Purinergic Signaling and Vascular Cell Proliferation and Death

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***Abstract*—**Evidence for the role of purinergic signaling (via P1 and P2Y receptors) in the proliferation of vascular smooth muscle and endothelial cells is reviewed. The involvement of the mitogen-activated protein kinase second-messenger cascade in this action is clearly implicated, although details of the precise intracellular pathways involved still remain to be determined. Synergistic actions of purines and pyrimidines with growth factors occur in promoting cell proliferation. Interaction between purinergic signaling for vascular cell proliferation and cell death mediated by $P2X_7$ receptors is discussed. There is evidence of the release of ATP from endothelial cells, platelets, and sympathetic nerves as well as from damaged cells in atherosclerosis, hypertension, restenosis, and ischemia; furthermore, there is evidence that vascular smooth muscle and endothelial cells proliferate in these pathological conditions. Thus, the involvement of ATP and its breakdown product, adenosine, is implicated; it is hoped that with the development of selective P1 (A_2) and P2Y receptor agonists and antagonists, new therapeutic strategies will be explored. **(*Arterioscler Thromb Vasc Biol*. 2002;22:364-373.)**

Key Words: ATP ■ apoptosis ■ purinergic signaling ■ proliferation ■ atherosclerosis

The roles of nucleotides and nucleosides as extracellular signaling molecules are now well established.[1,2] P1 receptors for adenosine, of which four subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been identified, have been distinguished from P2 receptors for ATP/ADP/UTP,[3] and P2 receptors have been divided into P2X ligand-gated ion channel and P2Y G protein–coupled receptor families. Seven subtypes of P2X receptors and 6 subtypes of P2Y receptors have been cloned and characterized.[4] The majority of studies involving purinergic signaling have been concerned with short-term events, such as neurotransmission or secretion. However, there is growing interest in the long-term trophic actions of extracellular nucleotides and nucleosides on cell growth, proliferation, and death.[5–9]

In the vascular system, short-term purinergic signaling events associated with the control of vascular tone by ATP released from nerves and endothelial cells have been clearly demonstrated.[10–15] However, the migration, proliferation, and death of vascular smooth muscle and endothelial cells play an important role in the development of intimal thickening during arterial diseases, such as arteriosclerosis and restenosis after angioplasty, and in the growth of new vessels that takes place during wound healing and in tumors.[16–18] Studies indicating that ATP, ADP, UTP, and adenosine play pivotal signaling roles in these long-term events will be discussed in the present review.[19–21]

Purines and Smooth Muscle Cell Proliferation

Adenosine (P1) Receptors

An early study reported that adenosine produces changes in cAMP and DNA synthesis in cultured arterial smooth muscle cells and suggested that this might result in the regulation of cell proliferation.[22] The authors speculated that adenosine could be one of several regulatory factors in the development of atherosclerosis and might also regulate the release of a smooth muscle mitogen, platelet-derived growth factor (PDGF), from platelets. There is now good evidence that adenosine, an ectoenzymatic breakdown product of ATP, does regulate smooth muscle cell proliferation, but as will be discussed, its properties differ from those for ATP and ADP.

Adenosine inhibits vascular smooth muscle cell proliferation by A_2 receptor activation via the elevation of cAMP,[22,23] and a selective A_2 receptor agonist, 2-octynyladenosine, reduced neointimal thickening in a rat femoral artery injury model.[24] Indeed, cAMP is a known pathway involved in smooth muscle cell growth arrest and in the maintenance of the contractile phenotype.[25] The possibility that a defect in local adenosine production within the vessel wall could contribute to vascular thickening and neointimal formation was explored,[23] and it was proposed that adenosine inhibits the growth of human aortic smooth muscle cells via A_{2B} receptors.[26] Later, it was demonstrated that adenosine, acting through A_{2B} receptors, inhibits collagen synthesis by smooth muscle cells, and it was suggested that drugs that modulate adenosine levels may protect against vaso-occlusive disorders by attenuating extracellular matrix synthesis and the cellular hypertrophy of smooth muscle cells.[27] It seems surprising that this role is not shared by A_{2A} adenosine receptors, which are coupled to the elevation of cAMP and are expressed on vascular smooth muscle, but it may be that the levels of expression of the A_{2A} receptors are low relative to A_{2B}

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receptors in those smooth muscle cells in which the trophic effects of adenosine were investigated. Other inhibitory pathways also exist, inasmuch as sodium butyrate (a small, naturally occurring molecule with demonstrated activity on cell growth and differentiation) and its more stable in vivo analogue, tributyrin, are potent DNA synthesis and cell proliferation inhibitors of vascular smooth muscle[28] by a mechanism not mediated by an elevation of cAMP.

P2 Receptors

ATP and ADP stimulate DNA synthesis and cell proliferation of cultured porcine artery vascular smooth muscle cells, an action that was shown to be mediated by P2Y receptors.[29] It was speculated that this mechanism was involved in the regulation of vascular smooth muscle cell proliferation during embryonic and early postnatal development, after injury, and in arteriosclerosis. It was further suggested that the ATP released from endothelial cells causes not only autocrine mitogenic stimulation of the endothelial cells themselves but also paracrine stimulation of the smooth muscle cells that migrate to the intima after injury. The mitogenic actions of ATP (but not those produced by adenosine) were reduced by indomethacin (indicating that part of the mechanism involves ATP-induced prostaglandin synthesis, as first proposed by Needleman et al, 1974,[30] by downregulation of protein kinase C [PKC], by long-term exposure to phorbol dibutyrate, and by the PKC inhibitor staurosporine). These results suggest that there is a dual mechanism involved in the trophic mitogenic actions of ATP and ADP, namely, arachidonic acid metabolism and PKC.

Exogenous ATP also appears to induce a limited cell cycle progression in arterial smooth muscle cells.[31,32] It was shown that stimulation of cultured, quiescent, smooth muscle cells induced chronological activation not only of immediate-early but also of delayed-early cell cycle–dependent genes. In contrast, ATP did not increase late G_1 gene mRNA. An increase in c-*fos* mRNA was also induced by ADP but not by AMP or adenosine. The fact that 2-methylthio-ATP but not α,β-methylene ATP mimicked these responses tends to favor P2Y rather than P2X receptor mediation.

Sympathetic nerves have been shown to exert a trophic influence on vascular smooth muscle.[33–35] From her studies of pulmonary artery denervated of sympathetic nerves, Bevan[35] concluded that sympathetic transmitters exert slow trophic as well as fast signaling actions on cell growth and division by influencing protein, DNA, and RNA synthesis. Since ATP as well as noradrenaline (NA) and neuropeptide Y (NPY) are known to be released as cotransmitters from sympathetic nerves,[36] this was consistent with the possibility that ATP and/or its breakdown product, adenosine, might be involved in these trophic actions. A study was initiated to examine the relative effects of ATP, NA, and NPY in the incorporation of [^{3}H]thymidine and the cell number and protein content of smooth muscle cells from the rat aorta and vena cava.[37] Compared with NA, NPY, epidermal growth factor, or insulin, ATP was shown to have considerably greater mitogenic effects on vascular smooth muscle. There is also evidence indicating that vascular smooth muscle has trophic actions on the pattern of sympathetic innervation of blood vessels.[34]

UTP, a pyrimidine, also has powerful mitogenic actions on vascular smooth muscle, suggesting that P2U receptors might be implicated.[38,39] Since the mitogenic effects of UTP and ATP were approximately equipotent, with the present knowledge of the pharmacology of P2 receptor subtypes, this would suggest that the receptor involved is either of the $P2Y_2$ or $P2Y_4$ subtype.[40] $P2Y_4$ receptors were identified on spontaneously hypertensive rat (SHR)-derived cultured rat aortic smooth muscle cells, perhaps coupled to mitogenesis via P42/P44 mitogen-activated protein kinase (MAPK).[41] Although these and other studies have reported that UTP is equipotent with ATP in producing mitogenesis of vascular smooth muscle,[37,42–45] a recent report has claimed that UTP, unlike ATP, has an antiproliferative action on human arterial and venous smooth muscle cells derived from internal mammary artery and saphenous vein.[46] There is no obvious explanation for this discrepancy. Either way, it is interesting that flow-induced release of UTP from vascular endothelial cells has been demonstrated,[47] as has ATP.[14,48]

ADP contributes significantly in synergy with the peptide growth factors PDGF, epidermal growth factor, and transforming growth factor-β, to the platelet-induced proliferation of vascular smooth muscle.[49] The mitogenic effect of ATP on vascular smooth muscle cells was synergistic with other mitogens, including insulin and insulin-like growth factor-1.[29] It is interesting in this respect that amiloride, which is known to inhibit the actions of several growth factors, also inhibited ATP-induced mitogenesis.[37] ATP has also been shown to be a mitogen for human vascular smooth muscle cells.[50] The molecular mechanisms underlying ATP and insulin synergistic stimulation of coronary artery smooth muscle proliferation have been examined.[51] ATP and insulin individually stimulated DNA synthesis 4- and 2-fold, respectively; however, they acted synergistically to stimulate a 17-fold increase. A similar synergistic stimulation of extracellular signal–regulated kinase (ERK) was demonstrated, whereas ATP dramatically reduced the insulin-stimulated AKT (also known as protein kinase B) activation. The authors concluded that their results were consistent with the relieving (by ATP) of an insulin-induced AKT-dependent inhibitory effect on the ERK signaling pathway, leading to synergistic stimulation of coronary artery smooth muscle cell proliferation.

In a study of the mechanisms involved in ATP-induced proliferation of vascular smooth muscle cells,[52] it was shown that P2Y receptor activation of smooth muscle was coupled to a pertussis toxin–insensitive G_q protein, triggering phosphoinositide hydrolysis and subsequent activation of PKC, Raf 1, and MAPK. Both 42- and 44-kDa MAPKs were activated, and tyrosine was phosphorylated. Western blot analysis, with the use of PKC isozyme–specific antibodies, indicated that the vascular smooth muscle cells express PKC-α and PKC-δ. P2Y receptor stimulation also caused synthesis of c-*fos* and c-*myc* mRNAs; Reactive blue 2 (a P2Y-selective antagonist) and staurosporine blocked this effect. A later study presented evidence indicating that ATP-stimulated vascular smooth muscle cell proliferation requires independent ERK and phosphatidylinositol 3-kinase–signaling pathways.[53] Tyrphostin, a specific inhibitor of tyrosine kinase, inhibited DNA synthesis, Fos-protein expression, and cell proliferation of vascular smooth muscle cells but not ATP-induced Ca^{2+}

influx or inositol phosphate production.[54] Stimulation of cultured aortic myocytes with P2Y agonists produced an increase in the amount of membrane-bound small GTPases of the RhoA family and stimulated actin cytoskeleton organization.[45] Cell proliferation and migration are also known to be induced by RhoA activation.[55,56]

There are 2 phenotypes of smooth muscle: the contractile phenotype and the synthetic (proliferative) phenotype.[57] In a study of cultures expressing these 2 phenotypes using quantitative reverse transcription–polymerase chain reaction, it was shown that $P2X_1$ receptors were strongly expressed in the contractile phenotype. In the synthetic phenotype, the mitogenic $P2Y_1$ and $P2Y_2$ receptor transcripts were upregulated 342- and 8-fold, respectively, whereas the contractile $P2X_1$ receptor was totally downregulated, and the $P2Y_4$ and $P2Y_6$ receptors were unchanged.[42] Furthermore, MAPK kinase–dependent growth factor induced the upregulation of $P2Y_2$ receptors in vascular smooth muscle cells, which the authors suggested may be of importance in atherosclerosis and neointimal formation after balloon angioplasty.[43] In a later study, this group showed that inflammatory cytokines, known to be released in atherosclerosis, upregulate $P2Y_2$ receptors through PKC and cyclooxygenase (but not cAMP), ERK-1 and -2, or P38-dependent pathways.[58] When the endothelial cells of the central ear artery were injured $\geq$2 times, the smooth muscle cells of the media migrated into the intima and proliferated there between 1 and 3 weeks after the last injury, despite restoration of the endothelium.[59] In rabbits pretreated with dipyridamole, an adenosine-uptake inhibitor, proliferation was limited.

Purines and Vascular Endothelial Cell Proliferation

Adenosine (P1) Receptors

Adenosine has been claimed to be an angiogenesis factor in chick chorioallantoic membrane and embryos.[60,61] In other early studies, long-term administration of adenosine was reported to induce capillary proliferation in the heart, although it was recognized that this effect might be secondary to mechanical factors resulting from an increased blood flow stimulating capillary growth.[62] We know from later studies that ATP is released from endothelial cells during the shear stress produced by changes in blood flow[14,48,63,64] and that there is an ectoenzymatic breakdown of ATP to adenosine. Electrical stimulation of skeletal muscle also resulted in capillary proliferation, as did long-term administration of adenosine.[62] Long-term local application of adenosine induces an increase of capillary diameter in skeletal muscle of anesthetized rabbits.[65] Adenosine has also been shown to induce dose-dependent proliferation of endothelial cells obtained from the aorta,[66] from coronary vessels,[67] and from human umbilical veins,[68] and it has been shown to stimulate canine retinal microvascular endothelial cell migration and tube formation.[69]

The action of adenosine in mediating endothelial cell proliferation is mediated by A_{2A} and A_{2B} receptors, although an action independent of adenosine receptors has also been suggested. It has been claimed by Sexl et al[70] that the adenosine receptor mediating endothelial cell proliferation of the human umbilical vein is an A_{2A} subtype acting via a mechanism that is independent of G_s and G_i. This group went on to show that stimulation of the A_{2A} receptor activates MAPK on these endothelial cells.[71] An investigation was carried out involving adenosine stimulation of DNA synthesis in endothelial cells by measuring [^{3}H]thymidine incorporation in cultures derived from human umbilical veins.[72] The authors concluded that the results suggest that Na^+-H^+ exchange and phospholipase A_2 are involved in adenosine-induced DNA synthesis independently of adenosine receptor, protein kinase A, or PKC activation. An 8-phenyltheophylline–resistant mitogenic action of adenosine, which was not mimicked by A_1- and A_2-selective agonists, was also described in bovine aortic endothelial cells.[66] An intracellular action of adenosine is possible.

Some of the mitogenic effects of adenosine are mediated via the modulation of vascular endothelial growth factor (VEGF) signaling via A_{2A} and A_{2B} receptors. Adenosine mediates growth factor expression through the A_{2B} receptor in human retinal endothelial cells.[73] A_{2B} activation results in sequential expression of VEGF mRNA, supporting a role for adenosine in initiating the autocrine production of a cascade of growth factors that facilitate new blood vessel formation. The addition of an antisense oligonucleotide complementary to the A_{2B} receptor mRNA inhibited VEGF production. Augmentation by adenosine of the expression of VEGF has been described in cerebral[74] and retinal[75] microvascular endothelial cells. In the retinal endothelial cells, this involved A_{2A} receptor activation of the cAMP-dependent protein kinase A pathway.[76] The initial decline in mRNA of receptors for VEGF and of VEGF binding sites during hypoxia was also shown to be antagonized by A_2 receptor blockade.[76] In the most recent study from Grant et al,[77] the selective A_{2B} receptor antagonists enprofylline and 3-isobutyl-8-pyrrolidinoxanthine inhibited 5′-(*N*-ethylcarboxamido)-adenosine (NECA)–stimulated proliferation of human retinal endothelial cells, ERK activation, cell migration, and capillary tube formation. The authors suggested that this may provide a novel approach to the treatment of diseases associated with aberrant neovascularization, such as diabetic retinopathy and the retinopathy of prematurity.

Hypoxia is a potent stimulus to vascular growth and adenosine, and the pyridine metabolite nicotinamide mimics these effects.[78,79] The P1 (adenosine) antagonist 8-phenyltheophylline prevented stimulation of the proliferation of bovine aortic and coronary vascular endothelial cells caused by hypoxia-conditioned medium or adenosine.[78] The proliferative response of endothelial cells to adenosine has been shown to depend on an increase in cAMP: consistent with actions of adenosine at A_2 receptors, pretreatment of endothelial cells with pertussis toxin blocked adenosine-induced proliferation, indicating that a G_i protein might be involved in the mechanism.[80]

P2 Receptors

ADP was shown to be one of several agonists that induced cultured endothelial cell migration and proliferation.[81] Angiogenesis (or neovascularization) begins with the migration of endothelial cells, originating from capillaries, into the tissue being vascularized. ADP and, to a lesser extent, adenosine and adenine showed strong chemotactic activity and were postulated to be angiogenesis factors in vivo.[82]

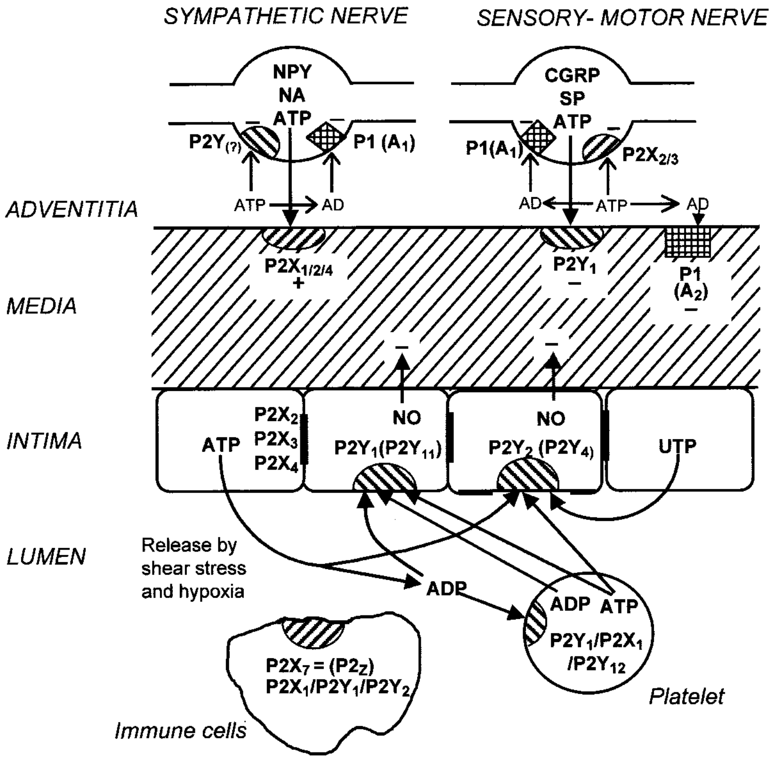

Figure 1. Short-term (acute) purinergic signaling controlling vascular tone. Schematic diagram illustrating the main receptor subtypes for purine and pyrimidines present in most blood vessels. Perivascular nerves in the adventitia release ATP as cotransmitter: ATP is released with NA and NPY from sympathetic nerves to act at smooth muscle $P2X_1$ receptors and, in some vessels, $P2X_2$ and $P2X_4$ purinoceptors, resulting in vasoconstriction; ATP is released with calcitonin gene-related peptide (CGRP) and substance P (SP) from sensory nerves during "axon reflex" activity to act on smooth muscle P2Y purinoceptors, resulting in vasodilatation. P1 (A_1) purinoceptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (arising from enzymatic breakdown of ATP) modulation of transmitter release. $P2X_3$ purinoceptors are present on a subpopulation of sensory nerve terminals. P1 (A_2) purinoceptors on vascular smooth muscle mediate vasodilatation. Endothelial cells release ATP and UTP during shear stress and hypoxia to act on $P2Y_1$, $P2Y_2$, and sometimes $P2Y_4$ purinoceptors, leading to the production of NO and subsequent vasodilatation. ATP, after its release from aggregating platelets, also acts on these endothelial receptors. Blood-borne platelets possess $P2Y_1$ and $P2Y_{12}$ ADP-selective purinoceptors as well as $P2X_1$ receptors, whereas immune cells of various kinds possess $P2X_7$ as well as $P2X_1$, $P2Y_1$, and $P2X_2$ purinoceptors. $P2X_2$, $P2X_3$, and $P2X_4$ receptors have also recently been identified on endothelial cell membranes. (Figure is modified from Burnstock,[150] 1996 with permission from Blackwell Science Ltd, UK).

Adenine nucleotides were shown to have a mitogenic action on aortic endothelial cells, probably via P2Y receptors; adenosine, inosine, and hypoxanthine also had mitogenic actions, but apparently they were not via A_1 or A_2 purinoceptor subtypes.[83] ATP has also been shown to produce proliferation of cultured bovine corneal endothelial cells.[84] The source of the purines involved in these trophic actions is largely from the endothelial cells, suggesting an autocrine mechanism.[85] ADP released from aggregating platelets may also play a role.[86]

When glomerular capillary or aortic endothelial cells were cultured in polypropylene hollow fibers perfused for 9 days, the endothelial cells formed adherent confluent monolayers with chronic flow, simulating shear stress, but not without flow.[87] Furthermore, the aortic, but not capillary, endothelial cells aligned themselves in the direction of flow. Since (as has been described earlier) ATP is released from endothelial cells by shear stress and because ATP can induce cell migration and proliferation, an involvement of ATP in these trophic changes is indicated. Similarly, mechanical scratching of cell monolayers of bovine pulmonary arterial endothelial cell cultures (which would lead to the release of ATP) induces surviving cells near the wound edge to move and proliferate.[88] Stretch-induced changes in endothelial cell shape[89] and changes produced by hypoxic stress[60] may be mediated by the ATP (and/or adenosine after ectoenzymatic breakdown) released from endothelial cells under both these conditions.

There is evidence at present for $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptor subtypes on endothelial cells mediating the release of NO, endothelium-derived hyperpolarizing factor, and prostanoids[4,15,90,91]; there is also recent evidence for the presence of $P2X_2$, $P2X_3$, and $P2X_4$ subtypes in the endothelium[92–95] (Figure 1).The functions of the P2X receptors are not yet clear, although they appear to be involved in cell adhesion and gap junction formation. Less is known about which P2 receptor subtypes are involved in the mitogenic actions of nucleotides or, indeed, about the mechanisms underlying their effects.

In a study of the EAhy 926 endothelial cell line, it has been shown that ATP and UTP activate the 42-kDa isoform of MAPK and that this activation is regulated by PKC, using both calcium-dependent and -independent mechanisms, but that G_i protein is not involved.[96] Regulation of rat brain capillary endothelial cells via P2Y receptors (probably $P2Y_2$ and/or $P2Y_4$, since UTP was equipotent with ATP) has been shown to be coupled to Ca^{2+}, phospholipase C (PLC), and MAPK.[97] In cultured endothelial cells from guinea pig cardiac vasculature, UTP and VEGF were mitogenic and chemotactic factors.[44] The possibility that UTP was acting indirectly via VEGF was not examined.

Activation of kinases (including the p42/44 MAPK and c-Jun N-terminal kinase [JNK]) may underlie the sustained effects of ATP and UTP on endothelial cells and smooth muscle, such as increased cell proliferation; by use of the EAhy 926 endothelial cell line, UTP and ATP, but not UDP, inhibited tumor necrosis factor-α (TNFα)–stimulated stress-activated protein kinase activity.[98]

Vascular Cell Death

There is increasing evidence that cell proliferation and programmed cell death (apoptosis) are linked. For example, VEGF turns on cell proliferation but inhibits apoptosis.[99] Distinct signal transduction cascades, composed of at least 3 protein kinases, mediate cell proliferation and differentiation, growth arrest, and apoptosis.[100] In diseases such as carcinogenesis, degenerative disorders, and ischemia/reperfusion injury, there is an imbalance between cell division and cell death.

Interactions between purinergic signaling for proliferation and cell death also occur.[101] An example is the turnover of keratinocytes in the squamous epithelium of the epidermis, where there is a continuous progression from cell proliferation in cells at the base of the stratum spinosum (labeled with $P2Y_1$ receptors) to differentiating keratinocytes (labeled with $P2X_5$ receptors), which gradually flatten as they reach the stratum corneum, where they become apoptotic (labeled with $P2X_7$ receptors), and the dead cells slough off at the skin surface.[102] A similar relationship between proliferation and differentiation ($P2X_5$ receptor–labeled cells) and apoptotic cell death ($P2X_7$-labeled cells) has been shown during the turnover of intestinal epithelial cells.[103] $P2X_7$ and P1 receptors have been linked to apoptosis in other cell types, particularly immune cells, astrocytes, and thymocytes.[104–106]

Extracellular ATP and adenosine have been shown to cause apoptosis of pulmonary artery endothelial cells.[107] Since the nucleoside transport inhibits dipyridamole, prevented ATP-induced DNA cleavage, it seems likely that apoptosis is mediated by the intracellular actions of adenosine rather than through surface receptors, as later reported for apoptosis in HL-60 cells.[108] The adenosine metabolites, inosine, hypoxanthine, and xanthine, do not cause apoptosis, although *S*-adenosylhomocysteine hydrolase inhibitors also cause DNA fragmentation that is typical of apoptosis. The authors speculate that ATP released from cells undergoing cytolysis or degranulation may cause endothelial cell death and that this may be important in acute vascular injury or in limiting angiogenesis. A later report from this group examined the mechanism of purine-induced apoptosis in pulmonary artery endothelial cells and showed that inhibition of methyltransferase activity is involved.[109]

ATP converts necrosis to apoptosis in oxidant-injured bovine pulmonary artery endothelial cells.[110] Apoptosis serves an important role in the economy of tissues by eliminating cells without the attendant risks of an acute inflammatory response associated with necrosis.[111]

In a study of porcine aortic endothelial cells, extracellular ATP and ADP, probably acting through $P2X_7$ receptors, were shown to activate nuclear factor-κB, a transcription factor, and induce apoptosis.[112] In another report, extracellular ATP was shown to activate nuclear factor-κB through the $P2X_7$ receptor by selectively targeting P35 (Rel A) in cells of the macrophage lineage.[113]

Implications for Vascular Disease

Vascular injury represents a critical initiating event in the pathogenesis of various vascular diseases, including organ transplantation, sepsis, and atherosclerosis, and the events that follow, ie, vascular cell growth, migration, proliferation, and death.[114,115] Since large amounts of ATP are released from injured cells and because ATP and its breakdown product, adenosine, have potent actions in smooth muscle and endothelial cell growth, migration, proliferation, and death, the possibility that purines are one of the factors involved in the development of vascular disease needs to be considered. Various models of vascular injury have been introduced, including denudation of the endothelium by mechanical injury (balloon or nylon catheters), diet-induced hypercholesterolemic injury, or immune injury. However, only a limited number of studies have been carried out to examine the possible roles of purines in the development of the pathology of vessels.

The growth of new blood vessels takes place in pathological events such as tumor growth, wound healing, psoriasis, and the ischemic retinopathies that occur in diabetes and sickle cell disease. In the adult, the development of new blood vessels, or neovascularization, occurs by budding from existing blood vessels and is referred to as angiogenesis (as distinct from vasculogenesis, which occurs in embryogenic development by vessel formation from mesenchyme precursor cells or angioblasts). Peptide growth factors such as fibroblast growth factor, transforming growth factor-α, and VEGF are clearly involved in angiogenesis, but as we have seen earlier in the present review, purines and pyrimidines also contribute to this process.[44] In rheumatoid arthritis, new capillary blood vessels invade the joint and destroy the cartilage. In diabetes, new capillaries in the retina invade the vitreous body, bleed, and cause blindness, and tumor growth and metastasis are angiogenesis dependent.[116] Anginal patients treated chronically with dipyridamole to increase adenosine levels showed an increase in coronary angiogenesis,[117] and dipyridamole has also been used for the prevention of stroke.[118] The former action may involve a preferential effect of adenosine on endothelial cells, since smooth muscle proliferation was inhibited in rabbits pretreated with dipyridamole.[59]

Apoptotic cell death is recognized to occur in a number of vascular diseases, including atherosclerosis, restenosis, and hypertension.[99,114] Vascular endothelial cells are continuously exposed to variations in blood flow, which plays an important role in vessel growth or regression and in the local development of atherosclerosis. The shear stress that occurs during changes in blood flow leads to a substantial release of ATP (and UTP) from endothelial cells,[14] and these purines might mediate alterations in the balance between proliferation and apoptosis.[119] Occupation of $P2X_7$ receptors leads to the production of proinflammatory cytokines,[101] and TNFα markedly increases endothelial cell apoptosis via the activation of caspase 3.[99]

Atherosclerotic damage results in the disappearance of endothelium-dependent responses to ATP,[120,121] whereas the relaxing action of smooth muscle is unimpaired. The release of ATP from endothelial cells has been claimed to be impaired in atherosclerotic rat caudal arteries.[122] Long-term supplementation with a high cholesterol diet decreases the release of ATP from the caudal artery of aged rats; there was a significant positive correlation between the unsaturation index of arterial fatty acids and the amount of ATP released and an inverse correlation between the amount of ATP released and blood pressure.[123] Although the roles of endothelial cells and smooth muscle in the pathogenesis of atherosclerosis are still not known precisely, it is known that smooth muscle cells migrate from the media to the intima, where they change to the proliferative phenotype, which leads to thickening of the intima.[124]

In restenosis following balloon angioplasty, there is a peak in the proliferation and apoptosis of vascular smooth muscle cells at ≈14 days.[125] The first balloon inflation during coronary angioplasty is a preconditioning stimulus leading to a decrease in ischemia during later inflations; intracoronary adenosine administration before coronary angioplasty modi-

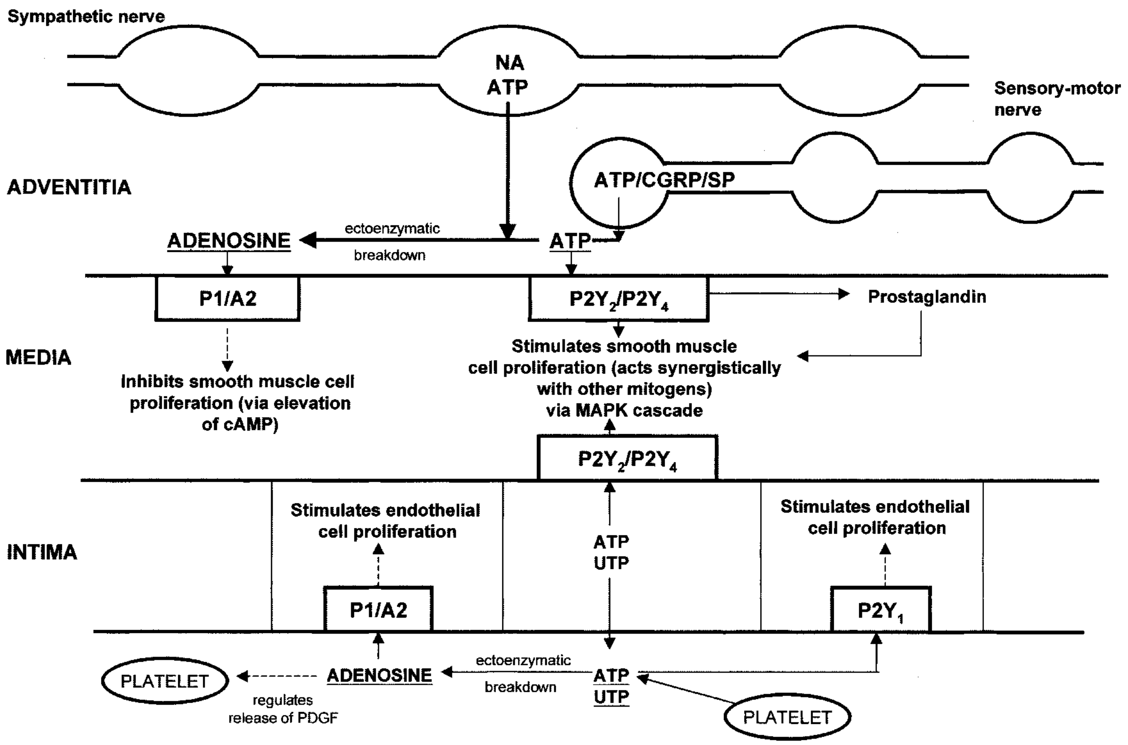

Figure 2. Schematic diagram of long-term (trophic) actions of purines released from nerves, platelets, and endothelial cells (which also release UTP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and sensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via P2Y$_2$ and/or P2Y$_4$ receptors via a MAPK cascade, whereas adenosine resulting from enzymatic breakdown of ATP acts on P1 (A$_2$) receptors to inhibit cell proliferation (via elevation of cAMP). ATP and UTP released from endothelial cells stimulate endothelial and smooth muscle cell proliferation via P2Y$_1$, P2Y$_2$, and P2Y$_4$ receptors. Adenosine resulting from ATP breakdown acts on P1 (A$_2$) receptors to stimulate endothelial cell proliferation and regulate the release of PDGF from platelets.

fies the preconditioning effect of the first inflation.[126] Further studies show that adenosine preconditions human myocardium against ischemia in vivo.[127]

The genetic defects underlying hypertension are unknown, but an increase in sympathetic nerve activity is well established, and there is an associated hyperplasia and hypertrophy of arterial walls.[128,129] An increased release of ATP as a cotransmitter with NA in sympathetic nerves is likely to occur in SHR[130,131] and may play a role in the trophic changes in the vessel wall. Also, sympathetic neurons innervating the vasculature are dependent on nerve growth factor (NGF) in development, and an increase in NGF gene expression and protein has been described in SHR.[132] α,β-Methylene ATP, an ATP agonist, was shown to increase NGF secretion by vascular smooth muscle cells in SHR.[133] In cultured aortic smooth muscle cells from SHR, responses to UTP and ATP were predominantly via P2Y$_4$ receptors, and Harper et al[41] have presented evidence to suggest that these receptors are coupled to mitogenesis via p42/p44 MAPK.

Pericytes partially envelop endothelial cells in most capillaries and have been implicated in capillary vasculogenesis and wound repair.[134] In addition, pericytes participate in the negative regulation of endothelial cell proliferation.[135] Along with its stimulating effect on bovine retinal capillary endothelial cells, adenosine has been shown to have an inhibitory effect on retinal pericytes, and it has been hypothesized that this dual function plays a role in the pathological neovascularization process that takes place in diabetes.[136] Diabetic microangiopathy has been implicated as a fundamental feature of the pathological complications of diabetes, including retinopathy, neuropathy, and foot ulceration.[137] Ischemia and hypoxia lead to a substantial release of ATP from endothelial cells,[64] and adenosine is released from hypoxic heart and skeletal muscle.[138] Adenosine has several cardiovascular protective effects in addition to vasodilation, including the promotion of endothelial cell proliferation and an increased expression of VEGF mRNA.[139] Adenosine also appears to play an important role in preconditioning.

When venous segments are transplanted into the arterial tree, the vein smooth muscle proliferates, and within ≈2 weeks, it resembles an artery and vice versa.[140] It is possible that ATP (and, subsequently, adenosine), which is released from the damaged cells during the operation and released from endothelial cells in response to the distension produced by increased blood pressure, is involved in the plasticity of change in vessel structure. Endothelial cells spread in response to localized injuries,[141] and ongoing localized injury leads to the release of purines, which might be involved in the repair process. High-velocity bolus doses of intracoronary adenosine have been used successfully as a technique to overcome the slow or "no-reflow" problem that complicates ≈10% to 15% of cases of catheter-based revascularization of degenerated saphenous vein bypass grafts. However, the mechanism involved seems likely to be largely the vasodilator actions of adenosine rather than trophic actions producing increased proliferation.

ATP is released from endothelial cells during hypoxia and, together with its breakdown product adenosine, produces

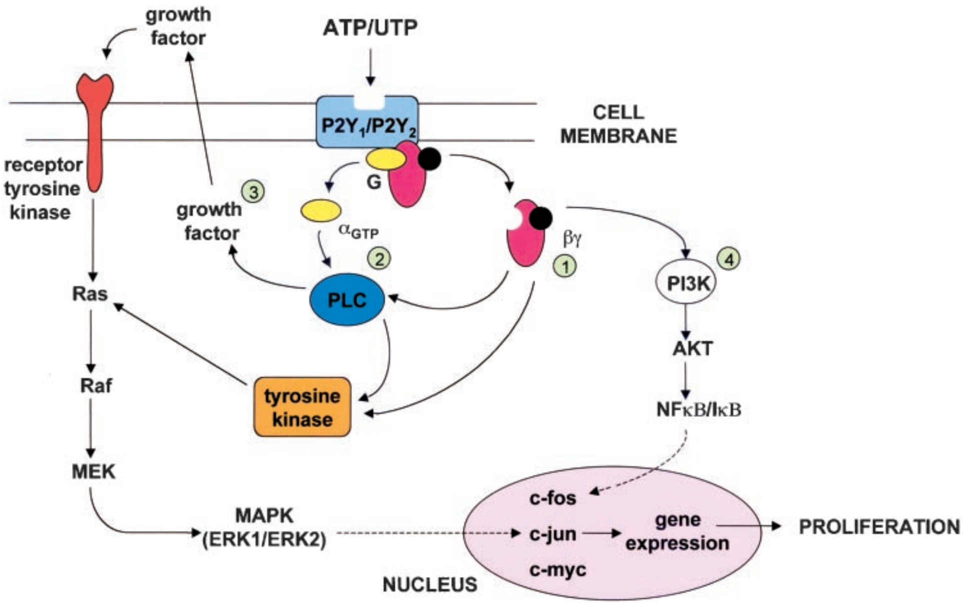

Figure 3. Schematic diagram illustrating possible MAPK-dependent and -independent pathways for P2Y receptor–activated mitogenesis. Pathway 1 is independent of PLC with direct activation of the $\beta\gamma$ subunit of the dissociated G protein. Pathway 2 indicates PLC-dependent events (eg, Ca^{2+} and PKC dependent) activating tyrosine kinase. Pathway 3 is dependent on the P2Y-regulated formation of growth factor, which acts via the extracellular compartment, to activate a receptor tyrosine kinase and, hence, the Ras-MAPK cascade (MAPK/ERK kinase, MAPK kinase). The control of mitogenesis by MAPK is illustrated. This schematic diagram implies that the 2 transcription factors, c-*fos* and c-*myc*, are synthesized in response to the MAPK cascade as well as other immediate early genes before downstream gene expression. Mitogenesis is also regulated by other events, including the action of phosphatidylinositol 3-kinase (PI3K), shown in pathway 4. (The figure was compiled from Boarder and Hourani,[20] 1998; Wilden et al,[53] 1998; and Neary,[100] 1997.)

vasodilatation and trophic actions on smooth muscle and endothelial cells. It has been proposed that adenosine released in this way may regulate the growth and spread of neoplastic tissues.[142] Evidence that has been presented in support of this hypothesis is that agents (such as dipyridamole) that increase the extracellular levels of adenosine also enhance tumor growth, whereas adenosine receptor antagonists reduce the size of primary tumors and the numbers of metastases. It is also known that tumor cells contain exceptionally high concentrations of ATP[143] and that the damage that occurs when tumors reach a size that leads to the breakage of cells during abrasive movements would release ATP, which might lead to apoptosis via $P2X_7$ receptors, resulting in tumor regression.[144,145]

Conclusions

A summary of the main trophic actions of purine nucleosides and nucleotides and of vascular cell proliferation is shown in Figure 2. There is compelling evidence that there is regulation of vascular smooth muscle and endothelial cell proliferation by P1 (A_2) and $P2Y_1$ and $P2Y_2$ receptors that acts through MAPK pathways. However, there is still much to learn about the precise pathways involved; eg, there is only preliminary evidence for the involvement of ERKs and c-Jun N-terminal kinases, and other pathways may also be involved[53,100,146] (Figure 3). Furthermore, there has been no exploration to determine whether the more recently cloned P2Y receptors, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$,[147–149] mediate the MAPK pathways that might be involved in vascular cell proliferation. Direct evidence for the involvement of these purinergic mechanisms in atherosclerosis, hypertension, and restenosis is awaited.

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References

1. Burnstock G. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology*. 1997;36:1127–1139.
2. Abbracchio MP, Williams M, eds. *Handbook of Experimental Pharmacology, Volume 15, I/II: Purinergic and Pyrimidinergic Signalling*. Berlin, Germany: Springer; 2001.
3. Burnstock G. A basis for distinguishing two types of purinergic receptor. In: Straub RW, Bolis L, eds. *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*. New York, NY: Raven Press; 1978:107–118.
4. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998;50:413–492.
5. Huang N, Wang D, Heppel LA. Extracellular ATP is a mitogen for 3T3, 3T6, and A431 cells and acts synergistically with other growth factors. *Proc Natl Acad Sci U S A*. 1989;86:7904–7908.
6. Rathbone MP, Deforge S, Deluca B, Gabel B, Laurenssen C, Middlemiss P, Parkinson S. Purinergic stimulation of cell division and differentiation: mechanisms and pharmacological implications. *Med Hypotheses*. 1992;37:213–219.
7. Abbracchio MP. P1 and P2 receptors in cell growth and differentiation. *Drug Dev Res*. 1996;39:393–406.
8. Neary JT, Rathbone MP, Cattabeni F, Abbracchio MP, Burnstock G. Trophic actions of extracellular nucleotides and nucleosides on glial and neuronal cells. *Trends Neurosci*. 1996;19:13–18.
9. Abbracchio MP, Burnstock G. Purinergic signalling: pathophysiological roles. *Jpn J Pharmacol*. 1998;78:113–145.
10. Burnstock G. Dual control of local blood flow by purines. *Ann N Y Acad Sci*. 1990;603:31–44.
11. Olsson RA, Pearson JD. Cardiovascular purinoceptors. *Physiol Rev*. 1990;70:761–845.
12. Ralevic V, Burnstock G. Roles of P_2-purinoceptors in the cardiovascular system. *Circulation*. 1991;84:1–14.

13. Burnstock G, Ralevic V. New insights into the local regulation of blood flow by perivascular nerves and endothelium. *Br J Plast Surg*. 1994;47: 527–543.
14. Burnstock G. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *J Anat*. 1999; 194:335–342.
15. Ralevic V. Roles of purines and pyrimidines in endothelium. In: Abbracchio MP, Williams M, eds. *Purinergic and Pyrimidines in Endothelium*. Berlin, Germany: Springer; 2001:101–120.
16. Schachter M. Endothelium and smooth muscle: trophic interactions and potential for therapeutic intervention. *J Hum Hypertens*. 1990;4:17–21.
17. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801–809.
18. Gibbons GH. Autocrine-paracrine factors and vascular remodeling in hypertension. *Curr Opin Nephrol Hypertens*. 1993;2:291–298.
19. Erlinge D. Extracellular ATP: a growth factor for vascular smooth muscle cells. *Gen Pharmacol*. 1998;31:1–8.
20. Boarder MR, Hourani SMO. The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Trends Pharmacol Sci*. 1998;19:99–107.
21. Neary JT, Abbracchio MP. Trophic roles of purines and pyrimidines. In: Abbracchio MP, Williams M, eds. *Handbook of Pharmacology: Purinergic and Pyrimidinergic Signalling*. Berlin, Germany: Springer; 2001: 305–338.
22. Jonzon B, Nilsson J, Fredholm BB. Adenosine receptor-mediated changes in cyclic AMP production and DNA synthesis in cultured arterial smooth muscle cells. *J Cell Physiol*. 1985;124:451–456.
23. Dubey RK, Gillespie DG, Osaka K, Suzuki F, Jackson EK. Adenosine inhibits growth of rat aortic smooth muscle cells: possible role of A2b receptor. *Hypertension*. 1996;27:786–793.
24. Takiguchi Y, Nagano M, Ikeda Y, Nakashima M. Early administration of YT-146, an adenosine A2 receptor agonist, inhibits neointimal thickening after rat femoral artery endothelium injury. *Eur J Pharmacol*. 1995;281:205–207.
25. Chamley-Campbell J, Campbell GR, Ross R. The smooth muscle cell in culture. *Physiol Rev*. 1979;59:1–61.
26. Dubey RK, Gillespie DG, Mi Z, Jackson EK. Adenosine inhibits growth of human aortic smooth muscle cells via A_{2B} receptors. *Hypertension*. 1998;31:516–521.
27. Dubey RK, Gillespie DG, Jackson EK. Adenosine inhibits collagen and total protein synthesis in vascular smooth muscle cells. *Hypertension*. 1999;33:190–194.
28. Feng P, Ge L, Akyhani N, Liau G. Sodium butyrate is a potent modulator of smooth muscle cell proliferation and gene expression. *Cell Prolif*. 1996;29:231–241.
29. Wang DJ, Huang NN, Heppel LA. Extracellular ATP and ADP stimulate proliferation of porcine aortic smooth muscle cells. *J Cell Physiol*. 1992;153:221–233.
30. Needleman P, Minkes MS, Douglas JR. Stimulation of prostaglandin biosynthesis by adenine nucleotides: profile of prostaglandin release by perfused organs. *Circ Res*. 1974;34:455–460.
31. Malam-Souley R, Campan M, Gadeau AP, Desgranges C. Exogenous ATP induces a limited cell cycle progression of arterial smooth muscle cells. *Am J Physiol*. 1993;264:C783–C788.
32. Miyagi Y, Kobayashi S, Ahmed A, Nishimura J, Fukui M, Kanaide H. P_{2U} purinergic activation leads to the cell cycle progression from the G_1 to the S and M phases but not from the G_0 to G_1 phase in vascular smooth muscle cells in primary culture. *Biochem Biophys Res Commun*. 1996; 222:652–658.
33. Hart MN, Heistad DD, Brody MJ. Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. *Hypertension*. 1980;2:419–423.
34. Southwell BR, Chamley-Campbell JH, Campbell GR. Tropic interactions between sympathetic nerves and vascular smooth muscle. *J Auton Nerv Syst*. 1985;13:343–354.
35. Bevan RD. Influence of adrenergic innervation on vascular growth and mature characteristics. *Am Rev Respir Dis*. 1989;140:1478–1482.
36. Burnstock G. Noradrenaline and ATP as cotransmitters in sympathetic nerves. *Neurochem Int*. 1990;17:357–368.
37. Erlinge D, Yoo H, Edvinsson L, Reis DJ, Wahlestedt C. Mitogenic effects of ATP on vascular smooth muscle cells vs. other growth factors and sympathetic cotransmitters. *Am J Physiol*. 1993;265:H1089–H1097.
38. Erlinge D, You J, Wahlestedt C, Edvinsson L. Characterisation of an ATP receptor mediating mitogenesis in vascular smooth muscle cells. *Eur J Pharmacol*. 1995;289:135–149.
39. Malam-Souley R, Seye C, Gadeau AP, Loirand G, Pillois X, Campan M, Pacaud P, Desgranges C. Nucleotide receptor P2u partially mediates ATP-induced cell cycle progression of aortic smooth muscle cells. *J Cell Physiol*. 1996;166:57–65.
40. Bogdanov YD, Wildman SS, Clements MP, King BF, Burnstock G. Molecular cloning and characterization of rat $P2Y_4$ nucleotide receptor. *Br J Pharmacol*. 1998;124:428–430.
41. Harper S, Webb TE, Charlton SJ, Ng LL, Boarder MR. Evidence that $P2Y_4$ nucleotide receptors are involved in the regulation of rat aortic smooth muscle cells by UTP and ATP. *Br J Pharmacol*. 1998;124: 703–710.
42. Erlinge D, Hou M, Webb TE, Barnard EA, Moller S. Phenotype changes of the vascular smooth muscle cell regulate P2 receptor expression as measured by quantitative RT-PCR. *Biochem Biophys Res Commun*. 1998;248:864–870.
43. Hou M, Moller S, Edvinsson L, Erlinge D, MAPKK-dependent growth factor-induced upregulation of $P2Y_2$ receptors in vascular smooth muscle cells. *Biochem Biophys Res Commun*. 1999;258:648–652.
44. Satterwhite CM, Farrelly AM, Bradley ME. Chemotactic, mitogenic, and angiogenic actions of UTP on vascular endothelial cells. *Am J Physiol*. 1999;276:H1091–H1097.
45. Sauzeau V, Le Jeune H, Cario-Toumaniantz C, Vaillant N, Gadeau AP, Desgranges C, Scalbert E, Chardin P, Pacaud P, Loirand G. P2Y(1), P2Y(2), P2Y(4), and P2Y(6) receptors are coupled to Rho and Rho kinase activation in vascular myocytes. *Am J Physiol*. 2000;278: H1751–H1761.
46. White PJ, Kumari R, Porter KE, London NJ, Ng LL, Boarder MR. Antiproliferative effect of UTP on human arterial and venous smooth muscle cells. *Am J Physiol*. 2000;279:H2735–H2742.
47. Saïag B, Bodin P, Shacoori V, Catheline M, Rault B, Burnstock G. Uptake and flow-induced release of uridine nucleotides from isolated vascular endothelial cells. *Endothelium*. 1995;2:279–285.
48. Bodin P, Burnstock G. ATP-stimulated release of ATP by human endothelial cells. *J Cardiovasc Pharmacol*. 1996;27:872–875.
49. Crowley ST, Dempsey EC, Horwitz KB, Horwitz LD. Platelet-induced vascular smooth muscle cell proliferation is modulated by the growth amplification factors serotonin and adenosine diphosphate. *Circulation*. 1994;90:1908–1918.
50. Erlinge D, Brunkwall J, Edvinsson L. Neuropeptide Y stimulates proliferation of human vascular smooth muscle cells: cooperation with noradrenaline and ATP. *Regul Pept*. 1994;50:259–265.
51. Agazie YM, Bagot JC, Trickey E, Halenda SP, Wilden PA. Molecular mechanisms of ATP and insulin synergistic stimulation of coronary artery smooth muscle growth. *Am J Physiol*. 2001;280:H795–H801.
52. Yu SM, Chen SF, Lau YT, Yang CM, Chen JC. Mechanism of extracellular ATP-induced proliferation of vascular smooth muscle cells. *Mol Pharmacol*. 1996;50:1000–1009.
53. Wilden PA, Agazie YM, Kaufman R, Halenda SP. ATP-stimulated smooth muscle cell proliferation requires independent ERK and PI3K signaling pathways. *Am J Physiol*. 1998;275:H1209–H1215.
54. Erlinge D, Heilig M, Edvinsson L. Tyrphostin inhibition of ATP-stimulated DNA synthesis, cell proliferation and fos-protein expression in vascular smooth muscle cells. *Br J Pharmacol*. 1996;118:1028–1034.
55. Narumiya S. The small GTPase Rho: cellular functions and signal transduction. *J Biochem (Tokyo)*. 1996;120:215–228.
56. Van Aelst L, D'Souza-Schorey C. Rho GTPases and signaling networks. *Genes Dev*. 1997;11:2295–2322.
57. Campbell GR, Campbell JH. Smooth muscle phenotypic changes in arterial wall homeostasis: implications for the pathogenesis of atherosclerosis. *Exp Mol Pathol*. 1985;42:139–162.
58. Hou M, Moller S, Edvinsson L, Erlinge D. Cytokines induce upregulation of vascular $P2Y_2$ receptors and increased mitogenic responses to UTP and ATP. *Arterioscler Thromb Vasc Biol*. 2000;20:2064–2069.
59. Ingerman-Wojenski CM, Silver MJ. Model system to study interaction of platelets with damaged arterial wall, II: inhibition of smooth muscle cell proliferation by dipyridamole and AH-P719. *Exp Mol Pathol*. 1988; 48:116–134.
60. Dusseau JW, Hutchins PM, Malbasa DS. Stimulation of angiogenesis by adenosine on the chick chorioallantoic membrane. *Circ Res*. 1986;59: 163–170.
61. Adair TH, Montani JP, Strick DM, Guyton AC. Vascular development in chick embryos: a possible role for adenosine. *Am J Physiol*. 1989; 256:H240–H246.
62. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. *Physiol Rev*. 1992;72:369–417.
63. Bodin P, Bailey DJ, Burnstock G. Increased flow-induced ATP release from isolated vascular endothelial but not smooth muscle cells. *Br J Pharmacol*. 1991;103:1203–1205.

64. Bodin P, Burnstock G. Purinergic signalling: ATP release. *Neurochem Res*. 2001;26:959–969.
65. Bosman J, Tangelder GJ, oude Egbrink MG, Reneman RS, Slaaf DW. Local application of adenosine induces an increase of capillary diameter in skeletal muscle of anesthetized rabbits. *J Vasc Res*. 1996;33:111–118.
66. Van Daele P, Van Coevorden A, Roger PP, Boeynaems JM. Effects of adenine nucleotides on the proliferation of aortic endothelial cells. *Circ Res*. 1992;70:82–90.
67. Ziche M, Parenti A, Morbidelli L, Meininger CJ, Granger HJ, Ledda F. The effect of vasoactive factors on the growth of coronary endothelial cells [in Italian]. *Cardiologia*. 1992;37:573–575.
68. Ethier MF, Chander V, Dobson JG Jr. Adenosine stimulates proliferation of human endothelial cells in culture. *Am J Physiol*. 1993;265: H131–H138.
69. Lutty GA, Mathews MK, Merges C, McLeod DS. Adenosine stimulates canine retinal microvascular endothelial cell migration and tube formation. *Curr Eye Res*. 1998;17:594–607.
70. Sexl V, Mancusi G, Baumgartner-Parzer S, Schutz W, Freissmuth M. Stimulation of human umbilical vein endothelial cell proliferation by A2-adenosine and beta 2-adrenoceptors. *Br J Pharmacol*. 1995;114: 1577–1586.
71. Sexl V, Mancusi G, Holler C, Gloria-Maercker E, Schutz W, Freissmuth M. Stimulation of the mitogen-activated protein kinase via the A2A-adenosine receptor in primary human endothelial cells. *J Biol Chem*. 1997;272:5792–5799.
72. Ethier MF, Dobson JG Jr. Adenosine stimulation of DNA synthesis in human endothelial cells. *Am J Physiol*. 1997;272:H1470–H1479.
73. Grant MB, Tarnuzzer RW, Caballero S, Ozeck MJ, Davis MI, Spoerri PE, Feoktistov I, Biaggioni I, Shryock JC, Belardinelli L. Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells. *Circ Res*. 1999;85:699–706.
74. Fischer S, Sharma HS, Karliczek GF, Schaper W. Expression of vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. *Brain Res Mol Brain Res*. 1995;28:141–148.
75. Takagi H, King GL, Robinson GS, Ferrara N, Aiello LP. Adenosine mediates hypoxic induction of vascular endothelial growth factor in retinal pericytes and endothelial cells. *Invest Ophthalmol Vis Sci*. 1996; 37:2165–2176.
76. Takagi H, King GL, Ferrara N, Aiello LP. Hypoxia regulates vascular endothelial growth factor receptor KDR/Flk gene expression through adenosine A2 receptors in retinal capillary endothelial cells. *Invest Ophthalmol Vis Sci*. 1996;37:1311–1321.
77. Grant MB, Davis MI, Caballero S, Feoktistov I, Biaggioni I, Belardinelli L. Proliferation, migration, and ERK activation in human retinal endothelial cells through A(2B) adenosine receptor stimulation. *Invest Ophthalmol Vis Sci*. 2001;42:2068–2073.
78. Meininger CJ, Schelling ME, Granger HJ. Adenosine and hypoxia stimulate proliferation and migration of endothelial cells. *Am J Physiol*. 1988;255:H554–H562.
79. Morris PB, Ellis MN, Swain JL. Angiogenic potency of nucleotide metabolites: potential role in ischemia-induced vascular growth. *J Mol Cell Cardiol*. 1989;21:351–358.
80. Meininger CJ, Granger HJ. Mechanisms leading to adenosine-stimulated proliferation of microvascular endothelial cells. *Am J Physiol*. 1990;258: H198–H206.
81. McAuslan BR, Reilly WG, Hannan GN, Gole GA. Angiogenic factors and their assay: activity of formyl methionyl leucyl phenylalanine, adenosine diphosphate, heparin, copper, and bovine endothelium stimulating factor. *Microvasc Res*. 1983;26:323–338.
82. Teuscher E, Weidlich V. Adenosine nucleotides, adenosine and adenine as angiogenesis factors. *Biomed Biochim Acta*. 1985;44:493–495.
83. Van Coevorden A, Roger P, Boeynaems J-M. Mitogenic action of adenine nucleotides and nucleosides on aortic endothelial cells. *Thromb Haemost*. 1989;62:190. Abstract.
84. Cha SH, Hahn TW, Sekine T, Lee KH, Endou H. Purinoceptor-mediated calcium mobilization and cellular proliferation in cultured bovine corneal endothelial cells. *Jpn J Pharmacol*. 2000;82:181–187.
85. LeRoy EC, Ager A, Gordon JL. Effects of neutrophil elastase and other proteases on porcine aortic endothelial prostaglandin I2 production, adenine nucleotide release, and responses to vasoactive agents. *J Clin Invest*. 1984;74:1003–1010.
86. Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS. Modulation of vascular smooth muscle contraction by the endothelium. *Annu Rev Physiol*. 1986;48:307–320.
87. Ott MJ, Olson JL, Ballermann BJ. Chronic in vitro flow promotes ultrastructural differentiation of endothelial cells. *Endothelium*. 1995;3: 21–30.
88. Sammak PJ, Hinman LE, Tran PO, Sjaastad MD, Machen TE. How do injured cells communicate with the surviving cell monolayer? *J Cell Sci*. 1997;110(pt 4):465–475.
89. Yamada T, Naruse K, Sokabe M. Stretch-induced morphological changes of human endothelial cells depend on the intracellular level of Ca^{2+} rather than of cAMP. *Life Sci*. 2000;67:2605–2613.
90. Motte S, Communi D, Pirotton S, Boeynaems JM. Involvement of multiple receptors in the actions of extracellular ATP: the example of vascular endothelial cells. *Int J Biochem Cell Biol*. 1995;27:1–7.
91. Malmsjö M, Edvinsson L, Erlinge D. P2X receptors counteract the vasodilatory effects of endothelium derived hyperpolarising factor. *Eur J Pharmacol*. 2000;390:173–180.
92. Hansen MA, Dutton JL, Balcar VJ, Barden JA, Bennett MR. P_{2X} (purinergic) receptor distributions in rat blood vessels. *J Auton Nerv Syst*. 1999;75:147–155.
93. Yamamoto K, Korenaga R, Kamiya A, Ando J. Fluid shear stress activates Ca^{2+} influx into human endothelial cells via $P2X_4$ purinoceptors. *Circ Res*. 2000;87:385–391.
94. Loesch A, Burnstock G. Ultrastructural localisation of ATP-gated $P2X_2$ receptor immunoreactivity in vascular endothelial cells in rat brain. *Endothelium*. 2000;7:93–98.
95. Glass R, Burnstock G. Immunohistochemical identification of cells expressing ATP-gated cation channels (P2X receptors) in the adult rat thyroid. *J Anat*. 2001;198:569–579.
96. Graham A, McLees A, Kennedy C, Gould GW, Plevin R. Stimulation by the nucleotides, ATP and UTP of mitogen-activated protein kinase in EAhy 926 endothelial cells. *Br J Pharmacol*. 1996;117:1341–1347.
97. Albert JL, Boyle JP, Roberts JA, Challiss RA, Gubby SE, Boarder MR. Regulation of brain capillary endothelial cells by P2Y receptors coupled to Ca^{2+}, phospholipase C and mitogen-activated protein kinase. *Br J Pharmacol*. 1997;122:935–941.
98. Paul A, Torrie LJ, McLaren GJ, Kennedy C, Gould GW, Plevin R. P2Y receptor-mediated inhibition of tumor necrosis factor alpha-stimulated stress-activated protein kinase activity in EAhy926 endothelial cells. *J Biol Chem*. 2000;275:13243–13249.
99. Mallat Z, Tedgui A. Apoptosis in the vasculature: mechanisms and functional importance. *Br J Pharmacol*. 2000;130:947–962.
100. Neary JT. MAPK cascades in cell growth and death. *News Physiol Sci*. 1997;12:286–293.
101. Di Virgilio F. Dr. Jekyll/Mr. Hyde: the dual role of extracellular ATP. *J Auton Nerv Syst*. 2000;81:59–63.
102. Gröschel-Stewart U, Bardini M, Robson T, Burnstock G. Localisation of $P2X_5$ and $P2X_7$ receptors by immunohistochemistry in rat stratified squamous epithelia. *Cell Tissue Res*. 1999;296:599–605.
103. Gröschel-Stewart U, Bardini M, Robson T, Burnstock G. P2X receptors in the rat duodenal villus. *Cell Tissue Res*. 1999;297:111–117.
104. Zheng LM, Zychlinsky A, Liu CC, Ojcius DM, Young JDE. Extracellular ATP as a trigger for apoptosis or programmed cell death. *J Cell Biol*. 1991;112:279–288.
105. Di Virgilio F, Zanovello P, Zambon A, Bronte V, Pizzo P, Murgia M. Cell membrane receptors for extracellular ATP: a new family of apoptosis-signalling molecules. *Fundam Clin Immunol*. 1995;3:80–81.
106. Jacobson KA, Hoffmann C, Cattabeni F, Abbracchio MP. Adenosine-induced cell death: evidence for receptor-mediated signalling. *Apoptosis*. 1999;4:197–211.
107. Dawicki DD, Chatterjee D, Wyche J, Rounds S. Extracellular ATP and adenosine cause apoptosis of pulmonary artery endothelial cells. *Am J Physiol*. 1997;273:L485–L494.
108. Kim KT, Yeo EJ, Choi H, Park SC. The effect of pyrimidine nucleosides on adenosine-induced apoptosis in HL-60 cells. *J Cancer Res Clin Oncol*. 1998;124:471–477.
109. Rounds S, Yee WL, Dawicki DD, Harrington E, Parks N, Cutaia MV. Mechanism of extracellular ATP- and adenosine-induced apoptosis of cultured pulmonary artery endothelial cells. *Am J Physiol*. 1998;275:L379–L388.
110. Lelli JL Jr, Becks LL, Dabrowska MI, Hinshaw DB. ATP converts necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic Biol Med*. 1998;25:694–702.
111. Kroemer G, Petit P, Zamzami N, Vayssiere JL, Mignotte B. The biochemistry of programmed cell death. *FASEB J*. 1995;9:1277–1287.
112. von Albertini M, Palmetshofer A, Kaczmarek E, Koziak K, Stroka D, Grey ST, Stuhlmeier KM, Robson SC. Extracellular ATP and ADP activate transcription factor NF-kappa B and induce endothelial cell apoptosis. *Biochem Biophys Res Commun*. 1998;248:822–829.

113. Ferrari D, Wesselborg S, Bauer MK, Schulze-Osthoff K. Extracellular ATP activates transcription factor NF-κB through the P2Z purinoreceptor by selectively targeting NF-κB p65. *J Cell Biol*. 1997;139:1635–1643.
114. Thomas WA, Reiner JM, Florentin FA, Lee KT, Lee WM. 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*. 1976;24:360–374.
115. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation. *J Am Coll Cardiol*. 1990;15:1667–1687.
116. Folkman J, Shing Y. Angiogenesis. *J Biol Chem*. 1992;267: 10931–10934.
117. Picano E, Michelassi C. Chronic oral dipyridamole as a "novel" antianginal drug: the collateral hypothesis. *Cardiovasc Res*. 1997;33:666–670.
118. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study, 2: dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *J Neurol Sci*. 1996;143:1–13.
119. Kaiser D, Freyberg MA, Friedl P. Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. *Biochem Biophys Res Commun*. 1997;231:586–590.
120. Verbeuren TJ, Jordaens FH, Zonnekeyn LL, Van Hove CE, Coene MC, Herman AG. Effect of hypercholesterolemia on vascular reactivity in the rabbit, I: endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits. *Circ Res*. 1986;58:552–564.
121. Ragazzi E, Froldi G, Pandolfo L, Chinellato A, De Biasi M, Prosdocimi M, Caparrotta L, Fassina G. Segmental impairment of endothelium-mediated relaxation in thoracic aortas from atherosclerotic rabbits: comparison to cholesterol infiltration and energy metabolism. *Artery*. 1989; 16:327–345.
122. Shinozuka K, Kitagawa S, Kunitomo M, Yamaguchi Y, Tanabe Y, Fujiwara M, Hattori K. Release of endogenous ATP from the caudal artery in rats with arteriosclerosis. *Eur J Pharmacol*. 1994;292:115–118.
123. Hashimoto M, Shinozuka K, Tanabe Y, Shahdat HM, Gamoh S, Kwon YM, Tanaka Y, Kunitomo M, Masumura S. Long-term supplementation with a high cholesterol diet decreases the release of ATP from the caudal artery in aged rats. *Life Sci*. 1998;63:1879–1885.
124. Burnstock G, Stewart-Lee AL, Brizzolara AL, Tomlinson A, Corr L. Dual control by nerves and endothelial cells of arterial blood flow in atherosclerosis. In: Wissler RW, Bond MG, Mercuri M, Tanganelli P, eds. *Atherosclerotic Plaques*. New York, NY: Plenum Press; 1991:285–292.
125. Han DK, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liau G. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am J Pathol*. 1995;147:267–277.
126. Kerensky RA, Kutcher MA, Braden GA, Applegate RJ, Solis GA, Little WC. The effects of intracoronary adenosine on preconditioning during coronary angioplasty. *Clin Cardiol*. 1995;18:91–96.
127. Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R. Preconditioning of human myocardium with adenosine during coronary angioplasty. *Circulation*. 1997;95:2500–2507.
128. Schwartz SM. Smooth muscle proliferation in hypertension: state-of-the-art lecture. *Hypertension*. 1984;6(suppl I):I-56–I-61.
129. Julius S, Nesbitt S. Sympathetic overactivity in hypertension: a moving target. *Am J Hypertens*. 1996;9:113S–120S.
130. Vidal M, Hicks PE, Langer SZ. Differential effects of α,β-methylene ATP on responses to nerve stimulation in SHR and WKY tail arteries. *Naunyn Schmiedebergs Arch Pharmacol*. 1986;332:384–390.
131. Brock JA, Van Helden DF. Enhanced excitatory junction potentials in mesenteric arteries from spontaneously hypertensive rats. *Pflugers Arch*. 1995;430:901–908.
132. Zettler C, Rush RA. Elevated concentrations of nerve growth factor in heart and mesenteric arteries of spontaneously hypertensive rats. *Brain Res*. 1993;614:15–20.
133. Spitsbergen JM, Stewart JS, Tuttle JB. Altered regulation of nerve growth factor secretion by cultured VSMCs from hypertensive rats. *Am J Physiol*. 1995;269:H621–H628.
134. Sims DE. The pericyte: a review. *Tissue Cell*. 1986;18:153–174.
135. D'Amore PA, Orlidge A. Growth factors and pericytes in microangiopathy. *Diabet Med*. 1988;14:495–504.
136. Jackson JA, Carlson EC. Inhibition of bovine retinal microvascular pericyte proliferation in vitro by adenosine. *Am J Physiol*. 1992;263: H634–H640.
137. Kamal K, Du W, Mills I, Sumpio BE. Antiproliferative effect of elevated glucose in human microvascular endothelial cells. *J Cell Biochem*. 1998;71:491–501.
138. Burnstock G. Hypoxia, endothelium and purines. *Drug Dev Res*. 1993; 28:301–305.
139. Hashimoto E, Kage K, Ogita T, Nakaoka T, Matsuoka R, Kira Y. Adenosine as an endogenous mediator of hypoxia for induction of vascular endothelial growth factor mRNA in U-937 cells. *Biochem Biophys Res Commun*. 1994;204:318–324.
140. Folkow B. Physiological aspects of primary hypertension. *Physiol Rev*. 1982;62:347–504.
141. Gotlieb AI. Endothelial and smooth muscle cell migration in the repair of the injured vessel wall. *Surv Synth Path Res*. 1983;1:5–22.
142. Phillis JW, Wu PH. Adenosine may regulate the vascular supply and thus the growth and spread of neoplastic tissues: a proposal. *Gen Pharmacol*. 1981;12:309–310.
143. Maehara Y, Kusumoto H, Anai H, Kusumoto T, Sugimachi K. Human tumor tissues have higher ATP contents than normal tissues. *Clin Chim Acta*. 1987;169:341–343.
144. Hopfner M, Maaser K, Barthel B, von Lampe B, Hanski C, Riecken EO, Zeitz M, Scherubl H. Growth inhibition and apoptosis induced by P2Y2 receptors in human colorectal carcinoma cells: involvement of intracellular calcium and cyclic adenosine monophosphate. *Int J Colorectal Dis*. 2001;16:154–166.
145. Janssens R, Boeynaems JM. Effects of extracellular nucleotides and nucleosides on prostate carcinoma cells. *Br J Pharmacol*. 2001;132:536–546.
146. Sellers LA, Simon J, Lundahl TS, Cousens DJ, Humphrey PP, Barnard EA. Adenosine nucleotides acting at the human $P2Y_1$ receptor stimulate mitogen-activated protein kinases and induce apoptosis. *J Biol Chem*. 2001;276:16379–16390.
147. Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *J Biol Chem*. 1997;272:31969–31973.
148. Communi D, Gonzalez NS, Detheux M, Brezillon S, Lannoy V, Parmentier M, Boeynaems JM. Identification of a novel human ADP receptor coupled to G(i). *J Biol Chem*. 2001;276:41479–41485.
149. Hollopeter G, Jantzen H-M, Vincent D, Li G, England L, Ramakrishnan V, Yang R-B, Nurden P, Nurden A, Julius D, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature*. 2001; 409:202–207.
150. Burnstock G. Development and perspectives of the purinoceptor concept. *J Auton Pharmacol*. 1996;16:295–302.