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Alteration of purinergic signaling in diabetes: Focus on vascular function

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Abstract

Diabetes is an important risk factor for the development of cardiovascular disease including atherosclerosis and ischemic heart disease. Vascular complications including macro- and micro-vascular dysfunction are the leading causes of morbidity and mortality in diabetes. Disease mechanisms at present are unclear and no ideal therapies are available, which urgently calls for the identification of novel therapeutic targets/agents. An altered nucleotide- and nucleoside-mediated purinergic signaling has been implicated to cause diabetes-associated vascular dysfunction in major organs. Alteration of both purinergic P1 and P2 receptor sensitivity rather than the changes in receptor expression accounts for vascular dysfunction in diabetes. Activation of $P2X_7$ receptors plays a crucial role in diabetes-induced retinal microvascular dysfunction. Recent findings have revealed that both ecto-nucleotidase CD39, a key enzyme hydrolyzing ATP, and CD73, an enzyme regulating adenosine turnover, are involved in the renal vascular injury in diabetes. Interestingly, erythrocyte dysfunction in diabetes by decreasing ATP release in response to physiological stimuli may serve as an important trigger to induce vascular dysfunction. Nucleot(s)ide-mediated purinergic activation also exerts long-term actions including inflammatory and atherogenic effects in hyperglycemic and diabetic conditions. This review highlights the current knowledge regarding the altered nucleot(s)ide-mediated purinergic signaling as an important disease mechanism for the diabetes-associated vascular complications. Better understanding the role of key receptor-mediated signaling in diabetes will provide more insights into their potential as targets for the treatment.

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None.

Keywords

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1. Introduction

Diabetes is an important risk factor for the development of cardiovascular disease including atherosclerosis and ischemic heart disease. Diabetes-induced macro- and micro-vascular dysfunction in heart, kidney, retinal microvasculature and peripheral vascular beds are the leading causes contributing to the increased morbidity and mortality in diabetes [1]. The pathogenesis of these vascular dysfunctions mainly comes from the imbalances between anti-inflammatory factors/vasodilators such as nitric oxide (NO) and adenosine triphosphate (ATP), and inflammatory factors/vasoconstrictors such as endothelin, reactive oxygen species (ROS) and ATP, as well as receptor-mediated signaling activated by certain endothelium-derived factors e.g. ATP and/or adenosine-activated purinergic signaling [2,3]. Despite the evidence showing that hyperglycemia has been a crucial factor driving vascular complications, recent clinical studies demonstrated that hyperglycemic control has not convincingly reduced the mortality among diabetic patients at high risk for cardiovascular events [4]. Apparently, the underlying causes of vascular dysfunction are multifactorial and complex, there is an unmet need to identify key disease mechanisms behind vascular complications in diabetes in order to develop novel therapies that specifically target such complications.

Increasing evidence suggests that extracellular nucleotides and nucleosides contribute to homeostasis of cardiovascular systems [5–7]. Nucleotides (such as ATP and UTP), nucleosides (such as adenosine), and dinucleotides (such as uridine adenosine tetraphosphate (Up_4A)), can be released from endothelial cells, adventitial nerves or circulating cells like platelets, immune cells and erythrocytes to regulate vascular tone, vascular remodeling, vascular permeability, and inflammation through purinergic receptors [3,8]. Of importance, nucleot(s)ide-mediated purinergic signaling is altered in diabetes, which accounts for the development of vascular dysfunction [3]. Therefore, the nucleot(s) ides and nucleot(s)ide-mediated purinergic signaling would represent an innovative and valuable target for the treatment of diabetes-associated vascular complications. In this review, we summarize important aspects of purinergic dysfunction involving not only the vascular effect of purinergic activation at the cellular and the intact organ levels but also the trophic effect of nucleotide-mediated purinergic signaling in hyperglycemic and diabetic conditions. The main focus is on the evidence addressing altered involvement of nucleot(s)ides and nucleot(s) ide-regulated purinergic network as key mediators of diabetes-associated vascular dysfunction in major organs.

1.1. Nucleot(s)ides and its mediated purinergic signaling

Nucleot(s)ides predominantly exert their biological effects through purinergic receptors. Purinergic receptors have been classified into P1 and P2 categories [9,10]. Four subtypes of P1 receptors (also termed adenosine receptors) have been identified, namely A1R, $A_{2A}R$,

$A_{2B}R$ and A3R. A1R and A3R are negatively coupled to adenylyl cyclase through the Gi/o protein alpha-subunits and activation of those receptors decreases cAMP levels, whereas $A_{2A}R$ and $A_{2B}R$ are positively coupled to adenylyl cyclase through Gs and enhance cAMP levels [11,12]. The P2 receptors are further classified into two major families: ionotropic P2XRs and metabotropic P2YRs [10,13]. At least 7 P2XRs ($P2X_{1\text{-}7}R$), and 8 P2YRs ($P2Y_1R$, $P2Y_2R$, $P2Y_4R$, $P2Y_6R$, $P2Y_{11}R$, $P2Y_{12}R$, $P2Y_{13}R$ and $P2Y_{14}R$) have been identified to date [14] (Fig. 1). Activation of P2XRs increases sodium and calcium permeability, while activation of P2YRs either affects adenylyl cyclase and alters cAMP levels or activates phospholipase C and releases intracellular calcium [14] (Fig. 1). Distribution and expression of purinergic receptors are ubiquitous throughout the body, with sub-type distributions varying regionally across tissues as well as across species [10,11,15]. In contrast to the activation of P1 receptors by adenosine only, P2 receptors can be activated by several nucleotides and have overlapping ligand preferences. $P2X_{1\_7RS}$ are mainly activated by ATP; $P2Y_4R$, $P2Y_{12}R$ and $P2Y_{13}R$ are activated by ADP, while $P2Y_4R$ are sensitive to both ATP and ADP. Additionally, $P2Y_2R$ and $P2Y_4R$ are preferably sensitive to UTP, while $P2Y_6R$ preferably responds to UDP stimulation [10] (Table 1). Upon stimulation by these extracellular nucleot(s)ides, purinergic signaling is initiated, resulting in various physiological responses, including platelet aggregation, cellular proliferation, angiogenesis, immune responses and vascular tone regulation [7,10–12,15]. Such purinergic action is regulated by ecto-nucleotidases such as nucleoside triphosphate diphosphohydrolase (NTPDase, also known as ecto-ATPDase CD39), ecto-5′-nucleotidase (CD73), nucleotide pyrophosphatase/phosphodiesterases (NPP) and adenosine deaminase [8]. For instance, CD39 phosphohydrolyzes ATP and ADP to AMP, which is further dephosphorylated to adenosine by CD73. Thus, ecto-enzymes play a critical role in maintaining extracellular nucleotides and adenosine homeostasis.

In the vascular system, all four P1 receptor subtypes are expressed in both endothelial cells and smooth muscle cells [11,12]. With respect to regulation of vascular tone, activation of A1R and A3R typically results in vascular contraction, whereas activation of $A_{2A}R$ and $A_{2B}R$ leads to vascular relaxation [11,12]. The P1 receptor-mediated vascular tone regulation appears to be independent of the locations of receptor subtypes [11], whereas the effects of the activation of P2 receptor subtypes are tissue–/cell-dependent. For example, activation of P2 receptor subtypes on endothelial cells typically leads to vasodilation, while activation of P2 receptor subtypes on smooth muscle cells results in vasoconstriction [7,16]. Thus, activation of $P2X_4R$, $P2X_2R$, $P2X_4R$, $P2Y_4R$, $P2Y_2R$ and $P2Y_6R$ on smooth muscle cells results in vasoconstriction [7] via a mechanism involving production of ROS or thromboxane (T_XA2) [8], while activation of $P2X_4R$, $P2X_2R$, $P2X_3R$, $P2X_4R$ $P2X_7R$, $P2Y_1R$, $P2Y_2R$, $P2Y_4R$ and $P2Y_{11}R$ on endothelial cells has been shown to produce NO, prostacyclin and endothelium-hyperpolarization factors leading to vasodilation [7,16] (Table 1), although there are also observations to suggest that activation of $P2X_1R$ on endothelial cells contributes to vascular contraction [17,18].

2. Purinergic dysfunction in diabetes

2.1. Alteration of purinergic signaling and endothelial/smooth muscle cell dysfunction

Endothelial dysfunction plays a crucial role in the development of vascular complications in diabetes [2]. Induction of endothelial dysfunction, caused by hyperglycemia, dyslipidemia and hyperinsulinemia results in impaired vascular regulation, oxidative stress, and inflammation. High glucose can stimulate the release of nucleotides thereby initiating purinergic signaling. Indeed, adenosine transport and adenosine receptor expression pattern have been found to be altered in human umbilical vein endothelial cells (HUVECs) derived from humans with gestational diabetes. HUVECs derived from gestational diabetic pregnancies exhibited a higher concentration of adenosine and higher protein levels for A1R but lower protein levels for $A_{2B}R$ [19,20]. Such alterations are attributed partially to the macro- and micro-vascular endothelial dysfunction in the placenta of humans with gestational diabetes [20,21]. Upon stimulation with high glucose and palmitate, ATP was released from HUVECs activating both $P2X_4R$ and $P2X_7R$ [22]. Such activation led to upregulation of various inflammatory genes, formation of ROS and reduction of NO bioavailability [22]. In aortas isolated from type 2 Goto-Kakizaki (GK) rats, endothelium-dependent relaxation was attenuated by not only the non-selective P1 and P2 receptor antagonists but also by the A1R, the $P2X_7R$ and the $P2Y_6R$ antagonists via a mechanism involving alteration of receptor sensitivity [23] (Table 2). Taken together, these studies indicate an altered purinergic signaling accounting for diabetes-associated endothelial dysfunction. In addition to endothelial cells, altered purinergic signaling may also be present in smooth muscle cells accounting for diabetes-induced vascular smooth muscle injury. Existing evidence demonstrated that increase in adenosine turnover affected iNOS levels in smooth muscle cells isolated from aortas of type 1 diabetic rats. The subsequent functional evidence has been observed in endothelium-denuded aortas in which NOS inhibition abolished adenosine-induced relaxation in diabetic but not control rats [24] (Table 2).

2.2. Alteration of purinergic signaling in the peripheral vasculature (aorta, mesenteric artery and femoral circulation)

As mentioned above, activation of purinergic receptors located on endothelium vs. smooth muscle cells leads to different vascular responses. Under diabetic conditions, the location and distribution of purinergic receptors are altered and can even be shifted between endothelial cells and smooth muscle cells, which may induce different net vascular reactivity [8]. In aortas isolated from type 1 diabetic mice without obvious endothelial dysfunction, the AlR-mediated vascular contraction was decreased, while the $A_{2A}R$-mediated vascular relaxation was impaired [25] (Table 2). The altered A1R-mediated vascular effect was likely due to a change in the receptor sensitivity, as the protein expression of A1R in aortas was comparable between diabetes and control [25]. In a type 1 diabetic rat model with endothelial dysfunction, adenosine-mediated NO production in endothelium was impaired [26]. Thus, the adenosine analogue- and the $A_{2A}R$ agonist-mediated aortic relaxations were reduced as compared to control rats [26] (Table 2). Such difference between diabetes and control rats in the adenosine analogue- and the $A_{2A}R$ agonist-mediated relaxations were abolished by endothelium denudation [26]. Likewise, NOS inhibition attenuated the adenosine analogue-induced relaxation more in aortas from control rats than those from

diabetic rats [26]. Further, upon stimulation by the novel dinucleotide Up_4A that activates both P1 and P2 receptors, Up_4A-enhanced aortic contraction in a type 2 diabetic GK rat model was attenuated by the non-selective P1 receptor and the A1R antagonists without alteration of the receptor expression [23]. Taken together, these observations indicate that the A1R-mediated vascular contraction and the $A_{2A}R$-mediated vascular relaxation in aortas are altered in diabetes likely through changes in receptor sensitivity.

In addition to altered P1 receptor-mediated vascular function in aortas, $P2X_7R$ inhibition attenuated Up_4A-enhanced vascular contraction in GK rats [23] (Table 2). This attenuation is through alteration of receptor sensitivity, as the $P2X_7R$ expression was not affected by diabetes [23]. Of interest, UDP activated vasoconstrictor prostanoids in aortas from control Long–Evans Tokushima Otsuka (LETO) rats limiting vascular relaxation as compared to type 2 diabetic model of obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats [27]. The UDP-induced relaxation in aortas from OLETF rats was partially attenuated by inhibition of MRS2578-sensitive $P2Y_6R$ [27]. On the other hand, UDP enhanced vascular contraction in endothelium-denuded aortas from OLETF rats vs. LETO rats likely through activation of MRS2578-insensitive $P2Y_6R$ [27] (Table 2).

In contrast to conduit aortas, purinergic signaling seems not to be affected in mesenteric arteries by diabetes in the same models mentioned above. Thus, adenosine-agonist (A1R, $A_{2B}R$ and A3R)-mediated vascular responses did not differ between type 1 diabetic mice and control mice [25] (Table 2). Moreover, impaired endothelium-dependent relaxation in mesenteric arteries isolated from GK rats was not affected by either the non-selective P1 or the non-selective P2 receptor antagonists [23]. On the other hand, the Up4A-induced vascular contraction was comparable in mesenteric arteries between GK and control rats [23] (Table 2). In an older type 1 diabetic rat model, $P2Y_1R$-mediated relaxation was impaired in superior mesenteric arteries from long-term type 1 diabetic rats [28]. Such impairment was caused by the reduced $P2Y_1R$-mediated NO signaling rather than reduced receptor expression [28] (Table 2). Similarly, in an aged and later-stage type 2 diabetic rat model, both ATP- and UTP-induced vascular contraction were increased in mesenteric arteries of GK rats [29] (Table 2). The enhancement in contraction was through endothelium-derived vasoconstrictor prostanoids/thromboxane and was mediated by activation of P2YR located on endothelial cells [29]. These observations suggest that aging and diabetic progression may play a determining role in regulation of purinergic signaling in resistance mesenteric arteries. In type 1 diabetic rats, perfusate of endothelium-denuded mesenteric arteries at basal condition or by electronic field stimulation as well as primary cultured endothelial cells by mechanical stimulation exhibited increased ATP and adenosine [30], providing insights into diabetes-induced neuropathy. However, the subsequent purinergic receptors activated by enhanced ATP and adenosine remain to be determined.

Alterations of vascular purinergic regulation observed in experimental diabetic models can be extrapolated into humans. Intrafemoral artery infusion of adenosine, ATP and UTP all resulted in impaired vasodilation in patients with type 2 diabetes as compared to control subjects [31]. Distribution and expression of the putative $P2X_1R$ and $P2Y_2R$ were comparable between patients with type 2 diabetes and control subjects [31], suggesting a

possible altered receptor sensitivity that accounts for impaired vascular responses in diabetes (Table 2).

2.3. Alteration of vascular purinergic signaling in the kidney

Purinergic activation is actively involved in blood flow regulation in the kidney [32–34]. Altered purinergic signaling in diabetes may result in renal vascular dysfunction. Increased adenosine levels have been observed not only in streptozotocin-induced type 1 diabetic rats (vs. control rats) [35,36] but also in diabetic patients with diabetic nephropathy vs. diabetic patients without nephropathy or healthy subjects [37]. Moreover, mice lacking adenosine deaminase exhibited increased circulating adenosine and developed renal dysfunction [38]. These studies suggest that increased adenosine may have detrimental effect on renal function in diabetes. This is supported by several observations that injection of adenosine decreased renal blood flow more in streptozotocin-induced type 1 diabetic rats than in control rats [39]. Likewise, renal vascular response to endogenous adenosine, assessed by post-occlusive reduction of renal blood flow following renal artery occlusion, was significantly blunted in this model [39]. The adenosine-mediated vasoconstrictor effect could be attenuated by the A1R antagonist in both groups [39] (Table 3). In contrast, i.p. injection of the adenosine analogue 5′-(N-ethylcarboxamido)-adenosine (NECA) into streptozotocin-induced type 1 diabetic rats reduced diabetes-induced oxidative stress, inhibited the expression of proinflammatory genes and deactivated JNK-MAPK signaling pathways [40]. Administration of adenosine into streptozotocin-induced type 1 diabetic rats prevented kidney histopathological changes, decreased plasma kidney injury factor-1 and tumor necrosis factor-α [41]. The different outcomes induced by adenosine or adenosine receptor stimulation may depend on which specific adenosine receptor subtypes are activated. Adenosine receptor expression and distribution have been shown to be altered in type 1 diabetic rats [42], implying a functional role of adenosine receptors in the control of renal vascular function in diabetes. In contrast to the blocking effect of the A1R antagonist on adenosine-induced renal vasoconstriction in diabetic rats [39], diabetic mice lacking A1R augmented hyperfiltration and glomerular injury [43]. However, no differences in glomerular filtration between alloxan-induced diabetic A1R knockout and wild-type mice were observed (Table 3) [44]. Thus, the exact role for A1R in regulation of renal function in diabetes remains unclear and warrants further investigations. Activation of $A_{2A}R$ with the $A_{2A}R$ agonist in streptozotocin-induced type 1 diabetic rats attenuated glomerular inflammation and injury [45,46] (Table 3), indicating a protective role of activation of $A_{2A}R$ in diabetes-induced renal injury. Adenosine-mediated renal injury in diabetes has been report to be linked to the activation of $A_{2B}R$, as $A_{2B}R$ inhibition prevented early alteration seen in diabetic glomerulopathy and fibrosis [47,48] (Table 3). In contrast, studies have reported that activation of endothelial $A_{2B}R$ corrected glomerular size and extracellular matrix deposition in type 1 diabetic mice [49] (Table 3). The role for $A_{2B}R$ is still controversial and future studies are needed to investigate the functional role of $A_{2B}R$ in the development of vascular dysfunction in diabetic kidney.

Ecto-nucleotidases regulating nucleot(s)ide concentrations and turnover play an important role in diabetes-induced renal injury. CD73 expression was shown to be increased in the kidney of both type 1 and 2 diabetic mice [49]. Deletion of CD73 was associated with more

severe nephropathy in streptozotocin-induced type 1 diabetic mice [49] (Table 3). Diabetic mice induced by streptozotocin lacking CD39 developed increased glomerular inflammation in association with higher levels of monocyte chemoattractant protein-1 (MCP-1) expression and showed much more glomerular fibrin deposition and stronger glomerular plasmingogen activator inhibitor-1 (PAI-1) staining as compared to control mice [50] (Table 3). CD39 phosphohydrolyzes ATP to ADP and AMP [8], the detrimental renal effects observed in diabetic mice with CD39 deficiency are likely related to increased accumulation of ATP/UTP, as PAI-1 and MCP-1 expression could be upregulated by ATP and UTP but not ADP or adenosine in vitro [50]. This may suggest an alteration of P2 receptor-mediated purinergic signaling in regulation of vascular function in diabetic kidney. Indeed, the renal vascular responses to UP4A stimulation were enhanced in type 2 diabetic GK rats via P2 receptor-mediated increase in thromboxane receptor sensitivity [51] (Table 3). Existing studies demonstrate that $P2X_7R$ levels were increased in the glomerular region of type 1 diabetic rats [52,53] and in both cortex and glomerular regions from patients with type 2 diabetes [54]. An alteration of $P2X_7R$-mediated increased cortical blood flow, and decreased glomerular filtration rate and glomerular insulin space in type 1 diabetic rats were also observed [36] (Table 3). $P2X_7R$ stimulation evoked and $P2X_7R$ deletion attenuated renal inflammation and injury induced by high-fat diet in type 2 diabetes [54] (Table 3). All evidence may suggest not only P2R but also ecto-nucleotidases as potential therapeutic targets for the treatment of diabetes-associated renal vascular disease.

2.4. Alteration of purinergic signaling in the retinal microvasculature

Diabetic retinopathy is the most common complication of diabetes and one of the leading causes of blindness worldwide [55]. One of the most important characteristics is the death of microvascular pericytes and endothelial cells [56,57]. During chronic hyperglycemia, multiple factors including increased oxidative stress, upregulation of VEGF signaling and formation of advanced glycation end products have been shown to account for diabetes-induced dysfunction in retinal microvasculature [55,58]. Regarding the role of purinergic regulation in the development of retinopathy in diabetes, much emphasis is put on $P2X_7R$ based on its ability to induce inflammation. High glucose induced a significant release of IL-1β and lactate dehydrogenase in human retinal pericytes, which was inhibited by the $P2X_7R$ antagonist [59]. Streptozotocin-induced type 1 diabetic rats exhibited higher mRNA and protein levels of $P2X_7R$ in retina [60]. In pericyte-containing microvessels of such model, both ATP and the $P2X_7R$ agonist induced microvascular cell death, which could be inhibited by the $P2X_7R$ antagonist [56] (Table 4). Diabetes increased the vulnerability of retinal microvessels to apoptosis induced by $P2X_7R$ activation [56]. Activation of $P2X_7R$ was also involved in extracellular nicotinamide adenosine dinucleotide-increased vulnerability of retinal microvascular death in such diabetic model [61] (Table 4). As a functional consequence, a significant lower concentration of the $P2X_7R$ agonist was enough to reduce retinal blood velocity in alloxan-induced diabetic rabbits to a similar extent as compared to control group [62] (Table 4), indicating a more vulnerable retinal microvascular function regulated by activation of $P2X_7R$ in diabetes. Of interest, chronic i.p. injection with either the potent small molecule $P2X_7R$ inhibitor A740003 or AZ10606120 in type 1 diabetic rats reversed the increased retina microvascular permeability, fully obliterated elevated VEGF mRNA and protein levels, as well as IL-6 levels in retina [60] (Table 4).

Strategies using various selective small molecule $P2X_7R$ inhibitors to combat chronic inflammatory diseases have been carried out in Phase I/II clinical studies [63] . With application of those small molecule inhibitors in the future, $P2X_7R$ may serve as a potential target for the treatment of retinal microvascular dysfunction in diabetic patients.

2.5. Alteration of vascular purinergic signaling in the heart

Purinergic activation plays a pivotal role in the regulation of coronary microcirculation [8,11]. In isolated hearts of type 1 diabetic mice, infusion of the non-selective adenosine receptor agonist and the selective $A_{2A}R$ agonist both increased coronary flow without obvious effects on heart rate and cardiac function when compared to the control mice [64]. Further, in vivo bolus injection of the $A_{2A}R$ agonist increased coronary blood flow more in type 1 diabetic mice than control mice [64] (Table 5). Based on the similar baseline coronary flow measured ex vivo and in vivo between the two groups, these findings indicate a greater cardiac reserve in type 1 diabetic mice. This functional evidence is in line with the higher levels of $A_{2A}R$ protein expression in the coronary arteries from type 1 diabetic mice compared to that of control mice [64]. These observations suggest that adenosine and its $A_{2A}R$ selective agonist (regadenoson, Lexiscan®), routinely used in clinics, may not be appropriate for the detection of coronary artery diseases in an early phase of diabetes in which the change in adenosine receptor-mediated vascular effect may mask an underlying disease state.

In a swine model with early-stage metabolic syndrome and hyperglycemia, despite both adenosine-induced increase in coronary blood flow in vivo and the adenosine analogue-mediated relaxation in isolated coronary arterioles did not differ from control swine [65] (Table 5), there was a shift from the $A_{2A}R$-mediated coronary relaxation, a primary mechanism for adenosine-induced vasodilation in healthy coronary microcirculation, to enhanced $A_{2B}R$-mediated coronary relaxation in swine with early-stage metabolic syndrome [65]. This shift is likely due to the increased sensitivity of $A_{2B}R$ upon stimulation by the adenosine analogue, as the $A_{2B}R$ expression level was lower in coronary arterioles isolated from swine with metabolic syndrome [65]. Activation of K_{ATP} and K_v channels are main final effectors through which adenosine acts to hyperpolarize and relax smooth muscle in coronary microcirculation [66–68]. Involvement of K_v channels in adenosine receptor-mediated coronary relaxation was not affected by early-stage metabolic syndrome, whereas there was a reduced K_{ATP} channel function [65]. We previously demonstrated that activation of A_{2A} receptors is coupled to K_{ATP} channel to regulate coronary microcirculation [68,69]. The reduced K_{ATP} function by early-stage metabolic syndrome is likely influenced by the shift of vasodilator $A_{2A}R$.

Up_4A produced potent coronary relaxation in normal swine, which was maintained in swine with metabolic derangement (diabetes induced by streptozotocin in combination with high fat diet), despite impaired coronary endothelial function in swine with metabolic derangement [70] (Table 5). The maintained relaxation by Up_4A results from a balanced but altered cross-talk among purinergic signaling and endothelium-derived vasoactive factors. Thus, mRNA expression for eNOS, as well as expression of the vasodilator $A_{2A}R$ and $P2X_7R$ was reduced in isolated coronary small arteries isolated from swine with metabolic

derangement [70]. Further, there was a decreased $P2X_7R$- and NO-mediated vasodilator effect of Up_4A, while a TxA2-mediated vasoconstrictor effect was unmasked [70]. All these vasoconstrictor effects were balanced by an increased vasodilator influence via $P2Y_4R$ and cytochrome P450 2C9 switched from producing vasoconstrictor to vasodilator metabolites in swine with metabolic derangement [70] (Table 5).

Taken together, existing evidence demonstrates that there is indeed an altered purinergic signaling regulating coronary microcirculation in diabetes. The vasodilator influence seems to be maintained by such altered purinergic signaling in early-phase of diabetes or metabolic syndromes. It is of interest and importance to further investigate the effect of disease progression e.g. late-stage of diabetes on the purinergic regulation of coronary microcirculation.

2.6. Alteration of purinergic signaling in other vascular beds

Existing evidence also demonstrated an altered purinergic signaling in diabetes accounting for vascular dysfunction in other vascular beds. Glucose-induced nucleotide-release increased L-type calcium channel activity and vasoconstriction in mouse cerebral arteries via activation of P2YnR [71] (Table 6). In perfused pancreas isolated from streptozotocin-induced type 1 diabetic rats, adenosine-mediated increase in vascular flow rate was impaired, which was restored by a chronic insulin treatment [72] (Table 6). Cutaneous vascular conductance during administration of ATP at three intradermal forearm skin sites of patients with type 2 diabetes was attenuated as compared to healthy subjects [73] (Table 6). Electrical stimulation in tail arteries isolated from streptozotocin-induced type 1 diabetic rats produced greater contraction, which was attenuated to a greater extent by the P2XR antagonist and the α-adrenergic blocker when comparing to control [74] (Table 6). Accordingly, ATP and norepinephrine produced a greater contraction in tail arteries of type 1 diabetic rats as compared to controls [74] (Table 6), providing evidence for diabetes-induced neuropathy in such arterial preparation.

2.7. Role of erythrocyte-mediated purinergic signaling

Emerging studies have revealed that erythrocytes not only act as regulators of normal physiological function to maintain cardiovascular homeostasis and integrity, but also act as important triggers for the development of various cardiovascular diseases [75–79]. Erythrocytes undergo functional alterations including reduced NO-like bioactivity and/or enhanced oxidative stress in various diseases including diabetes [77,80]. Such altered function of erythrocytes may subsequently affect cardiovascular function thereby accounting for cardiovascular complications. Indeed, recent studies have shown that erythrocytes from patients with type 2 diabetes induce endothelial dysfunction and aggravate cardiac injury following ischemia reperfusion [75,79,81], indicating erythrocyte as a novel disease mediator for the development of cardiovascular complications in diabetes.

Erythrocytes serve as an ATP pool in the circulation. The release of ATP from erythrocytes, particularly when subjected to reduced oxygen tension or shear stress, has been suggested to regulate the matching of oxygen delivery with need in skeletal muscle [82,83]. ATP release from erythrocytes in response to low oxygen tension or mechanical deformation requires

increases in cAMP, resulting from the activity of Gistimulated adenylyl cyclase. In these pathways, cAMP can be hydrolyzed by phosphodiesterase (PDE) 3 [84,85]. ATP is suggested to be exported in response to exposure of erythrocytes to reduced oxygen tension via pannexin 2 channels [86]. In contrast, ATP release in response to activation of prostacyclin receptors on erythrocytes was reported to occur via the voltage-dependent anion channel [87]. Once released into the microcirculation, ATP activates purinergic receptors e.g. $P2Y_4R$ on vascular walls to generate vasodilators such as NO and PGI_2 [82]. These vasodilators are released extraluminally where they act on vascular smooth muscle to induce vasodilation. NO and PGI_2 are also released into the vascular lumen where they interact with erythrocytes. NO has been shown to inhibit hypoxia-induced ATP release (negative feedback regulation), while prostacyclin stimulates receptor-mediated ATP release (positive feedback regulation) [82]. Further, once released in the circulation, ATP can be degraded by various ecto-nucleotidases to adenosine contributing to vasodilation [8]. Of note, ATP release in response to exposure of erythrocytes to low oxygen tension was markedly impaired in patients with type 2 diabetes as compared to healthy subjects [88–90]. As a functional consequence, erythrocytes from patients with type 2 diabetes failed to dilate resistance vessels under hypoxic conditions in contrast to erythrocytes from healthy subjects [88,90] (Table 6). The impairment in ATP release was associated with deceased Gi2 expression and cAMP production [89], the latter of which was restored by PDE3 inhibition in erythrocytes from patients with type 2 diabetes [89–91]. Further, the same group found that the impairment in ATP release could be rescued by addition of insulin and c-peptide in erythrocytes from patients with type 2 diabetes via a mechanism stimulating PKC and cGMP pathways [92,93]. Of functional importance, PDE3 inhibition in erythrocytes from patients with type 2 diabetes rescued vasodilator ability in hamster skeletal muscle arterioles [90], indicating a detrimental effect of erythrocytes in diabetes on vascular function due to attenuated ATP release. All together, these findings strongly suggest that erythrocytes and erythrocyte-mediated purinergic signaling may serve as potential therapeutic targets for the treatment of vascular complications in diabetes.

2.8. The trophic action of purinergic signaling in diabetes

As mentioned above, diabetes provokes an exaggerated stimulation of endothelium resulting in increased ROS and decreased NO. Diabetic patients also experience vascular remodeling characterized by increased wall-lumen ratio, mainly reflecting an increase in vascular smooth muscle cells. Together with involvement of other inflammatory cells, this remodeling eventually leads to the development of vascular disease including atherosclerosis [94]. Diabetic patients are at increased atherothrombotic risk and have elevated rates of ischemic recurrence [95]. Activation of both P1R and P2R by adenosine, ATP or UTP exerts various long-term actions resulting in vascular proliferation and growth [3,8]. These purinergic receptor-mediated long-term actions may be dysregulated in diabetes accounting for diabetic vascular disease. HUVECs derived from gestational diabetic pregnancies exhibited a higher concentration of adenosine and higher protein levels for A1R but lower protein levels for $A_{2B}R$ [19,20] (Table 7). Further, high extracellular glucose induced release of ATP and/or UTP and reduced adenosine transport in endothelial cells [22,96]. The high glucose-induced ATP release led to upregulation of various inflammatory genes, formation of ROS and reduction of NO bioavailability via activation of $P2X_4R$ and $P2X_7R$ [22] (Table

7). High glucose was also shown to induce UTP release in smooth muscle cells which subsequently activated $P2Y_2R$ and $P2Y_6R$ resulting in increases in the proatherogenic nuclear factor of activated T-cells (NFAT) [97] (Table 7). Aortic smooth muscle cells obtained from rats with streptozotocin-induced type 1 diabetes exhibited a greater susceptibility to the inhibitory effects of adenosine on cell proliferation, suggesting a role for adenosine regulation on diabetic atherosclerosis [98]. It was also reported that there is an association of increased $P2X_7R$ expression in monocytes of patients with type 2 diabetes with high plasma C-reactive protein that may play a role in the vascular dysfunction in type 2 diabetes [99] (Table 7). $P2Y_{12}R$ inhibition has been recommended in the management of myocardial infarction. $P2Y_{12}R$ inhibition combined with aspirin reduced ischemic events also in diabetic patients with acute coronary syndrome undergoing percutaneous coronary intervention. More clinical trials are currently under investigations and will provide understandings into optimizing the beneficial effects and minimizing bleeding risks [95]. Collectively, these studies indicate that extracellular nucleotide release is a potential metabolic sensor for the vascular trophic response to hyperglycemia and may provide a link between diabetes and diabetic vascular disease.

3. Conclusions and perspectives

Emerging evidence has revealed the importance of nucleot(s)ide-mediated purinergic signaling in diabetes-associated vascular complications in major organs including heart, kidney, retinal microvasculature and many other peripheral vascular beds. Hyperglycemia plays a role in initiating disease progress, while aging and diabetes progression may further affect purinergic signaling and deteriorate vascular complications particularly in resistance vessels. A1R, $A_{2A}R$ and $A_{2B}R$ among P1 receptors, $P2X_4R$, $P2X_7R$, $P2Y_4R$, $P2Y_6R$, $P2Y_{41}R$ and $P2Y_{12}R$ among P2 receptors are mainly involved in diabetes-associated vascular dysfunction. It seems that the altered receptor sensitivity rather than the changes in receptor expression accounts for vascular dysfunction in diabetes. Activation of $P2X_7R$ plays a crucial role in diabetes-induced retinal microvascular injury. Application of the small molecule $P2X_7R$ inhibitor A740003 and AZ10606120 has already provided promising outcome for the treatment of retinal microvascular dysfunction in diabetes and future clinical studies are needed to confirm the therapeutic potential. Recent findings have elucidated CD39 as a key enzyme protecting the kidney from renal vascular injury in diabetic animals, while CD73 is induced in diabetic kidney and deletion of CD73 is associated with severe nephropathy. Furthermore, erythrocyte dysfunction in diabetes acts as a trigger by decreasing ATP release that affects vascular function. Nucleot(s)ide-mediated purinergic activation also exerts long-term vascular actions including inflammatory and atherogenic effects in hyperglycemic and diabetic conditions. However, the role of vascular purinergic signaling in the development of atherosclerosis, hypertension and calcification secondary to diabetes remains unclear, which warrants future investigations. Targeting nucleod(s)ide-mediated purinergic signaling may serve as a potential therapeutic target for the treatment of vascular complications in diabetes.

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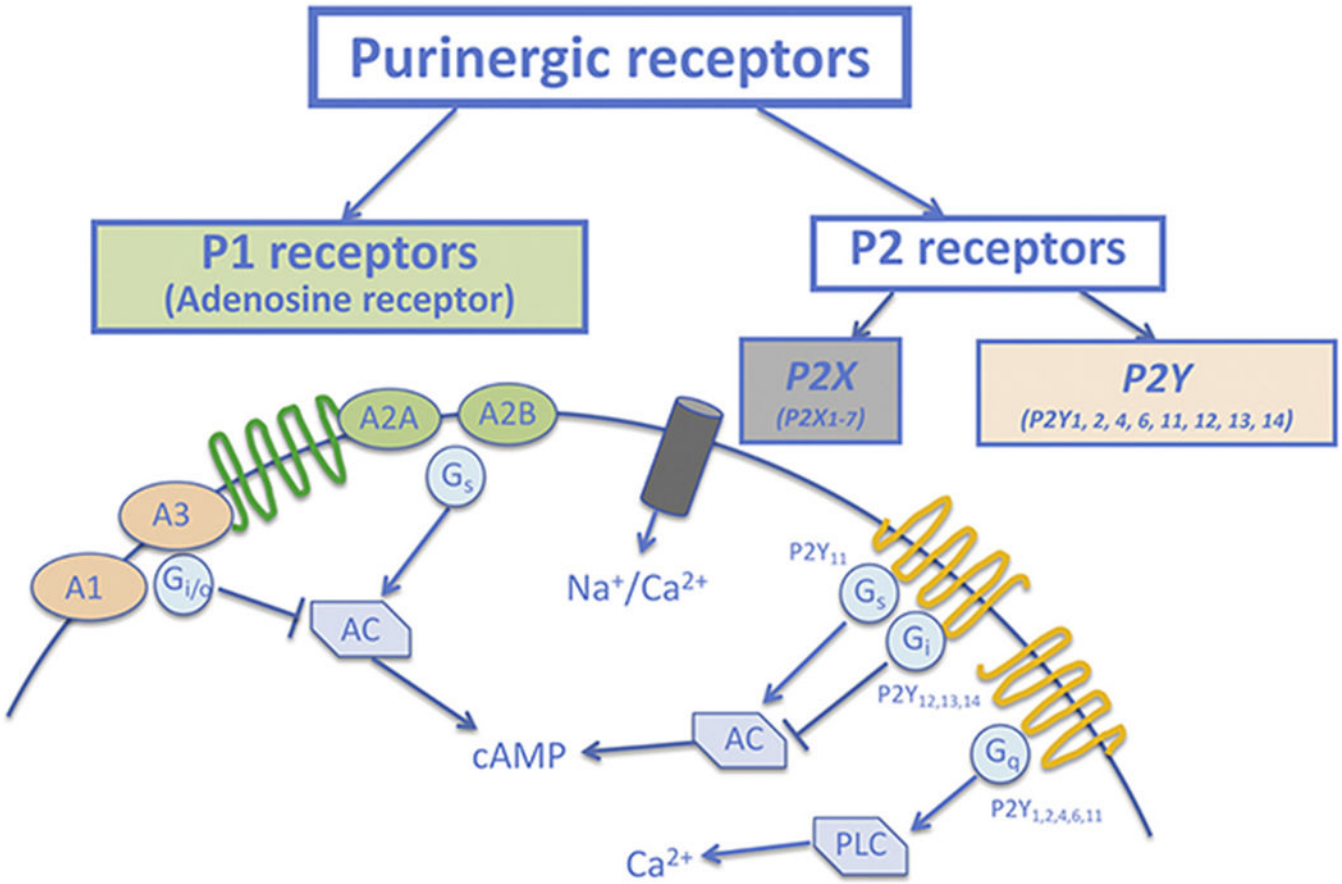

Fig. 1.

Purinergic receptor classification and their activated intracellular components. Purinergic receptors are divided to P1 and P2 classes. P1 receptors consist of A1, A_{2A}, A_{2B} and A3 receptors. A1 and A3 receptors are negatively coupled to adenylyl cyclase (AC) through the Gi/o and activation of those receptors decreases cAMP levels, whereas A_{2A} and A_{2B} receptors are positively coupled to AC through Gs and enhance cAMP levels. The P2 receptors are further classified into two major families: ionotropic P2X and metabotropic P2Y receptors. Activation of P2X receptors increases sodium and calcium permeability, while activation of P2Y receptors either affects AC and alter cAMP levels or activates phospholipase C (PLC) and release intracellular calcium.

Table 1

Nucleot(s)ide ligand-receptor preference and purinergic receptor-mediated vascular action.

Ligand	Preferable receptors	Vascular effect	Post-receptor signaling	Reference
ATP	$P2X_{1\text{-}7}R$, $P2Y_1R$	Relaxation (endothelial activation: $P2X_1R$, $P2X_2R$, $P2X_3R$, $P2X_4R$, $P2X_7R$, $P2Y_1R$, $P2Y_2R$, $P2Y_4R$, $P2Y_{11}R$); Contraction (SMC activation: $P2X_1R$, $P2X_2R$, $P2X_4R$, $P2Y_1R$, $P2Y_2R$, $P2Y_6R$)	NO, Prostacyclin, EDHF (relaxation); ROS, TxA2 (contraction)	[10]
ADP	$P2Y_1R$, $P2Y_{12}R$, $P2Y_{13}R$			[10]
UTP	$P2Y_2R$, $P2Y_4R$			[7,16]
UDP	$P2Y_6R$			[10]
ADO	A1, A_{2A}, A_{2B}, A3	Relaxation ($A_{2A}R$, $A_{2B}R$) and contraction (A1R, A3R) independent of receptor location		[11]

ADO: adenosine; EDHF: endothelium-derived hyperpolarization factor; NO: nitric oxide; ROS: reactive oxygen species; TxA2: thromboxane.

Table 2

Purinergic receptor-mediated vascular function in the peripheral vasculature.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
	Rat	STZ with endothelium-denudation	ADO+LNMA		Vascular relaxation↓	[24]
Aorta	Rat	GK	Up_4A	A1R, $P2X_7R$	Vascular contraction↑	[23]
			ACh	A1R, $P2X_7R$, $P2Y_6R$	EDR↓	
	Rat	STZ with endothelial dysfunction	ADO	$A_{2A}R$	EDR↓	[26]
			ADO analogue/ the $A_{2A}R$ agonist		Vascular relaxation↓	
	Rat	OLETF	UDP	$P2Y_6R$-sensitive	Vascular relaxation↑	[27]
				$P2Y_6R$-insensitive	Vascular contraction↑	
	Mouse	STZ without endothelial dysfunction	The A1R agonist	A1R	Vascular contraction↓	[25]
			The $A_{2A}R$ agonist	$A_{2A}R$	Vascular relaxation ↓	
Mesenteric artery	Rat	GK	Up_4A		Vascular contraction -	[23]
			ACh		EDR↓	
	Rat	STZ	ADP	$P2Y_1R$	EDR↓	[28]
	Rat	GK	ATP, UTP	$P2Y_6R$	Vascular contractionf	[29]
	Mouse	STZ without endothelial dysfunction	The A1R agonist The $A_{2B}R$ agonist The A3R agonist		Vascular contraction/relaxation -	[25]
Femoral circulation	Human	Type 2 diabetes	ATP, UTP, ADO	$P2X_1R$, $P2Y_2R$	LBF↓	[31]

ADO: adenosine; EDR: endothelium-dependent relaxation; GK: Goto Kakizaki; LBF: leg blood flow; LNMA: N^G-monomethyl-L-arginine; OLETF: Otsuka Long-Evans Tokushima Fatty; STZ: streptozotocin; Up_4A: uridine adenosine tetraphosphate; ↑: increased effect; ↓: decreased effect; −: no effect vs. control group.

Table 3

Purinergic receptor-mediated vascular function in kidney.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
Kidney	Rat	STZ	ADO/renal occlusion	A1R	RBF↓	[39]
			The $A_{2B}R$ antagonist	$A_{2B}R$	Glomerulopathy and fibrosis↓	[47,48]
			The $A_{2A}R$ agonist	$A_{2A}R$	Glomerular inflammation and injury↓	[45,46]
			The $P2X_7R$ agonist	$P2X_7R$	Cortical blood flow↑, GFR↓	[36]
	Rat	GK	Up_4A	P2R	vascular contraction↑	[51]
	Mouse	$Ins2^{+/-}/A_{1A}R^{-/-}$ mutants		A1R	GFR↑, Glomerular Injury↑	[43]
	Mouse	alloxan-induced $A_{1A}R^{-/-}$		A1R	Glomerular Injury -	[44]
	Mouse	STZ	The $A_{2B}R$ agonist	Endothelial $A_{2B}R$	Glomerular Injury↓	[49]
	Mouse	STZ with $CD73^{-/-}$	Adenosine		Glomerular Injury↑	[49]
	Mouse	STZ with $CD39^{-/-}$	ATP/UTP		Glomerular inflammation↑	[50]
	Mouse	HFD	The $P2X_7R$ agonist	$P2X_7R$	Renal inflammation and injury↑	[54]
			$P2X_7R^{-/-}$		Renal inflammation and injury↓	

ADO: adenosine; CD39: ectonucleoside triphosphate diphosphohydrolase; CD73: ecto-5′-nucleotidase; GFR: glomerular filtration rate; GK: Goto Kakizaki; HFD: high fat diet; Ins2: also known as $Akita^{+/-}$, a naturally occurring diabetic model; RBF: renal blood flow; STZ: streptozotocin; Up_4A: uridine adenosine tetraphosphate; ↑: increased effect; ↓: decreased effect; −: no effect vs. control group.

Table 4

Purinergic receptor-mediated vascular function in the retinal microvasculature.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
Retinal microvasculature	Rat	STZ	ATP, the $P2X_7R$ agonist	$P2X_7R$	Micro vascular death↑	[56]
			NAD +	$P2X_7R$	Micro vascular death↑	[61]
			The $P2X_7R$ antagonist	$P2X_7R$	Micro vascular permeability↓	[60]
	Rabbit	Alloxan	The $P2X_7R$ agonist	$P2X_7R$	Retinal blood velocity↓	[62]

NAD+: nicotinamide adenosine dinucleotide; STZ: streptozotocin; ↑: increased effect; ↓: decreased effect.

Table 5

Purinergic receptor-mediated vascular function in the heart.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
Heart	Swine	STZ and high-fat diet	Up_4A	$A_{2A}R$, $P2X_7R$, $P2Y_1R$	Coronary relaxation -	[70]
	Ossabaw miniature swine	Metabolic syndrome	ADO/the ADO analogue	$A_{2B}R$	CBF/coronary relaxation -	[65]
	Mouse	STZ	The $A_{2A}R$ agonist	$A_{2A}R$	CBF/coronary relaxation↑	[64]

ADO: adenosine; CBF: coronary blood flow; STZ: streptozotocin; Up_4A: uridine adenosine tetraphosphate; ↑: increased effect; ↓: decreased effect; −: no effect vs. control group.

Table 6

Purinergic receptor-mediated vascular function in other vascular beds.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
Cerebral artery	Mouse	Hyperglycemia	Glucose	$P2Y_{11}R$	Vascular contraction↑	[71]
Pancreas	Rat	STZ	ADO		Vascular flow rate↓	[72]
Tail artery	Rat	STZ	ATP/Electrical stimulation	P2XR	Contraction↑	[74]
Skin	Human	Type 2 diabetes	ATP		Vascular conductance↓	[73]
Muscle arteriole	Hamster	RBC from patients with type 2 diabetes	Decreased ATP release under hypoxia		Vascular relaxation↓	[88,90]

ADO: adenosine; RBC: red blood cells; STZ: streptozotocin; ↑: increased effect; ↓: decreased effect.

Table 7

Purinergic receptor-mediated vascular trophic action.

Organ	Species	Diabetic model	Nucleot(s)ide/stimuli	Purinergic receptor	Vascular effect	Reference
HUVECs	Human	GDP		A1R, $A_{2B}R$	ADO↑	[19,20]
		High glucose	ATP, UTP	$P2X_4R$, $P2X_7R$	Inflammatory genes↑; ROS↑; NO↓	[22,96]
Monocytes	Human	Type 2 diabetic patients		$P2X_7R$	Plasma C-reactive protein↑	[99]
Aorta SMCs	Mouse	High glucose	UTP	$P2Y_2R$, $P2Y_6R$	NFAT↑	[97]

ADO: adenosine; GDP: gestational diabetic pregnancy; HUVECs: human umbilical endothelial cells; NFAT: nuclear factor of activated T-cells; NO: nitric oxide; ROS: reactive oxygen species; ↑: increased effect; ↓: decreased effect.