Review

Purinergic Signaling, Dyslipidemia and Inflammatory Disease

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Key Words

Nucleotides • Lipoprotein • Autophagy • Inflammation • Insulin receptor • Purinergic signaling

Abstract

Metabolic syndrome is a compound obesity disorder, wherein the abnormal metabolism of glucose and lipid is associated with the development of chronic inflammatory diseases. The prevalence of this disease is increasing in the developed world, but the causative linkage between these metabolic disorders has remained obscure. Metabolic disease may be associated with chronic nucleotide secretion, purinergic signaling and activation of inflammatory pathways. Purinergic signaling has been implicated in impaired glucose metabolism and inflammatory disease and may contribute to dyslipidemia. Our research shows that purinergic signaling disrupts hepatic lipoprotein metabolism by blocking insulin receptor signaling and by activating cellular autophagic pathways. Chronic stimulation of purinergic signaling may therefore be causative to glucose and lipid metabolic disorders and associated with the development of cardiovascular disease.

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Introduction

While nucleotides are well known for their important role in intracellular energy metabolism, it is now established that they also play a role as extracellular messengers to modulate the immune and inflammatory response [1-3]. In healthy tissues, extracellular

nucleotide concentration is maintained at low concentrations to minimize purinergic signaling [4]. In fresh blood samples, nucleotide levels are normally in low μM concentrations [5, 6], but can increase both acutely and chronically in various disease states [2, 7, 8]. Extracellular nucleotide levels are controlled by cellular secretion and extracellular degradation. Nucleotide secretion is affected by intracellular $[\text{Ca}^{2+}]$ and P2X7 [9]. The gated ion channel, P2X7 receptor, has been shown to stimulate nucleotide secretion and activate both the immune and inflammatory response [3, 10]. High blood glucose levels directly stimulate the release of adenosine triphosphate (ATP) from endothelial tissues and circulating blood cells [11-13], but ATP is unstable in the circulation and is rapidly degraded to ADP, AMP and adenosine, by unique ecto-enzymes. Nucleotides are degraded by membrane ATP metabolizing proteins and specific ectonucleotidases, including NTPDase1, 2, 3, 8 (ATPase and ADPase) and CD73 (AMPase) [14, 15]. The extracellular nucleotide milieu acts through specific P2X and P2Y receptors to promote a purinergic signaling response (Fig. 1). Short-lived nucleotide signaling may positively affect glucose metabolism by stimulating insulin secretion from pancreatic beta cells [16, 17]. Sustained purinergic signaling appears to have the opposite effect and inhibits insulin receptor (IR- β) signaling [18, 19] and insulin secretion [20, 21]. The acute release of nucleotides, by stress or injury, also activates nuclear factor kappa B [1, 22] and triggers the release of pro-inflammatory cytokines [8, 23]. Chronic nucleotide signaling may therefore be involved in the pathophysiology of both metabolic and cardiovascular diseases [2, 24].

Nucleotides and Disease

Extracellular nucleotides are implicated in the development of coronary artery disease through a number of putative mechanisms [2, 7, 25] and therapeutic inhibition of purinergic signaling has a well-established utility in the treatment of cardiovascular disease [24, 26]. Nucleotides activate thrombosis pathways in the bloodstream and inhibition of nucleotide-dependent platelet activation has shown significant cardiovascular therapeutic value [26, 27]. Abnormal nucleotide metabolism may also contribute to the development of type 2 diabetes (T2D), metabolic syndrome, and dyslipidemia. Fibroblasts from T2D patients show 2-3 fold increase in ATP secretion [28] and enhanced inflammatory and cytotoxic responses through the P2X7 receptor [29]. P2X7 expression appears to be elevated in peripheral blood mononuclear cells from T2D patients and significantly correlated with LDL-cholesterol [30]. Adipocyte P2X7 expression is elevated in patients with metabolic syndrome and associated with an enhanced inflammatory response [31]. Nucleotide degradation may also be impaired in diabetes, as polymorphisms in NTPDase1/CD39 are associated with T2D and diabetic nephropathy [32]. *In vivo* murine studies corroborate this view and show that a deficiency in CD39 can cause insulin resistance and hypertriglyceridemia [33]. Alterations in nucleotide secretion and/or degradation therefore appear to be associated with dyslipidemia, which suggests that purinergic signaling influences lipoprotein metabolism.

A role for extracellular nucleotides in plasma lipoprotein metabolism was proposed with the discovery of a plasma membrane form of F_1 -ATP synthase, now called ecto- F_1 -ATP synthase or F_1 -ATPase. F_1 -ATPase was identified to be an HDL receptor that functions to bind with apoA-I and to regulate HDL endocytosis [34]. F_1 -ATPase was also shown to stimulate extracellular ADP production [35, 36] and promote purinergic signaling through the G-protein coupled receptor, P2Y_{13} [37, 38]. F_1 -ATPase is inhibited by mitochondrial inhibitory factor 1 (IF1) [34] and serum IF1 levels have been shown to be positively correlated with HDL-cholesterol levels and negatively correlated with serum triglyceride levels in normolipidemic subjects [39]. This is consistent with other work, which suggested that therapeutic modulation of circulating HDL levels may be associated with the expression of F_1 -ATPase, ADP production and purinergic signaling [19, 40, 41].

Fig. 1. Extracellular nucleotide levels are modulated by ecto-nucleotidases (ATPase and ADPase). Adenosine triphosphate (ATP) and adenosine diphosphate (ADP) act through P2X and P2Y receptors to promote inflammation by activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB). Nucleotides can also affect glucose metabolic pathways by inhibiting insulin receptor-β (IR-β) and protein kinase B (Akt) signaling.

Nucleotides, Lipoproteins, and Cellular Protein Degradation

Lipoprotein secretion is affected by proteolytic degradation pathways, which appear to be controlled by purinergic signaling [19]. Nucleotides therefore affect lipoprotein secretion by regulating intracellular protein degradation (Fig. 2). Perturbations in the lipidation or folding of the LDL protein, apoB100, result in the ubiquitination and transport of the protein for proteasomal degradation [42-44]. Inhibitors of proteasomal degradation are known to stimulate apoB100 secretion from liver cells and extracellular ADP acts much like a proteasomal inhibitor to affect lipoprotein secretion [19]. Both ADP and the proteasomal inhibitor, ALLN, stimulate apoB100 secretion and inhibit HDL secretion at 4h. This appears due to a co-regulation of proteasomal and autophagic protein degradation, since proteasomal inhibitors are known to stimulate autophagy [45-48]. Proteasomal inhibitors activate autophagy and increase expression of the autophagic proteins, Atg5, Beclin-1 and Atg7 [47, 48], which then gives rise to the transport and accumulation of Atg8/LC3 in autophagic vacuoles [45]. Much like proteasomal inhibitors, ADP also stimulates autophagy and significantly increases cellular LC3 levels [19].

Autophagy is a cellular stress response that promotes the lysosomal degradation of cytosolic components when stimulated by stressors, i.e. nutrient deprivation, extracellular signals, cytokines and pathogens [49, 50]. ADP stimulates autophagy, increases autophagic protein levels and decreases HDL secretion from liver cells (Fig. 2) in similar fashion to that observed by serum deprivation [19]. Serum deprivation is known to stimulate autophagy [49, 50] and to inhibit HDL/apoA-I secretion from liver cells [51]. Both ADP and serum deprivation stimulate an autophagic response in hepatic cells and increase LC3-II and p62 levels over a three to six hour period. Confocal micrographs of ADP-treated liver cells show increased LC3 levels in punctate autophagosomes and significant colocalization of apoA-I with LC3 [19]. The work shows that HDL secretion from liver cells is inhibited by cellular autophagic pathways, which may suggest that HDL secretion and lipidation are oppositely regulated by autophagy. Autophagy is also known to stimulate ABCA1-mediated cholesterol efflux from macrophages and promote the transport and clearance of cholesterol [52].

Fig. 2. Extracellular nucleotides act through P2Y₁₃ receptors to activate MAPK, inhibit Akt and regulate cellular proteolytic pathways. A purinergic stimulation in autophagy blocks HDL secretion from liver cells and stimulates VLDL-apoB secretion.

Purinergic Signaling and Autophagy

Extracellular nucleotides act through membrane P2 receptors to stimulate purinergic signaling. ADP activates the P2Y receptor class and affects cellular metabolism through a stimulation of mitogen-activated protein kinase (MAPK) and inhibition of adenylate cyclase [53]. Lipoprotein secretion is directly affected by MAPK, but is less sensitive to cAMP-dependent pathways, since chemical inhibition of adenylate cyclase had no effect on HDL secretion, while blocking MAPK pathways affects both autophagy and HDL secretion [19]. MAPK signaling is known to affect cellular autophagy [50] and ADP directly stimulates MAPK pathways [19, 37, 54]. Insulin receptor (IR-β) signaling is also known to regulate cellular autophagic pathways [49] and ADP inhibits IR-β signaling [19]. IR-β signaling is negatively associated with autophagy, since the phosphorylation of protein kinase B (Akt) has been shown to inhibit autophagy [49, 55]. ADP blocks insulin signaling and reduces both IR-β and Akt phosphorylation by ~50%, similar to that observed with tumor necrosis factor alpha. ADP therefore regulates autophagy and lipoprotein secretion through both MAPK and Akt signaling pathways (Fig. 2).

Human liver cells contain two ADP-receptors, P2Y₁ and P2Y₁₃, but lipoprotein metabolism appears to be primarily affected by P2Y₁₃ [37, 54, 56]. Chemical inhibitors of P2Y₁ have no effect on HDL secretion, while modulation of P2Y₁₃ expression directly affects HDL secretion from liver cells. P2Y₁₃ overexpression increases cellular LC3-II levels and decreases HDL secretion, while P2Y₁₃ gene silencing decreases LC3-II levels and increases HDL secretion [19]. Consistent with the view that P2Y₁₃ expression regulates HDL secretion through cellular autophagic signaling pathways, P2Y₁₃ expression also regulates both MAPK and Akt signaling. A reduction in P2Y₁₃ expression causes a parallel reduction in ERK1/2 phosphorylation, but increases the phosphorylation of IR-β, IGF-1R and Akt. Increasing P2Y₁₃ expression has the opposite effect. Nucleotide signaling through P2Y₁₃ therefore blocks insulin receptor signaling [19] (Fig. 1).

Conclusion

Lipoprotein metabolism is directly affected by nucleotides and cellular autophagy and therefore inhibition of hepatic purinergic signaling should directly affect circulating lipoprotein levels. Niacin may impact cardiovascular disease and lipoprotein metabolism through purinergic and G-protein coupled signaling pathways [40, 57, 58]. Niacin has been

shown to increase circulating HDL levels by blocking hepatic apoA-I degradation [59]. Niacin reduces cell surface levels of F_1 -ATPase in hepatocytes and thereby blocks the production of extracellular ADP [40]. Linoleic acid phospholipids also block ADP production by inhibiting the cell surface expression of F_1 -ATPase and thereby stimulate HDL secretion from liver cells [41]. These phospholipids appear to mute purinergic signaling and cellular autophagy by stimulating Akt phosphorylation and blocking MAPK activation [19]. An activation of Akt and inhibition of autophagy may therefore be important in increasing plasma HDL levels. Metformin and sulfonylurea drugs are popular anti-diabetic drugs that have been well described to reduce plasma glucose levels and improve circulating HDL and triglycerides [60]. Both classes of drugs are known to stimulate Akt phosphorylation and may therefore also block cellular autophagy [61, 62]. This may suggest that augmentation of insulin receptor signaling and inhibition of cellular autophagy may positively affect both glucose and lipoprotein metabolism. This dual metabolic property may represent a common mechanistic feature of both the insulin-sensitizing drugs and some therapeutics that are utilized to treat patients with disorders in lipid metabolism.

Abbreviations

ADP (adenosine diphosphate); Akt (protein kinase B); apoA-I (apolipoprotein A-I); ATP (adenosine triphosphate); CD73 (ecto-5'-nucleotidase); F_1 -ATP synthase (F_1 -ATPase); HDL (high density lipoprotein); IF1 (mitochondrial inhibitory factor 1); IR- β (insulin receptor β); LC3 (microtubule-associated protein 1 light chain 3); LDL (low density lipoprotein); MAPK (mitogen-activated protein kinase); NF- κ B (nuclear factor kappa B); NTPDase (nucleoside triphosphate diphosphohydrolase); P2X (ion channel purinergic receptor); P2Y (G-protein-coupled purinergic receptor); T2D (type 2 diabetes).

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