REVIEW

P2 receptors in cardiovascular regulation and disease

David Erlinge · Geoffrey Burnstock

Abstract The role of ATP as an extracellular signalling molecule is now well established and evidence is accumulating that ATP and other nucleotides (ADP, UTP and UDP) play important roles in cardiovascular physiology and pathophysiology, acting via P2X (ion channel) and P2Y (G protein-coupled) receptors. In this article we consider the dual role of ATP in regulation of vascular tone, released as a cotransmitter from sympathetic nerves or released in the vascular lumen in response to changes in blood flow and hypoxia. Further, purinergic long-term trophic and inflammatory signalling is described in cell proliferation, differentiation, migration and death in angiogenesis, vascular remodelling, restenosis and atherosclerosis. The effects on haemostasis and cardiac regulation is reviewed. The involvement of ATP in vascular diseases such as thrombosis, hypertension and diabetes will also be discussed, as well as various heart conditions. The purinergic system may be of similar importance as the sympathetic and renin-angiotensin-aldosterone systems in cardiovascular regulation and pathophysiology. The extracellular nucleotides and their cardiovascular P2 receptors are now entering the phase of clinical development.

Keywords ATP · Cardiovascular · Heart · Pathophysiology · Purine · Pyrimidine · P2X · P2Y · Receptor

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Introduction

Ever since the first proposition of cell surface receptors for nucleotides [1, 2], it has become increasingly clear that, in addition to functioning as an intracellular energy source, the purines and pyrimidines ATP, adenosine diphosphate (ADP), uridine triphosphate (UTP) and uridine diphosphate (UDP) can serve as important extracellular signalling molecules [3, 4] acting on 13 P2X homo- and heteromultimer ionotropic and 8 P2Y metabotropic receptor subtypes [5, 6] (Table 1). To terminate signalling, ectonucleotidases are present in the circulation and on cell surfaces, rapidly degrading extracellular ATP into ADP, AMP and adenosine [7, 8]. Evidence is accumulating suggesting an important role for the purinergic system in cardiovascular regulation [9–15]. It stimulates vasoconstriction and vasodilatation, growth of vascular smooth muscle cells and endothelial cells, angiogenesis, is involved in vascular remodelling, stimulates platelet aggregation, regulates coagulation, inflammation and several aspects of cardiac function. It is involved in blood pressure regulation, development of myocardial infarction, heart failure and xenograft rejection. The physiological effects of the purinergic signalling system are dependent on the release of extracellular nucleotides, the degradation by ectonucleotides, the type of P2 receptors expressed on the cells, their desensitisation rates and their second messengers. P2 receptors have highly specific organ distributions and they can be rapidly up- or downregulated. The purinergic system may be of similar importance as the sympathetic and renin-angiotensin-aldosterone systems in cardiovascular regulation and pathophysiology. The involvement of purinergic signalling in a variety of different cardiovascular clinical conditions has been addressed to a limited extent previously [15–19] and the large number of new discoveries calls for a review to summarise the most

Table 1 Receptor classification, intracellular signalling, ligands and selective agonists and antagonists

P2 subtype	I.c. signalling	Ligand	Selective agonist	Selective antagonist	Non-selective antagonist
P2Y$_1$	↑IP3	ADP (ATP)	MRS2365	MRS 2179, MRS2500	
P2Y$_2$	↑IP3	UTP = ATP	MRS2498, UTPγS, INS3717		Suramin > RB2
P2Y$_4$	↑IP3	UTP (=ATP in rodents)	UTPγS, INS3717	–	RB2 > Suramin
P2Y$_6$	↑IP3	UDP	MRS2666, MRS2633, UDPβS	MRS2578	
P2Y$_{11}$	↑IP3, ↑cAMP	ATP	AR-C67085MX, NF546	NF157	Suramin > RB2
P2Y$_{12}$	↓cAMP	ADP	–	Clopidogrel, prasugrel, AZD6140, INS50589, AR-C9931 (cangrelor)	
P2Y$_{13}$	↓cAMP	ADP	–	MRS2211	
P2Y$_{14}$	IP3	UDP-glucose, UDP-galactose	UDP-glucose, UDP-galactose	–	
P2X$_1$	Positive ion channel	ATP	α,β-mATP	NF023, NF449	TNP-ATP, Ip$_5$I
P2X$_2$	Positive ion channel	ATP	–	NF770	Suramin, isoPPADS, RB2
P2X$_3$	Positive ion channel	ATP	α,β-mATP	A317491, NF110	Suramin
P2X$_4$	Positive ion channel	ATP	Ivermectin potentiates	–	TNP-ATP
P2X$_5$	Positive ion channel	ATP	–	–	Suramin, PPADS
P2X$_6$	Positive ion channel	ATP	–	–	–
P2X$_7$	Positive ion channel	ATP	–	KN62, KN04, MRS2427	Coomassie brilliant blue G
Ectonucleotidase			Apyrase, human SolCD39	ARC67156	

important roles of the purinergic system in cardiovascular regulation and disease.

The research field has grown rapidly since the term P2 receptor was coined [2]. In preparation of this review more than 2,700 references on P2 receptors and cardiovascular regulation were found in PubMed. Among the 15 receptor subtypes, one is the target for one of the most widely used medical drugs—the platelet inhibitor clopidogrel (Plavix). As will be reviewed here, several of the other 15 receptors are promising drug targets to prevent cardiovascular disease.

Regulation of vascular tone

Vasoconstriction produced by ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves was recognised early [20, 21]. However, following the seminal discovery of endothelium-dependent vasodilatation by Furchgott in the early 1980s, it was shown that ATP acted on endothelial cells to release endothelial derived relaxing factor [later shown to be largely nitric oxide (NO)]

resulting in vasodilatation [22] and dual purinergic neural and endothelial control of vascular tone established [23, 24].

Neuronal regulation of vascular tone

ATP is, together with NA and neuropeptide Y (NPY), a cotransmitter in sympathetic neurons [11, 25, 26], and sensory-motor nerves during "axon reflex" activity release ATP to dilate or constrict vessels [27, 28] (Fig. 1). The contribution of ATP to sympathetic contraction varies between vascular beds [25]. For example, it may mediate up to 50% of the neurogenic vasoconstriction via P2X$_1$ receptors as seen in mesenteric arteries [29], about 70% in rabbit saphenous arteries and 100% in rabbit jejunal artery, while NA acts as a prejunctional modulator [30]. In the renal vasculature both P2X$_1$ and P2Y$_2$ receptors are important for neurogenic contraction. P2X$_1$ receptors mediate contraction in afferent but are absent in the efferent renal arterioles [31, 32]. The result is that extracellular nucleotides selectively influence preglomerular resistance without having an effect on postglomerular tone. ATP is also released locally in the
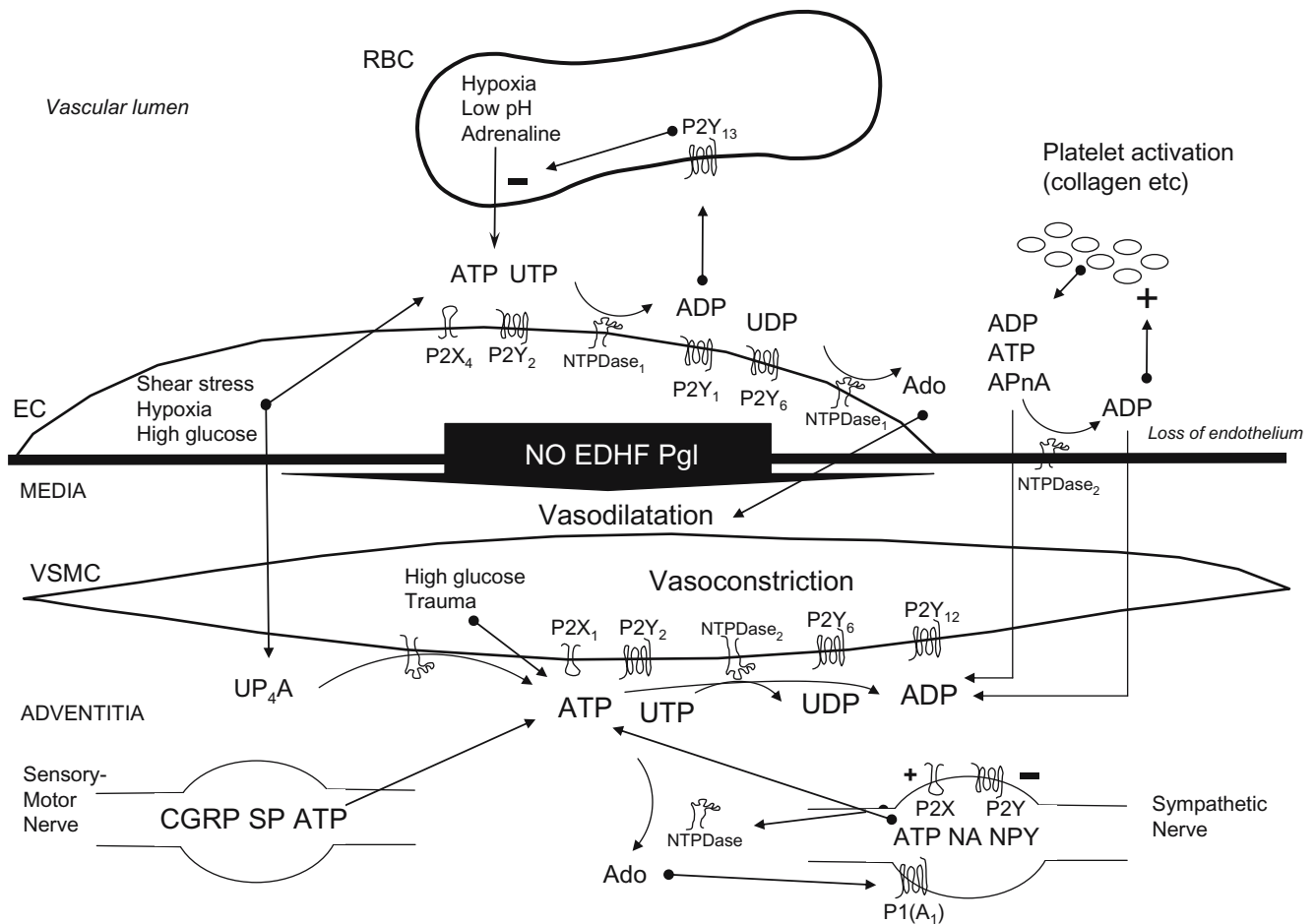
Fig. 1 P2 receptor-mediated regulation of the circulation. See text for details. Purines and pyrimidines are released on the luminal side from endothelial cells, platelets and red blood cells (*RBC*) in response to hypoxia, acidosis, adrenaline, shear stress and other stimuli. When the endothelial cell layer is intact, the response is vasodilatation by the endothelial release of nitric oxide (*NO*), endothelium-derived hyperpolarising factor (*EDHF*) and prostaglandins (*Pgl*). When the endothelium is damaged, platelets accumulate, release ATP and ADP and mediate vasoconstriction via P2 receptors on the vascular smooth muscle cell (*VSMC*). On the adventitial side sympathetic and sensory nerves mediate vasoconstriction. Extracellular nucleotides are rapidly degraded by NTPDase1 on endothelium reducing ATP to AMP, followed by conversion to adenosine by CD73 (not shown). In the subendothelium NTPDase2 is present, degrading ATP to ADP, maintaining the platelet activating and contractile effects. *Ado* adenosine, *CGRP* calcitonin gene-related peptide, *SP* substance P

kidney in response to increased perfusion pressure, and in $P2X_1$ receptor knockout mice auto-regulatory responses in kidney afferent arterioles are abolished, indicating an important role in renal glomerular pressure regulation [32, 33].

Sympathetic nerves express inhibitory P2Y and P1 (A_1) receptors indicating a P2 receptor-mediated negative feedback loop both directly by ATP and its degradation product adenosine [34, 35]. Ectonucleotidases are released from sympathetic nerves together with its substrate ATP, as a termination mechanism for the signalling [36]. Another balancing mechanism is the sympatholytic effect seen in exercising skeletal muscle, which is important for increased blood flow in the working skeletal muscle during exercise, despite sympathetic NA release that normally would reduce blood flow. This sympatholytic effect is mimicked by injection in the arterial lumen of ATP and UTP, inducing a vasodilatation that overrides sympathetic vasoconstrictor activity in human

skeletal muscle, an effect not obtained by injection of adenosine [37], suggesting that ATP and UTP may mediate this important physiological mechanism.

The first evidence of the presence of P2 receptor subtypes suggested P2X receptors on vascular smooth muscle cells (VSMC) and P2Y receptors on endothelial cells [38]. Rat blood vessel immunohistochemistry and human mRNA quantification have shown the $P2X_1$ receptor to be the highest expressed subtype in smooth muscle cells [39, 40]. It has been difficult to prove the importance of other contractile P2X receptors besides $P2X_1$ due to the paucity of specific agonists and antagonists. However, contractile effects of UTP suggested that P2Y receptors were present on VSMCs and mediated contraction [41]. Using selective pyrimidines resistant to degradation, it has been possible to show that contractile effects are mediated by both UTP-sensitive $P2Y_2$ and UDP-sensitive $P2Y_6$ receptors [42]. Ectonucleotidases in

the vessel wall rapidly degrade nucleotides and markedly reduce their contractile effects. Pyrimidine analogues more resistant to ectonucleotidases are powerful vasoconstrictors and up to 1,000-fold more potent than the endogenous ligands [42]. Similarly, a prominent more potent UDP contraction has been seen in NTPDase1 knockout mice, demonstrating the protective effect of ectonucleotidases against nucleotide contraction [43]. In human saphenous vein grafts, used during coronary bypass, a stable UDP analogue stimulates a strong contraction lasting for hours, which is not desensitised [44]. The reason why the $P2Y_6$ receptor is not desensitised is the lack of serine residues in the C-terminal part of the $P2Y_6$ receptor [45]. Neither α_1-adrenoceptors, nor $P2Y_6$ receptors, are present in human coronary arteries, possibly to avoid deleterious vasospasm during ischaemia [44].

VSMC express $P2Y_{12}$ receptors that mediate contraction after stimulation with ADP [46]. At the mRNA level, the $P2Y_{12}$ receptor is the highest expressed ADP receptor and the second highest expressed P2 receptor in human VSMC [46]. The contractions are not inhibited in patients medicated with clopidogrel. However, drugs with antagonistic effects on $P2Y_{12}$ receptors that reach the peripheral circulation (AZD6140 and INS50589), affecting both platelets and VSMC, could be of double therapeutic benefit in their prevention of both thrombosis and vasospasm [46].

In conclusion, extracellular nucleotides mediate vaso-constriction when released from nerves on the adventitial side, or when released in the lumen when the endothelium is damaged (Fig. 1). The most important contractile receptors on the VSMC are the ATP $P2X_1$ receptor, the ATP/UTP $P2Y_2$ receptor, the UDP $P2Y_6$ receptor and the ADP $P2Y_{12}$

receptor (Fig. 1 and Table 2). To determine the relative physiological importance of these receptor subtypes in different human vascular beds is an important task for the future.

Endothelial regulation

Shear stress and hypoxia are important stimuli of both ATP and UTP release from endothelial cells [47] (Fig. 1). Extracellular nucleotides have several important effects mediated by activation of endothelial cells. Vasodilatation and decreased blood pressure by release of prostaglandins and NO has been demonstrated in several studies [5], but P2 receptors also mediate release of endothelium-derived hyperpolarising factor (EDHF), which relaxes VSMC by activation of potassium channels, with subsequent hyper-polarisation [48–50]. Both UTP and ATP reduce forearm vascular resistance in a prostaglandin and NO independent way [51], indicating an important role for EDHF in P2 receptor-mediated vasodilatation in man.

The $P2Y_1$ receptor seems to be of major importance in most vascular beds [11]. However, several other P2 receptors are important for endothelial regulation. Pharmacology of vasodilatation and mRNA quantification in man indicates that $P2Y_2$ and to a lesser degree $P2Y_6$ also are important endothelial P2Y receptors [40, 52]. Knockout mice experiments recently confirmed this picture and demonstrated that the $P2Y_4$ receptor does not mediate dilatation [53]. UTP and ATP are equipotent as vasodilators when infused in the human forearm circulation, indicating a role for both purinergic and pyrimidinergic receptors.

Table 2 P2 receptor expression in the cardiovascular system

P2 subtype	Endothelium	VSMC	Heart	Platelets	Red blood cells	Inflammatory cells	Perivascular sensory nerves	Perivascular sympathetic nerves
$P2Y_1$	High	–	–	High	(Turkey)		Medium	Medium (neg. feedback)
$P2Y_2$	High	High	High	–	–	High	Low	Possible
$P2Y_4$	–	Possible	–	–	–			
$P2Y_6$	Medium	Medium	High	–	–			
$P2Y_{11}$	Possible	–	High	–	–	High		
$P2Y_{12}$	–	Medium	–	High	–	Low		
$P2Y_{13}$	–	–	–	–	High	Low		
$P2Y_{14}$	–	–	–	–	–			
$P2X_1$	–	High	Medium	High	–	Medium		
$P2X_2$	–	–	–	–	–		High	Medium (pos. feedback)
$P2X_3$	–	–	–	–	–		High	
$P2X_4$	High	–	Low	–	–	High		
$P2X_5$	–	–	–	–	–			
$P2X_6$	–	–	–	–	–			
$P2X_7$	–	Possible	–	–	Low	High		

Unless specified data refer to human tissue. High, medium or low expression represents an effort to summarise the results of a large number of mRNA, protein and pharmacological studies. Dash (–) denotes lack of convincing evidence for expression. *VSMC* vascular smooth muscle cell

The $P2X_1$ receptor is not expressed on the vascular endothelium and the evidence for other P2X receptors has been scarce, except for the $P2X_4$ receptor which is the highest expressed P2 receptor in endothelium [40, 54]. Using antisense oligonucleotides the $P2X_4$ receptor was shown to be important for shear stress-dependent Ca^{2+} influx via an ATP-dependent mechanism [55]. This indicates that ATP and P2 receptors may be of importance for shear stress-mediated effects, which is in agreement with the well-established release of ATP from endothelial cells during shear stress [56]. Vessel dilation induced by acute increases in blood flow is markedly suppressed in $P2X_4$ receptor knockout mice [57]. Thus, endothelial $P2X_4$ channels are crucial to flow-sensitive mechanisms that regulate blood pressure and vascular remodelling [57].

Extracellular ATP in the circulation is rapidly degraded into ADP, AMP and adenosine by ectonucleotidases. Vascular NTPDase1 (CD39) is an endothelial cell membrane protein with both ecto-ATPase and ecto-ADPase activities [8, 58]. Ectonucleotidases are also released by shear stress from endothelial cells [59].

Reactive hyperaemia is the massive increase in blood flow that starts when a blood vessel is opened after a period of ischaemia. It is well known that ATP is released during ischaemia [7, 60]. Adenosine only mediates the late phase of the reactive hyperaemia as has been shown by infusion of adenosine deaminase or a selective A_{2A} antagonist [61, 62]; ADP has been shown to mediate the mid-portion and peak of coronary reactive hyperaemia via endothelial $P2Y_1$ receptors [63]. It has been proposed that different purines may mediate three phases of the reactive hyperaemia [63]. According to this hypothesis, ATP would mediate the first part of the hyperaemia via endothelial $P2Y_2$ or $P2X_4$ receptors. ATP would then be degraded to ADP, which mediates peak hyperaemia via endothelial $P2Y_1$ receptors, followed by degradation of ADP to adenosine resulting in late phase hyperaemia mediated via A_{2A} receptors on smooth muscle cells.

Red blood cells as regulators of vascular tone

The matching of oxygen supply with demand requires a mechanism that increases blood flow in response to decreased tissue oxygen levels. Several reports suggest that the red blood cell (RBC) acts as a sensor for hypoxia and different mechanisms have been suggested by which the deoxygenated RBC stimulates vasodilatation [37, 64–66]. RBCs contain millimolar amounts of ATP and possess the membrane-bound glycolytic enzymes necessary for its production [67–69]. ATP is released in response to reductions in oxygen tension and pH [37, 64] (see Table 3). It has been shown in vitro that vessels dilate in response to low O_2 levels only when blood vessels are perfused with RBCs [70]. ATP is released in working human skeletal muscle circulation depending on the number of unoccupied haemoglobin O_2 binding sites [64, 71]. Similar results have been shown in the coronary circulation of dogs [72]. The released ATP then binds to P2Y receptors on the endothelium and stimulates vasodilatation. Thus, the RBC functions as an O_2 sensor, contributing to the regulation of blood flow and O_2 delivery, by releasing ATP depending on the oxygenation state of haemoglobin.

ADP activates a negative feedback pathway for ATP release from human RBCs via $P2Y_{13}$ receptors [73]. Since blood consists of approximately 40% RBCs that contain a 1,000-fold higher ATP concentration than plasma (mmol/

Table 3 Release of purines and pyrimidines in the cardiovascular system

Source	Endothelium	VSMC	Heart	Platelets	Red blood cells	Inflammatory cells	Vascular nerves
Stimuli	Shear stress, hypoxia, high glucose	High glucose, hypoxia	Hypoxia	Collagen, thromboxane, other platelet activators	Hypoxia, adrenaline, deformation	Infl. activators	Sympathetic nerves, sensory nerve collaterals
Mechanism	Vesicular	Unknown	Unknown	Vesicular	Unknown	Unknown or vesicular	Vesicular
Nucleotide	ATP, UTP	ATP, UTP	ATP, UTP	ADP > ATP	ATP	ATP	ATP
Target	Endothelium VSMC	VSMC	Cardiomyocytes, blood vessels, intrinsic cardiac neurons	Platelets; endothelium or VSMC	Endothelium	VSMC, endothelium	VSMC
Degradation	NTPDase1	NTPDase2, NTPDase1	NTPDase1	NTPDase1	NTPDase1	NTPDase1	NTPDase1 (co-released)

Stimuli represents stimuli leading to release. *Mechanism* refers to mechanism of release at the cellular level. *Target* represents the cells that are regulated by the released purine or pyrimidine. *Degradation* refers to the degrading enzyme responsible for removing the purine or pyrimidine after the release. *VSMC* vascular smooth muscle cell

l vs μmol/l), even a minor release of ATP from the high intracellular concentrations could have major circulatory effects. A negative feedback system may therefore be of great physiological importance to mitigate ATP release. Another negative feedback system is the mechanism by which NO inhibits ATP release from erythrocytes, illustrating how NO may turn off ATP release [74]. NO is then scavenged by haemoglobin.

Furthermore, the previous view of the RBC as a "passive bag that transports oxygen" is challenged. It now turns out that it releases ATP in response to stimuli and, as with most important signalling systems, it has a negative feedback system to terminate its release. The described negative feedback pathway may be important to avoid high extracellular concentrations of ATP. At levels above 100 μmol/l, ATP concentrations may exceed the catalytic capacity of ectonucleotidases and could, in fact, stimulate ATP release by increasing permeability of the RBC [75], probably via $P2X_7$ receptors [76]. ATP may even release ATP from endothelial cells [77]. At high concentrations of ATP, a self-sustaining process may thus be instigated which may contribute to the irreversible stage of circulatory shock that can develop rapidly in severely ill patients. Similar mechanisms may be of importance in malaria because induction of the osmolyte permeability in *Plasmodium*-infected erythrocytes involves purinoceptor signalling [78].

A mechanism of ATP release from RBCs, which has eluded researchers for many years, has recently been suggested by Locovei and co-workers as being mediated via the gap junction protein pannexin-1 [79]. Erythrocytes do not form gap junctions, instead pannexin-1 forms a mechanosensitive ATP-permeable channel that mediates osmotically induced ATP release.

Hypertension

Blood pressure regulation by purinergic signalling is the net result of balancing contractile and dilatory effects as described above. ATP and UTP released on the luminal side, from endothelial cells and erythrocytes, stimulate vasodilatation, in contrast to release from nerves on the adventitial side, which results in vasoconstriction. ATP may also regulate blood pressure via renal mechanisms or brain stem regulation [31, 32].

ATP plays a significantly greater role as a sympathetic cotransmitter in spontaneously hypertensive rats (SHR) [80, 81], and there is increased responsiveness of the renal vasculature of isolated perfused rat kidneys to α,β-methylene ATP in SHR [82], while mesenteric vascular contractile reactivity to ATP via $P2X_1$ and $P2Y_2$ receptors is not altered in deoxycorticosterone acetate (DOCA)-salt hypertension [83]. In the aorta of SHR, endothelium-dependent relaxation to ATP is impaired because of the concomitant generation of an endothelium-derived contracting factor. Contractions to ATP were significantly potentiated in SHR aorta [84]. In hypertensive mature stroke-prone rats, NPY modulation of release of NA and ATP from sympathetic perivascular nerves in kidney vessels is impaired, which may account for the increased nerve-mediated responses [85]. Thus, animal models of hypertension reveal several alterations in purinergic signalling that may contribute to the development of hypertension.

Diadenosine polyphosphates such as Ap_4A, Ap_5A and Ap_6A are combinations of two adenosine molecules connected with four to six phosphate groups. They have been identified as vasocontractile agents [86], probably via actions on $P2X_1$ and $P2Y_2$ receptors. Ap_5A and Ap_6A are stored at higher levels in platelets from patients with hypertension and may contribute to their increased peripheral vascular resistance [87]. Up_4A is a novel endothelium-derived vasoconstrictive factor more potent than endothelin in renal vasoconstriction [88]. It is released upon stimulation of the endothelium by acetylcholine, thrombin and mechanical stress and can be cleaved into either ATP or UTP to stimulate both $P2X_1$ and $P2Y_2$ receptors on VSMC resulting in increased blood pressure [88].

As mentioned above, the $P2X_4$ receptor is the most abundantly expressed P2 receptor in the endothelium and mediates shear stress-stimulated vasodilatation. $P2X_4$ receptor knockout mice have higher blood pressures and excrete smaller amounts of NO products in their urine than do wild-type mice [57]. The $P2X_4$ receptor protein is upregulated in the placenta in preeclampsia [89]. Thus, shear stress-stimulated release of ATP acting on $P2X_4$ receptors may be important for blood pressure regulation.

The importance of P2 receptors for hypertension will not be proven until tested in man. It would be of great interest to perform a clinical study on the effects of $P2X_1$, $P2Y_2$ or $P2Y_6$ receptor antagonists in the treatment of hypertension.

Pulmonary hypertension

Erythrocyte release of ATP appears to regulate pulmonary resistance under some conditions [90], and patients with pulmonary hypertension have been found with impaired release of ATP from RBC [69]. Endothelium-dependent relaxation to ATP has been demonstrated in human pulmonary arteries [91]. On the other hand, ATP is a mitogen for pulmonary artery smooth muscle cells, which may be relevant for the pathophysiological basis of pulmonary hypertension [92]. Again, the balance between endothelial versus smooth muscle stimulation by ATP may regulate blood pressure in different directions and we are far from a full understanding of the role of the purinergic system in pulmonary hypertension.

Migraine and vascular pain

Classic migraine is associated with two distinct vascular changes: an initial vasoconstriction, followed by vasodilatation associated with pain. It has been proposed that purinergic signalling is involved in these changes. ATP released from perivascular sympathetic nerves may participate in producing the initial vasospasm mediated by P2 receptors in vascular smooth muscle. Cerebral arteries are strongly contracted by UTP and especially UDP via $P2Y_6$ receptors. A UDP agonist might therefore have similar effects as the 5-HT_{1D} agonists frequently used to treat migraine [93]. ATP released by endothelial cells acting on P2 receptors on endothelial cells mediating release of NO and producing vasodilatation may contribute to the phase of reactive hyperaemia following vasospasm [94]. It has been suggested that antagonists to $P2X_3$ receptors, which are located on nociceptive sensory nerve endings in cerebral vessels, may be promising candidates for anti-migraine drug development [95]. A recent report demonstrates selective upregulation of nociceptive $P2X_3$ receptors on trigeminal neurons by calcitonin gene-related peptide and suggests that this mechanism might contribute to pain sensitisation in migraine [96]. P2Y receptors on trigeminal sensory nerves have also been implicated in pain in migraine. It has been claimed that migraine attacks are characterised by a relative depletion of sympathetic NA stores in conjunction with an increase in release of ATP [97]. In a review in the *Lancet* in 1996, Burnstock proposed that vascular pain, as in angina, ischaemic muscle and pelvic pain in women, may be initiated by ATP released from microvascular endothelial cells during reactive hyperaemia, diffusing a short distance to activate $P2X_3$ nociceptive receptors on sensory nerves in the adventitia [26].

Atherosclerosis

Atherosclerosis is the main cause of ischaemic stroke and cardiovascular disease and is now considered to be an inflammatory disease [98]. The formation of a plaque starts with the accumulation of cholesterol followed by invasion of macrophages taking up cholesterol and becoming foam cells. The plaque can be stabilised by smooth muscle cell formation of a fibrous cap that covers the lipid-rich region. However, stimulation of inflammation by oxidised low-density lipoprotein activates macrophages and dendritic cells into antigen-presenting cells, activating T lymphocytes resulting in release of cytokines and metalloproteinases degrading the fibrous cap. The end result is a vulnerable plaque and, when it ruptures, its highly thrombogenic contents activates platelets and causes the formation of a local thrombus occluding the artery or embolising, resulting

in ischaemic stroke or myocardial infarction. Evidence both from basic research and from clinical studies indicates important involvement on several levels for purinergic signalling in the atherosclerotic process (Fig. 2). Interestingly, fish oil components increase the release of ATP [99] and a high cholesterol diet decreases ATP release from arteries [100, 101].

Atherosclerosis—P2 receptor-mediated effects on inflammatory cells

Inflammatory cells express a large number of P2 receptors with multiple effects [102]. The final result is difficult to predict depending on the subtype of receptor expressed by a particular cell type and on the differentiation stage of the cell. The most important inflammatory cells for atherosclerosis are monocytes that differentiate into macrophages or dendritic cells in the plaque, and the T-helper and suppressor lymphocytes that coordinate the inflammatory reaction in the plaque [98].

A large number of P2 receptors are expressed on T lymphocytes and macrophages [14, 103] and have been suggested to be important players in atherosclerosis [14]. Strong evidence for a functional role exists for $P2X_7$, $P2Y_2$ and $P2Y_{11}$ receptors. The $P2X_7$ receptor is mitogenic and anti-apoptotic for T lymphocytes [104, 105]. $P2X_7$ is important for release of interleukin (IL)-1 [106], tumour necrosis factor [107] and L-selectin, an adhesion molecule important for lymphocyte binding to endothelium [108]. All of these effects are known to be important for atherosclerosis development. The severity of arthritis is reduced in $P2X_7$ receptor knockout mice [109] and rheumatoid arthritis is coupled to increased incidence of myocardial infarction. It would be of great interest to examine the $P2X_7$ receptor knockout mouse in an atherosclerosis model. ATP and UTP are chemotactic for dendritic cells probably via the $P2Y_2$ receptor and may attract inflammatory cells to the vascular lesion [110]. The $P2Y_2$ receptor enhances the oxidative burst in human macrophages [111]. $P2Y_1$ receptor deletion in knockout mice reduces the atherosclerotic lesions and the plaque area occupied by macrophages in ApoE knockout mice [112]. Whether this is due to platelet inhibition, endothelial or an inflammatory mechanism requires further studies.

ATP inhibits CD4+ T cell activation via an increase in cyclic AMP (cAMP) probably via a $P2Y_{11}$ receptor [113]. ATP acting on $P2Y_{11}$ receptors regulates the maturation of human monocyte-derived dendritic cells and induces immunosuppression by inhibiting T-helper 1 cytokines and promoting T-helper 2 cytokines [114, 115].

A polymorphism in the $P2Y_{11}$ receptor has been shown to have clinical importance by increasing the risk of myocardial infarction [116]. The G-459-A polymorphism, carried by one fifth of the population, causes an Ala-87-Thr
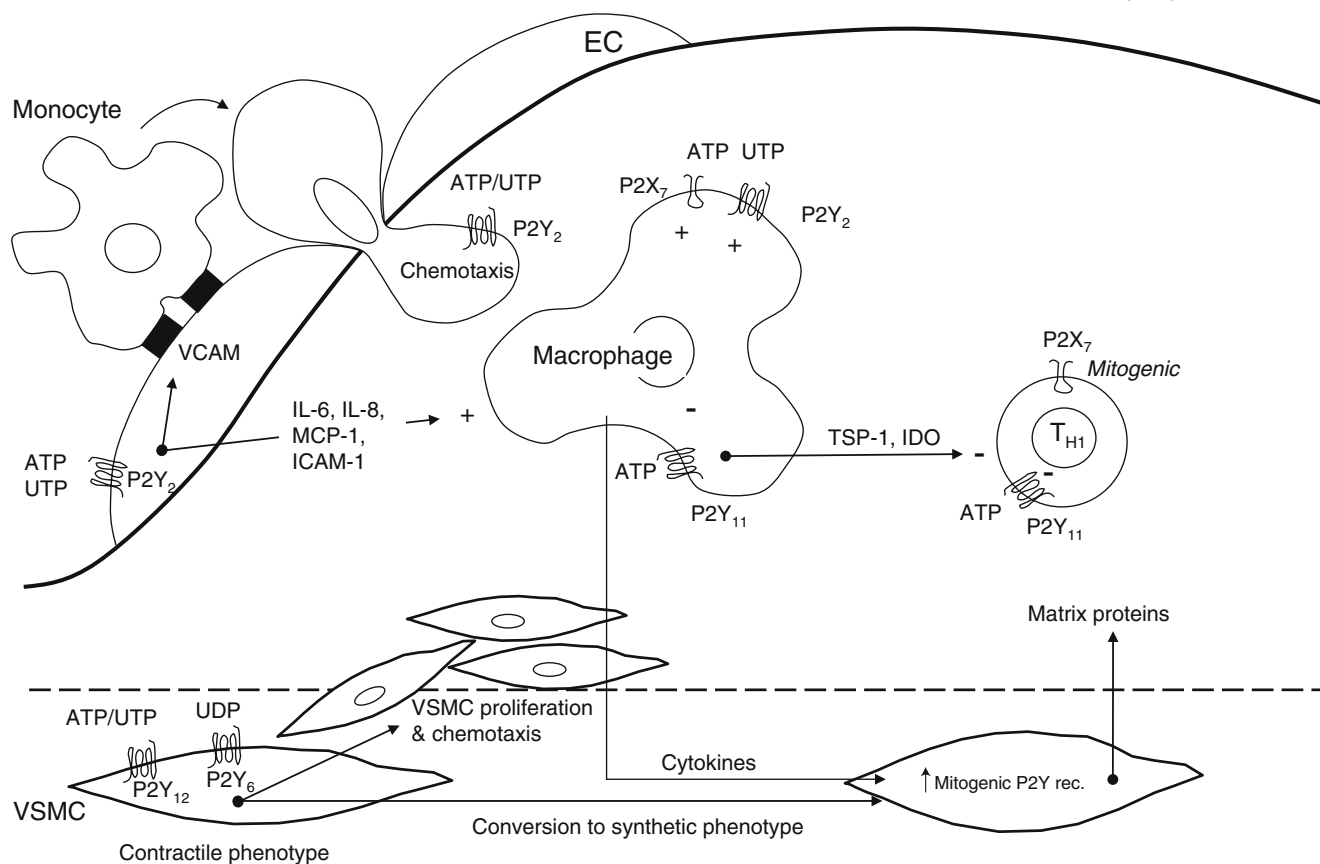
Atherosclerotic plaque

Fig. 2 Functional roles of P2 receptors in the atherosclerotic inflammatory plaque and during restenosis. See text for details. Purines and pyrimidines acting on P2 receptors stimulate vascular inflammation both by actions on the endothelial cell (*EC*) and by effects on inflammatory cells. Furthermore, they stimulate vascular smooth muscle cell (*VSCM*) proliferation, the conversion to synthetic phenotype and production of matrix proteins. Mitogenic P2 receptors are upregulated by growth factors and cytokines. *IL* interleukin, *MCP-1* monocyte chemoattractant protein-1, *ICAM-1* intercellular adhesion molecule-1, *TSP* thrombospondin, *IDO* indoleamine 2,3-dioxygenase

substitution in the $P2Y_{11}$ ATP receptor and increases the risk of myocardial infarction by 21%. The odds ratio increased stepwise depending on the number of Thr-87 alleles, and in subgroups in which the genetic influence is known to be of increased importance, family history of acute myocardial infarction (AMI), early onset AMI or the combined group of early onset AMI with family history. The mechanism by which the polymorphism causes AMI seems to be coupled to increased inflammation because the Thr-87 variant of the $P2Y_{11}$ receptor was coupled to elevated C-reactive protein (CRP) levels. CRP is a marker of inflammation and an independent prognostic risk factor for the development of AMI [98]. Thus, the $P2Y_{11}$ receptor is important in the development of atherosclerosis via modulation of inflammation either via effects on T lymphocytes or macrophage cells.

Proinflammatory effects on the endothelium

Even though P2 receptor-mediated activation of the endothelium stimulates release of NO, which inhibits inflammatory cells, it is now well established that important proinflammatory endothelial effects may also be triggered. A proinflammatory dysfunctional endothelium is crucial in the recruitment of monocytes to the atherosclerotic plaque and in the general extravasation and loss of peripheral resistance seen in sepsis. ATP stimulates neutrophil adherence to cultured endothelial cells [117]. ATP stimulates release of IL-6, IL-8, monocyte chemoattractant protein-1, growth-regulated oncogene α and increased expression of intercellular adhesion molecule-1 in human microvascular endothelial cells [118]. UTP and ATP stimulate expression of proinflammatory vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells through activation of the $P2Y_2$ receptor and increases the adherence of monocytic cells to human coronary endothelial cells, an effect that was inhibited by anti-VCAM-1 antibodies [119]. VCAM is important for the recruitment of monocytes and lymphocytes. The VCAM stimulation is caused by $P2Y_2$ receptor-induced transactivation of the vascular endothelial growth factor receptor-2 [120]. These effects have been confirmed in an in vivo neointima model, in which perivascular infusion of UTP enhanced infiltration by macrophages [121].

In conclusion, ATP and UTP stimulate several inflammatory responses known to be important for atherosclerosis development (Fig. 2).

Restenosis and vascular smooth muscle cell proliferative disease

VSMC proliferation contributes to plaque development, but it is probably beneficial by stabilising the plaque and forming a stronger fibrinous cap. However, there are several situations in which VSMC proliferation is important in vascular disease development: restenosis after balloon angioplasty, diabetic microvascular disease, chronic allograft rejection, pulmonary hypertension and possibly systemic hypertension. The latter is suggested since sympathetic nerves exert a trophic effect on vascular smooth muscle [122]. ATP is a cotransmitter from sympathetic nerves [25] and has been shown to be a more potent mitogen than the other cotransmitters NA and NPY [123].

Extracellular ATP is a potent growth factor for VSMC by activation of G protein-coupled P2Y receptors [12, 123–125]. UTP is also mitogenic, acting on $P2Y_2$ receptors [123], as is UDP acting on $P2Y_6$ receptors [124, 126, 127]. ATP is synergistic with polypeptide growth factors (e.g. platelet-derived growth factor, basic fibroblast growth factor) and insulin [123, 124]. The signal transduction is mediated via Gq proteins, phospholipase C and D, diacylglycerol, protein kinase C, extracellular signal-regulated kinase, phosphatidy-linositol-3 kinase, MAPK/ERK activity kinase, mitogen-activated protein kinases and Rho [12, 128, 129]. Several immediate early genes are activated and the cell is taken through different phases of the cell cycle [125, 130]. Sometimes, as with UDP acting on $P2Y_6$ receptors, progression is stimulated to both the S and G_2 phases, that is through the whole cell cycle [127].

Mitogenic effects have been demonstrated in rat, porcine, and bovine VSMC and cells from human coronary arteries, aorta, and subcutaneous arteries and veins [12, 13, 124, 131]. The trophic effects on VSMC and the abundant sources for extracellular ATP in the vessel wall make a pathophysiological role probable in the development of atherosclerosis, neointima formation after angioplasty, chronic allograft rejection, pulmonary hypertension, diabetic microvascular disease and possibly hypertension. In these processes interaction between the VSMC and the matrix is important. It is therefore interesting that the human $P2Y_2$ receptor contains an integrin-binding domain (RGD) in its first extracellular loop and that interaction with integrins influences $P2Y_2$ receptor-mediated activation of G proteins [132]. ATP stimulates release of matrix metalloproteinase-2 (MMP-2) from human aortic smooth muscle cells and MMP-2 has been implicated in aortic aneurysm pathogenesis [133].

MMPs open up the matrix for migrating VSMC. ATP and UTP are potent chemotactic agents stimulating VSMC migration via $P2Y_2$ and $P2Y_6$ receptors [134]. These mechanisms, together with growth-stimulating effects, explain why perivascular infusion of UTP in a neointima model enhanced neointimal development [121]. Overexpression of the ATP- and UTP-degrading enzyme NTPDase1 reduces neointima development in rat aorta [135]. Augmentation of NTPDase1 activity is an important adaptive response for cardiac allograft survival, with increased inflammation, platelet deposition and infarction rates in NTPDase-deficient mice [136]. The mechanism is dependent on increased expression of the atherosclerotic matrix protein osteopontin [134, 137].

Diabetic microvascular disease

Sympathetic vascular dysfunction in early experimental juvenile diabetes was recognised many years ago. Two weeks after induction of streptozotocin diabetes in rats, there was prejunctional impairment of sympathetic neurotransmission and impaired ATP-mediated endothelial function in mesenteric arteries [138]. Altered relaxant responses to ATP in the corpus cavernosum of men and rats with diabetes have also been reported [139].

As mentioned above, red blood cells may stimulate vasodilatation via release of ATP, which may be important in maintaining perfusion in the microcirculation. Interestingly, in erythrocytes from humans with type 2 diabetes ATP release is impaired, which is consistent with the hypothesis that the defect in erythrocyte physiology could contribute to the vascular disease associated with this clinical condition [140].

High extracellular glucose releases ATP and/or UTP in endothelial cells and pancreatic β cells [141, 142]. An increase in glucose from 5 to 15 mmol/l results in a marked increase in the proatherogenic nuclear factor of activated T cells signalling pathway in VSMC [143]. The effect is mediated via glucose-induced release of ATP and UTP, which subsequently activate $P2Y_2$ but also $P2Y_6$ receptors (after degradation to UDP). Thus, nucleotide release is a potential metabolic sensor for the arterial smooth muscle response to high glucose. Diabetic patients experience microvascular disease characterised by increased wall-lumen ratio, mainly because of an increase in VSMC and have higher rates of restenosis after coronary angioplasty. High glucose-induced release of extracellular nucleotides, acting on P2Y receptors to stimulate VSMC growth via NFAT (nuclear factor of activated T cells) activation, may provide a link between diabetes and diabetic vascular disease [143].

In conclusion, ATP/UTP/UDP stimulate VSMC growth, migration, release of MMPs and osteopontin that may contribute to the development of restenosis, diabetic microvascular disease, chronic allograft rejection, pulmo-

nary hypertension and possibly systemic hypertension (Fig. 2).

Vascular remodelling and angiogenesis

Vascular remodelling

P2 receptors may regulate VSMC phenotype and vice versa, and P2 receptor expression is markedly altered during phenotype changes [13]. The shift from a specialised contractile VSMC phenotype into a proliferating, matrix-producing, synthetic phenotype is a prerequisite for VSMC pathogenesis in vascular disease. Pacaud and co-workers found that the Ca^{2+}-mobilising effects of 2-methylthioATP increased in VSMC during culture in serum, indicating upregulation of $P2Y_1$ receptors in the transition from contractile to synthetic phenotype [144]. This was confirmed at the mRNA level where $P2Y_2$ receptors were found to be upregulated, while $P2X_1$ receptors were downregulated [145]. Mitogenic P2Y receptors are upregulated while ion channel receptors, with only contractile effects, are down-regulated in the synthetic phenotype [145]. Growth factors, cytokines and interestingly also ATP are potent stimulators of $P2Y_2$ receptor expression [146, 147]. Thus, factors of importance in the development of vascular disease increase mitogenic $P2Y_2$ receptors; this is further supported by their upregulation in neointima after balloon angioplasty [148, 149]. ATP has a dual concentration-dependent effect on VSMC phenotype. Low ATP concentrations stimulate expression of genes specific for the contractile phenotype, while high ATP concentrations cause a phenotypic shift from the contractile to the synthetic phenotype, and this shift is dependent on a transient activation of protein kinase A, which inhibits activation of a serum response factor [150]. This previously unrecognised mechanism also appears to be important for the profound mitogenic effect of ATP. In intact human blood vessels examined in vitro, shear stress decreased contractile $P2X_1$ receptor expression, but increased the expression of mitogenic $P2Y_2$ and $P2Y_6$ receptors in VSMC [151]. This mechanism could promote vascular growth and remodelling induced by shear stress as an adaptive response to increased flow. $P2X_4$ receptor knockout mice are incapable of adaptive vascular remodelling, that is, a decrease in vessel size in response to a chronic decrease in blood flow [57].

Angiogenesis

Very little is known about the role of P2 receptors in angiogenesis, but there are some studies suggesting their involvement. Ischaemia stimulates release of ATP [7, 60] and it has been shown to be a growth factor for endothelial cells [13, 152]. ATP and UTP stimulate cytoskeletal rearrangements with consequent cell migration of human endothelial cells [153] and ATP stimulates vasa vasorum neovascularisation in pulmonary arteries [154]. Newly developed vascular endothelia express very high levels of NTPDase1, also seen under hypoxic conditions [155]. NTPDase1 knockout mice exhibit disordered cellular migration and angiogenesis [156].

Varicose veins

It has been suggested that cell lysis, consequent to $P2X_7$ receptor-induced pore formation, contributes to the disorganisation and decrease in contractile myocytes in the media of varicose veins [157]. Upregulation of $P2Y_1$ and $P2Y_2$ receptors and downregulation of $P2X_1$ receptors on smooth muscle of varicose veins is associated with a shift from contractile to synthetic and/or proliferative roles; this phenotypic change in smooth muscle leads to weakening of vein walls and may be a causal factor in the development of varicose veins [158], an interesting parallel to the receptor changes seen in response to increased shear stress (see above) [151].

Heart

ATP is released in the heart as a cotransmitter together with catecholamines from sympathetic nerves, but it may also be released from other sources in the heart such as endothelium, platelets, RBC and ischaemic myocardium (Table 3) [7, 60, 159]. P2 receptors are abundantly expressed in the foetal human heart [160] as well as in the adult human heart [60, 161].

Inotropy

In cardiomyocytes, ATP stimulates a pronounced positive inotropic effect and may also act in synergy with β-adrenergic agonists to augment myocyte contractility [60, 162–165]. ATP stimulates an increase in cytosolic calcium and evidence for the involvement of inositol 1,4,5-trisphosphate (IP_3)-coupled $P2Y_2$ receptors and ion channel P2X receptors has been presented [162, 164–167]. Ap_4A, probably after degradation to ATP, increases force of contraction in human ventricular trabeculae [168].

The inotropic effects of ATP are dependent on both IP_3 and cAMP [169]. The inotropic effects of catecholamines acting on β-receptors are mediated by an increase of cAMP and antagonists of these receptors are important drugs for the treatment of hypertension and reduce mortality in congestive heart failure. Similarly, ATP stimulates an increase in cAMP in cardiac myocytes and may act in

synergy with the β_1-adrenergic agonist, isoproterenol, by differential activation of adenylyl cyclase isoforms [60, 170, 171]. However, the ATP receptor mediating this increase in cAMP has not been related to any particular P2 receptor subtype in cardiac cells [60]. It could be the P2Y$_{11}$ receptor that is additionally coupled to G$_s$ and activates adenylyl cyclase [60, 172–174]. mRNA for the P2Y$_{11}$ receptor has been detected in the human heart at high levels [161, 175].

The unstable agonist, UTP, has been shown to induce a positive inotropic effect in rat atria and in rat and guinea pig ventricular cardiomyocytes [164, 166, 176, 177]. Stable UTP and UDP analogues induce a pronounced inotropic effect on mouse cardiomyocytes [161]. The P2Y$_2$ receptor is the most abundantly expressed receptor with very low levels of the P2Y$_4$ receptor in the human heart [161], suggesting that the inotropic effects of UTP are mediated via P2Y$_2$ receptors, while the UDP effects are mediated via the P2Y$_6$ receptor [161]. These mechanisms are mediated via IP$_3$-mediated signalling.

In conclusion, the inotropic effects in man are mediated via P2Y$_2$ and P2Y$_6$ receptors and a P2Y$_{11}$-like receptor (Fig. 3). P2X receptors are probably also involved, since several subtypes are expressed [60, 160], but it has not been possible to perform a clear receptor characterisation due to lack of selective antagonists.
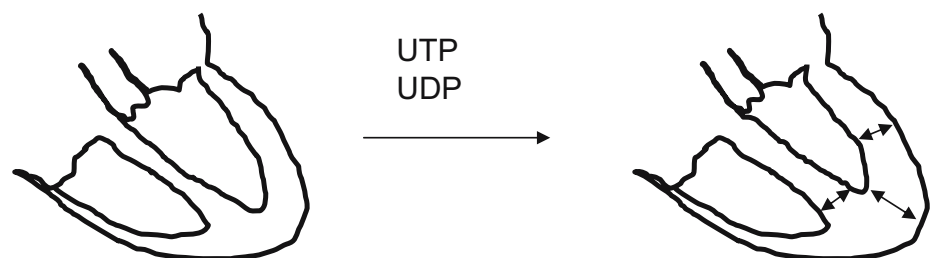
Myocardial infarction

Using microdialysis, ATP in the interstitial space has been estimated to be 40 nmol/l, but the levels may increase markedly during electrical stimulation, ischaemia, challenge with cardiotonic agents, increase in blood flow, mechanical stretch and increased work load [60]. ATP is released from

Fig. 3 Functional roles of purines and pyrimidines acting on P2 receptors in the regulation of the heart. See text for details. **a** ATP, UTP and UDP exert inotropic effects on cardiomyocytes leading to increased cardiac output. **b** UTP and UDP stimulate hypertrophy of cardiomyocytes, while ATP can have apoptotic effects. **c** UTP protects against ischaemic injury and cardiomyocyte cell death. ATP-degrading enzymes preserve endothelial integrity and protect against allograft rejection. *NTPDase* nucleoside triphosphate diphosphohydrolase
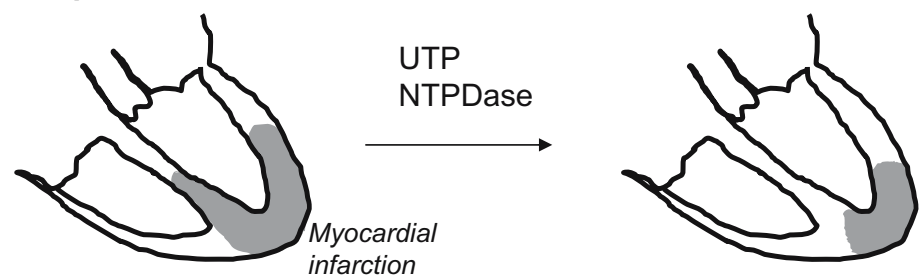
a *Inotropic effects*

Cardiac output

ATP
UTP
UDP

Cardiac output
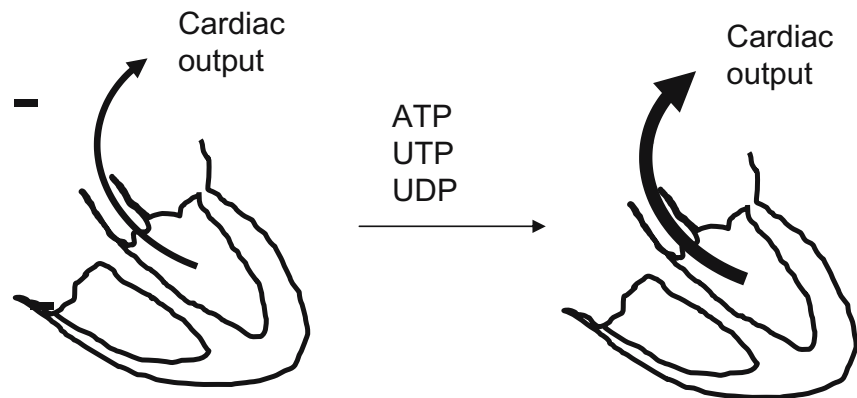
b *Hypertrophic effects*

UTP
UDP

c *Cardioprotective effects*

UTP
NTPDase

Myocardial infarction

cardiomyocytes during reduced oxygen tension [60]. UTP and ATP are released from the heart during cardiac ischaemia [178] and patients with myocardial infarction have higher plasma levels of both ATP and UTP [161, 179]. There is a significant increase in ectonucleotidase activity (NTPDase1) in the hearts of patients with ischaemic heart disease [180] that could represent a compensatory mechanism against increased nucleotide levels during chronic ischaemia.

Ischaemia-reperfusion provokes barrier failure of the coronary microvasculature, leading to myocardial oedema, ATP may protect against reperfusion-induced coronary endothelial barrier damage and inhibition of ATP degradation enhances the stabilising effect of ATP on barrier function [181].

The common $P2Y_{11}$ ATP receptor polymorphism, Ala-87-Thr, is associated with both increased CRP and increased risk of developing myocardial infarction [116]. Based on this association, we hypothesise that the $P2Y_{11}$ receptor plays an important role in cardiovascular biology and in inflammatory disease (see above), causing myocardial infarction via a proinflammatory effect. However, increased inotropic effects may also contribute.

An interesting yin and yang situation for ATP and UTP has been revealed regarding hypertrophic effects. UTP but not ATP causes hypertrophic growth in neonatal cardiomyocytes [182] (Fig. 3). In contrast, ATP inhibits hypertrophy and may even induce apoptosis and necrosis [163, 183]. The reason for the difference in effects could be due to activation of P2X receptors or cAMP stimulation by ATP [60, 161]. Both UTP and ATP transactivate epidermal growth factor receptors, but only ATP stimulates the hypertrophic marker genes atrial natriuretic peptide and myosin light chain 2 [184]. Similarly, UTP but not ATP protects cultured cardiomyocytes against hypoxic stress [185]. Since UTP is released during preconditioning [178], a role for UTP in the protective effects of preconditioning is plausible. Recently, Yitzhaki and co-workers were able to demonstrate prominent reductions in myocardial infarction size and improved rat heart function in vivo, by a single intravenous bolus dose of UTP before ischaemia [186] (Fig. 3).

Congestive heart failure

There are several reports of alterations or adaptations of purinergic signalling during congestive heart failure. The positive inotropic effect of ATP is impaired in heart failure, but reversed by the angiotensin-converting enzyme (ACE) inhibitor imidapril [187]. The contractile responses for the $P2Y_{11}$ receptor agonist AR-C67085 are decreased in heart failure, suggesting a downregulation of this receptor function in cardiomyocytes in a similar manner as seen for β_1-receptors in congestive heart failure [169]. In human

hearts, only the $P2X_6$ receptor was altered in congestive heart failure [188]. In congestive heart failure $P2X_1$ receptors are downregulated in VSMC in resistance arteries, which could represent a protective response against the increased sympathetic nerve activity and peripheral resistance seen in congestive heart failure [189].

Several P2 receptors have been suggested as targets for pharmacological treatment of congestive heart failure. Overexpression of $P2X_4$ receptors has a beneficial, life-prolonging effect in a heart failure model [190]. Both ATP and catecholamines are released from sympathetic nerves, acting through cAMP-stimulating receptors to mediate positive inotropic effects, stimulating the same intracellular mechanisms as adrenergic β-receptors. It is possible that agonists for the $P2Y_{11}$ receptor could be used to improve cardiac output in patients with circulatory shock. However, an even more important drug candidate would be a $P2Y_{11}$ receptor antagonist that may be beneficial in patients with congestive heart failure. The extracellular pyrimidines UTP and UDP may be inotropic factors in man, acting on $P2Y_2$ and $P2Y_6$ receptors stimulating the same intracellular pathways as angiotensin II. Synthetic agonists could thus be used as inotropic agents during circulatory shock and antagonists may have effects similar to angiotensin II receptor blockers, being beneficial in the treatment of hypertension and congestive heart failure.

Chronotropy and arrhythmia

It has been difficult to determine the chronotropic effects of ATP due to the dominating inhibitory effects of adenosine on the AV node. We know that ATP increases the contractile rate in neuron-myocyte co-cultures but the effect is markedly reduced in non-innervated myocyte cultures [191]. ATP could induce arrhythmia based on its increased automaticity and early after-depolarisations [60]. The $P2X_1$ receptor is colocalised with connexin 43 in gap junctions that transmit the contractile stimulus between cardiomyocytes [192]. However, so far no firm evidence exists of an important role for P2 receptors for chronotropy or arrhythmia.

Platelets and coagulation

Platelets

ADP, released from erythrocytes or produced after ectonucleotidase breakdown of ATP released from erythrocytes and endothelial cells, was found to cause platelet aggregation as early as 1961, before the concept of P2 receptors was conceived [193]. Later, the effects were attributed to a single receptor, designated P2T, but it was not until 1998 that the three receptors involved were characterised [194–196].

Two ADP receptors ($P2Y_{12}$ and $P2Y_1$) and one ion channel ATP receptor ($P2X_1$) are expressed on platelets [197, 198] (Fig. 4). The $P2Y_{12}$ receptor is coupled to inhibition of cAMP. The molecular identifications of the $P2Y_{12}$ receptor and generation of knockout mice revealed highly prolonged bleeding times, and their platelets aggregate poorly in response to ADP and display a reduced sensitivity to thrombin and collagen [199]. The $P2Y_1$ receptor is responsible for ADP-induced shape change and weak, transient aggregation [200–202], while the $P2Y_{12}$ receptor is responsible for the completion and amplification of the response to ADP and to all platelet agonists, including thromboxane A_2, thrombin and collagen. The platelets also express $P2Y_1$ receptors that have been shown in knockout mice to be of similar importance as $P2Y_{12}$ receptors [200, 203].

The physiological importance of the $P2X_1$ receptor is not fully elucidated. However, recent studies have demonstrated that $P2X_1$ receptors can generate significant functional platelet responses, independently and in synergy with other receptor pathways. In addition, studies in transgenic animals indicate an important role for $P2X_1$ receptors in platelet activation, particularly under conditions of shear stress and thus during arterial thrombosis [204]. Synergistic interaction between ATP and NA in stimulating platelet aggregation may have significant clinical implications and suggests a prothrombotic role for ATP in stress [205].

NTPDase1 is an important inhibitor of platelet activation. Unexpectedly, NTPDase1-deficient mice had prolonged bleeding times with minimally perturbed coagulation parameters. Platelet interactions with injured mesenteric vasculature were considerably reduced in vivo and purified mutant platelets failed to aggregate to standard agonists in vitro [206]. This platelet hypofunction was reversible and associated with $P2Y_1$ receptor desensitisation. In keeping with deficient vascular protective mechanisms, fibrin deposition was found at multiple organ sites in NTPDase1-deficient mice and in transplanted cardiac grafts. Endothelial ADPase activity is lost following ischaemia-reperfusion injury, xenograft rejection and inflammation resulting in increased levels of ATP and ADP [58, 207]. This may cause platelet aggregation but also other effects, such as mitogenic effects on VSMC. Infusion of systemic apyrase inhibits platelet aggregation and prolongs xenograft survival [208]. This has also been shown with adenoviral transfer of NTPDase1 [209]. NTPDase1 is also lost in vascular cardiac grafts subjected to oxidant stress. NTPDase2 only degrades ATP to ADP and has a prothrombotic effect. It is present in the subendothelium and can shift the balance in a prothrombotic direction after endothelial injury [210] (Fig. 1).

ADP acting on $P2Y_{12}$ receptors is not only important for platelet activation, it also stimulates vasoconstriction. Stable drugs with antagonistic effects on $P2Y_{12}$ receptors, affecting
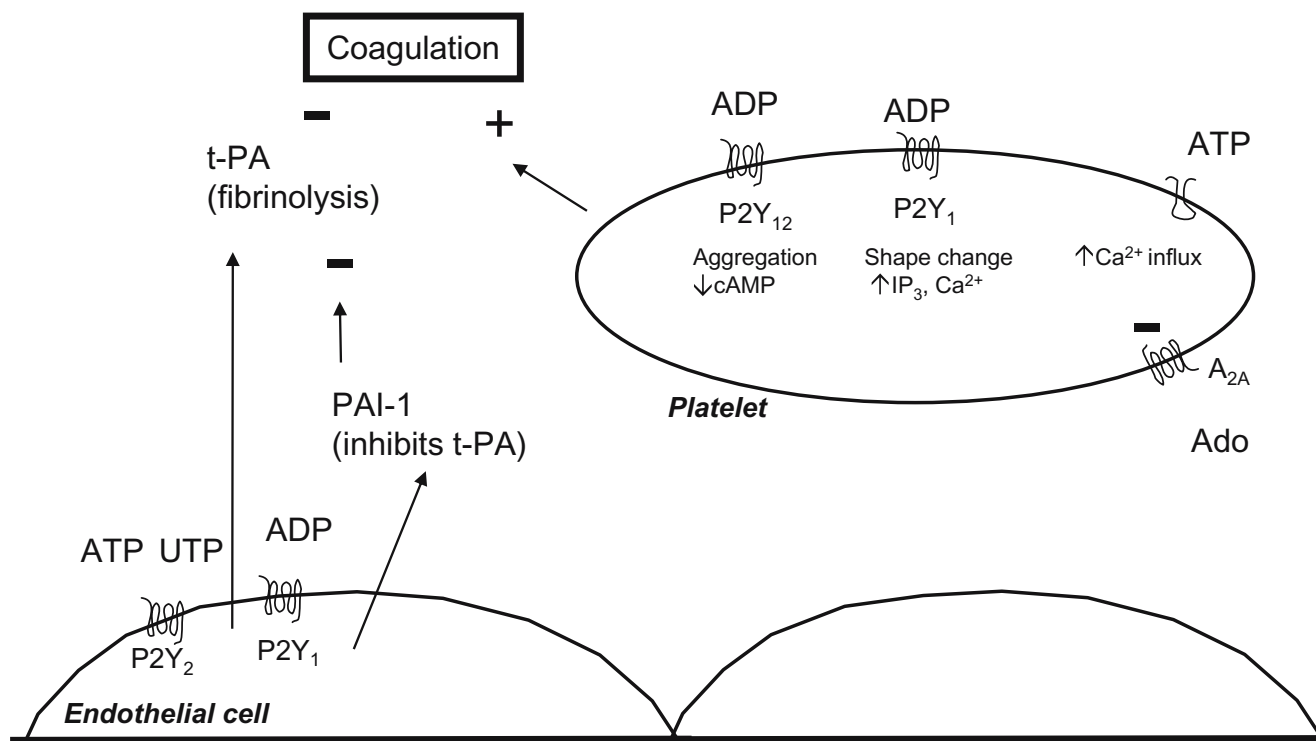

Fig. 4 P2 receptor-mediated regulation of coagulation and platelet aggregation. See text for details. ADP is an important mediator and positive feedback mechanism for platelet aggregation. Activated platelets stimulate coagulation, i.e. formation of a fibrin network. In contrast, ATP, ADP and UTP release tissue plasminogen activator (*t-PA*) from endothelial cells, resulting in degradation of the fibrin network. At the same time plasminogen activator inhibitor (*PAI-1*) is also released from endothelial cells by ATP stimulation, in turn inhibiting t-PA. Thus, P2 receptors regulate several balancing factors in haemostasis. *Ado* adenosine

both platelets and VSMC, could be of double therapeutic benefit in their prevention of both thrombosis and vasospasm [46].

Coagulation

The prothrombotic effect of ADP on the platelets is counteracted by the effects of UTP and ATP on the endothelium that stimulates a substantial release of tissue-type plasminogen activator (tPA) with fibrinolytic effects [51, 211] (Fig. 4). To make the picture even more complex, ATP also releases plasminogen activator inhibitor (PAI-1) which in turn inhibits tPA [212]. Thus, P2 receptors are involved at several stages of haemostasis, which is important for the

development of atherosclerosis and thrombotic occlusions leading to myocardial infarction and stroke.

Clinical importance of P2 receptor-mediated regulation of platelets

The first ADP inhibitors, ticlopidine and clopidogrel, were developed before the cloning and identification of the platelet ADP receptors. Later, it was concluded that they were prodrugs, converted in the liver to a metabolite that binds irreversibly to the $P2Y_{12}$ receptor resulting in non-competitive antagonism.

Ticlodipine has the disadvantage of a small risk of neutropaenia, so that clinically so far the most important

Table 4 Cardiovascular disease targets

Receptor target	Agonist/antagonist activity of therapeutic drug	Disease
Vascular smooth muscle cell		
$P2Y_2$	Antagonist	Hypertension, restenosis, diabetic microvascular disease, subarachnoidal bleeding
$P2Y_6$	Antagonist	Hypertension, restenosis, diabetic microvascular disease, subarachnoidal bleeding
$P2Y_{12}$	Antagonist	Hypertension, vasospasm
$P2X_1$	Antagonist	Hypertension, migraine, subarachnoidal bleeding
$P2Y_6$	Agonist	Migraine
SolNTPDase1	Ectonucleotidase	Restenosis, xenograft rejection
Endothelial cell		
$P2Y_2$	Antagonist	Atherosclerosis, inflammation
$P2X_4$	Antagonist	Vascular remodelling (cancer?), preeclampsia
Heart		
$P2Y_2$	Antagonist	Congestive heart failure
$P2Y_6$	Antagonist	Congestive heart failure
$P2Y_{11}$	Antagonist	Congestive heart failure, myocardial infarction
$P2Y_2$	Agonist	Shock, reduced cardiac output, cardiac protection
$P2Y_6$	Agonist	Shock, reduced cardiac output
$P2Y_{11}$	Agonist	Shock, reduced cardiac output
SolNTPDase1	Ectonucleotidase	Heart transplantation, myocardial infarction
Inflammatory cells		
$P2Y_2$	Antagonist	Atherosclerosis
$P2Y_{11}$	Antagonist	Atherosclerosis
$P2X_7$	Antagonist	Atherosclerosis
Platelets		
$P2Y_1$	Antagonist	Myocardial infarction, stroke, peripheral artery disease
$P2Y_{12}$	Antagonist	Myocardial infarction, stroke, peripheral artery disease
$P2X_1$	Antagonist	Myocardial infarction, stroke, peripheral artery disease
SolNTPDase1	Ectonucleotidase	Myocardial infarction, stroke, peripheral artery disease
Red blood cell		
$P2Y_{13}$	Antagonist	Improved peripheral circulation
$P2X_7$	Antagonist	Shock
Perivascular nociceptive sensory nerves		
$P2Y_1$	Agonist	Migraine, vascular pain
$P2X_3$	Antagonist	Migraine, vascular pain
Perivascular sympathetic nerves		
$P2Y_1$	Agonist	Hypertension
$P2X_2$	Antagonist	Hypertension

The table presents possible targets for the development of new pharmaceutical compounds for the treatment of cardiovascular diseases

contribution of drug development aimed at P2 receptors has been the beneficial effects of the platelet ADP receptor antagonist clopidogrel in atherosclerotic disease. In the CAPRIE trial, clopidogrel was even more effective compared to aspirin in preventing myocardial infarctions [213]. Clopidogrel, as an addition to aspirin, turned out to be necessary to prevent acute in-stent thrombosis in percutaneous coronary interventions. The CURE trial demonstrated that the addition of clopidogrel to aspirin reduced clinical events by 20% in acute coronary syndromes [213, 214]. This has also been shown for ST-elevation myocardial infarctions (COMMIT, CLARITY). However, long-term secondary prevention in high-risk groups with clopidogrel added to aspirin did not have significant benefits (CHARISMA). Clopidogrel combined with aspirin reduces cerebral emboli in patients undergoing carotid endarterectomy [215]. Clopidogrel (Plavix®) is now a blockbuster drug and one of the most sold drugs worldwide to the benefit of atherosclerotic patients. Nevertheless, clopidogrel is a rather weak antagonist at $P2Y_{12}$ receptors with variable effects, often referred to as clopidogrel resistance. Newer $P2Y_{12}$ receptor antagonists, such as prasugrel and AZD6140, have a stronger more consistent effect and are currently being studied in major clinical trials (TRITON and PLATO).

Clinical importance of purinergic signalling in cardiovascular disease: future directions

The P2 receptor family provides opportunities for drug development (see Table 4). The large number of receptor subtypes and the increasing knowledge of their tissue distributions may make it possible to target a specific cardiovascular organ with limited side effects. So far, only $P2Y_{12}$ receptor antagonists have been explored in patients, but with extraordinary success. Clopidogrel is one of the most prescribed drugs in the world for the treatment of thrombosis, stroke and myocardial infarctions in millions of patients. The following compounds, prasugrel and AZD6140, are even more potent $P2Y_{12}$ receptor antagonists with clinical benefits currently being tested in phase III trials. Improved platelet inhibition can also be achieved with antagonists against the other two P2 receptors on platelets. Studies in $P2Y_1$ and $P2X_1$ receptor knockout mice and experimental thrombosis models using selective $P2Y_1$ and $P2X_1$ receptor antagonists have shown that, depending on the conditions, these receptors could also be potential targets for new antithrombotic drugs.

ATP, UTP and UDP, acting on $P2Y_2$ and $P2Y_6$ receptors, are potent stimulators of inflammation, VSMC proliferation and migration. Antagonists to these receptors may be explored to protect against atherosclerosis, restenosis after balloon angioplasty, chronic transplant rejection and diabetic microvascular disease.

It is possible that hypertension and pulmonary hypertension could be treated with $P2X_1$, $P2Y_2$, $P2Y_6$ or $P2Y_{12}$ receptor antagonists. In the heart $P2Y_2$, $P2Y_6$ and $P2Y_{11}$ receptor antagonists may have similar beneficial roles as angiotensin inhibition or β-blockers in the treatment of congestive heart failure. In the acute phase of a myocardial infarction, a UTP analogue could protect cardiomyocytes and limit infarct size. A polymorphism in $P2Y_{11}$ receptors could be considered as a prognostic marker to identify patients at risk of myocardial infarction and drugs aimed at the receptor could be explored to prevent myocardial infarction.

A promising strategy would be to use soluble NTPDase to prevent thrombosis, restenosis and xenograft transplantation. SolNTPDase1 protects against brain damage in a stroke model and a human genetically engineered solNTPDase has been developed that could be used in clinical trials. Possible clinical indications could be stroke, myocardial infarction and transplantation. $P2X_3$ receptor antagonists could be used against migraine and vascular pain [see 19, 26].

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