G protein–coupled receptors (GPCRs) are proteins in the plasma membrane harboring seven transmembrane spanning domains. They are activated by a wide variety of moieties including peptides, lipids, purines, chemokines, odorants, and light. Based on sequence homology to known receptors, the sequencing of the human genome accelerated the identification of many novel GPCRs, now numbering >800 family members. Many of these novel receptors have no known ligands and therefore are known as orphan GPCRs.

In 2001, a cluster of six GPCRs was identified on chromosome 3q24 –3q25, including the four orphan receptors GPR86, GPR87, H963, and GPR91.1 The latter shares high sequence homology with the family of P2Y purinoreceptors. Shortly thereafter, another novel receptor of this subfamily was identified on chromosome 13q32.2, GPR99,2 showing high sequence homology with GPR91 and P2Y receptors (Figure 1). Thus, GPR91 and GPR99 might be expected to activate on contact with purinergic compounds. Surprisingly, however, GPR91 and GPR99 activate specifically through the Krebs cycle intermediates succinate and α-ketoglutarate (also called oxoglutarate), respectively.3 Therefore, the gene encoding GPR91 was renamed SUCNR1 (succinate receptor 1), whereas the gene encoding GPR99 was renamed OXGR1 (for oxoglutarate receptor 1). The tissue distribution of SUCNR1 and OXGR1 are highly expressed in the kidney.3,4 Although mRNA encoding OXGR1 is detected in the renal distal convoluted and connecting tubule, no function is known as yet. Several physiologic roles SUCNR1 have emerged, and we will focus here on its signaling in the kidney and its involvement in the development of hypertension in diabetes mellitus.

REGULATION OF SUCCINATE LEVELS

Succinate is a well-known intermediate in the tricarboxylic acid (Krebs) cycle, where it is formed from succinyl-CoA by succinyl-CoA synthetase and subsequently converted by succinate dehydrogenase to generate fumarate. Because the succinate dehydrogenase complex is part of the electron transport chain in the mitochondrial membrane (complex II; Figure 2), its activity indirectly depends on the availability of oxygen. As such, in situations when oxygen tension is low, succinate accumulates because of low activity of succinate dehydrogenase or other enzymes in the electron transport chain that affect its activity.5–7 Low oxygen states, such as ischemia or exercise also increase circulating levels of succinate. The effect of low oxygen states on succinate levels is also obvious in rats anesthetized with 100% CO2 instead of oxygenated isoflurane; succinate levels increased from 7 to 40 μM in the left ventricle and up to 173 μM from low-oxygen blood collected from the vena cava.10

Alternatively, other changes in energy balance affect the production and release of succinate, particularly in animal models of diabetes mellitus,11 metabolic disease,10 and liver damage.12 During chronic hyperglycemia, the high activity of the Krebs cycle increases the H+ gradient across the mitochondrial membrane (Fig-
Figure 1. Phylogenetic tree of the family of P2Y purinergic receptors. Amino acid sequences encoding human P2Y receptors were aligned, resulting in a phylogenetic tree based on closest sequence homology.

SUCNR1 IS A LOCAL SENSOR OF STRESS

Quantitative PCR assays show mRNA encoding SUCNR1 in kidney, liver, and spleen, and a subsequent study confirmed its expression in kidney and liver, as well as in white adipose tissue. Subsequently, several studies described the function of SUCNR1 in specific cell types in these tissues. Although its detailed function remains to be established in most settings, it is clear this receptor is a detector of disturbances in energy balance.

Regulation of Lipolysis in White Adipose Tissue

In states of hypoglycemia, hormones such as glucagon trigger adipocytes in white adipose tissue to degrade triglycerides into free fatty acids for energy production. Stimulatory Gs proteins mediate this process of lipolysis. In SUCNR1+ adipocytes, succinate inhibits lipolysis in a pertussis toxin–dependent manner, showing that SUCNR1 signaling inhibits adenylyl cyclase to form cAMP. Because increased succinate levels are found in rodent models of diabetes mellitus and metabolic syndrome, high succinate levels may prevent lipolysis in states when fuels such as glucose and free fatty acids are abundant.

Stellate Cell Activation in Liver Pathology

The liver is crucial for regulating the body’s metabolism by storing fuel molecules such as glycogen and plays a major role in lipid and amino acid conversion or synthesis, as well as the degradation of toxic compounds. Likely, therefore, this organ is subject to multiple stressors primarily related to an unhealthy lifestyle. In the liver, SUCNR1+ adipocytes, succinate inhibits lipolysis in a pertussis toxin–dependent manner, showing that SUCNR1 signaling inhibits

Figure 2. Generation of succinate in mitochondria. Succinate is an intermediate in the citric acid cycle and is converted by succinate dehydrogenase (also called complex II) to fumarate. When high H+ gradients over the inner membrane are present, or when the oxygen pressure is low, complexes I, II, III, and IV will be inhibited, leading to accumulation of succinate. See text for more details.
the downstream effectors of SUCNR1 signaling in different tissues and cell types are summarized in Table 1.

**Apoptosis of Cardiomyocytes**

A recent study by Aguiar et al. showed the presence of mRNA encoding SUCNR1 and protein in freshly isolated preparations of ventricular cardiomyocytes, where it localizes in the sarcolemmal membrane and T-tubules. In these cardiomyocytes, succinate leads to increased protein kinase A activity that subsequently releases intracellular calcium transients. Moreover, succinate-stimulated cardiomyocytes show increased maximum peak height and higher frequency of calcium transients, which affect contraction of these cells. Importantly, prolonged incubation of cardiomyocytes with succinate induces apoptosis, most likely caused by a combination of protein kinase A activation and increased intracellular calcium levels or by the release of prostaglandins and the subsequent transduction of these stimuli. Together, this explains how administration of succinate in a mouse model of chemotherapy-induced myelosuppression leads to increased levels of hemoglobin, platelets, and neutrophils; succinate therefore may be beneficial to patients recovering from chemotherapy.

In contrast, Rubic et al. did not detect mRNA encoding SUCNR1 in monocytes, T cells, or B cells, but only in immature dendritic cells (DCs), suggesting that SUCNR1 expression is induced when monocytes develop into immature DCs. In these cells, succinate stimulates migration in a concentration-dependent manner and thus mediates chemotaxis. Moreover, by phosphorylation of ERK1/2, SUCNR1 and Toll-like receptors act in synergy to potentiate the production of the inflammatory cytokines TNFα and IL-1β. On activation, immature DCs will mature to antigen-presenting DCs that can subsequently activate T cells. Succinate treatment of DCs promotes IFNγ production of activated CD4+ T cells.

The prostimulatory effects of succinate on immature DCs are subject to a self-induced negative feedback loop, which downregulates SUCNR1 expression when DCs achieve maturity. Furthermore, underscoring the fact that the above observations are SUCNR1-mediated, mice challenged with tetanus toxin accumulate higher levels of mature DCs in their lymph nodes compared with SUCNR1−/− mice. Grafts from SUCNR1−/− mice show improved outcome during skin graft rejection. As such, interfering with SUCNR1 signaling by specific receptor antagonists or preventing succinate accumulation may be beneficial for patients receiving organ transplants. However, specific inhibitors of the SUCNR1 remain to be developed.

**Vascularization of the Retina**

In the retina, SUCNR1 is predominantly expressed in the cell bodies of the retinal ganglion cell layer. To study the role of SUCNR1 in developing retina, siRNA against mRNA encoding SUCNR1 was injected into the eye of newborn rat pups, which decreases the vascularization of the retina at day 4 postpartum compared with controls. In contrast, injection of succinate increases vessel numbers in the retina, clearly showing a positive role for SUCNR1 in retinal vascularization. In addition, SUCNR1 regulates vessel growth through the production and release of proangiogenic hormones. Moreover, the retinal ganglion cells expressing SUCNR1 are essential for proper vascularization of the eye. However, in diabetes mellitus or retinal ischemia, increased levels of succinate induce high rates of neovascularization, leading to retinopathy.

### Table 1. Tissue distribution of SUCNR1 and its signaling effects in specific cell types

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type</th>
<th>Effectors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>HEK293</td>
<td>Ca²⁺, ERK1/2, prostaglandins</td>
<td>3</td>
</tr>
<tr>
<td>Kidney</td>
<td>Vascular endothelium, GENC</td>
<td>Ca²⁺, NO, PGE₂, renin release</td>
<td>11</td>
</tr>
<tr>
<td>Kidney</td>
<td>Macula densa, MMDD1</td>
<td>p38, ERK1/2, COX-2, PGE₂, renin release</td>
<td>20</td>
</tr>
<tr>
<td>Kidney</td>
<td>MDCK, collecting duct principal cells</td>
<td>Ca²⁺, ERK1/2, PGE₂, PG₁₂</td>
<td>19</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatic Stellate cells</td>
<td>α-SMA</td>
<td>12</td>
</tr>
<tr>
<td>Heart</td>
<td>Cardiomyocytes</td>
<td>PKA, Ca²⁺</td>
<td>14</td>
</tr>
<tr>
<td>Bone marrow/Blood</td>
<td>CD34 + progenitor cells, megakaryocytes, erythroid progenitor cells</td>
<td>IP, ERK1/2, proliferation, anti-apoptotic</td>
<td>15</td>
</tr>
<tr>
<td>Blood</td>
<td>T cells, B cells, monocytes, platelets</td>
<td>Potentiates platelet aggregation</td>
<td>16</td>
</tr>
<tr>
<td>Blood</td>
<td>Immature dendritic cells</td>
<td>Ca²⁺, chemotaxis, potentiates cytokine production</td>
<td>17</td>
</tr>
<tr>
<td>Retina</td>
<td>Retinal ganglion neurons</td>
<td>VEGF</td>
<td>18</td>
</tr>
<tr>
<td>White adipose</td>
<td>Adipocytes</td>
<td>Inhibition of lipolysis</td>
<td>4</td>
</tr>
</tbody>
</table>

respect, inhibitors of SUCNR1 may provide a potential treatment.

REGULATION OF BP AND THE RENIN-ANGIOTENSIN SYSTEM IN THE DIABETIC KIDNEY

Together with deorphanizing SUCNR1, He et al. observed that injection of succinate in mice induces the release of renin from the juxtaglomerular apparatus (JGA) in the kidney, resulting in hypotension. Therefore, expression of SUCNR1 may occur in the JGA, although this was not confirmed at the time. Since then, we and others determined the localization of SUCNR1 in the kidney to establish its role in renal pathophysiology. The SUCNR1 localizes to the renal vascular lumen, in particular the afferent arteriole and the glomerular vasculature. Moreover, SUCNR1 expresses on the luminal membrane of multiple segments of the renal tubules: the cortical thick ascending limb (cTAL) of Henle’s loop, including the macula densa (MD), and the cortical and medullary collecting duct (CD). The renal distribution of SUCNR1 and its proposed actions (see below) are summarized in Figure 3A.

Recent work by the Peti-Peterdi group showed that SUCNR1 mediates the release of renin from the JGA through SUCNR1 located in the vascular luminal membrane or along the apical membrane of MD cells. Elegant microperfusion studies combined with live imaging of isolated glomeruli showed that perfusion with a succinate-containing buffer induces renin release from the granular cells of the JGA, which rapidly induce vasodilation of the afferent arteriole. This shows that SUCNR1 plays a dynamic role in development of glomerular hyperfiltration and activation of the renal renin-angiotensin system.

The release of renin from the JGA is mediated in part by the formation of NO. Moreover, activation of SUCNR1 increases levels of cyclooxygenase (COX)-2, leading to the production and release of prostaglandin E2 that subsequently transactivates EP2 and/or EP4 receptors on granular cells. Subsequently, it was shown that activation of SUCNR1 on the luminal membrane of MD cells triggers renin release from the JGA through a similar mechanism, although in this case, SUCNR1 serves as a sensor for succinate in tubular fluid rather than in blood. The SUCNR1-mediated release of renin described above is shown in Figure 3B.

Figure 3. Localization of SUCNR1 and its signaling effect in the nephron. (A) This schematic overview of the nephron shows the segments expressing SUCNR1 in dark gray: glomerular vasculature, cTAL, MD, and CD. SUCNR1 activation in the AA and MD induces the release of NO and PGE2, resulting in secretion of renin from the granular cells of the JGA, leading to activation of the renin-angiotensin system that affects AA width, BP, and renal sodium and water handling. Moreover, in cTAL and CD, SUCNR1 may also directly affect sodium and water reabsorption. Finally, SUCNR1 in the CD promotes ERK1/2 phosphorylation, which may contribute to the development of diabetic nephropathy. (B) SUCNR1 activation triggers the release of renin. SUCNR1 located in the luminal membrane of MD cells, or in the plasma membrane of AA cells, induces the release of PGE2 and NO. This will promote the release of renin from the JGA granular cells. Glom, glomerular vasculature; AA, afferent arteriole; EA, efferent arteriole.

Analogous to the development of hypertension on administration of succinate to mice, plasma levels of succinate elevate in several rodent models of hypertension and metabolic disease. Spontaneous hypertensive rats, fatty Zucker fa/fa rats, db/db diabetic mice, and ob/ob mice have two- to four-fold elevated suc-
cinate levels compared with their nonhypertensive or lean controls. However, serum levels of succinate in hypertensive or diabetic patients are similar to healthy age-matched controls. The source of this discrepancy between rodent models and patients remains unknown.

As SUCNR1 along the renal tubules sense the availability of succinate, measurements of succinate in excreted urine may provide an easy, noninvasive way to determine SUCNR1 activity in kidney compared with circulating succinate levels. Moreover, because of concentrating mechanisms along the nephron, the more distal parts of the tubule may be exposed to increased succinate levels compared with endothelial cells along the afferent arteriole that sense only circulating and regional succinate. Indeed, urinary succinate concentrations in diabetic mice are approximately 5 to 10 times higher than plasma succinate levels in mice with a similar genetic background. In control mice, however, urinary succinate concentrations are similar to plasma levels, in which the concentrating effect of the nephron awaits more specialized ground. In control mice, however, urinary succinate concentrations are similar to plasma levels, in which the concentrating effect of the nephron may be partially counteracted by reabsorption of succinate by a variety of dicarboxylate transporters along the proximal tubule. Recent studies also suggested the SLCO4C1 transporter eliminates uremic toxins, including succinate, and upregulation of this transporter attenuates hypertension and renal inflammation; statins stimulate this effect. Thus, determination of the exact filtration fraction of succinate and the amount of succinate locally produced by tubular cells of the nephron awaits more specialized clearance studies.

Although these succinate measurements indicate that SUCNR1 might play a role in diabetes and metabolic syndrome, the relationship between diabetes and development of hypertension was first suggested by work in SUCNR1 knockout mice. The JGA and whole kidney renin content of diabetic mice are elevated compared with non-diabetic controls, and renin release is stimulated by perfusion of the afferent arteriole or the MD-containing cTAL with a high glucose or succinate buffer. The observed release of renin combined with the aforementioned dilatation of the afferent arteriole resulting in hyperfiltration are hallmarks of the diabetic kidney.

In healthy individuals, the release of renin from the JGA is subject to a negative feedback loop through angiotensin II, which activates its receptor on granular cells to inhibit renin release by the Ca2+-protein kinase C pathway. However, in kidneys of diabetic mice, renin levels are increased, especially in the cortical areas around the JGA, where SUCNR1 is found, whereas no upregulation of renin is observed in SUCNR1 knockout mice. As such, SUCNR1 may allow the body to escape from the angiotensin II–negative feedback loop and maintain high levels of (pro)renin, thereby contributing to sustained hypertension.

Nowadays, it is well established that the production and release of renin is no longer restricted to the JGA, and individual components of the renin-angiotensin system have been detected throughout the nephron. The function of this paracrine tubular renin-angiotensin system is slowly emerging. In the kidney of diabetic mice, activation of SUCNR1 in the cTAL and CD leads to increased ERK1/2 phosphorylation, whereas this effect is absent in SUCNR1 knockout mice. Sustained tubular ERK1/2 phosphorylation associates with proliferation of tubular cells and the development of tubulointerstitial fibrosis, and SUCNR1 may be instrumental in the development of fibrosis in diabetic nephropathy and diabetes-induced hypertension. ERK1/2 phosphorylation downstream of SUCNR1 activation in kidney cells is rapid and transient. Therefore, direct SUCNR1 signaling, or by release of JGA renin and the subsequent formation of angiotensin II, may induce the tubular formation of (pro)renin, as has recently been shown for the CD in diabetes and other tubular segments in hypertension. The released (pro)renin subsequently binds to its receptor, leading to sustained ERK1/2 phosphorylation. However, the exact role of SUCNR1 activation and mechanisms underlying these processes requires further investigation.

**FUTURE PERSPECTIVES**

As shown above and summarized in Table 2, signaling through SUCNR1 is involved in the pathophysiology of disease in multiple organs. These processes are linked particularly to local stress factors that affect the energy balance of a tissue, such as ischemia, hypoxia, metabolic syndrome, and diabetes mellitus; SUCNR1 senses local damage and increases inflammatory responses. Therefore, this receptor acts a sensor of local stress that affects cellular metabolism, as reflected by increased formation and release of succinate. It is also clear that SUCNR1 is a regulator of BP in diabetes mellitus and may contribute to the development of tubulointerstitial fibrosis in diabetic nephropathy. Future challenges lie in elucidating the

**Table 2. SUCNR1 in pathophysiology**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Model System</th>
<th>SUCNR1 KO/ No SUCNR1</th>
<th>Stimulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Diabetes mellitus</td>
<td>BP not increased</td>
<td>Renin release, hypertension</td>
<td>11,20</td>
</tr>
<tr>
<td>Liver</td>
<td>(Ischemic) stress</td>
<td></td>
<td>Stellate cell activation</td>
<td>12</td>
</tr>
<tr>
<td>Heart</td>
<td>Ischemic stress</td>
<td></td>
<td>Apoptosis</td>
<td>14</td>
</tr>
<tr>
<td>Blood</td>
<td>Skin transplantation</td>
<td>Improved graft survival</td>
<td>Chemotaxis + maturation of dendritic cells</td>
<td>17</td>
</tr>
<tr>
<td>Blood</td>
<td>Chemotherapy</td>
<td></td>
<td>Improved blood cell recovery</td>
<td>15</td>
</tr>
<tr>
<td>Retina</td>
<td>Development</td>
<td>Reduced vascularization, vascular density</td>
<td>Accelerated retinal vascularity</td>
<td>18</td>
</tr>
<tr>
<td>Retina</td>
<td>Ischemic stress</td>
<td>Reduced retinopathy</td>
<td>Angiogenesis, retinopathy</td>
<td>18</td>
</tr>
</tbody>
</table>

KO, knockout.
cellular and molecular mechanisms responsible for these effects and identifying specific receptor antagonists to prevent or ameliorate this pathophysiology.

Besides the SUCNR1 found in the kidney, its presence on immune cells could also affect renal pathology. Succinate’s role as a chemotactic signal through SUCNR1 on immature DCs may induce infiltration of immune cells in the kidney. In renal transplantation, ischemia and hypoxia will likely increase renal succinate formation as similarly observed in ischemic retinopathy. Analogous to the skin transplantation effects described earlier, this may promote maturation of immature DCs in the kidney. In renal ischemia-reperfusion experiments, which serve as a window to some transplantation responses, DCs are the major source of TNFα produced early in the inflammatory response. The synergistic effect of SUCNR1 and Toll-like receptors contributes significantly to the release of high levels of TNFα, thus increasing inflammation, renal epithelial apoptosis, and recruitment, binding, and migration of leukocytes. Eventually, this may lead to graft injury and rejection.

Besides promoting retinal vascularization during development, no clear role yet exists for SUCNR1 in normal physiology. This in part may be because of the relatively recent discovery that succinate can act as a signaling molecule. Alternatively, its normal role may be very subtle or redundant, and extracellular succinate acts exclusively as a stress or damage signal, as we have shown here. Although more details are needed, it is clear that SUCNCR1 is a highly promising drug target in a multitude of disorders.

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