P2X_6$ subunits in *Xenopus* oocytes. *J. Neurosci*. **2000**, *20***,** 4871-4877.

[30] Torres, G.E.; Egan, T.M.; Voigt, M.M. Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners. *J. Biol. Chem*. **1999**, *274***,** 6653-6659.

[31] North, R.A.; Surprenant, A. Pharmacology of cloned P2X receptors. Annu. Rev. *Pharmacol. Toxicol*. **2000**, *40***,** 563-580.

[32] 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. *Brain Res. Mol. Brain Res*. **1999**, *64***,** 246-254.

[33] Zimmermann, H. Extracellular purine metabolism. *Drug Dev. Res*. **1996**, *39***,** 337-352.

[34] 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.

[35] Boyer, J.L.; Mohanram, A.; Camaioni, E.; Jacobson, K.A.; Harden, T.K. Competitive and selective antagonism of $P2Y_1$ receptors by N^6-methyl 2'-deoxyadenosine 3', 5'-bisphosphate. Br. *J. Pharmacol*. **1998**, *124***,** 1-3.

[36] Brown, S.G.; King, B.F.; Kim, Y.-C.; Jang, S.Y.; 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.

[37] Bogdanov, Y.D.; Wildman, S.S.; Clements, M.P.; King, B.F.; Burnstock, G. Molecular cloning and characterization of rat $P2Y_4$ nucleotide receptor. Special Report. Br. *J. Pharmacol*. **1998**, *124***,** 428-430.

[38] King, B.F.; Townsend-Nicholson, A.; Burnstock, G. Metabotropic receptors for ATP and UTP: exploring the correspondence between native and recombinant nucleotide receptors. *Trends Pharmacol. Sci*. **1998**, *19***,** 506-514.

[39] Bogdanov, Y.D.; Dale, L.; King, B.F.; Whittock, N.; Burnstock, G. Early expression of a novel nucleotide receptor in the neural plate of *Xenopus* embryos. *J. Biol. Chem*. **1997**, *272***,** 12583-12590.

[40] 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.

[41] 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.

[42] 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 G(i). *J. Biol. Chem*. **2001**, *276***,** 41479-41485.

[43] 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.

[44] Freeman, K.; Tsui, P.; Moore, D.; Emson, P.C.; Vawter, L.; Naheed, S.; Lane, P.; Bawagan, H.; Herrity, N.; Murphy, K.; Sarau, H.M.; Ames, R.S.; Wilson, S.; Livi, G.P.; Chambers, J.K. Cloning, Pharmacology, and Tissue Distribution of G-Protein-Coupled Receptor GPR105 (KIAA0001) Rodent Orthologs. *Genomics* **2001**, *78***,** 124-128.

[45] Collo, G.; North, R.A.; Kawashima, E.; Merlo-Pich, E.; Neidhart, S.; Surprenant, A.; Buell, G. 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.

[46] 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 imunocytochemistry. *Proc. Natl. Acad. Sci.* USA **1996**, *93***,** 8063-8067.

[47] Bradbury, E.J.; Burnstock, G.; McMahon, S.B. The expression of $P2X_3$ purinoceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. *Mol. Cell. Neurosci*. **1998**, *12***,** 256-268.

[48] Chan, C.M.; Unwin, R.J.; Bardini, M.; Oglesby, I.B.; Ford, A.P.D.W.; Townsend-Nicholson, A.; Burnstock, G. Localization of the $P2X_1$ purinoceptors by autoradiography and immunohistochemistry in rat kidneys. *Am. J. Physiol*. **1998**, *274***,** F799-F804.

[49] Xiang, Z.; Bo, X.; Burnstock, G. Localization of ATP-gated P2X receptor immunoreactivity in rat sensory and sympathetic ganglia. *Neurosci. Lett*. **1998**, *256***,** 105-108.

[50] Xiang, Z.; Bo, X.; Oglesby, I.B.; Ford, A.P.D.W.; Burnstock, G. Localization of ATP-gated $P2X_2$ receptor immunoreactivity in the rat hypothalamus. *Brain Res*. **1998**, *813***,** 390-397.

[51] Oglesby, I.B.; Lachnit, W.G.; Burnstock, G.; Ford, A.P.D.W. Subunit specificity of polyclonal antisera to the carboxy terminal regions of P2X receptors, $P2X_1$ through $P2X_7$. *Drug Dev. Res*. **1999**, *47***,** 189-195.

[52] Bo, X.; Alavi, A.; Xiang, Z.; Oglesby, I.; Ford, A.; Burnstock, G. Localization of ATP-gated $P2X_2$ and $P2X_3$ receptor immunoreactive nerves in rat taste buds. *Neuroreport* **1999**, *10***,** 1107-1111.

[53] Gröschel-Stewart, U.; Bardini, M.; Robson, T.; Burnstock, G. Localisation of $P2X_5$ and $P2X_7$ receptors by immunohistochemistry in rat stratified squamous epithelia. *Cell Tissue Res*. **1999**, *296***,** 599-605.

[54] Gröschel-Stewart, U.; Bardini, M.; Robson, T.; Burnstock, G. P2X receptors in the rat duodenal villus. *Cell Tissue Res*. **1999**, *297***,** 111-117.

[55] Bardini, M.; Lee, H.Y.; Burnstock, G. Distribution of P2X receptor subtypes in the rat female reproductive tract at late pro-oestrus/early oestrus. *Cell Tissue Res*. **2000**, *299***,** 105-113.

[56] Brouns, I.; Adriaensen, D.D.; Burnstock, G.; Timmermans, J.-P. Intraepithelial vagal sensory nerve terminals in rat pulmonary neuroepithelial bodies express $P2X_3$ receptors. Am. J. Respir. *Cell Mol. Biol*. **2000**, *23***,** 52-61.

[57] Lee, H.-Y.; Bardini, M.; Burnstock, G. Distribution of P2X receptors in the urinary bladder and ureter of the rat. *J. Urol*. **2000**, *163***,** 2002-2007.

[58] 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.

[59] Llewellyn-Smith, I.J.; Burnstock, G. Ultrastructural localization of $P2X_3$ receptors in rat sensory neurons. *Neuroreport* **1998**, *9***,** 2245-2250.

[60] Loesch, A.; Burnstock, G. Electron-immunocytochemical localization of the $P2X_1$ receptors in the rat cerebellum. *Cell Tissue Res*. **1998**, *294***,** 253-260.

[61] Loesch, A.; Burnstock, G. Ultrastructural localisation of ATP-gated $P2X_2$ receptor immunoreactivity in vascular endothelial cells in rat brain. *Endothelium* **2000**, *7***,** 93-98.

[62] Loesch, A.; Burnstock, G. Immunoreactivity to $P2X_6$ receptors in the rat hypothalamo-neurohypophysial system: an ultrastructural study with ExtrAvidin and colloidal gold-silver immunolabelling. *Neuroscience* **2001**, *106***,** 621-631.

[63] Loesch, A.; Miah, S.; Burnstock, G. Ultrastructural localisation of ATP-gated $P2X_2$ receptor immunoreactivity in the rat hypothalamo-neurohypophysial system. J. Neurocytol. **1999**, *28***,** 495-504.

[64] Nori, S.; Fumagalli, L.; Bo, X.; Bogdanov, Y.; Burnstock, G. Coexpression of mRNAs for $P2X_1$, $P2X_2$ and $P2X_4$ receptors in rat vascular smooth muscle: an *in situ* hybridization and RT-PCR study. *J. Vasc. Res*. **1998**, *35***,** 179-185.

[65] Hansen, M.A.; Dutton, J.L.; Balcar, V.J.; Barden, J.A.; Bennett, M.R. P_{2X} (purinergic) receptor distributions in rat blood vessels. *J. Auton. Nerv. Syst*. **1999**, *75***,** 147-155.

[66] Lewis, C.J.; Evans, R.J. Comparison of P2X receptors in rat mesenteric, basilar and septal (coronary) arteries. *J. Auton. Nerv. Syst*. **2000**, *81***,** 69-74.

[67] Lewis, C.J.; Surprenant, A.; Evans, R.J. 2', 3'-O-(2, 4, 6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP)-a nanomolar affinity antagonist at rat mesenteric artery P2X receptor ion channels. *Br. J. Pharmacol*. **1998**, *124***,** 1463-1466.

[68] King, B.F.; Liu, M.; Pintor, J.; Gualix, J.; Miras-Portugal, M.T.; Burnstock, G. Diinosine pentaphosphate (IP_5I) is a potent antagonist at recombinant rat $P2X_1$ receptors. *Br. J. Pharmacol*. **1999**, *128***,** 981-988.

[69] 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 P2X1 receptors. Naunyn Schmiedebergs Arch. *Pharmacol*. **2001**, *364***,** 285-290.

[70] Brown, S.G.; Kim, Y.-C.; Kim, S.-A.; Jacobson, K.A.; Burnstock, G.; King, B.F. Actions of a series of PPADS analogs at $P2X_1$ and $P2X_3$ receptors. *Drug Dev. Res*. **2001**, *53***,** 281-291.

[71] King, B.F.; Ziganshina, L.E.; Pintor, J.; Burnstock, G. Full sensitivity of $P2X_2$ purinoceptor to ATP revealed by changing extracellular pH. Br. J. *Pharmacol*. **1996**, *117***,** 1371-1373.

[72] Wildman, S.S.; King, B.F.; Burnstock, G. Modulation of ATP-responses at recombinant $rP2X_4$ receptors by extracellular pH and zinc. *Br. J. Pharmacol*. **1999**, *126***,** 762-768.

[73] Burnstock, G. P2X receptors in sensory neurones. *Br. J. Anaesth*. **2000**, *84***,** 476-488.

[74] Rong, W.; Burnstock, G.; Spyer, K.M. P2X purinoceptor-mediated excitation of trigeminal lingual nerve terminals in an *in vitro* intra-arterially perfused rat tongue preparation. *J. Physiol*. **2000**, *524***,** 891-902.

[75] Alavi, A.M.; Dubyak, G.R.; Burnstock, G. Immunohistochemical evidence for ATP receptors in human dental pulp. *J. Dental Res*. **2001**, *80***,** 476-483.

[76] Vlaskovska, M.; Kasakov, L.; Rong, W.; Bodin, P.; Bardini, M.; Cockayne, D.A.; Ford, A.P.D.W.; Burnstock, G. $P2X_3$ knockout mice reveal a major sensory role for urothelially released ATP. *J. Neurosci*. **2001**, *21***,** 5670-5677.

[77] Glass, R.; Townsend-Nicholson, A.; Burnstock, G. P2 receptors in the thymus: expression of P2X and P2Y receptors in adult rats, an immunohistochemical and i*n situ* hybridisation study. *Cell Tissue Res*. **2000**, *300***,** 295-306.

[78] Glass, R.; Burnstock, G. Immunohistochemical identification of cells expressing ATP-gated cation channels (P2X receptors) in the adult rat thyroid. *J. Anat.* **2001**, *198***,** 569-579.

[79] Burnstock, G. Expanding field of purinergic signaling. *Drug Dev. Res*. **2001**, *52***,** 1-10.

[80] Meyer, M.P.; Gröschel-Stewart, U.; Robson, T.; Burnstock, G. Expression of two ATP-gated ion channels, $P2X_5$ and $P2X_6$, in developing chick skeletal muscle. *Dev. Dyn*. **1999**, *216***,** 442-449.

[81] Ryten, M.; Hoebertz, A.; Burnstock, G. Sequential expression of three receptor subtypes for extracellular ATP in developing rat skeletal muscle. *Dev. Dyn*. **2001**, *221***,** 331-341.

[82] Surprenant, A.; Rassendren, F.; Kawashima, E.; North, R.A.; Buell, G. The cytolytic P_{2Z} receptor for extracellular ATP identified as a P_{2X} receptor ($P2X_7$). *Science* **1996**, *272***,** 735-738.

[83] 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. $P2X_1$ receptor membrane redistribution and down-regulation visualized by using receptor-coupled green fluorescent protein chimeras. *Neuropharmacology* **2000**, *39***,** 2054-2066.

[84] Burnstock, G. Purinergic cotransmission. *Brain Res. Bull*. **1999**, *50***,** 355-357.

[85] Burnstock, G. & Ralevic, V. Cotransmission. In *The Pharmacology of Vascular Smooth Muscle* Oxford University Press, Oxford. (Garland, C.J. & Angus, J.A., eds), **1996,** pp. 210-232.

[86] Burnstock, G. Purinergic signalling in lower urinary tract. In Handbook of Experimental Pharmacology, Volume 151/I. Purinergic and Pyrimidinergic Signalling I - Molecular, Nervous and Urinogenitary System Function (Abbracchio, M.P. & Williams, M., eds), Springer-Verlag, Berlin. pp. **2001**, 423-515.

[87] Burnstock, G. Purinergic signalling in gut. In Handbook of Experimental Pharmacology, Volume 151/II. Purinergic and Pyrimidinergic Signalling II - Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function (Abbracchio, M.P. & Williams, M., eds), Springer-Verlag, Berlin. **2001**, pp. 141-238.

[88] Evans, R.J.; Derkach, V.; Surprenant, A. ATP mediates fast synaptic transmission in mammalian neurons. *Nature* **1992**, *357***,** 503-505.

[89] Silinsky, E.M.; Gerzanich, V.; Vanner, S.M. ATP mediates excitatory synaptic transmission in mammalian neurones. *Br. J. Pharmacol*. **1992**, *106***,** 762-763.

[90] Edwards, F.A.; Gibb, A.J.; Colquhoun, D. ATP receptor-mediated synaptic currents in the central nervous system. *Nature* **1992**, *359***,** 144-147.

[91] Nieber, K.; Poelchen, W.; Illes, P. Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurones of the rat. *Br. J. Pharmacol*. **1997**, *122***,** 423-430.

[92] Pankratov, Y.; Lalo, U.; Castro, E.; Miras-Portugal, M.T.; Krishtal, O. ATP receptor-mediated component of the excitatory synaptic transmission in the hippocampus. Prog. *Brain Res*. **1999**, *120***,** 237-249.

[93] Nörenberg, W.; Illes, P. Neuronal P2X receptors: localisation and functional properties. Naunyn Schmiedebergs Arch. *Pharmacol*. **2000**, *362***,** 324-339.

[94] Masino, S.A. & Dunwiddie, T.V. Role of purines and pyrimidines in the central nervous system. In Handbook of Experimental Pharmacology. Purinergic and Pyrimidinergic Signalling I - Molecular, Nervous and Urinogenitary System Function (Abbracchio, M.P. & Williams, M., eds), Springer-Verlag, Berlin. **2001**, pp. 251-288.

[95] Dunn, P.M.; Zhong, Y.; Burnstock, G. P2X receptors in peripheral neurones. Prog. *Neurobiol*. **2001**, *65***,** 107-134.

[96] Abbracchio, M.P., Ceruti, S., Bolego, C., Puglisi, L., Burnstock, G., & Cattabeni, F. Trophic roles of P2-purinoceptors in central nervous system astroglial cells. In *P2 Purinoceptors: Localization, Function and Transduction Mechanisms. Ciba Foundation Symposium* John Wiley and Sons, Chichester. **1996**, *198* pp. 142-148.

[97] 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.

[98] Loubatières-Mariani, M.M.; Chapal, J. Purinergic receptors involved in the stimulation of insulin and glucagon secretion. *Diabete Metab*. **1988**, *14***,** 119-126.

[99] Schöfl, C.; Ponczek, M.; Mader, T.; Waring, M.; Benecke, H.; von zur Mühlen A.; Mix, H.; Cornberg, M.; Böker, K.H.W.; Manns, M.P.; Wagner, S. Regulation of cytosolic free calcium concentration by extracellular nucleotides in human hepatocytes. *Am. J. Physiol*. **1999**, *276***,** G164-G172.

[100] Kunapuli, S.P.; Daniel, J.L. P2 receptor subtypes in the cardiovascular system. *Biochem. J.* **1998**, *336***,** 513-523.

[101] Mayer, C.; Quasthoff, S.; Grafe, P. Differences in the sensitivity to purinergic stimulation of myelinating and non-myelinating Schwann cells in peripheral human and rat nerve. *Glia* **1998**, *23***,** 374-382.

[102] Chen, Z.-P.; Kratzmeier, M.; Levy, A.; McArdle, C.A.; Poch, A.; Day, A.; Mukhopadhyay, A.K.; Lightman, S.L. Evidence for a role of pituitary ATP receptors in the regulation of pituitary function. *Proc. Natl. Acad. Sci.* USA **1995**, *92***,** 5219-5223.

[103] Lee, P.S.; Squires, P.E.; Buchan, A.M.; Yuen, B.H.; Leung, P.C. P2-purinoreceptor evoked changes in intracellular calcium oscillations in single isolated human granulosa-lutein cells. *Endocrinology* **1996**, *137***,** 3756-3761.

[104] Tomic, M.; Jobin, R.M.; Vergara, L.A.; Stojilkovic, S.S. Expression of purinergic receptor channels and their role in calcium signaling and hormone release in pituitary gonadotrophs. Integration of P2 channels in plasma membrane- and endoplasmic reticulum-derived calcium oscillations. *J. Biol. Chem.* **1996**, *271***,** 21200-21208.

[105] Sperlágh, B.; Mergl, Z.; Jurányi, Z.; Vizi, E.S.; Makara, G.B. Local regulation of vasopressin and oxytocin secretion by extracellular ATP in the isolated posterior lobe of the rat hypophysis. *J. Endocrinol.* **1999**, *160***,** 343-350.

[106] Vainio, M.; Törnquist, K. The role of adenosine A_1 receptors in the ATP-evoked Ca^{2+} response in rat thyroid FRTL-5 cells. Eur. *J. Pharmacol.* **2000**, *390***,** 43-50.

[107] Eltze, M.; Ullrich, B. Characterization of vascular P2 purinoceptors in the rat isolated perfused kidney. Eur. *J. Pharmacol.* **1996**, *306***,** 139-152.

[108] Huber-Lang, M.; Fischer, K.G.; Gloy, J.; Schollmeyer, P.; Kramer-Guth, A.; Greger, R.; Pavenstadt, H. UTP and ATP induce different membrane voltage responses in rat mesangial cells. *Am. J. Physiol* **1997**, *272***,** F704-F711.

[109] Cha, S.H.; Sekine, T.; Endou, H. P2 purinoceptor localization along rat nephron and evidence suggesting existence of subtypes P2Y1 and P2Y2. *Am. J. Physiol* **1998**, *274***,** F1006-F1014.

[110] van der, Weiden.L.; Adams, D.J.; Morris, B.J. Capacity for purinergic control of renin promoter via P2Y(11) receptor and cAMP pathways. *Hypertension* **2000**, *36***,** 1093-1098.

[111] Bailey, M.A.; Imbert-Teboul, M.; Turner, C.; Marsy, S.; Srai, K.; Burnstock, G.; Unwin, R.J. Axial distribution and characterization of basolateral P2Y receptors along the rat renal tubule. *Kidney Int*. **2000**, *58***,** 1893-1901.

[112] Cuffe, J.E.; Bielfeld-Ackermann, A.; Thomas, J.; Leipziger, J.; Korbmacher, C. ATP stimulates Cl^- secretion and reduces amiloride-sensitive Na^+ absorption in M-1 mouse cortical collecting duct cells. *J. Physiol* 2000, *524* Pt 1 **,** 77-90.

[113] Dockrell, M.E.; Noor, M.I.; James, A.F.; Hendry, B.M. Heterogeneous calcium responses to extracellular ATP in cultured rat renal tubule cells. *Clin. Chim. Acta* **2001**, *303***,** 133-138.

[114] Webb, T.E.; Barnard, E.A. Molecular biology of P2Y receptors expressed in the nervous system. Prog. *Brain Res.* **1999**, *120***,** 23-31.

[115] Neary, J.T.; Kang, Y.; Bu, Y.; Yu, E.; Akong, K.; Peters, C.M. Mitogenic signaling by ATP/P2Y purinergic receptors in astrocytes: involvement of a calcium-independent protein kinase C, extracellular signal-regulated protein kinase pathway distinct from the phosphatidylinositol-specific phospholipase C/calcium pathway. *J. Neurosci.* **1999**, *19***,** 4211-4220.

[116] Schafer, R.; Reiser, G. ATPαS is a ligand for P2Y receptors in synaptosomal membranes: solubilization of [^{35}S]ATPαS binding proteins associated with G- proteins. *Neurochem. Int.* **1999**, *34***,** 303-317.

[117] Moore, D.; Chambers, J.; Waldvogel, H.; Faull, R.; Emson, P. Regional and cellular distribution of the $P2Y_1$ purinergic receptor in the human brain: striking neuronal localisation. *J. Comp. Neurol.* **2000**, *421***,** 374-384.

[118] Morán-Jiménez, M.; Matute, C. Immunohistochemical localization of the $P2Y_1$ purinergic receptor in neurons and glial cells of the central nervous system. Brain Res. Mol. *Brain Res*. **2000**, *78***,** 50-58.

[119] Zundorf, G.; Schafer, R.; Vohringer, C.; Halbfinger, E.; Fischer, B.; Reiser, G. Novel modified adenosine 5'-triphosphate analogues pharmacologically characterized in human embryonic kidney 293 cells highly expressing rat brain P2Y(1) receptor: Biotinylated analogue potentially suitable for specific P2Y(1) receptor isolation. *Biochem. Pharmacol.* **2001**, *61***,** 1259-1269.

[120] Meyer, M.P.; Clarke, J.D.W.; Patel, K.; Townsend-Nicholson, A.; Burnstock, G. Selective expression of purinoceptor $cP2Y_1$ suggests a role for nucleotide signalling in development of the chick embryo. *Dev. Dyn.* **1999**, *214***,** 152-158.

[121] Müller, C.E.; Stein, B. Adenosine receptor antagonists: structures and potential therapeutic applications. *Curr. Pharm. Des.* **1996**, *2***,** 501-530.

[122] Broadley, K.J. Drugs modulating adenosine receptors as potential therapeutic agents for cardiovascular diseases. Exp. Opin. Ther. *Patents* **2000**, *10***,** 1169-1692.

[123] Impagnatiello, F.; Bastia, E.; Ongini, E.; Monopoli, A. Adenosine receptors in neurological disorders. *Emerging Therapeutic Targets* **2001**, *4***,** 635-664.

[124] Kaiser, S.M.; Quinn, R.J. Adenosine receptors as potential therapeutic targets. *Drug Discov. Today* **1999**, *4***,** 542-551.

[125] Agteresch, H.J.; Dagnelie, P.C.; van den Berg, J.W.O.; Wilson, J.H. Adenosine triphosphate: established and potential clinical applications. *Drugs* **1999**, *58***,** 211-232.

[126] Williams, M. Clinical opprortunities in purinergic neuromodulation. In *Handbook of Experimental Pharmacology: Purinergic and Pyrimidinergic Signalling II* (Abbracchio, M.P. & Williams, M., eds), Springer, Berlin. **2001**, pp. 407-434.

[127] Burnstock, G.; Williams, M. P2 purinergic receptors: modulation of cell function and therapeutic potential. *J. Pharmacol. Exp. Ther.* **2000**, *295***,** 862-869.

[128] Fischer, B. Therapeutic applications of ATP-(P2)-receptors agonists and antagonists. Exp. Opin. Ther. *Patents* **1999**, *9***,** 385-399.

[129] Burnstock, G.; Ralevic, V. New insights into the local regulation of blood flow by perivascular nerves and endothelium. *Br. J. Plast. Surg.* **1994**, *47***,** 527-543.

[130] Erlinge, D. Extracellular ATP: a growth factor for vascular smooth muscle cells. *Gen. Pharmacol.* **1998**, *31***,** 1-8.

[131] Brass, S. Cardiovascular biology. Small cells, big issues. *Nature* **2001**, *409***,** 145, 147.

[132] CAPRIE Steering Committee A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events. *Lancet* **2001**, *348***,** 1329-1339.

[133] Yusuf, S.; Zhao, F.; Mehta, S.R.; Chrolavicius, S.; Tognoni, G.; Fox, K.; The Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N. Engl. J. Med.* **2001**, *345***,** 494-502.

[134] Vidal, M.; Hicks, P.E.; Langer, S.Z. Differential effects of α, β-methylene ATP on responses to nerve stimulation in SHR and WKY tail arteries. Naunyn Schmiedebergs *Arch. Pharmacol.* **1986**, *332***,** 384-390.

[135] Hou, M.; Malmsjo, M.; Moller, S.; Pantev, E.; Bergdahl, A.; Zhao, X.H.; Sun, X.Y.; Hedner, T.; Edvinsson, L.; Erlinge, D. Increase in cardiac $P2X_1$- and $P2Y_2$-receptor mRNA levels in congestive heart failure. *Life Sci.* **1999**, *65***,** 1195-1206.

[136] 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-Liss, New York. **1997,** pp. 423-447.

[137] Burnstock, G. A unifying purinergic hypothesis for the initiation of pain. *Lancet* **1996**, *347***,** 1604-1605.

[138] Cockayne, D.A.; Hamilton, S.G.; Zhu, Q.-M.; Dunn, P.M.; Zhong, Y.; Novakovic, S.; Malmberg, A.B.; Cain, G.; Berson, A.; Kassotakis, L.; Hedley, L.; Lachnit, W.G.; Burnstock, G.; McMahon, S.B.; Ford, A.P.D.W. Urinary bladder hyporeflexia and reduced pain-related behaviour in $P2X_3$-deficient mice . *Nature* **2000**, *407***,** 1011-1015.

[139] Biaggioni, I.; Feoktistov, I.; Sheller, J.R.; Grant, M.; Belardinelli, L.; Wells, J.N. Development of new selective A2B antagonists with greater potency than enprofylline. *Drug Dev. Res.* **2000**, 50**,** 104.

[140] Schulman, E.S.; Glaum, M.C.; Post, T.; Wang, Y.; Raible, D.G.; Mohanty, 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.

[141] Yerxa, B.R. Therapeutic use of nucleotides in respiratory and ophthalmic diseases. *Drug Dev. Res.* **2000**, *52***,** 196-201.

[142] 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. **1999**, pp. 3-25.

[143] Brook, M.M.; Fineman, J.R.; Bolinger, A.M.; Wong, A.F.; Heymann, M.A.; Soifer, S.J. Use of ATP-$MgCl_2$ in the evaluation and treatment of children with pulmonary hypertension secondary to congenital heart defects. *Circulation* **1994**, *90***,** 1287-1293.

[144] Burnstock, G. Purine-mediated signalling in pain and visceral perception. Trends *Pharmacol*. *Sci*. **2001**, *22***,** 182-188.

[145] 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.

[146] Knight, G.E., Bodin, P., de Groat, W.C., & Burnstock, G. Ureter epithelium on distension. *Am. J. Physiol*. **2002**, *282*, F281-F288.

[147] Jackson, E.K. P1 and P2 receptors in the renal system. In *Purinergic and Pyrimidinergic Signalling II: Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function* (Abbracchio, M.P. & Williams, M., eds),. Springer, Berlin. **2001**, pp. 33-71

[148] Yiangou, Y.; Facer, P.; Baecker, P.A.; Ford, A.P.; Knowles, C.H.; Chan, C.L.; Williams, N.S.; Anand, P. ATP-gated ion channel $P2X_3$ is increased in human inflammatory bowel disease. *Neurogastroenterol*. *Motil.* **2001**, *13***,** 365-369.

[149] Hoebertz, A.; Meghji, 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.

[150] 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.

[151] Di Virgilio, F.; Falzoni, S.; Mutini, C.; Sanz, J.M.; Chiozzi, P. Purinergic $P2X_7$ receptor: a pivotal role in inflammation and immunomodulation. *Drug Dev*. *Res*. **1998**, *45***,** 207-213.

[152] Burnstock, G. Overview of P2 receptors: possible functions in immune cells. *Drug Dev*. *Res*. **2001**, *53***,** 53-59.

[153] Calvert, R.C., Moules, E.W., Thompson, C.S., Mikhailidis, D.P., Morgan, R.J., & Burnstock, G. ATP inhibits proliferation of human prostate cancer cell line (PC-3) via P2-purinergic receptors. *Prostate Cancer and Prostatic Diseases* 4, 17. **2001**.

[154] Höpfner, M.; Maaser, K.; Barthel, B.; von Lampe, B.; Hanski, C.; Riecken, E.O.; Zeitz, M.; Scherubl, H. Growth inhibition and apoptosis induced by $P2Y_2$ receptors in human colorectal carcinoma cells: involvement of intracellular calcium and cyclic adenosine monophosphate. *Int*. *J*. *Colorectal Dis.* **2001**, *16***,** 154-166.

[155] Loubatières-Mariani, M.M., Hillaire-Buys, D., Chapal, J., Bertrand, 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.

[156] Housley, G.D. Physiological effects of extracellular nucleotides in the inner ear. *Clin*. *Exp*. *Pharmacol*. *Physiol*. **2000**, *27***,** 575-580.