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Regulation of Vascular and Renal Function by Metabolite Receptors*

János Peti-Peterdi¹, Bellamkonda K. Kishore², and Jennifer L. Pluznick³

János Peti-Peterdi: petipete@usc.edu; Bellamkonda K. Kishore: BK.Kishore@hsc.utah.edu; Jennifer L. Pluznick: jpluznick@jhmi.edu

¹Department of Physiology and Biophysics and Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, California 90033

²Department of Internal Medicine and Center on Aging, University of Utah Health Sciences Center, Department of Veterans Affairs Salt Lake City Health Care System, Salt Lake City, Utah 84148

³Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Abstract

To maintain metabolic homeostasis, the body must be able to monitor the concentration of a large number of substances, including metabolites, in real time and to use that information to regulate the activities of different metabolic pathways. Such regulation is achieved by the presence of sensors, termed metabolite receptors, in various tissues and cells of the body, which in turn convey the information to appropriate regulatory or positive or negative feedback systems. In this review, we cover the unique roles of metabolite receptors in renal and vascular function. These receptors play a wide variety of important roles in maintaining various aspects of homeostasis—from salt and water balance to metabolism—by sensing metabolites from a wide variety of sources. We discuss the role of metabolite sensors in sensing metabolites generated locally, metabolites generated at distant tissues or organs, or even metabolites generated by resident microbes. Metabolite receptors are also involved in various pathophysiological conditions and are being recognized as potential targets for new drugs. By highlighting three receptor families—(a) citric acid cycle intermediate receptors, (b) purinergic receptors, and (c) short-chain fatty acid receptors—we emphasize the unique and important roles that these receptors play in renal and vascular physiology and pathophysiology.

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DISCLOSURE STATEMENT

The use of selective antagonists of P2Y₂, P2Y₁₂, or related receptors for the treatment of nephrogenic diabetes insipidus is proprietary to the US Department of Veterans Affairs and the University of Utah Research Foundation and is protected by the pending or issued patents PCT/US2005/03231 and PCT/US2012/052819, respectively. The measurement of dicarboxylate in urine samples as a novel biomarker of kidney damage is proprietary to the University of Southern California and is protected by US patent 8,652,771. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Keywords

mitochondria; vascularization; nucleotides; short-chain fatty acids; SCFA; gut microbiota

INTRODUCTION

In complex biological systems, there are multiple levels of feedback communications between alterations in cell metabolism and the physiological maintenance of organ functions and whole-body homeostasis. In metabolic stress, when a cell's energy demand and supply are out of balance, various emergency signals, including metabolites (e.g., ATP, adenosine, succinate), can induce rapid adjustments in, for example, blood supply, tissue metabolism, energy conservation, ischemia protection, or hypoxia genes, in the interest of cell survival (1–5). On a longer timescale, changes in diet may lead to alterations in tissue environment and cell metabolism, which in turn can activate compensatory mechanisms in excretory organs to maintain body homeostasis (e.g., pH balance) (6). Also, the gut microbiota via its metabolites is increasingly recognized as a critical player in regulating multiple organ functions of the host organism (7).

Specific intracellular metabolites, which are produced and released by the stressed cells and accumulate in the surrounding tissue environment, are important messengers in these feedback communications between cells, organs, and the whole body. In most cases, these messenger metabolites are sensed in an autocrine or a paracrine manner by their binding to specific G protein–coupled receptors (GPCRs) expressed at the plasma membrane of the target cell. Metabolite GPCRs can activate a variety of intracellular signaling cascades that target, for example, nuclear transcription, enzyme activation, and cell membrane ion transport mechanisms. Such activation ultimately results in the metabolic regulation and adaptation of key cellular, organ, and systemic functions (Figure 1).

In this review, we focus on three metabolite receptor families that received considerable attention in recent years: (a) receptors of citric acid cycle (also known as the tricarboxylic acid or Krebs cycle) intermediates, which can signal mitochondrial stress to whole cells and tissues; (b) purinergic receptors that detect the energy molecule ATP and its metabolites in the extracellular milieu; and (c) receptors for short-chain fatty acids (SCFAs) derived from the metabolism of the gut microbiota. Although numerous other metabolite receptors—including the hydroxy-carboxylic acid (e.g., lactate) receptors G protein–coupled receptor 81 (GPR81) (8), GPR109A, and GPR109B (9) and various free fatty acid receptors (10)—have been identified and characterized, we focus on the three aforementioned receptor families to exemplify and emphasize the importance of direct metabolic regulation of renal and vascular functions via these metabolite GPCRs. The unique cell metabolism, the hypoxic tissue environment, and the importance to whole-body metabolism and homeostasis (in the kidney) and the direct control of organ blood supply (in the vasculature) make the kidney and the vasculature highly relevant for addressing the function of metabolite receptors.

MITOCHONDRIAL CITRIC ACID CYCLE INTERMEDIATES AND THEIR GPCRS

In the classic biochemical view, the role of the citric acid cycle intermediates succinate and α -ketoglutarate is to generate energy in cells' mitochondria from carbohydrate, fat, and protein metabolism. However, this old paradigm has changed in the last decade, and a nontraditional, hormone-like mediator and signaling role for these metabolites has been established. Interestingly, new functions of succinate have recently emerged in epigenetics and in the posttranslational modification (succinylation) of proteins (11), which are not discussed here.

In their pioneering 2005 work, He et al. identified succinate and α -ketoglutarate (also known as oxoglutarate) as the specific ligands for GPR91 and GPR99, which had been previously thought to be orphan receptors (12). Thus, these receptors are also known as SUCNR1 and OXGR1, respectively. This study provided the initial characterization of receptor pharmacology and signaling, mutational analysis, 3D modeling, and tissue distribution and function (12) and was an important milestone for a subsequent and rapidly developing new field of GPR91 research. Since then, a regulated, high level of GPR91 expression has been confirmed in different tissues, including white adipose tissue (13), the liver (14), and the kidney (15–17)—organs of great importance in whole-body metabolism. Other tissues with significant, functional GPR91 expression include the retina (18), immune system (19), bone marrow (20), heart (21), and brain (22). Kidney-specific expression of GPR99 as originally proposed (12) was later confirmed (6). GPR91 and GPR99 are cell surface receptors and can be rapidly internalized like other GPCRs (12). A variety of intracellular signaling mechanisms—including inositol trisphosphate (IP_3) and Ca_2^+ (12, 15), cAMP (13, 23), and mitogen-activated protein (MAP) kinases p38 and ERK1/2 (15–17, 24)—have been linked to GPR91 and GPR99 activation via either the pertussis-toxin-sensitive G_i/G_o pathway or the pertussis-toxin-insensitive G_q pathway (Figure 2). The detailed tissue distribution, signaling pathways, and (patho)physiological roles of GPR91 and GPR99 were recently reviewed (25, 26).

GPR91 activation and signaling may be involved in normal tissue maintenance and protective mechanisms and in the cell stress response to hypoxia and to various metabolic stimuli. For example, succinate signaling via GPR91 is part of the cell's hypoxia response that works in parallel with, but independently of, hypoxia-inducible factor (HIF) to activate classic hypoxia genes such as the gene encoding vascular endothelial growth factor (VEGF) (Figure 2) (18). However, GPR91 activation appears to be a double-edged sword because the dysfunction or overactivation of this pathway may lead to the development of various pathologies (18, 22, 27, 28). He et al. (12), in their pioneering report, proposed that GPR91 plays an important role in the succinate-induced hypertensive effect and may be involved in renovascular hypertension, a disease linked to diabetes, renal failure, and atherosclerosis.

Sensing Energy Imbalance and Mitochondrial Stress via Succinate and GPR91

Succinate accumulates extracellularly in the local interstitium, when the cell's energy demand and metabolic supply (e.g., nutrients, oxygen, blood) are out of balance. Such

imbalance leads to the activation of GPR91 signaling (Figure 2) (1). As established by Krebs and others >50 years ago, acute cessation of blood flow and the resulting hypoxia quickly put mitochondria under stress, causing a selective rise in succinate levels (compared with levels of other citric acid cycle intermediates) (29, 30). This increase is due to a variety of mitochondrial metabolite- and enzyme-regulatory pathways (e.g., those involving reactive oxygen species) and due to an accelerated, abbreviated citric acid cycle (31). Still, over the years, the importance of succinate was overlooked and was not rediscovered until its role in the regulation of HIF and the hypoxia response (3) and in GPR91 activation (12) was established.

More recently, the accumulation and role of succinate were also established in diabetes and metabolic disease. In contrast to the normally low plasma levels (1–5 μ M) of succinate, elevated circulating succinate levels were found in conditions of hypertension and metabolic disease, at least in rodents (32). However, succinate accumulation in the peripheral, local tissue environment is likely more significant and relevant to GPR91 signaling, although interstitial succinate levels are difficult to measure. Because the urine is a special transcellular fluid from the kidney tissue, urinary, rather than plasma, succinate measurements may better reflect mitochondrial stress in renal tissues. Indeed, high urinary succinate levels were found in diabetic mice compared with levels in healthy controls (16), suggesting the diagnostic potential of urinary succinate measurements for metabolic and kidney diseases.

Succinate and GPR91 Signaling and Function in the Kidney

The kidney is one of the organs that express GPR91 most highly (12, 13), a fact that is not un-expected considering the high energy demand of tubular ion transport and very low pO_2 values normally present in renal tissues (approximately 50 mm Hg in the cortex, near zero in the inner medulla) (33). The first identified function of GPR91 was highly relevant and exciting for renal (patho)physiology: Succinate via GPR91 activation and signaling caused increased blood pressure that involved renin release and activation of the renin-angiotensin system (RAS) (12). Functional GPR91 expression was subsequently described in the glomerular endothelium (16) and distal nephron and collecting duct tubular epithelia (15, 17). Consistent with the initial report of succinate- and GPR91-induced renin release (12), cell membrane GPR91 expression was found in two cell types in the juxtaglomerular apparatus (JGA) area, where renin, the rate-limiting enzyme step of RAS activation, is synthesized and released. At the luminal membranes of macula densa (MD) cells, GPR91 can detect succinate present in tubular fluid; such succinate is most likely released from the mitochondria-rich and actively salt reabsorbing but hypoxic thick ascending limb (Figure 3). MD GPR91 is involved in the normal physiological mechanisms that induce JGA renin release and RAS activation in response to low dietary salt (reduced extracellular volume) (17), as well as in the pathological overactivation of both renin release and the RAS in the diabetic environment (17).

In addition to being expressed in MD cells, GPR91 is expressed in the (juxta)glomerular endothelium, which is directly adjacent to renin-producing JG cells, where GPR91 may detect plasma succinate (16). GPR91 signaling in both the vascular endothelium and MD

cells triggers paracrine cell-to-cell cross talk to the JG cell to induce renin release. In various isolated JGA tissue preparations, high glucose or succinate administration induced the renin release via GPR91 activation within 1–2 min (16, 17). The paracrine signaling mechanisms involved increases in MD cell and vascular endothelium–derived prostaglandin E₂ (PGE₂) and nitric oxide (NO) synthesis and release mediated by (a) intracellular Ca²⁺ in the endothelium (16) and (b) p38, pERK1/2, and cyclooxygenase-2 (COX-2) activation in MD cells (Figure 3) (17). PGE₂ is a classic, well-established paracrine mediator in the JGA, and via its EP₄ receptor on the target JG cell, it causes renin synthesis and release (34, 35). PGE₂ and NO also cause afferent arteriole vasodilation, which may contribute to glomerular hyperfiltration in early diabetes (16). On the systemic, whole-body level, the elevations in renal and plasma renin and prorenin that were observed in diabetes were abolished by GPR91 deficiency (16). Therefore, local effects of hyperglycemia in the JGA, which involve succinate and GPR91 signaling, appear to be critically important in triggering renin and RAS activation and glomerular hyperfiltration, the two renal hallmarks of early diabetes (28, 36).

The renal tubular segment with the highest GPR91 expression is the collecting duct (15), which is also actively reabsorbing and is localized in the most hypoxic area of the kidney (especially the medullary part). The function of GPR91 in the collecting duct, e.g., whether GPR91 directly regulates fluid and electrolyte transport, remains to be established.

GPR91-Mediated Tissue Vascularization

Due to the pivotal role of GPR91 in the cell's hypoxia response, which includes VEGF expression (Figure 2), GPR91 is involved in the maintenance of normal tissue vasculature and in neovascularization in response to hypoxia. Recent studies have characterized this GPR91 function mainly in retinal blood vessels, and the results show another example of how metabolite signaling via succinate and GPR91 can act as a sensor of ischemic stress and can govern important tissue functions such as angiogenesis. In the pioneering study in this emerging field, Sapieha et al. (18) showed that succinate accumulates in the hypoxic retina and, via GPR91, acts as a potent mediator of vessel growth in the settings of both normal retinal development and proliferative ischemic retinopathy. The autocrine/paracrine mechanisms of GPR91 activation and signaling in the retina were similar to those in the kidney and JGA detailed above. The effects of GPR91 in the retina were mediated by retinal ganglion neurons (RGCs), which in response to succinate triggered the production and release of numerous paracrine-acting angiogenic factors such as VEGF (18). Importantly, this effect was independent of HIF (18), and the signaling pathway involved ERK1/2, COX-2, and PGE₂ release and binding to the target cell's EP₄ receptor (22, 24, 37). Therefore, the mechanisms of GPR91 signaling in RGCs in the retina that promote angiogenesis, and those in MD cells in the JGA and kidney that cause renin release, are essentially identical. In addition to RGCs, another retinal cell type, the retinal pigment epithelium, also shows functional GPR91 expression; GPR91 plays a role in the maintenance of normal tissue cellular composition (27, 38).

Most recently, similar succinate- and GPR91-dependent angiogenic mechanisms were described in other vascular beds, including those in the brain (22). This GPR91 signaling

pathway appears to be relevant to other pathologies, for example, diabetic retinopathy (37) and age-related macular degeneration (27).

Regulation of Acid-Base Balance by α -Ketoglutarate and GPR99

In contrast to the well-established mechanism and role of succinate and GPR91 signaling, little has been known about the highly related GPR99 metabolic receptor and its ligand, α -ketoglutarate. Although He et al. demonstrated the predominant expression of GPR99 in the renal distal tubules (12), the (patho)physiological function of this receptor remained unknown until very recently. As an important new contribution to the field of metabolic receptors, the Firsov group discovered an unexpected, nontraditional role of GPR99 in the adaptive regulation of renal HCO_3^- secretion and salt reabsorption and therefore in the maintenance of body fluid, electrolyte, and pH homeostasis (6). The studies of this group established the elements of GPR99 signaling in the distal nephron and collecting duct: (a) GPR99 expression in type B and non-A–non-B intercalated cells, special cells that secrete HCO_3^- into the urine, and in other emerging functions and (b) the presence of the ligand α -ketoglutarate in physiologically and functionally relevant concentrations in the urine under alkali loading conditions (6).

In terms of its functional role, α -ketoglutarate/GPR99 signaling is part of a paracrine regulatory loop between the proximal nephron and the distal nephron that controls acid-base balance. Using a number of elegant experimental approaches, Tokonami et al. (6) established that GPR99 can detect α -ketoglutarate secreted by proximal tubular and loop segments and can regulate Cl^- -dependent HCO_3^- secretion and electroneutral transepithelial NaCl reabsorption in the distal nephron and collecting ducts (Figure 3). Increased urinary HCO_3^- excretion via this new mechanism reestablishes acid-base balance under dietary alkali loading conditions. These results also suggested that GPR99-mediated distal NaCl reabsorption is required to compensate for the altered proximal reabsorption of salt under a body acid-base challenge (6). This novel α -ketoglutarate/GPR99 paracrine mechanism may act along with the related and above-described succinate/GPR91 pathway to maintain body fluid, electrolyte, and acid-base homeostasis (39).

Due to a common mitochondrial origin and presence in the extracellular environment that reflects the level of mitochondrial stress and the cell's energy status, it is not surprising that the GPCRs of the highly related metabolites succinate (GPR91), α -ketoglutarate (GPR99), and ATP/metabolites (P2Ys) share a high level of homology (Figure 4) (40). In fact, GPR91 was formerly known as the P2U_2 receptor (41). Therefore, in the next section we discuss the receptors of ATP and its degradation products.

RECEPTORS FOR NUCLEOTIDES, NUCLEOSIDES, AND NUCLEOBASES

In the 1970s, Dr. Geoffrey Burnstock first used the term purinergic to distinguish a set of neurons that did not respond to either adrenergic or cholinergic stimuli but responded to a purine nucleotide, ATP, thus ushering in what now appears to be a vast and rapidly expanding field of purinergic signaling (42, 43). Although first used in the context of ATP, today the term purinergic signaling encompasses signaling mediated by extracellular purine and pyrimidine nucleotides (ATP, ADP, UTP, and UDP) and their derivatives and

degradation products, such as adenosine and adenine. Since the early 1990s, renal physiologists have made rapid strides in this new field by uncovering the breadth and depth of purinergic signaling in renal vascular and tubular functions in health and disease. This section focuses on three types of purinergic receptors: the P2 (or extracellular nucleotide) receptors; the P1 (or extracellular adenosine) receptors; and the newly identified receptors for extracellular adenine, a nucleobase, tentatively termed the P0 receptors. This section also highlights the physiological and pathophysiological importance of the two distinctive and dynamic pathways that regulate the availability of ligands for purinergic signaling in the extracellular milieu, namely the CD39-adenosinergic axis and the extracellular cAMP–adenosine pathway. Because purinergic regulation of renal functions was extensively reviewed recently, the emphasis here is on providing a comprehensive analysis of purinergic signaling as an intrinsic mechanism in the kidney, rather than on presenting a fund of knowledge with a glossary of publications in the field.

P2 Receptors

P2 receptors are nucleotide receptors that respond to ATP, ADP, UTP, or UDP, but not to AMP or adenosine. P2 receptors are divided into two distinctive groups, namely the P2X receptors (comprising subtypes 1–7) and the P2Y receptors (comprising subtypes 1, 2, 4, 6, and 11–14) (Figure 5). The P2X receptors, which bind only ATP, are ATP-gated, nonselective cation channels that open up rapidly to allow influx or efflux of ions and that rapidly desensitize. In contrast, the P2Y receptors, which bind a variety of nucleotides, are metabotropic GPCRs, with distinctive downstream intracellular signaling pathways that increase intracellular Ca^{2+} or cAMP (Figure 5). The structure, molecular physiology, and pharmacology of P2 receptors have been reviewed (44–47). Both P2X and P2Y receptor subtypes are expressed in vascular and tubular structures of the kidney, where they exert physiological actions (4, 48). Signaling through P2 receptors potentially regulates or modulates, for example, tubular transport of water and sodium, tubuloglomerular feedback (TGF) and autoregulation, microcirculation, blood pressure, cell proliferation, necrosis, apoptosis, and oxidative stress.

P2Y Receptors in the Kidney

Among the P2Y receptors, P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₂ are expressed in different structures of the kidney at varying levels. The UTP/ATP-activated P2Y₂ receptor, which is expressed in several kidney structures (48, 49), is the most widely studied one in renal physiology thanks to the availability of a gene knockout mouse model. In the collecting duct, the P2Y₂ receptor is localized to both the basolateral and apical domains (49), where it has distinctive and apparently exclusive roles. Activation of the basolateral, but not the apical, P2Y₂ receptor opposes the action of arginine vasopressin (AVP) on the collecting duct and thus reduces free water absorption (50–52). In contrast, activation of the apical P2Y₂ receptor opposes the effect of aldosterone on ENaC (epithelial sodium channel), thus decreasing sodium absorption (53–55). Although the signal input, regulation, and termination of this fine-tuning mechanism are yet to be deciphered, there is a general consensus that extracellular nucleotides, acting through the P2Y₂ receptor, exert a tonic inhibitory effect on the actions of both AVP and aldosterone (56–60). This notion is exemplified in P2Y₂ receptor knockout mice, which manifest significantly higher urinary-

concentrating ability and salt-sensitive hypertension due to facilitated renal Na⁺ and water absorption (54, 58, 60–62).

Comparatively less is known about the physiological roles of the P2Y₂ receptor in the thick ascending limb, although it is the site of absorption of a significant proportion of filtered sodium through the bumetanide-sensitive Na-K-2Cl cotransporter (54, 62). The role of the P2Y₂ receptor in thick-ascending-limb function needs further study.

Pathophysiologically, P2Y₂ receptor knockout mice are significantly resistant to lithium-induced polyuria, natriuresis, and kaliuresis (61, 62). Furthermore, P2Y₂ receptor deficiency reportedly aggravates chronic kidney disease progression (63). These reports exemplify the critical roles played by the P2Y₂ receptor in water and sodium homeostasis and renal pathophysiology.

The expression and activity of the ADP-activated P2Y₁₂ receptor in the kidney were recently reported (64). Although the P2Y₁₂ receptor is phylogenetically distinct from the P2Y₂ receptor, signaling through the P2Y₁₂ receptor is also mediated through G_i, which inhibits adenylyl cyclase (AC). Accordingly, when the activity of the P2Y₁₂ receptor was blocked in rats by the administration of its selective and irreversible antagonist, clopidogrel bisulfate, there was increased urinary-concentrating ability and increased aquaporin 2 (AQP2) protein abundance in the kidney, as well as amelioration of lithium-induced polyuria (64). Thus, the P2Y₂ and P2Y₁₂ receptors, which apparently have a tonic inhibitory effect on the action of AVP in the collecting duct, are potential targets for the treatment of lithium-induced nephrogenic diabetes insipidus (65).

P2X Receptors in the Kidney

The P2X receptors play specialized roles in the regulation of ion and water transport in tubular cells and in the regulation of cell proliferation, fibrosis, and inflammation. Of particular interest is the critical role of the P2X₁ receptor in renal autoregulation, as elegantly demonstrated by Inscho and associates in a series of studies (reviewed in References 66 and 67). Furthermore, the pathophysiological roles of the P2X₇ receptor in hypertension, glomerulonephritis, and renal cyst development are being uncovered (68–70). Because comprehensive reviews of P2X receptor function in the kidney are available, we do not elaborate further on these receptors (69, 71–76).

P1 (Adenosine) Receptors in the Kidney

There are four types of G protein-coupled adenosine receptors (A₁, A_{2A}, A_{2B}, and A₃), of which two stimulate AC, whereas the other two inhibit AC (Figure 5). The molecular physiology, the pathophysiological roles, and the therapeutic potential of adenosine receptors are well studied and reported (77–80). Of particular interest are the critical roles played by adenosine in TGF (81), renal ischemic-reperfusion injury, acute kidney injury, hypertension, and diabetic nephropathy (82–85).

CD39-Adenosinergic Axis

Cellular ATP is released into the extracellular milieu by regulated exocytosis, by diffusion through membrane channels (e.g., anion channels, connexins, or pannexins), and possibly through multidrug resistance gene products (86–90). It appears that cells, even at rest, constantly release ATP in small quantities, thus establishing a basal level of activation, the so-called set point, for signal transduction pathways (91). Released ATP and related nucleotides are sequentially hydrolyzed to ADP, AMP, and adenosine by the activities of ectonucleotidases (92–95). Adenosine is converted to inosine by the action of adenosine deaminase. Thus, by critically limiting the availability of the ligands in the extracellular milieu, the ectonucleotidases and adenosine deaminase can regulate the activities of the purinergic receptors. In addition, in many tissues or cells, the activity of P1 receptors (whose ligand is adenosine) opposes the activities of P2 receptors (whose ligands are nucleotides), thus establishing a feedback regulation of autocrine/paracrine signaling (92).

CD39, also known as NTPDase1 (nucleoside triphosphate diphosphohydrolase 1), is a vascular ectonucleotidase expressed in several structures of the rodent kidney (96, 97). It hydrolyzes ATP to ADP and then to AMP. AMP is converted to adenosine by CD73 (also known as 5'-ectonucleotidase). Although the regulation of the expression and activities of these ectonucleotidases is not clearly understood, the CD39-adenosinergic axis is considered to play critical roles in renal physiology and pathophysiology, such as TGF, renin secretion, water and sodium excretion, ischemic-reperfusion injury, and renal fibrosis (84, 98–100). CD39 is a major generator of adenosine during ischemia-reperfusion in the kidney, liver, heart, lung, brain, and bowel. Some beneficial effects of adenosine in renal pathophysiology are mediated through Tregs (regulatory T cells) (101, 102).

Extracellular cAMP–Adenosine Pathway

Although the sequential hydrolysis of ATP by ectonucleotidases represents the major pathway for the generation of extracellular adenosine, accumulated evidence shows that extracellular cAMP can also be a precursor of adenosine. During the receptor-mediated heightened activity of AC, some intracellular cAMP is released into the extracellular milieu through multidrug resistance gene products or connexin hemichannels (103–105). The released cAMP is first converted to AMP by ectophosphodiesterases and/or tissue-nonspecific alkaline phosphatases. AMP is then converted to adenosine by CD73, which acts as the convergence point for both the CD39-adenosinergic and extracellular cAMP–adenosine pathways (104, 106).

Both pathways for the production of extracellular adenosine have advantages and disadvantages. The local generation of adenosine from extracellular ATP through the CD39-adenosinergic axis has the advantage of tight local regulation of autocrine/paracrine purinergic signaling. However, because ATP is not stable in blood or other body fluids, this axis has a limited range. In contrast, as a stable molecule in blood and other body fluids, extracellular cAMP can reach distant organs and exert actions therein. This is exemplified in the pancreato-hepato-renal axis, by which glucagon secreted from the pancreas stimulates AC in the liver, resulting in the release of cAMP into the hepatic vein. The filtered cAMP in the kidney gets concentrated in the proximal tubular fluid, where it inhibits proximal tubular

reabsorption of sodium and phosphate (107) and may thus account for the well-documented natriuretic and phosphaturic actions of glucagon. Furthermore, the cAMP reaching the kidney is converted to adenosine through the local extracellular cAMP–adenosine pathway (106). In this respect, the extracellular cAMP–adenosine pathway functions more as a hormonal system without feedback regulation than as an autocrine/paracrine signaling mechanism with feedback regulation. This fact is well illustrated by the excessive secretion of glucagon in animals and humans with certain pathophysiological conditions, such as obesity, insulin resistance, and hyperlipidemia, resulting in chronically elevated extracellular cAMP in the kidney. Such elevated levels in turn increase extracellular adenosine concentrations in the kidney, contributing to the development of hypertension (108).

Adenine (P0) Receptors in the Kidney

The nucleobase adenine is formed in cells during the purine salvage pathway and polyamine synthesis. Unlike adenosine, adenine is not generated by the extracellular degradation of nucleotides by ectonucleotidases. The transport systems for the release of intracellular adenine into extracellular milieu are not known. But adenine may be transported through the same or similar transport systems existing for nucleotides and may thus accumulate in the extracellular fluids. In normal humans, plasma adenine levels are very low (~70 nM) but rise severalfold (to the micromolar range) in patients with chronic kidney disease (109). An orphan GPCR originally cloned from dorsal root ganglia binds adenine, but not adenosine, with high affinity (110). It was recognized as the adenine receptor (AdeR) and was tentatively named the P0 receptor. AdeR expression and function were recently reported in the rat kidney (111). The AdeR protein is expressed in the vasculature in the cortical region, including glomerular arterioles, and throughout the collecting duct system. In the collecting duct, the AdeR protein is colocalized with AQP2. Functionally, the AdeR, a G_i-coupled receptor, inhibited desmopressin-induced cAMP production in acutely isolated medullary collecting ducts, and such inhibition was prevented in the presence of PSB-08162, a selective and potent antagonist of the AdeR (111). Unlike in the rat, in the mouse two different AdeRs have been cloned (112), and thus the investigation of the functional roles of the AdeR in the mouse is complex. The human ortholog of the AdeR is yet to be cloned, but there is evidence for the presence of functional AdeR in cultured human cell lines (112). The AdeR is an interesting addition to the repertoire of purinergic receptors in the kidney, and its physiological and pathophysiological functions have to be deciphered.

METABOLITE RECEPTORS FOR SHORT-CHAIN FATTY ACIDS

Recent studies have highlighted important roles that sensory receptors, such as taste receptors and olfactory receptors (ORs), play in a variety of tissues (12, 113–122), including the kidney and cardiovascular system (17, 123–126). In particular, multiple ORs, taste receptors, and other orphan receptors (of the GPR family) are found in the kidney (125, 126). Although some of these receptors are expressed at relatively high levels relative to well-characterized and extensively studied renal GPCRs (126), few studies have examined their functional roles. In this section, we focus on a role for two sensory receptors, olfactory receptor 78 (Olfr78) and Gpr41, which are found in the renal and cardiovascular systems.

Both Olfr78 and Gpr41 are receptors for SCFAs and play a novel role in modulating blood pressure control.

Physiological Roles for Short-Chain Fatty Acid Receptors

SCFAs have recently emerged as important signaling molecules that regulate a variety of responses. Intriguingly, SCFAs are found in the bloodstream as a by-product of metabolic production by the gut microbiota (127). The gut microbiota are an important element of human physiology and have been referred to as the forgotten organ. In humans, these microbes have been estimated to weigh between 175 g and 1.5 kg (127, 128), and they produce SCFAs at such a rate that the concentration in the colon is approximately 100 mM (127). Subsequently, SCFAs are absorbed into the bloodstream, where they are found in the low-millimolar (0.1–10 mM) range (129–132). SCFAs produced via the gut microbiota are now understood to act as signaling molecules to modulate host physiology, and they are known to modulate parameters as varied as metabolism, immune responses, and susceptibility to HIV infection (129, 130, 133, 134).

Receptors for Short-Chain Fatty Acids

In this subsection, we focus on a recently uncovered role for two SCFA receptors, Gpr41 and Olfr78, in the regulation of both renal and cardiovascular function. Gpr41 and Olfr78 are seven-transmembrane GPCRs that are activated by SCFAs. Gpr41 was an orphan receptor until 2003, when two different groups reported that it is a receptor for SCFAs (129, 135). Gpr41 couples to G_i (129, 135), and although it responds to multiple SCFAs (including formate, acetate, butyrate, and isobutyrate), its strongest ligand appears to be propionate. Gpr41 is activated by SCFAs with an EC_{50} in the low- to mid-micromolar range (129, 135). Olfr78 was identified as a SCFA receptor in 2013 (124), and it responds only to acetate and propionate, with an EC_{50} in the low-millimolar range (e.g., 0.92 mM for propionate). Both of these receptors have similar ligand profiles in both rodents and humans (124, 135, 136), implying that their function is evolutionarily conserved. In addition, although this topic is beyond the scope of this review, there are two other known SCFA receptors: Gpr109A (responds to butyrate with an EC_{50} of ~1 mM but does not respond to acetate or propionate) (137) and Gpr43 [responds to acetate, propionate, and butyrate with EC_{50} s generally in the low- to mid-micromolar range (129, 135)].

Localization of Short-Chain Fatty Acid Receptors in the Kidney and Cardiovascular System

Olfr78 was initially reported to be expressed in the whole kidney by RT-PCR (125), and a subsequent study (124) used a reporter gene mouse to more specifically localize this receptor. In this mouse model, the entire single coding exon for Olfr78 was deleted and replaced with a LacZ reporter. Localization data showed that LacZ expression (as a surrogate for Olfr78) was found in the renal afferent arteriole, as well as in a variety of resistance vascular beds (124) in the peripheral circulation (where it localized to vascular smooth muscle cells). Because both the renal afferent arteriole and resistance vascular beds are classically associated with blood pressure regulation, it was speculated that this receptor might modulate blood pressure control. Unfortunately, antibodies were not available for either Gpr41 or Olfr78. Therefore, Gpr41 was also localized by RT-PCR, which showed that Gpr41 is consistently expressed in the murine vasculature (124) on the mRNA level.

Intriguingly, Gpr43 localized to blood vessels as well, implying that SCFA receptors in general may play a role in vascular responses.

Short-Chain Fatty Acids and Blood Pressure Regulation

Because SCFA receptors localized to cell types and tissues important in blood pressure regulation (the renal afferent arteriole and vascular resistance beds), subsequent studies investigated a potential role of these receptors in blood pressure regulation. As Olfr78 is found in the afferent arteriole, SCFA-induced renin release from glomeruli *ex vivo* was monitored. SCFAs induced renin release from wild-type glomeruli, but not from Olfr78 knockout glomeruli. These data imply that Olfr78 mediates SCFA-induced renin secretion; consistent with this hypothesis, Olfr78 knockouts had lower plasma renin and lower baseline blood pressure (124).

Olfr78 and Gpr41 also localized to vasculature beds outside of the kidney. Previous studies showed that SCFAs cause vasodilation in blood vessels *ex vivo* (138–140). To determine whether there is an *in vivo* consequence for this reported *ex vivo* vasodilation, mice were treated with SCFAs (via intravenous infusion) while blood pressure was monitored by catheter under anesthesia. Upon SCFA delivery, there was a rapid, reproducible, and dose-dependent drop in blood pressure (124); this hypotensive response peaked in 1–2 min and recovered over 5–10 min. SCFAs are found in plasma in the 0.1–10 mM range (129–132); this concentration matches well with the dose response of the hypotensive response *in vivo*. The hypotensive effect after SCFA delivery is consistent with previous reports that SCFAs cause rapid vasodilation *ex vivo* (138–140) and is consistent with reports that the use of acetate in hemodialysis solutions causes hypotension in patients (141, 142).

Because SCFA receptors localize to the resistance vessels that presumably mediate this effect, subsequent studies determined whether this hypotensive response was altered in mice that were null for either Olfr78 or Gpr41 (124). The hypotensive response of SCFAs was attenuated in Gpr41^{+/-} mice and was largely absent in Gpr41^{-/-} mice. In contrast, in Olfr78-null animals, the hypotensive response to SCFAs was accentuated, especially at lower doses. These data imply that the hypotensive response to SCFAs is mediated by Gpr41 and that Olfr78 acts as a brake that opposes the hypotensive response (Figure 6). The dual involvement of two SCFA receptors in the vascular response is in contrast to the case for renin release, which appears to be mediated solely by Olfr78 (143). In both of these cases, Olfr78 increases blood pressure.

Involvement of the Gut Microbiota

The primary source of SCFAs in the bloodstream is production by the gut microbiota and their subsequent absorption from the gut; however, there is also negligible production of SCFAs by native host metabolism (127). Therefore, to more directly implicate gut microbiota in blood pressure regulation, mice were treated with antibiotics in their drinking water to drastically reduce gut microbiota (124). This treatment resulted in a slight increase in the blood pressure of wild-type mice but in a much more dramatic (~15–20 mm Hg) increase in the blood pressure of Olfr78-null animals. These data are consistent with the model that Gpr41 promotes hypotension, whereas Olfr78 promotes hypertension (Figure 6).

In wild-type mice, both the Gpr41 and Olfr78 pathways are at work, and antibiotic treatment simultaneously decreases the activation of both pathways; therefore, there is little effect in a wild-type animal (the slight increase in blood pressure in the wild type is likely due to the fact that the Gpr41/hypotensive pathway predominates). In contrast, in an Olfr78-null animal, the situation is actually much simpler: Gpr41 acts alone to lower blood pressure (hence, these animals have decreased baseline blood pressure) (Figure 6). Therefore, in these animals, antibiotic treatment attenuates Gpr41 activation, and the result is a much larger increase in blood pressure.

The metabolic products of the gut microbiota (including SCFAs) have been tied to various aspects of physiology and pathophysiology (130, 144, 145); however, in many cases we do not yet fully understand why it is advantageous for these parameters in the host to be coupled to changes in gut microbial activity. In the case of blood pressure, one can speculate that SCFA-induced vasodilation would enhance nutrient absorption after a meal. Although most nutrient absorption occurs in the small intestine, a significant quantity of nutrients is absorbed from the large intestine, where the microbiota reside (127, 146–154). Following a meal, SCFA levels in gut circulation would peak, promoting vasodilation and therefore the efficient absorption of nutrients. This effect would complement the role of intestinal vasoactive peptides and would ensure that nutrients are not lost in the stool. Although it is not clear why this effect would necessitate a change in systemic blood pressure, there is evidence for a drop in blood pressure following a meal (when SCFA levels would be expected to rise): Postprandial hypotension is common in the elderly, even among healthy populations (155).

Two Receptors, One Ligand?

The involvement of both Gpr41 and Olfr78 begs the question: What is the purpose of having two different GPCRs that respond to the same ligand (SCFAs) but mediate opposing responses (with Olfr78 mediating hypertension and Gpr41 mediating hypotension)? The answer to this apparent conundrum may reside in the different EC_{50} s of these two receptors. Whereas plasma SCFA concentrations are 0.1–10 mM (129–132), Gpr41 has an EC_{50} (for propionate) of ~100–300 μ M (129, 135), and Olfr78 has an EC_{50} (for propionate) of ~900 μ M (124). Therefore, at a resting SCFA level of approximately 0.1 mM, one would expect Gpr41 to be relatively active. Thus, Gpr41 is likely to be tonically active under basal conditions. As SCFA levels further elevated, Gpr41 would become further activated, promoting a hypotensive response. However, when SCFA levels reached a sufficiently high concentration, they would also activate Olfr78, which would act as a brake on the primary hypotensive response. Therefore, Olfr78 could be a safety mechanism for this response, preventing elevations in SCFA levels from causing inappropriate hypotension.

Microbiota and Blood Pressure: Evidence for a Link

Numerous studies support the concept that microbial SCFAs may be involved in blood pressure control. It is well established that increased dietary fiber intake decreases blood pressure (reviewed in Reference 156); increased dietary fiber would be expected to increase microbial SCFA production. Might SCFA production contribute to the cardioprotective effects of high-fiber diets? Similarly, a meta-analysis of randomized controlled trials showed that the use of probiotics (which elevate SCFA production) is associated with decreased

blood pressure (157). A study examining >4,000 humans from both Asia and Europe found that lower urinary formate (a microbially produced SCFA) correlates with increased blood pressure (158), implying that decreased microbial SCFA production may contribute to blood pressure elevation. The above evidence is primarily correlative. However, two recent studies evaluated the gut microbiota in rodent models of hypertension and found that hypertension is associated with microbial dysbiosis and that the transfer of gut microbiota can alter blood pressure (159, 160). Going forward, it is critical that future studies carefully and systematically evaluate the potential role of gut microbiota in blood pressure regulation, both so that we can more correctly understand the underlying physiological pathways and so that potential future therapeutic approaches can be evaluated.

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SUMMARY POINTS

1. Succinate and GPR91 signaling is involved in the classic hypoxia response of cells; this response includes the stimulation of VEGF synthesis independently of HIF.
2. Mitochondrial citric acid cycle intermediates and their novel metabolic receptors, including succinate/GPR91 and α -ketoglutarate/GPR99, may help maintain body fluid, electrolyte, and acid-base homeostasis.
3. Extracellular nucleotides, nucleosides, and nucleobases, through their receptors, potentially regulate renal transport function and play significant roles in the development of hypertension and other pathophysiological conditions.
4. The CD39-adenosinergic axis and extracellular cAMP–adenosine pathway, by acting like bridges between the extracellular catabolism of nucleotides and the activities of purinergic receptors, modulate and fine-tune purinergic signaling.
5. The metabolic products of the gut microbiota have been tied to various aspects of physiology and pathophysiology, including blood pressure regulation.

FUTURE ISSUES

1. Is succinate and GPR91 signaling involved in the regulation of aging; longevity; and metabolism-related diseases such as diabetes, obesity, and cancer?
2. What signaling pathways are involved in GPR99-mediated HCO_3^- secretion and NaCl reabsorption in the renal collecting duct?
3. Future studies should identify the local regulatory mechanisms that trigger release of nucleotides by the kidney cells and identify any coordinated or synchronized regulatory mechanisms.
4. Future studies should identify connections or cross talk points between the local regulation of purinergic signaling in the kidney and systemic regulation by hormones.
5. Future studies need to carefully and systematically evaluate the potential role of gut microbiota in blood pressure regulation.
6. If gut microbial metabolites contribute to blood pressure regulation, might purposeful manipulation of the gut microbiota predictably alter blood pressure?

Figure 1.

Illustration of the autocrine and paracrine effects of metabolites on the regulation of cell, organ, and whole-body functions. Certain intracellular metabolites that accumulate in response to cell stress and activation and are released to the interstitium play a hormone-like mediator and signaling role. They bind to their specific G protein–coupled receptors (GPCRs) and activate signaling mechanisms that in turn regulate several functions in the kidneys and the vasculature. SCFAs denote short-chain fatty acids.

Figure 2.

Succinate and αKG accumulation and signaling via GPR91 and GPR99 in response to cell or mitochondrial stress. An accelerated citric acid (TCA) cycle leads to significant succinate and αKG accumulation in the mitochondria and to the backleak of succinate and αKG into the cytosol and local interstitium via dicarboxylate carriers and organic anion transporters for both the mitochondrial and plasma membranes. GPR91 and GPR99 activation is linked to G_{i/o}- and G_q-mediated intracellular signaling pathways that involve, for example, IP₃ and Ca²⁺, cAMP, and mitogen-activated protein kinases p38 and ERK1/2. Such activation may be involved in the cell stress response to hypoxia (parallel to and independent of the classic HIF pathway) and various metabolic stimuli. However, the overactivation of these normally protective signaling pathways may lead to the development of pathology. Abbreviations: αKG, α-ketoglutarate; EPO, erythropoietin; GLUT1, glucose transporter 1; HIF, hypoxia-inducible factor; IP₃, inositol trisphosphate; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor. Adapted with permission from Peti-Peterdi (28).

Figure 3.

The presence and paracrine effects of mitochondrial citric acid cycle intermediates in the renal tubular fluid. Normally, low amounts of plasma α KG and succinate are filtered in the glomerulus. However, the proximal tubule can release α KG into tubular fluid (e.g., during dietary alkali load), which mediates paracrine cross talk between the proximal tubule and distal nephron segments (the CNT and CD) via α KG's receptor, GPR99. GPR99 is exclusively expressed in type B (and also in non-A–non-B) ICs in the CNT and CD and regulates HCO_3^- secretion and electroneutral transepithelial NaCl reabsorption (magnified in *right inset*). Succinate may be released from the hypoxic thick-ascending-limb segment of the loop of Henle, and via its receptor GPR91, which is expressed in the luminal membranes of macula densa cells, succinate can induce renin release from the JGC. Paracrine signaling between these JGA cells involves GPR91 activation, COX-2-mediated PGE₂ synthesis, and PGE₂ release from the macula densa (magnified in *left inset*). These intrarenal mechanisms appear to play important, newly recognized roles in the metabolic regulation of body fluid and electrolyte balance, pH homeostasis, and blood pressure control. Abbreviations: α KG, α -ketoglutarate; CD, collecting duct; COX-2; cyclooxygenase-2; CNT, connecting tubule; IC, intercalated cell; JGA, juxtaglomerular apparatus; JGC, juxtaglomerular cell; PGE₂,

prostaglandin E₂; RAS, renin-angiotensin system. Adapted with permission from Peti-Peterdi (39).

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Figure 4.

Phylogenetic relation of human P2Y receptors. GPR91 and GPR99 are most related to P2Y₁, P2Y₁₁, P2Y₂, P2Y₄, and P2Y₆ (framed in *gray*). Adapted with permission from Schöneberg et al. (40).

Figure 5.

Schematic representation of the components of the complex purinergic signaling pathways mediated by extracellular nucleotides. Intracellular ATP (3–5 mM) is released from the cells in small quantities into the extracellular milieu through specific transport processes, such as nucleotide transporters (NT) or pannexin (PNX) or connexin (CNX) hemichannels.

Extracellular ATP, even at low-micromolar (5–20 μM) concentrations, interacts with P2X or P2Y receptors. P2X receptors (subtypes 1–7) are ATP-gated channels that open up, allowing ions (Na⁺, K⁺, or Ca²⁺) to either influx or efflux. P2Y receptors (subtypes 1, 2, 4, 6, and 11–14) are G protein-coupled receptors with downstream effector signaling pathways that cause an increase in either intracellular free Ca²⁺ or cyclic AMP (cAMP). The extracellular ATP and related nucleotides are sequentially hydrolyzed to ADP and AMP by CD39 [nucleoside triphosphate diphosphohydrolase 1 (NTPDase1)] and/or nucleotide pyrophosphatase (NPP). AMP is converted to adenosine by CD73 (5'-ectonucleotidase). Adenosine is a potent agonist of P1 receptors (subtypes A₁, A_{2A}, A_{2B}, and A₃), the activation of which results in increased or decreased activity of adenylyl cyclase (AC). Similar to P2Y receptors, P1 receptors are G protein-coupled receptors with complex downstream signaling pathways. This pathway leading from ATP to the generation of adenosine, known as the CD39-adenosinergic axis, is active locally and apparently regulates the balance between P2 and P1 receptor activity in cells. Adenosine can also be generated from extracellular cAMP, which has two sources. Being stable in blood and body fluids, cAMP released from distant organs, such as the liver, can reach kidney cells. cAMP can also be released locally within the kidney during heightened receptor-mediated activation of AC. Intracellular cAMP reaches the extracellular milieu through the same transport systems used by ATP and other nucleotides. Extracellular cAMP is converted to AMP by the action of ectophosphodiesterase (ePDE) or tissue-nonspecific alkaline phosphatases (ns-AP). The AMP thus formed is converted to adenosine by CD73. This pathway, which connects the cAMP formed distantly with the local formation of adenosine from cAMP, is known as the

extracellular cAMP–adenosine pathway. Another purinergic ligand, the nucleobase adenine, is not generated in these two pathways. Instead, extracellular adenine either comes from the bloodstream or is released locally from cells through NT. Adenine selectively binds to P0 or adenine receptor (AdeR), a G protein–coupled receptor. In most species, the activation of the AdeR results in decreased AC activity. But there is evidence that some AdeRs may activate AC. ADA denotes adenosine deaminase.

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Figure 6.

Working model for the acute hypotensive effect of SCFA delivery on BP. Gpr41 acts to decrease BP in response to SCFAs, whereas Olfr78 acts in opposition to increase BP. In WT animals, the hypotensive response of Gpr41 prevails. When Olfr78 is genetically deleted, the hypotensive response is further accentuated, as the brake on the pathway has been removed. However, when Gpr41 is genetically deleted, the hypotensive response is absent, and instead a slight hypertensive response (presumably mediated by Olfr78) remains. Abbreviations: BP, blood pressure; KO, knockout; SCFA, short-chain fatty acid; WT, wild type. Reprinted with permission from Pluznick (161).