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P2 receptors in atherosclerosis and postangioplasty restenosis

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Abstract Atherosclerosis is an immunoinflammatory process that involves complex interactions between the vessel wall and blood components and is thought to be initiated by endothelial dysfunction [1–3]. Extracellular nucleotides that are released from a variety of arterial and blood cells [4] can bind to P2 receptors and modulate proliferation and migration of smooth muscle cells (SMC), which is known to be involved in intimal hyperplasia that accompanies atherosclerosis and postangioplasty restenosis [5]. In addition, P2 receptors mediate many other functions, including platelet aggregation, leukocyte adherence, and arterial vasomotoricity. A direct pathological role of P2 receptors is reinforced by recent evidence showing that up-regulation and activation of $P2Y_2$ receptors in rabbit arteries mediates intimal hyperplasia [6]. In addition, up-regulation of functional P2Y receptors also has been demonstrated in the basilar artery of the rat double-hemorrhage model [7] and in coronary arteries of diabetic dyslipidemic pigs [8]. It has been proposed that up-regulation of P2Y receptors may be a potential diagnostic indicator for the early stages of atherosclerosis [9]. Therefore, particular effort must be made to understand the consequences of nucleotide release from cells in the cardiovascular system and the subsequent effects of P2 nucleotide receptor activation in blood vessels, which may reveal novel therapeutic strategies for atherosclerosis and restenosis after angioplasty.

Key words atherosclerosis · inflammation · migration · nucleotide receptors · proliferation · restenosis · smooth muscle cell

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Introduction

Atherosclerosis is a pathological phenomenon primarily affecting the large conduit arteries, for example, the aorta and coronary, carotid iliac, and femoral arteries. Development of atherosclerotic lesions in arteries involves intimal recruitment of smooth muscle cells (SMC) within the blood-vessel wall and also infiltration of blood-derived cells [1]. This process necessitates the proliferation and migration of SMC from the underlying media and the endothelial adhesion of leukocytes and their infiltration into the subendothelium. A similar intimal accumulation of SMC also takes place during the postangioplasty restenotic process. Although the factors involved in intimal-cell recruitment are not clearly identified, it is becoming evident that endothelial dysfunction is a key factor in the development of vascular disease. Experimental evidence suggests that an intact endothelium plays a central role in maintaining a low proliferative state of SMC under normal conditions [10]. In arterial injury, endothelial cells, SMC, and various blood cells can release chemotactic factors and mitogens, including ATP and other nucleotides [4]. Activation of P2 nucleotide receptors has been shown to induce not only the proliferation and migration of vascular SMC but also apoptosis (programmed cell death), a process involved in the evolution of atherosclerotic plaque [11]. In

addition, P2 receptors mediate both vasorelaxation and vasoconstriction of arteries that may be involved in the vascular remodeling accompanying atherosclerosis and postangioplasty restenosis [5]. A better understanding of the causative agents and mechanisms of proliferation and migration of vascular SMC as well as recruitment of blood-derived cells by the endothelium could lead to prevention, attenuation, or even reversal of intimal thickening, which may dramatically reduce morbidity and mortality from vascular diseases such as atherosclerosis and restenosis after angioplasty. In this respect, a better understanding of the physiological role of P2 receptors in both normal and pathological blood vessels could potentially lead to a breakthrough in the fight against vascular disease.

P2 receptors in the cardiovascular system

Extracellular nucleotides bind to cell-surface receptors known as P2 receptors, which are present in many tissues. To date, these receptors have been classified into two main families: the P2X receptors that are ligand-gated ion channels comprised of homo- or hetero-oligomers [12], and P2Y receptors that are seven-membrane-spanning receptors coupled via G proteins ($G_{q/11}$ or $G_{i/o}$) to phospholipase C (PLC) and/or adenylate cyclase [12–14]. In turn, PLC activation generates inositol 1,4,5-triphosphate (IP_3), a mediator of Ca^{2+} release from intracellular stores, and diacylglycerol, an activator of protein kinase C (PKC) whereas adenylate cyclase generates cyclic AMP, an activator of protein kinase A (PKA). The cloning of seven P2X ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, $P2X_7$) and eight P2Y ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) receptor subtypes has made it possible to use molecular and pharmacological approaches to study the distribution and functional properties of specific P2 receptor subtypes at the tissue and cellular level.

P2 receptors in vascular cells

The normal arterial wall consists of three layers: intima, media, and adventitia. The single layer of endothelial cells facing the vessel lumen is a very important component of the vascular wall in terms of releasing both vasodilators such as nitric oxide (NO) and prostacyclin (PGI_2), and vasoconstrictors such as thromboxane A_2 and endothelin. The principal P2Y receptor subtypes that have been functionally characterized in endothelial cells are $P2Y_1$ and $P2Y_2$, but mRNAs for $P2Y_4$ and $P2Y_6$ receptors have also been detected [4]. Endothelium-dependent vasorelaxation has been attributed to the release of NO and PGI_2 after binding of nucleotides to $P2Y_1$ and $P2Y_2$ receptors in endothelial cells [15] whereas vasoconstrictor effects in SMC result from the action of nucleotides on $P2Y_2$ and P2X receptors [16, 17]. In most blood vessels, $P2Y_1$ receptors for ADP are present on the endothelium and regulate vasodilatation by Ca^{2+}-dependent (PLC-mediated) activation of NO synthase (NOS) and generation of endothelial-dependent relaxing factor (EDRF) [4]. Endothelial prostacyclin production is also stimulated by $P2Y_1$ and $P2Y_2$ receptors, but this seems to play a minimal role in vasodilatation, at least under physiological conditions [18]. Recent studies have indicated that in the aorta of $P2Y_2$-null mice, the endothelium-dependent relaxation by ATP and ATPγS was inhibited, demonstrating the role of the $P2Y_2$ receptors, but that a relaxation by UTP and UDP was maintained, suggesting the additional involvement of $P2Y_6$ receptors [19]. The majority of cells in intact blood vessels are SMC, which occupy most of the media and are involved in vessel vasoconstriction and vasorelaxation. $P2Y_2$Rs in SMC mediate the induction of immediate-early and delayed-early cell-cycle-dependent genes, consistent with a role for $P2Y_2$Rs in vascular proliferation of SMC [20, 21]. A recent study demonstrated that $P2Y_2$ is the predominant functional receptor that responds to ATP and UTP in rat aortic SMC [22]. In human cerebral arteries, $P2Y_6$ seems to be the predominant subtype and induces vasoconstriction when activated by UDP/UTP [23, 24]. This is consistent with findings from rat pulmonary and mesenteric arteries [25, 26]. In addition, a recent study on $P2X_1$ knock-out mice further supports the prominent contractile effect of the $P2Y_6$ subtype in mesenteric arterial trees [27]. Taken together, it seems that the principal receptor mediating UTP/UDP-induced contractile responses in blood vessels might be the $P2Y_6$ subtype. Other studies have reported the presence of both $P2Y_4$ and $P2Y_6$ receptors in rat aortic SMC [21, 28, 29]. $P2Y_1$ receptors are expressed in SMC of a number of blood vessel types and, like their endothelial cell counterparts, mediate vasodilatation most likely through the activation of K^+ channels [18]. The presence of several P2X receptor subtypes also has been reported in human saphenous vein SMC, including $P2X_1$, $P2X_2$, $P2X_4$, and $P2X_7$ receptors [30]. The outermost layer of the blood vessel consists of connective tissue and fibroblasts, which have not been appreciated in the regulation of vascular tone. A recent study showed that fibroblasts can migrate into the neointima, suggesting their possible involvement in the development of vascular diseases such as atherosclerosis and restenosis after angioplasty [31]. Since human and rat fibroblasts are known to express $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2X_3$, $P2X_4$, and $P2X_7$ receptors [32], further investigation is needed to establish the role of fibroblast P2 receptors in either physiological or pathophysiological conditions.

P2 receptors in blood cells

G-protein-coupled P2Y receptors the activation of which leads to intracellular calcium mobilization have been observed in neutrophils [4, 33, 34], and turkey erythrocytes express both $P2Y_1$ and $P2X_7$ receptors [35, 36]. ATP and UTP act as secretagogues by binding to $P2Y_2$Rs to enhance exocytosis of primary granules in neutrophils [37]. In macrophages, ATP activates a $P2X_7$ receptor that differs from other ligand-gated ion-channel P2X receptors by its ability to generate plasma membrane pores when activated [38]. Human T lymphocytes also have been shown to express $P2X_7$ receptors [39]. Human monocytes and macrophages coexpress $P2X_1$, $P2X_4$, and $P2X_7$ receptors whereas granulocytes only express $P2X_7$ receptors [40]. $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors also are expressed in monocytes, B lymphocytes, and polymorphonuclear granulocytes [41]. Human platelets express $P2Y_1$, $P2Y_{12}$, and $P2X_1$ receptors [42–44]. Thus, the diversity of P2-receptor expression in blood cells, as well as endothelial cells, SMC and fibroblasts, suggests that this receptor superfamily plays a significant role in the regulation of cardiovascular functions.

P2 receptors regulate nucleotide-induced vascular smooth muscle cell proliferation

Proliferation of SMC is a hallmark of vascular diseases such as atherosclerosis and restenosis following angioplasty. ATP and other nucleotides are released by aggregating platelets and damaged vascular cells, such as endothelial cells and SMC in pathological or stress conditions [45]. Extracellular nucleotides can act via P2X and P2Y receptors to induce acute responses such as regulation of vascular tone [46]. However, it is generally believed that the ionotropic P2X receptors do not mediate the chronic responses of nucleotides, such as cell proliferation. The mitogenic effect of extracellular nucleotides on vascular SMC (VSMC) has been known for years [47]. However, a potent antiproliferative effect of UTP on VSMC also has been reported in human arterial and venous SMC [48]. In either case, the P2 receptor subtype(s) responsible for these effects on the proliferation of VSMC has not been determined. Earlier studies have shown that ATP or UTP increases DNA and protein synthesis in subcultured rat aortic VSMC [49, 50]. In the same cell-culture model, however, Malam-Souley et al. [51] were unable to detect increases in DNA synthesis after ATP/UTP stimulation although ATP or UTP up-regulated the expression of mRNA to several cell-cycle, progression-related genes. Because P2X receptor agonists were essentially inactive, it was concluded that a P_{2U}-like receptor (now termed $P2Y_2$) was responsible for the mitogenic effects of ATP/UTP. However, the role of a $P2Y_4$ receptor cannot be excluded because the nucleotide agonist profile between rat $P2Y_2$ and $P2Y_4$ receptors is essentially indistinguishable [52]. Indeed, Harper et al. [53] suggested that the $P2Y_4$ receptor mediated ATP/UTP-induced proliferation of rat aortic VSMC. Recent studies indicated that ATP, UTP, or ITP, three agonists of the cloned porcine $P2Y_2$ receptor, increased DNA and protein synthesis and cell number in coronary artery SMC [54]. Indeed, treatment of pig coronary artery SMC with UTP, ATP, or ITP caused a concentration-dependent increase in DNA and protein synthesis and cell number whereas UDP only caused a small increase in protein synthesis. Intriguingly, ATP was much more potent and efficacious than UTP, ITP, and UDP in increasing DNA synthesis and expression of PCNA, a protein marker of cell proliferation, suggesting that another receptor may contribute to the proliferative response [54]. In vivo experiments have shown that intimal thickening of collared rabbit carotid arteries was greatly enhanced by in situ UTP application and was closely associated with osteopontin expression in medial SMC [6]. Osteopontin is chemotactic for SMC and is associated with arterial SMC proliferation [55]. Moreover, both UTP and ATP increased osteopontin expression in cultured SMC whereas ADP, UDP, and 2-MeSATP were ineffective, which suggests a role for the $P2Y_2$R in which ATP and UTP are equipotent. Direct evidence for involvement of the $P2Y_2$R was provided by inhibition of UTP-induced osteopontin expression in cultured SMC by $P2Y_2$ antisense oligonucleotides [6]. $P2Y_6$ receptors also have been shown to mediate proliferation of SMC in rat aorta [56].

Role of P2 receptors in the migration of vascular SMC

Recent studies indicate that the extracellular nucleotides ATP, ADP, UTP, and UDP serve as directional cues for migration of rat aortic SMC [57]. At identical concentrations, the most powerful migratory response induced by these nucleotides was elicited by UTP. Nucleotide-induced migration of SMC is the consequence of both chemotaxis and chemokinesis and may result either from activation of one particular P2 nucleotide receptor subtype or of several P2 receptor subtypes. The ability of UTP at submicromolar levels to stimulate migration of SMC supports the hypothesis that this response could have physiological consequences and is essentially mediated by $P2Y_2$ receptor activation without excluding participation of other P2Y receptor subtypes. The difference in the capacity of UTP and ATP to elicit migration of SMC could be due to inhibition of nucleotide-induced cell migration by adenosine generated from ATP catabolism by cell-surface ectonucleotidases.

Indeed, ATP and UTP were equally effective in causing migration of SMC when ATP was prevented from degradation by addition of the ectonucleotidase inhibitor α,β-methylene-ATP [57]. Several P2Y receptor subtypes could be involved in nucleotide-induced migration of SMC. It has been shown in rat aortic SMC that the $P2Y_2$ receptor is the predominant P2Y receptor subtype [28, 58] whereas lower levels of $P2Y_4$ and $P2Y_1$ receptor mRNA were detected [58]. The very low level of $P2Y_1$ receptor mRNA expression was consistent with the absence of ADP-induced migration of cultured rat aortic SMC, demonstrating that $P2Y_1$ is not involved in this process [58]. In addition, the same study showed that a commercially available solution of hexokinase-treated UDP (UTP-free) induced cell migration equally as well as untreated UDP, thereby demonstrating that UDP is chemotactic for aortic SMC by activation of the $P2Y_6$ receptor. Conversely, migration of rat aortic SMC induced by UTP occurred even when UTP degradation by nucleoside diphosphate kinase was inhibited, demonstrating the involvement of $P2Y_2$ and/or $P2Y_4$ receptor(s) [58].

The increased migration of SMC in response to extracellular nucleotides could be related to increases in extracellular matrix (ECM) protein expression. Indeed, previous studies have shown that UTP induces osteopontin expression in rat and rabbit aortic SMC [6, 51]. Increased expression of osteopontin, an RGD-containing ECM protein, is associated with the activation of rat arterial SMC in vitro and in vivo [57]. The increase in osteopontin expression plays a key role in UTP-induced migration of rat aortic SMC since a monoclonal antibody against osteopontin fully abolished UTP-induced migration [57] whereas an antibody against vitronectin, another ECM protein also involved in migration of human SMC [59], had no effect on the migration of rat aortic SMC [57]. UTP induces increases in osteopontin mRNA expression by increasing both osteopontin mRNA stabilization and osteopontin promoter activity [60]. Recent studies have shown that activation of an AP-1 binding site located 76 bp upstream of the transcription start in the rat osteopontin promoter is involved in UTP-induced osteopontin expression. Using a luciferase promoter deletion assay, Renault et al. [61] identified a new region of the rat osteopontin promoter (−1837 to −1757) that is responsive to UTP. This region contains an NFB site located at −1800 and an Ebox located at −1768. Supershift electrophoretic mobility shift and chromatin immunoprecipitation assays identified NFB and USF-1/USF-2 as DNA-binding proteins induced by UTP. Using dominant negative mutants of IB kinase and USF transcription factors, it was confirmed that NFB and USF-1/USF-2 are involved in the UTP-induced expression of osteopontin.

This ability of nucleotides to act as chemoattractants for rat arterial SMC in a concentration range potentially found in pathological vessels [62] and the findings of previous studies demonstrating the mitogenic activity of extracellular nucleotides for these cells suggest that nucleotides released from mechanically stretched vascular or damaged cells during the angioplasty process may participate in arterial-wall remodeling.

Role of P2 receptors in nucleotide-induced vascular inflammation

In addition to their mitogenic effects, extracellular nucleotides may also cause cell recruitment by inducing lymphocyte and macrophage adhesion to human pulmonary artery endothelial cells, as demonstrated in vitro [63]. Nucleotides can also modulate rat aortic SMC adhesion and migration by increasing the expression of osteopontin [51, 57], a protein involved in both processes. Moreover, extracellular nucleotides may play a role in intra-arterial attraction of monocytes by inducing an increased expression of monocyte-chemoattractant protein-1 by arterial SMC [21]. Stimulation of P2 receptors is coupled to the release of the proinflammatory cytokines IL-1β, IL-1α, IL-8, and TNF-α [4] that are of obvious relevance to inflammation in atherosclerosis. Activation of $P2X_7$ receptors on monocytes/macrophages enhances release of proinflammatory cytokines that modulate NO production and expression of inducible NO synthase (iNOS) [64], mediators of immune cell activation that is an early step in atherosclerotic lesion development.

Monocyte recruitment into the vessel wall is a complex process that includes cell rolling, firm attachment, and directed migration. It is now becoming evident that adhesion molecules such as VCAM-1 play an important role in leukocyte adherence to vascular endothelial cells [65, 66]. VCAM-1 expression is induced or up-regulated by proinflammatory cytokines such as TNF-α and IL-1β in cellular components of the arterial wall, including endothelial cells, SMC, and fibroblasts [67–69]. ATP and UTP have been shown to induce cell–cell adhesion in a human monocyte/macrophage lineage and neutrophil adherence to human endothelial cell monolayers [63, 70].

Recent studies have shown that local UTP delivery via an osmotic pump to collared rabbit carotid arteries induced intimal accumulation of macrophages, similar to oxidized low-density lipoprotein (LDL), a response that was mediated by activation of $P2Y_2$ receptors [6]. Leukocyte migration depends on the activities of adhesion proteins (e.g., selectins and integrins) on leukocytes and vascular endothelial cells. We demonstrated that activation of $P2Y_2$ receptors in endothelial cells causes expression of VCAM-1 that mediates adherence of monocytes to vascular endothelium [71], leading to their penetration into the

vessel wall to promote arterial inflammation associated with atherosclerosis.

Recent studies revealed that a Src homology-3 (SH3)-binding domain in the C-terminal tail of the $P2Y_2$ receptor promotes nucleotide-induced association of Src with the $P2Y_2$ receptor, leading to transactivation of growth factor receptors, such as the EGF and VEGF receptors, and nucleotide-induced up-regulation of VCAM-1 [72, 73]. Since leukocyte infiltration and migration are key processes involved in atherosclerosis, these findings suggest that $P2Y_2$ receptors represent a novel target for reducing arterial inflammation associated with cardiovascular disease.

P2 receptors in vascular apoptosis

Apoptosis has been reported to occur in various vascular diseases, such as atherosclerosis, restenosis, and hypertension [74, 75]. The major cell types undergoing apoptosis in human atherosclerotic lesions are arterial SMC [11, 76–78] and macrophages [79]. In restenosis following balloon angioplasty, there is a peak in the proliferation and apoptosis of rat vascular SMC 14 days postangioplasty [76]. Furthermore, apoptosis of arterial SMC has been described in animal models of intimal thickenings [80] and probably takes part in the normal process involved in the control of hyperplasia. In contrast, apoptosis of SMC in advanced human atherosclerotic plaques may destabilize the fibrous lesion to promote plaque rupture and its clinical consequences.

As a mediator of cell-to-cell communication, ATP can trigger a variety of biological responses after being rapidly released in large amounts from various sources, including activated platelets, endothelial cells, nerve terminals, antigen-stimulated T cells, and other cell types following hypoxia, stress, and tissue damage. For example, in human umbilical cord vein endothelial cells (HUVEC), substantial release of ATP (and UTP) is induced by shear stress [81], which may lead to alterations in the balance between proliferation and apoptosis regulated by P1 adenosine and P2 (particularly $P2X_7$) nucleotide receptors [82]. $P2X_7$ and P1 receptors have been previously linked to apoptosis in other cell types, including immune cells, astrocytes, and thymocytes [83–85]. The $P2X_7R$ also has been shown to mediate ATP-induced cell death in human embryonic kidney cells [86], human cervical epithelial cells [87], and primary rat cortical neurons [88]. In human arterial SMC, adenosine-induced apoptosis is essentially mediated via the A_{2b}-adenosine receptor subtype and involves a cAMP-dependent pathway [89].

As an important constituent of atherosclerotic plaques, fibroblasts share several features with SMC. In human fibroblasts, $P2X_7$ was identified as the main nucleotide receptor involved in the high glucose concentration-dependent responses modulated by ATP, including morphological changes, enhanced apoptosis, caspase-3 activation, and IL-6 release [90]. In the immune system, ATP also plays important roles through nucleotide receptors in leukocyte functions. $P2X_7$-receptor-mediated apoptosis has been demonstrated in various types of leukocytes, including a lymphocytic cell line, murine thymocytes, murine peritoneal macrophages, human macrophages, mesangial cells, dendritic cells, and microglial cells [83, 91–96]. Extracellular ATP acting via the $P2X_7$ receptor activates the transcription factor NF-κB by selectively targeting NF-κB p65 (Rel A) in the N9 mouse microglial cell line [97]. It also has been reported that the $P2X_7$ receptor modulates macrophage production of TNF-α, IL-1β and NO following LPS exposure [98], consistent with a role for the $P2X_7$ receptor in inflammation. In HUVEC, TNF-α markedly increases apoptotic cell death via the activation of caspase-3 [74]. Recent reports indicate that ATP/ADP activate NF-κB and induce apoptosis, probably through $P2X_7$ receptors in porcine aortic endothelial cells [99]. These studies have provided compelling evidence suggesting a role for P2- and P1-receptor-mediated apoptosis in vascular diseases; however, further studies are needed to determine the precise pathways involved and to accumulate direct evidence that these pathways contribute significantly to the development of atherosclerosis, hypertension, and restenosis.

Modulation of P2 receptors in vascular injury

Experimental arterial intimal hyperplasia can be induced by balloon angioplasty or by perivascular placement of a silicone collar around an artery. An influx of leukocytes precedes migration and proliferation of vascular SMC into the intima in both these models [100]. In normal adult rat aorta, $P2Y_2$ mRNA was found in endothelial cell lining while a sustained expression of $P2Y_2$ mRNA was detected in a few medial SMC [20]. In contrast, $P2Y_2$ mRNA was detected in all medial SMC of rat fetal aortas and in most aortic SMC of intimal lesions after balloon angioplasty, with an overexpression in cells lining the lumen both 1 and 3 weeks after injury.

In the collar model, neointimal formation appears to be triphasic [100]. The first phase is characterized by vascular infiltration of leukocytes beginning 2 h after collar placement around a rabbit carotid artery. The second phase begins within 12 h of collar placement and is characterized by medial replication of SMC. The third phase is characterized by the appearance, beginning at day 3 after collar placement, of subendothelial SMC. In situ hybridization with sham-operated rabbit carotid arteries indicated that $P2Y_2$ mRNA expression was localized to CD31-

positive aortic endothelial cells and not medial SMC [6]. High levels of $P2Y_2$ mRNA were detected in medial SMC 3 days after collar placement, before appearance of neointima. At day 14, all intimal and medial SMC were $P2Y_2$ positive. Fura-2 digital imaging of single SMC, used to measure changes in myoplasmic calcium concentration in response to P2Y receptor agonists, confirmed an increase in $P2Y_2$ receptor activity. However, the same study showed that $P2Y_4$ mRNA was equivalently expressed in sham-operated and collared arteries or cultured rabbit carotid SMC whereas $P2Y_6$ mRNA was not detected in carotid arteries or cultured SMC. In a more recent study, it was shown that $P2Y_2$ receptor up-regulation occurs in stented porcine coronary artery, a clinically relevant model of arterial injury [54]. $P2Y_2$ receptor mRNA levels were significantly increased in coronary SMC dispersed from stented segments of coronary arteries 3 weeks after stent angioplasty compared with SMC from unstented segments. There was no significant difference observed in levels of $P2Y_6$ mRNA in the stented and unstented artery segments whereas $P2Y_4$ receptor mRNA was undetectable.

Up-regulation of functional P2Y receptors also occurs in the basilar artery of the rat double-hemorrhage model [7], in the coronary artery of diabetic dyslipidemic pigs [8], and in human atherosclerotic lesions (Seye and Desgranges, unpublished data). It has been proposed that up-regulation of P2Y receptors could be a potential diagnostic indicator for the early stages of atherosclerosis [9]. Interestingly, a more recent study showed that high shear stress associated with vascular diseases can selectively up-regulate $P2Y_2$ and $P2Y_6$ receptors in perfused arterial SMC [101]. $P2X_1$ and $P2X_4$ receptors have been shown to be up-regulated in rabbit intimal thickenings [102]. Taken together, these findings strongly suggest that at least some P2 receptor subtypes (most notably $P2Y_2$) are implicated in the development of vascular disease.

Pathophysiological significance of P2 receptor modulation in vascular injury

Migrating and proliferating SMC in the arterial media, together with infiltrating macrophages and T lymphocytes, are the main cell types that comprise atherosclerotic and restenotic lesions [1]. In the early stages of intimal hyperplasia, SMC are modified from a differentiated, contractile phenotype to an immature, synthetic phenotype, and this enables them to migrate into the intima, proliferate, and secrete extracellular matrix components. In many respects, this shift in phenotype is a reversal of the normal differentiation pattern of vascular SMC during fetal and early postnatal life [103, 104]. However, there is still a lack of knowledge concerning the phenotypic regulation of SMC during vasculogenesis and vascular disease. A high level of P2 receptor expression could be related to the altered phenotype of SMC in intimal thickenings. Partially dedifferentiated SMC are found in rat arterial intimal lesions after balloon angioplasty [105, 106], and their phenotype has been compared with that of newborn rat aortic SMC [107]. Interestingly, it has been reported that medial SMC of rat embryonic aorta also exhibit high $P2Y_2$ expression similar to intimal thickenings [20]. Others studies have shown that $P2Y_1$ and $P2Y_2$ receptor transcripts are strongly up-regulated with phenotypic changes in rat SMC whereas $P2X_1$ mRNA is completely down-regulated and $P2Y_4$ and $P2Y_6$ mRNA levels are unchanged [28, 58]. Taken together, these and other results suggest that $P2Y_2$ receptor expression is up-regulated in the entire cell population of intimal thickenings and is closely associated with a poorly differentiated phenotype of SMC. The dramatic increase in $P2Y_2$ mRNA expression observed in balloon angioplasty-induced intimal lesions would suggest increased activity of extracellular nucleotides with consequent enhancement of cell proliferation and vasoreactivity. Indeed, extracellular nucleotides, particularly ATP and UTP, have been shown to induce cell-cycle progression and proliferation of cultured arterial SMC [21, 49, 50, 51] and vasoconstriction in the absence of endothelial cells [17, 108, 109]. Since both neointimal hyperplasia and vasoconstrictive remodeling have been found to be involved in postangioplasty restenosis [1, 5, 107, 110], these findings suggest that extracellular nucleotides may play a significant role in this process, at least as long as functional endothelial cells, which regulate intimal thickening [111, 112] and nucleotide-induced vasorelaxation [113, 114], are not regenerated.

Increased $P2Y_2$ receptor expression in the neointima may by itself be sufficient to enhance local effects of extracellular nucleotides on proliferation of SMC. Although expression of other P2 receptors has been described in arterial SMC [21, 29, 115], the $P2Y_2$ receptor seems to be more specifically involved in the response of SMC to ATP and UTP [116], particularly in potentiation of proliferation [21, 50]. Effects of extracellular nucleotides are not only dependent on the nature and number of P2 receptors present on target cells but also on local concentrations of nucleotide agonists. Although in vivo concentrations of extracellular nucleotides are difficult to measure, various in vitro experiments suggest that extracellular nucleotides are released from blood and vascular cells exposed to various physicochemical conditions, e.g., stress, hypoxia, and other factors [117–119] associated with the angioplasty process.

$P2Y_2$ receptors in SMC are involved in nucleotide-induced constriction of normal arteries [17, 108, 109]. Long-lasting alterations in vasomotricity after endothelial-cell denudation, resulting in increased sensitivity to

vasoconstrictive substances, have previously been demonstrated [113, 114]. It appears that, like other receptors for vasoconstrictive factors such as angiotensin II [120], endothelin [121], and PDGF [122], which are overexpressed in neointima, $P2Y_2$ receptors may play an important role in controlling vasoactive properties of pathological arteries, particularly in chronic constriction at the lesion site that is postulated to be one of the processes leading to postangioplasty restenosis [5, 110].

Conclusion

P2 receptor subtypes, including $P2Y_2$, $P2X_1$ and $P2X_4$, appear to play a role in responses to endothelial injury that are thought to be key events in the initiation of atherosclerosis and restenosis after angioplasty. $P2Y_2R$ up-regulation and activation in endothelial cells and SMC promote leukocyte transmigration and intimal thickening in arteries of animal models of vascular injury, suggesting a possible regulatory role for this receptor in mechanisms leading to neointimal hyperplasia after angioplasty. Although there are no selective $P2Y_2$ receptor antagonists yet available, recent progress in small interfering RNA (siRNA) technology has made it possible to design small RNA interference molecules that can selectively inhibit P2 receptor subtype expression. Such molecules can be efficiently delivered into the vessel wall using adenoviral vectors. In addition, P2 receptor transgenic mice (i.e., mice in which the relevant receptor subtype has been deleted or overexpressed) will be valuable tools to substantiate the role that nucleotides and P2 receptors play in the etiology of cardiovascular disease. Further delineation of signaling pathways involved in these P2 receptor-mediated processes may help limit or prevent vascular diseases such as atherosclerosis and restenosis after angioplasty.

References

1. Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362(6423):801–809
2. Fuster V, Badimon L, Badimon JJ, Chesebro JH (1992) The pathogenesis of coronary artery disease and the acute coronary syndromes (1). N Engl J Med 326(4):242–250
3. Davies MJ, Woolf N (1993) Atherosclerosis: what is it and why does it occur? Br Heart J 69(1):S3–S11
4. Di Virgilio F, Solini A (2002) P2 receptors: new potential players in atherosclerosis. Br J Pharmacol 135(4):831–842
5. Lafont A, Guzman LA, Whitlow PL, Goormastic M, Cornhill JF, Chisolm GM (1995) Restenosis after experimental angioplasty: intimal, medial, and adventitial changes associated with constrictive remodeling. Circ Res 76(6):996–1002
6. Seye CI, Kong Q, Erb L, Garrad RC, Krugh B, Wang M, Turner JT, Sturek M, Gonzalez FA, Weisman GA (2002) Functional $P2Y_2$ nucleotide receptors mediate uridine 5'-triphosphate-induced intimal hyperplasia in collared rabbit carotid arteries. Circulation 106(21):2720–2726
7. Carpenter RC, Miao L, Miyagi Y, Bengten E, Zhang JH (2001) Thesis Altered expression of P_2 receptor mRNAs in the basilar artery in a rat double hemorrhage model. Stroke 32(2):516–522
8. Hill BJ, Wamhoff BR, Sturek M (2001) Functional nucleotide receptor expression and sarcoplasmic reticulum morphology in dedifferentiated porcine coronary smooth muscle cells. J Vasc Res 38(5):432–443
9. Elmaleh DR, Narula J, Babich JW, Petrov A, Fischman AJ, Khaw BA, Rapaport E, Zamecnik PC (1998) Rapid noninvasive detection of experimental atherosclerotic lesions with novel 99mTc-labeled diadenosine tetraphosphates. Proc Natl Acad Sci USA 95(2):691–695
10. Casscells W (1992) Migration of smooth muscle and endothelial cells. Critical events in restenosis. Circulation 86(3):723–729
11. Isner JM, Kearney M, Bortman S, Passeri J (1995) Apoptosis in human atherosclerosis and restenosis. Circulation 91(11): 2703–2711
12. North RA, Surprenant A (2000) Pharmacology of cloned P2X receptors. Ann Rev Pharmacol Toxicol 40:563–580
13. Cooper DM, Rodbell M (1979) ADP is a potent inhibitor of human platelet plasma membrane adenylate cyclase. Nature 282 (5738):517–518
14. Communi D, Gonzales NS, Detheux M, Brezillon S, Lannoy V, Parmentier M, Boeynaems J-M (2001) Identification of a novel human ADP receptor coupled to G1. J Biol Chem 276(44): 41479–41485
15. Ralevic V, Burnstock G (1991) Roles of P2-receptors in the cardiovascular system. Circulation 84(1):1–14
16. Saiag B, Milon D, Shacoori V, Allain H, Rault B, van den Driessche J (1992) Newly evidenced pyrimidinoceptors and the P2X receptors are present on the vascular smooth muscle and respectively mediate the UTP- and ATP-induced contractions of the dog maxillary internal vein. Res Commun Chem Pathol Pharmacol 76(1):89–94
17. von Kügelgen I, Starke K (1990) Evidence for two separate vasoconstriction-mediating nucleotide receptors, both distinct from the P2X-receptor, in rabbit basilar artery: a receptor for pyrimidine nucleotides and a receptor for purine nucleotides. Naunyn-Schmiedeberg's Arch Pharmacol 341(6):538–546
18. Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidine. Pharmacol Rev 50(3):413–492
19. Guns PJ, Van Assche T, Fransen P, Robaye B, Boeynaems JM, Bult H (2006) Endothelium-dependent relaxation evoked by ATP and UTP in the aorta of $P2Y_2$-deficient mice. Br J Pharmacol 147(5):569–574
20. Seye CI, Gadeau AP, Daret D, Dupuch F, Alzieu P, Capron L, Desgranges C (1997) Overexpression of $P2Y_2$ receptor in intimal lesions of the rat aorta. Arterioscler Thromb Vasc Biol 17(12): 3602–3610
21. Malam-Souley R, Seye CI, Gadeau AP, Loirand G, Pillois X, Campan M, Pacaud P, Desgranges, C (1996) Nucleotide receptor P2u partially mediates ATP-induced cell cycle progression of aortic smooth muscle cells. J Cell Physiol 166(1):57–65
22. Kumari R, Goh G, Ng LL, Boarder MR (2003) ATP and UTP responses of cultured rat aortic smooth muscle cells revisited: dominance of $P2Y_2$ receptors. Br J Pharmacol 140(7): 1169–1176
23. Malmsjo M, Hou M, Pendergast W, Erlinge D, Edvinsson L (2003) The stable pyrimidines UDPβS and UTPγS discriminate between contractile cerebrovascular P2 receptors. Eur J Pharmacol 458(3):305–311
24. Malmsjo M, Hou M, Pendergast W, Erlinge D, Edvinsson L (2003) Potent $P2Y_6$ receptor mediated contractions in human cerebral arteries. BMC Pharmacol 3:4

25. Hartley SA, Kato K, Salter KJ, Kozlowski RZ (1998) Functional evidence for a novel suramin-insensitive pyrimidine receptor in rat small pulmonary arteries. Circ Res 83(9):940–946
26. Malmsjo M, Adner M, Harden TK, Pendergast W, Edvinsson L, Erlinge D (2000) The stable pyrimidines UDPβS and UTPγS discriminate between the P2 receptors that mediate vascular contraction and relaxation of the rat mesenteric artery. Br J Pharmacol 131(1):51–56
27. Vial C, Evans RJ (2002) $P2X_1$ receptor-deficient mice establish the native P2X receptor and a $P2Y_6$-like receptor in arteries. Mol Pharmacol 62(6):1438–1445
28. Erlinge D, Hou M, Webb TE, Barnard EA and Moller S (1998) Phenotype changes of the vascular smooth muscle cell regulate P2 receptor expression as measured by quantitative RT-PCR. Biochem Biophys Res Commun 248(3):864–870
29. Chang K, Hanaoka K, Kumada M, Takuwa Y (1995) Molecular cloning and functional analysis of a novel P2 nucleotide receptor. J Biol Chem 270(44):26152–26158
30. Cario-Toumaniantz C, Loirand G, Ladoux A, and Pacaud P (1998) $P2X_7$ receptor activation-induced contraction and lysis in human saphenous vein smooth muscle. Circ Res 83(2):196–203
31. Zalewski A, Shi Y, Johnson AG (2002) Diverse origin of intimal cells: smooth muscle cells, myofibroblasts, fibroblasts, and beyond? Circ Res 91(8):652–655
32. von Kugelgen I, Wetter A (2000) Molecular pharmacology of P2Y receptors. Naunyn Schmiedeberg's Arch Pharmacol 362(4–5):310–323
33. Kuhns DB, Wright DG, Nath J, Kaplan SS, Basford RE (1988) ATP induces transient elevations of $[Ca^{2+}]_i$ in human neutrophils and primes these cells for enhanced O_2^- generation. Lab Invest 58(4):448–453
34. Cowen DS, Lazarus HM, Shurin SB, Stoll SE, Dubyak GR (1989) Extracellular adenosine triphosphate activates calcium mobilization in human phagocytic leukocytes and neutrophil/monocyte progenitor cells. J Clin Invest 83(5):1651–1660
35. Parker JC, Snow RL (1972) Influence of external ATP on permeability and metabolism of dog red blood cells. Am J Physiol 223(4):888–893
36. Boyer JL, Downes CP, Harden TK (1989) Kinetics of activation of phospholipase C by P2 purinergic agonists and guanine nucleotides. J Biol Chem 264(2):884–890
37. Cockcroft S, Stutchfield J (1989) ATP stimulates secretion in human neutrophils and HL60 cells via a pertussis toxin-sensitive guanine nucleotide-binding protein coupled to phospholipase C. FEBS Lett 245(1–2):25–29
38. Steinberg TH, Newman AS, Swanson JA, Silverstein, SC (1987) ATP^{4-} permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. J Biol Chem 262(18):8884–8888
39. Baricordi OR, Ferrari D, Melchiorri L, Chiozzi P, Hanau S, Chiari E, Rubini M, Di Virgilio F (1996) An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. Blood 87(2):682–690
40. Suh BC, Kim JS, Namgung U, Ha H, Kim KT (2001) $P2X_7$ nucleotide receptor mediation of membrane pore formation and superoxide generation in human promyelocytes and neutrophils. J Immunol 166(11):6754–6763
41. Di Virgilio F, Chiozzi P, Ferrari D, Falzoni S, Sanz JM, Morelli A, Torboli M, Bolognesi G, Baricordi OR (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97(3):587–600
42. Sun B, Li J, Okahara K, Kambayashi J (1998) $P2X_1$ receptor in human platelets. J Biol Chem 273(19):11544–11547
43. Leon C, Hechler B, Vial C, Leray C, Cazenave JP, Gachet C (1997) The $P2Y_1$ receptor is an ADP receptor antagonized by ATP and expressed in platelets and megakarioblastic cells. FEBS Lett 403(1):26–30
44. Hollopeter G, Jantzen H-M, Vincent D, Li G, England L, Ramakrishnan V, Yang R-B, Nurden P, Nurden A, Julius D, Conley PB (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409(6817):202–207
45. Hamada K, Takuwa N, Yokoyama K, Takuwa Y (1998) Stretch activates Jun N-terminal kinase/stress-activated protein kinase in vascular smooth muscle cells through mechanisms involving autocrine ATP stimulation of receptors. J Biol Chem 273 (11):6334–6340
46. Dubyak GR, El-Moatassim C (1993) Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 265(3 Pt 1):C577–C606
47. Wang DJ, Huang NN, Heppel LA (1992) Extracellular ATP and ADP stimulate proliferation of porcine aortic smooth muscle cells. J Cell Physiol 153(2):221–233
48. White PJ, Kumari R, Porter KE, London NJ, Ng LL, Boarder MR (2000) Antiproliferative effect of UTP on human arterial and venous smooth muscle cells. Am J Physiol Heart Circ Physiol 279(6):H2735–H2742.
49. Erlinge D, Yoo H, Edvinsson L, Reis DJ, Wahlestedt C (1993) Mitogenic effects of ATP on vascular smooth muscle cells vs. other growth factors and sympathetic cotransmitters. Am J Physiol 265(4 Pt 2):H1089–H1097
50. Erlinge D, You J, Wahlestedt C, Edvinsson L (1995) Characterisation of an ATP receptor mediating mitogenesis in vascular smooth muscle cells. Eur J Pharmacol 289:135–149
51. Malam-Souley R, Campan M, Gadeau AP, Desgranges C (1993) Exogenous ATP induces a limited cell cycle progression of arterial smooth muscle cells. Am J Physiol 264(4 Pt 1):C783–C788
52. Wildman SS, Unwin RJ, King BF (2003) Extended pharmacological profiles of rat $P2Y_2$ and rat $P2Y_4$ receptors and their sensitivity to extracellular H^+ and Zn^{2+} ions. r J Pharmacol 40 (7):1177–1186
53. Harper S, Webb TE, Charlton SJ, Ng LL, Boarder MR (1998) Evidence that $P2Y_4$ nucleotide receptors are involved in the regulation of rat aortic smooth muscle cells by UTP and ATP. Br J Pharmacol 124(4):703–710
54. Shen J, Seye CI, Wang M, Weisman GA, Wilden PA, Sturek M (2004) Cloning, up-regulation, and mitogenic role of porcine $P2Y_2$ receptor in coronary artery smooth muscle cells. Mol Pharmacol 66(5):1265–1274
55. Gadeau AP, Campan M, Millet D, Candresse T, Desgranges C (1993) Osteopontin overexpression is associated with arterial smooth muscle cell proliferation in vitro. Arterioscler Thromb 13 (1):120–125
56. Hou M, Harden TK, Kuhn CM, Baldetorp B, Lazarowski E, Pendergast W, Moller S, Edvinsson L, Erlinge D (2002) UDP acts as a growth factor for vascular smooth muscle cells by activation of $P2Y_6$ receptors. Am J Physiol Heart Circ Physiol 282(2):H784–H792
57. Chaulet H, Desgranges C, Renault MA, Dupuch F, Ezan G, Peiretti F, Loirand G, Pacaud P, Gadeau AP (2001) Extracellular nucleotides induce arterial smooth muscle cell migration via osteopontin. Circ Res 89(9):772–778
58. Pillois X, Chaulet H, Belloc I, Dupuch F, Desgranges C, Gadeau AP (2002) Nucleotide receptors involved in UTP-induced rat arterial smooth muscle cell migration. Circ Res 90 (6):678–681
59. Dufourcq P, Louis H, Moreau C, Daret D, Boisseau MR, Lamaziere JM, Bonnet J (1998) Vitronectin expression and interaction with receptors in smooth muscle cells from human atheromatous plaque. Arterioscler Thromb Vasc Biol 18(2): 168–176
60. Renault MA, Jalvy S, Belloc I, Pasquet S, Sena S, Olive M, Desgranges C, Gadeau AP (2003) AP-1 is involved in UTP-

induced osteopontin expression in arterial smooth muscle cells. Circ Res 93(7):674–681
61. Renault MA, Jalvy S, Potier M, Belloc I, Genot E, Dekker LV, Desgranges C, Gadeau AP (2005) UTP induces osteopontin expression through a coordinate action of NFκB, activator protein-1, and upstream stimulatory factor in arterial smooth muscle cells. J Biol Chem 280(4):2708–2713
62. Communi D, Janssens R, Suarez-Huerta N, Robaye B, Boeynaems JM (2000) Advances in signalling by extracellular nucleotides. The role and transduction mechanisms of P2Y receptors. Cell Signal 12(6):351–360
63. Parker AL, Likar LL, Dawicki DD, Rounds S (1996) Mechanism of ATP-induced leukocyte adherence to cultured pulmonary artery endothelial cells. Am J Physiol 270:L695–L703
64. Weisman GA, Yu N, Liao Z, Gonzalez F, Erb L, Seye CI (2005) P2 Receptors in health and disease. Biotechnology and genetics. 22:171–195
65. Faruqi RM, DiCorleto PE (1993) Mechanisms of monocyte recruitment and accumulation. Br Heart J 69:S19–S29
66. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS (2001) A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 107(10):1255–1262
67. Rice GE, Munro JM, Bevilacqua MP (1990) Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. J Exp Med 171(4):1369–1374
68. Iademarco MF, Barks JL, Dean DC (1995) Regulation of vascular cell adhesion molecule-1 expression by IL-4 and TNF-alpha in cultured endothelial cells. J Clin Invest 95 (1):264–271
69. Braun M, Pietsch P, Felix SB, Baumann G (1995) Modulation of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 on human coronary smooth muscle cells by cytokines. J Mol Cell Cardiol 27(12):2571–2579
70. Ventura MA, Thomopoulos P (1995) ADP and ATP activate distinct signaling pathways in human promonocytic U-937 cells differentiated with 1,25-dihydroxy-vitamin D3. Mol Pharmacol 47(1):104–107
71. Seye CI, Yu N, Jain R, Kong Q, Minor T, Newton J, Erb L, Gonzalez FA, Weisman GA (2003) The $P2Y_2$ nucleotide receptor mediates UTP-induced vascular cell adhesion molecule-1 expression in coronary artery endothelial cells. J Biol Chem 278(27):24960–24965
72. Liu J, Liao Z, Camden J, Griffin KD, Garrad RC, Santiago-Perez LI, Gonzalez FA, Seye CI, Weisman GA, Erb L (2004) Src homology 3 binding sites in the $P2Y_2$ nucleotide receptor interact with Src and regulate activities of Src, proline-rich tyrosine kinase 2, and growth factor receptors. J Biol Chem 279 (9):8212–8218
73. Seye CI, Yu N, Gonzalez FA, Erb L, Weisman GA (2004) The $P2Y_2$ nucleotide receptor mediates vascular cell adhesion molecule-1 expression through interaction with VEGF receptor-2 (KDR/Flk-1). J Biol Chem 279(34):35679–35686
74. Mallat Z, Tedgui A (2000) Apoptosis in the vasculature: mechanisms and functional importance. Br J Pharmacol 130 (5):947–962
75. Thomas WA, Reiner JM, Florentin FA, Lee KT, Lee WM (1976) Population dynamics of arterial smooth muscle cells, V: cell proliferation and cell death during initial 3 months in atherosclerotic lesions induced in swine by hypercholesterolemic diet and intimal trauma. Exp Mol Pathol 24(3):360–374
76. Han DK, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liau G (1995) Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. Am J Pathol 147(2): 267–277
77. Geng YJ, Libby P (1995) Evidence for apoptosis in advanced human atheroma: colocalization with interleukin-1β-converting enzyme. Am J Pathol 147(2):251–266
78. Crisby M, Kallin B, Thyberg J, Zhivotovsky B, Orrenius S, Kostulas V, Nilsson J (1997) Cell death in human atherosclerotic plaques involves both oncosis and apoptosis. Atherosclerosis 130(1–2):17–27
79. Björkerud S, Björkerud B (1996) Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. Am J Pathol 149(2):367–380
80. Bochaton-Piallat ML, Gabbiani F, Redard M, Desmoulière A, Gabbiani G (1995) Apoptosis participates in cellularity regulation during rat aortic intimal thickening. Am J Pathol 146 (5):1059–1064
81. Burnstock G (1999) Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J Anat 194:335–342
82. Kaiser D, Freyberg MA, Friedl P (1997) Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. Biochem Biophys Res Commun 231(3):586–590
83. Zheng LM, Zychlinsky A, Liu CC, Ojcius DM, Young JD (1991) Extracellular ATP as a trigger for apoptosis or programmed cell death. J Cell Biol 112(2):279–288
84. Di Virgilio F, Zanovello P, Zambon A, Bronte V, Pizzo P, Murgia M (1995) Cell membrane receptors for extracellular ATP: a new family of apoptosis-signalling molecules. Fundam Clin Immunol 3:80–81
85. Jacobson KA, Hoffmann C, Cattabeni F, Abbracchio MP (1999) Adenosine-induced cell death: evidence for receptor-mediated signalling. Apoptosis 4(3):197–211
86. Wen LT, Caldwell CC, Knowles AF (2003) Poly (ADP-ribose) polymerase activation and changes in Bax protein expression associated with extracellular ATP-mediated apoptosis in human embryonic kidney 293-$P2X_7$ cells. Mol Pharmacol 63(3): 706–713
87. Wang Q, Wang L, Feng YH, Li X, Zeng R, Gorodeski GI (2004) $P2X_7$ receptor-mediated apoptosis of human cervical epithelial cells. Am J Physiol Cell Physiol 287(5):C1349–C1358
88. Kong Q, Wang M, Liao Z, Camden JM, Yu S, Simonyi A, Sun GY, Gonzalez FA, Erb L, Seye CI, Weisman GA (2005) $P2X_7$ nucleotide receptors mediate caspase-8/9/3-dependent apoptosis in rat primary cortical neurons. Purinergic Signalling 1(4):337–347
89. Peyot M, Gadeau A, Dandré F, Belloc I, Dupuch F, Desgranges C (2000) Extracellular adenosine induces apoptosis of human arterial smooth muscle cells via A_{2b}-receptor. Circ Res 86(1): 76–85
90. Solini A, Chiozzi P, Falzoni S, Morelli A, Fellin R, Di Virgilio F (2000) High glucose modulates $P2X_7$ receptor-mediated function in human primary fibroblasts. Diabetologia 43(10): 1248–1256
91. Zanovello P, Bronte V, Rosato A, Pizzo P, and Di Virgilio F (1990) Responses of mouse lymphocytes to extracellular ATP. II. Extracellular ATP causes cell type-dependent lysis and DNA fragmentation. J Immunol 145(5):1545–1550
92. Hogquist KA, Nett MA, Unanue ER, Chaplin DD (1991) Interleukin 1 is processed and released during apoptosis. Proc Natl Acad Sci USA 88(19):8485–8491
93. Lammas DA, Stober C, Harvey CJ, Kendrick N, Panchalingam S, Kumararatne DS (1997) ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z ($P2X_7$) receptors. Immunity 7(3):433–444
94. Schulze-Lohoff E, Hugo C, Rost S, Arnold S, Gruber A, Brune B, Sterzel RB (1998) Extracellular ATP causes apoptosis and

necrosis of cultured mesangial cells via P2Z/P2X$_7$ receptors. Am J Physiol 275:F962–F971
95. Coutinho-Silva R, Persechini PM, Da Cunha Bisaggio R, Perfettini J, Torres de Santo AC, Kanellopoulos JM, Mottaly I, Dautry-Varsat A, Ojcius DM (1999) P2Z/P2X$_7$ receptor-dependent apoptosis of dendritic cells. Am J Physiol 276:C1139–C1147
96. Ferrari D, Los M, Bauer MK, Vandenabeele P, Wesselborg S, Schulze-Osthoff K (1999) P2Z purinoreceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death. FEBS Lett 447(1):71–75
97. Ferrari D, Wesselborg S, Bauer MK, Schulze-Osthoff K (1997) Extracellular ATP activates transcription factor NF-κB through the P2Z purinoreceptor by selectively targeting NF-κB p65. J Cell Biol 139(7):1635–1643
98. Hu Y, Fisette PL, Denlinger LC, Guadarrama AG, Sommer JA, Proctor RA, Bertics PJ (1998) Purinergic receptor modulation of lipopolysaccharide signaling and inducible nitric-oxide synthase expression in RAW 264.7 macrophages. J Biol Chem 273 (42):27170–27175
99. Von Albertini M, Palmetshofer A, Kaczmarek E, Koziak K, Stroka D, Grey ST, Stuhlmeier KM, Robson SC (1998) Extracellular ATP and ADP activate transcription factor NF-κB and induce endothelial cell apoptosis. Biochem Biophys Res Commun 248(3):822–829
100. De Meyer GR, Van Put DJ, Kockx MM, Van Schil P, Bosmans R, Bult H, Buyssens N, Vanmaele R, Herman AG (1997) Possible mechanisms of collar-induced intimal thickening. Arterioscler Thromb Vasc Biol 17(10):1924–1930
101. Wang L, Andersson M, Karlsson L, Watson MA, Cousens DJ, Jern S, Erlinge D (2003) Increased mitogenic and decreased contractile P2 receptors in smooth muscle cells by shear stress in human vessels with intact endothelium. Arterioscler Thromb Vasc Biol 23(8):1370–1376
102. Pulvirenti TJ, Yin JL, Chaufour X, McLachlan C, Hambly BD, Bennett MR, Barden JA (2000) P2X (purinergic) receptor redistribution in rabbit aorta following injury to endothelial cells and cholesterol feeding. J Neurocytol 29(9):623–631
103. Katoh Y, Periasamy M (1996) Growth and differentiation of smooth muscle cells during vascular development. Trends Cardiovasc Med 6:100–106
104. Owens GK (1995) Regulation of differentiation of vascular smooth muscle cells. Physiol Rev 75(3):487–517
105. Gabbiani G, Kocher O, Bloom WS, Vandekerckhove J, Weber K (1984) Actin expression in smooth muscle cells of rat aortic intimal thickening, human atheromatous plaque, and cultured rat aortic media. J Clin Invest 73(1):148–152
106. Kocher O, Gabbiani F, Gabbiani G, Reidy MA, Cokay M, Petters H, Huttner I (1991) Phenotypic features of smooth muscle cells during the evolution of experimental carotid artery intimal thickening: biochemical and morphologic studies. Lab Invest 65(4):459–470
107. Schwartz SM, deBlois D, O'Brien ER (1995) The intima: soil for atherosclerosis and restenosis. Circ Res 77(3):445–465
108. Ralevic V, Burnstock G (1991) Effects of purines and pyrimidines on the rat mesenteric arterial bed. Circ Res 69(6):1583–1590
109. Saïag B, Milon D, Allain H, Rault B, Van Den Driessche J (1990) Constriction of the smooth muscle of rat tail and femoral arteries and dog saphenous vein is induced by uridine triphosphate via `pyrimidinoceptors', and by adenosine triphosphate via P2X receptors. Blood Vessels 27(6):352–354
110. Andersen HR, Mæng M, Thorwest M, Falk E (1996) Remodeling rather than neointimal formation explains luminal narrowing after deep vessel wall injury: insights from a porcine coronary (re)stenosis model. Circulation 93(9):1716–1724
111. Clowes AW, Clowes MM, Reidy MA (1986) Kinetics of cellular proliferation after arterial injury. III. Endothelial and smooth muscle growth in chronically denuded vessels. Lab Invest 54(3):295–303
112. Haudenschild CC, Schwartz SM (1979) Endothelial regeneration. II. Restitution of endothelial continuity. Lab Invest 41 (5):407–418
113. Shimokawa H, Flavahan NA, Vanhoutte PM (1989) Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium removal in porcine coronary arteries: possible dysfunction of a pertussis toxin-sensitive G protein. Circ Res 65(3):740–753
114. Weidinger FF, McLenachan JM, Cybulsky MI, Gordon JB, Rennke HG, Hollenberg NK, Fallon JT, Ganz P, Cooke JP (1990) Persistent dysfunction of regenerated endothelium after balloon angioplasty of rabbit iliac artery. Circulation 81(5):1667–1679
115. Valera S, Hussy N, Evans RJ, Adami N, North RA, Surprenant A, Buell G (1994) A new class of ligand gated ion channel defined by P2X receptor for extracellular ATP. Nature 371 (6497):516–519
116. Nicholas RA, Watt WC, Lazarowski ER, Li Q, Harden TK (1996) Uridine nucleotide selectivity of three phospholipase C-activating P2 receptors: identification of a UDP-selective, a UTP-selective, and an ATP- and UTP-specific receptor. Mol Pharmacol 50(2):224–229
117. Gordon JL (1986) Extracellular ATP: effects, sources and fate. Biochem J 233(2):309–319
118. Bergfeld GR, Forrester T (1992) Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. Cardiovasc Res 26(1):40–47
119. Bodin P, Burnstock G (1995) Synergistic effect of acute hypoxia on flow-induced release of ATP from cultured endothelial cells. Experientia 51(3):256–259
120. Viswanathan M, Strömberg C, Seltzer A, Saavedra JM (1992) Balloon angioplasty enhances the expression of angiotensin II AT1 receptors in neointima of rat aorta. J Clin Invest 90 (5):1707–1712
121. Winkles JA, Alberts GF, Brogi E, Libby P (1993) Endothelin-1 and endothelin receptor mRNA expression in normal and atherosclerotic human arteries. Biochem Biophys Res Commun 191(3):1081–1088
122. Majesky MW, Reidy MA, Bowen-Pope DF, Hart CE, Wilcox JN (1990) PDGF ligand and receptor gene expression during repair of arterial injury. J Cell Biol 111:2149–2158