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Review

Dual control of vascular tone and remodelling by ATP released from nerves and endothelial cells

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Abstract:
Purinergic signalling is important both in short-term control of vascular tone and in longer-term control of cell proliferation, migration and death involved in vascular remodelling. There is dual control of vascular tone by ATP released from perivascular nerves and by ATP released from endothelial cells in response to changes in blood flow (shear stress) and hypoxia. Both ATP and its breakdown product, adenosine, regulate smooth muscle and endothelial cell proliferation. The involvement of these regulatory mechanisms in pathological conditions, including hypertension, atherosclerosis, restenosis, diabetes and vascular pain, are discussed.

Key words:
atherosclerosis, ATP, hypertension, pain, purinergic, restenosis

Abbreviations: NA – noradrenaline, NGF – nerve growth factor, NO – nitric oxide, PDGF – platelet-derived growth factor, SHR – spontaneously hypertensive rats, VEGF – vascular endothelial growth factor

Introduction

The concept of purinergic signalling, i.e. ATP acting as an extracellular signalling molecule, was proposed by Burnstock in 1972 when evidence was presented that ATP was the neurotransmitter in non-adrenergic, non-cholinergic neuromuscular transmission in the gut and urinary bladder [21]. Later, it was recognised as a cotransmitter in sympathetic, parasympathetic, sensory-motor and enteric nerves [10]. There was early resistance to this concept (see [12, 22, 26]), but the roles of nucleotides and nucleosides as extracellular signalling molecules are now well established in both neural and non-neural tissues [2, 18, 27]. P1 receptors for adenosine, of which four subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been cloned and characterised, have been distinguished from P2 receptors for ATP/ADP/UTP [8], 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 8 subtypes of P2Y receptors have been cloned and characterized [19, 79]. The majority of studies involving purinergic signalling 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 proliferation and death [1, 75].

ATP and adenosine are very much involved in the mechanisms underlying local control of vessel tone

[14, 15, 28] as well as cell migration, proliferation, differentiation, and death during angiogenesis, atherosclerosis, and restenosis following angioplasty [19, 39, 40].

Short-term purinergic signalling regulating vascular tone

In the vascular system, short-term purinergic signalling events associated with the control of vascular tone by ATP released from nerves and endothelial cells have been clearly demonstrated [11, 24, 28] (see Fig. 1).

ATP released as a cotransmitter with noradrenaline (NA) from perivascular sympathetic nerves acts mainly on $P2X_1$ receptors on medial vascular smooth muscle to produce constriction, whereas ATP released as a cotransmitter from sensory-motor nerves during 'axon reflex' activity dilates some vessels [28].

ATP released from endothelial cells during changes in blood flow (producing shear stress) or hypoxia acts on P2 receptors on endothelial cells to release nitric oxide (NO), resulting in relaxation [4, 24]. $P2Y_1$ receptors are dominant in some vessels activated selectively by ADP [79], in other vessels $P2Y_2$ receptors are present that are activated equally by ATP and the pyrimidine UTP [72]. UTP has also been shown to be released from endothelial cells by shear stress [85]. Endothelial cells also express other types of purinergic receptors ($P2Y_4$ and $P2Y_6$) [78]; and yet another P2Y receptor subtype ($P2Y_{11}$) is present on endothelial cells of human mammary artery and umbilical vein [104]. Endothelial cells of arteries and veins express different levels of $P2X_4$ receptors – high levels on saphenous veins and low levels on mammary arteries, although the function of this receptor was unknown [81]. However, Yamamoto et al. [107] reported that, in some blood vessels, $P2X_4$ receptors induced vascular dilatation in an NO-dependent manner. This finding contrasts with another study that examined $P2X_1$ receptors on the endothelium of rat mesenteric arteries. $P2X_1$ receptors mediated endothelial-dependent vasodilation, but a NO synthase inhibitor had no effect on dilation [50]. $P2X_4$ and $P2X_6$ receptors (perhaps as heteromultimers) were reported to be associated with VE-cadherin in human endothelial cells, suggesting a role for these receptors in modulating adhesion junctions between endothelial cells [46].

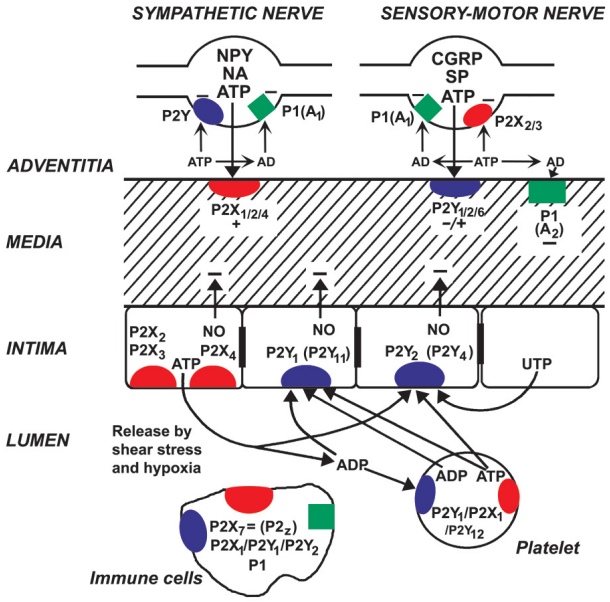

Fig. 1. Schematic diagram illustrating the main receptor subtypes for purines and pyrimidines present in blood vessels involved in control of vascular tone. ATP is released as a cotransmitter with noradrenaline (NA) and neuropeptide Y (NPY) from sympathetic nerves in the adventitia to act at smooth muscle $P2X_1$ receptors and, in some vessels, $P2X_2$, $P2X_4$ and $P2Y_2$ and $P2Y_6$ receptors, resulting in vasoconstriction; ATP is released with calcitonin gene related peptide (CGRP) and substance P (SP) from sensory-motor nerves during "axon reflex" activity to act on smooth muscle P2Y receptors, resulting in either vasodilatation or vasoconstriction. P1 (A_1) receptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (arising from ecto-enzymatic breakdown of ATP) modulation of transmitter release. $P2X_{2/3}$ receptors are present on a subpopulation of sensory nerve terminals. P1 (A_2) receptors 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 $P2Y_4$, $P2Y_{11}$ and $P2X_4$ receptors, leading to the production of nitric oxide (NO) and subsequent vasodilatation. ATP, after its release from aggregating platelets, also acts, together with its breakdown product ADP, on these endothelial receptors. Blood-borne platelets possess $P2Y_1$ and $P2Y_{12}$ ADP-selective receptors as well as $P2X_1$ receptors, whereas immune cells of various kinds possess $P2X_7$ as well as P1, $P2X_1$, $P2Y_1$ and $P2Y_2$ receptors. (Figure is modified from Burnstock G: J Auton Pharmacol, 1996, 16, 295–302 with permission from Blackwell Science Ltd, UK)

Adenosine produced after breakdown of released ATP from nerves and endothelial cells causes vasodilatation *via* smooth muscle P1 (usually A_2 subtype) receptors. ATP release from red blood cells is increased in pathological conditions such as subarachnoid hemorrhage, largely because there is widespread blood cell lysis [92]. This leads to transient constriction of arterioles via P2X receptors and sustained constriction of large cerebral vessels, largely through $P2Y_2$ receptors. The differences in purinergic receptor

distribution between macro- and microvessels in the cerebral circulation are likely to have important consequences in pathological conditions.

Long-term purinergic signalling involved in proliferation of smooth muscle and endothelial cells

Smooth muscle

An early study reported that adenosine produces changes in cyclic AMP and DNA synthesis in cultured arterial smooth muscle cells and suggested that this might result in the regulation of cell proliferation [57]. 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 its properties differ from those for ATP and ADP. Adenosine inhibits vascular smooth muscle cell proliferation by A_{2B} receptor activation via the elevation of cyclic AMP [37] and a selective A_2 receptor agonist, 2-octynyladenosine, reduced neointimal thickening in a rat femoral artery injury model [95].

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 [103]. 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. Exogenous ATP and UTP also induce a limited cell cycle progression in arterial smooth muscle cells as well as having powerful mitogenic actions, probably via $P2Y_2$ or $P2Y_4$ receptors [41, 68, 71].

Sympathetic nerves have been shown to exert a trophic influence on vascular smooth muscle [3, 90]. From her studies of pulmonary artery denervated of sympathetic nerves, Bevan [3] concluded that sympathetic transmitters exert slow trophic as well as fast signalling actions on cell growth and division by influencing protein, DNA, and RNA synthesis. Since ATP as well as NA and neuropeptide Y (NPY) are known to be released as cotransmitters from sympathetic nerves [15], this was consistent with the possibility that ATP and/or its breakdown product, adenosine, might be involved in these trophic actions.

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 [33]. The mitogenic effect of ATP on vascular smooth muscle cells is synergistic with other mitogens, including insulin and insulin-like growth factor-1 [103]. In a study of the mechanisms involved in ATP-induced proliferation of vascular smooth muscle cells [108], 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. A later study presented evidence indicating that ATP-stimulated vascular smooth muscle cell proliferation requires independent ERK and phosphatidylinositol 3-kinase–signalling pathways [105]. There are 2 phenotypes of smooth muscle: the contractile phenotype and the synthetic (proliferative) phenotype [30]. 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 (proliferative) 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 down-regulated, while the $P2Y_4$ and $P2Y_6$ receptors were unchanged [40]. 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 [53].

Endothelial cells

Long-term administration of adenosine was reported to induce capillary endothelial cell proliferation in the heart [54]. Adenosine has also been shown to induce dose-dependent proliferation of endothelial cells obtained from the aorta [98], from coronary vessels

[111] and from human umbilical veins [42]. It has also been shown to stimulate canine retinal microvascular endothelial cell migration and tube formation [67]. The action of adenosine in producing endothelial cell proliferation is mediated by A_{2A} and A_{2B} receptors partly by the modulation of vascular endothelial growth factor (VEGF) [48, 88]. 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 [43] and retinal [94] microvascular endothelial cells. The selective A_{2B} receptor antagonists enprofylline and 3-isobutyl-8-pyrrolidinoxanthine inhibited 5'-(*N*-ethylcarboxamido)-adenosine-stimulated proliferation of human retinal endothelial cells, ERK activation, cell migration, and capillary tube formation [47].

ADP was shown to be one of several agonists that induced cultured endothelial cell migration and proliferation [70]. 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* [96]. Adenine nucleotides were shown to have a mitogenic action on aortic endothelial cells, probably via P2Y receptors [97]. ATP has also been shown to produce proliferation of cultured bovine corneal endothelial cells [31]. The source of the purines involved in these trophic actions is largely from the endothelial cells, suggesting an autocrine mechanism [66]. ADP released from aggregating platelets may also play a role [99]. Stretch-induced changes in endothelial cell shape [106] and changes produced by hypoxic stress may be mediated by the ATP (and adenosine after ectoenzymatic breakdown) released from endothelial cells under both these conditions (see [5]).

There is increasing evidence that cell proliferation and programmed cell death (apoptosis) are linked [35]. For example, VEGF turns on cell proliferation, but inhibits apoptosis [69]. Distinct signal transduction cascades, composed of at least 3 protein kinases, mediate cell proliferation and differentiation, growth arrest, and apoptosis [73]. In diseases such as carcinogenesis, degenerative disorders, and ischemia/reperfusion injury, there is an imbalance between cell division and cell death. Extracellular ATP and adenosine have been shown to cause apoptosis of pulmonary artery endothelial cells [34]. It has been speculated that ATP released from cells undergoing cytolysis or degranulation may cause endothelial cell death, perhaps by inhibition of methyltransferase activity and that this may be important in acute vascular injury or in limiting angiogenesis [84]. ATP converts necrosis to apoptosis in oxidant-injured bovine pulmonary artery endothelial cells [65]. 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 [102].

Purinergic signalling in vascular diseases

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 [45, 83, 87]. ATP, ADP, UTP, and adenosine play pivotal signalling roles in these long-term events [23, 38, 39, 74, 80].

Hypertension

ATP plays a significant cotransmitter role in sympathetic nerves supplying hypertensive blood vessels. The purinergic component is increased in spontaneously hypertensive rats (SHR) [6, 101]. The increase in sympathetic nerve activity in hypertension is well established, and there is an associated hyperplasia and hypertrophy of arterial walls [58]. 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 spontaneously hypertensive rats (SHR) [109]. α,β-Methylene ATP has been shown to increase NGF secretion by vascular smooth muscle cells in SHR [91]. ATP is a rapidly acting hypotensive agent that compares favourably with sodium nitroprusside [63]. ATP-$MgCl_2$ is a safe, effective, and preferential pulmonary vasodilator in children with pulmonary hypertension secondary to congenital heart defects; it has also been used for treating pulmonary hypertension after cardiac surgery [7]. ATP ex-

erts mitogenic actions on human pulmonary artery smooth muscle cells, which may be relevant to the pathophysiological basis of severe pulmonary hypertension [59, 110]. Eicosapentaenoic acid, one of the active components in fish oil that has antihypertensive effects, increases the release of ATP from vascular endothelial cells, leading to reduction of the blood pressure rise characteristic of aging [51]. $P2X_4$ receptors are localized in the syncytiotrophoblast, stroma, and foetal capillary endothelial cells of human placenta. Placental $P2X_4$ receptors are significantly upregulated in mild preeclampsia [82].

Angiogenesis

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 purines and pyrimidines also contribute to this process [86]. 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 [44]. Anginal patients treated chronically with dipyridamole to increase adenosine levels showed an increase in coronary angiogenesis [76], and dipyridamole has also been used for the prevention of stroke [36]. The former action may involve a preferential effect of adenosine on endothelial cells, since smooth muscle proliferation was inhibited in rabbits pre-treated with dipyridamole [55].

Atherosclerosis

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, i.e., vascular cell growth, migration, proliferation, and death. 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 has been 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. A limited number of studies have been carried out to examine the possible roles of purines in the development of the pathology of vessels.

Atherosclerotic damage results in the disappearance of endothelium-dependent vasodilator responses to ATP [77, 100], 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 [89]. Long-term supplementation with a high cholesterol diet decreases the release of ATP from the caudal artery of aged rats [52]. Changes in the dual nervous and endothelial control of blood flow in hypocholesteraemic rabbits have been described [29].

Apoptotic cell death is recognized to occur in a number of vascular diseases, including atherosclerosis, restenosis, and hypertension [69]. 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 [24], and these purines might mediate alterations in the balance between proliferation and apoptosis [60]. Occupation of $P2X_7$ receptors leads to the production of proinflammatory cytokines [35] and tumor necrosis factor-α markedly increases endothelial cell apoptosis via the activation of caspase 3 [69].

Restenosis

In restenosis following balloon angioplasty, there is a peak in the proliferation and apoptosis of vascular smooth muscle cells at ~14 days [49]. 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 modifies the preconditioning effect of the first inflation [62]. Further studies show that adenosine preconditions hu-

man myocardium against ischemia *in vivo* [64]. Ischemia and hypoxia lead to a substantial release of ATP from endothelial cells [5] and ATP and adenosine are released from hypoxic heart and skeletal muscle [13].

Diabetes

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 neovascularisation process that takes place in diabetes [56]. Diabetic microangiopathy has been implicated as a fundamental feature of the pathological complications of diabetes, including retinopathy, neuropathy, and foot ulceration [61].

Vascular pain

$P2X_3$ receptors are found on nociceptive sensory nerve fibres and local release of ATP activates these fibres sending messages to the pain centres in the brain (see [20]). Burnstock speculated in 1996 [9] that vascular pain, such as angina, migraine, ischemic muscle pain, lumbar pain and pelvic pain, may involve purinergic signalling. It was suggested that following local vasospasm, ATP released from microvessel endothelial cells during the reactive hyperaemia that follows and is known to be associated with pain, reaches $P2X_3$ receptors on the nociceptive sensory fibres in the adventitia to initiate pain. This concept was first proposed for the pain occurring in migraine [17, 25]. In anginal pain, cardiac myocytes as well as coronary microvessel endothelial cells may be the source of ATP and adenosine reaching purinergic nociceptors [32, 93].

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