Succinate Receptors in the Kidney

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ABSTRACT

The G protein–coupled succinate and α-ketoglutarate receptors are closely related to the family of P2Y purinoreceptors. Although the α-ketoglutarate receptor is almost exclusively expressed in the kidney, its function is unknown. In contrast, the succinate receptor, SUCR1N1, is expressed in a variety of tissues, including blood cells, adipose tissue, liver, retina, and the kidney. Recent evidence suggests SUCR1N1 and its succinate ligand are novel detectors of local stress, including ischemia, hypoxia, toxicity, and hyperglycemia. Local levels of succinate in the kidney also activate the renin-angiotensin system and together with SUCR1N1 may play a key role in the development of hypertension and the complications of diabetes mellitus, metabolic disease, and liver damage. This makes the succinate receptor a promising drug target to counteract an expanding number of interrelated disorders.


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 ischemia5 or exercise9 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 (Figure 2), inhibiting individual complexes of the electron transport chain, and the succinate dehydrogenase complex is no exception. As such, succinate accumulates in states of low oxygen, ischemia, and metabolic disease, which may be detected by the succinate receptor.
the electron transport chain including succinate dehydrogenase.6 This results in the intracellular accumulation and eventually release of succinate.

**SUCNR1 IS A LOCAL SENSOR OF STRESS**

Quantitative PCR assays show mRNA encoding SUCNR1 in kidney, liver, and spleen,3 and a subsequent study confirmed its expression in kidney and liver, as well as in white adipose tissue.4 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.4 Because increased succinate levels are found in rodent models of diabetes mellitus and metabolic syndrome,10,11 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 is exclusively expressed in quiescent hepatic stellate cells (HSCs), but on activation of HSCs, the expression of SUCNR1 decreases rapidly,12,13 suggesting that SUCNR1 serves as an early detector of hepatic stress or damage. Application of ischemia in a perfused liver model increases succinate levels of the perfusate 14-fold to approximately 1 mM.12 Moreover, HSCs treated with succinate show increased levels of a myofibroblastic marker compared with inactive control cells, indicating that succinate independently stimulates HSC activation. SUCNR1 signaling, therefore, plays a role in HSC activation to restore damaged tissue in the ischemic liver or contributes to the formation of fibrosis. The signaling pathways involved in this HSC activation remain obscure. In contrast to adipocytes or renal cells (see below), administration of succinate to HSCs fails to induce an intracellular
Ca$^{2+}$ response, nor does it decrease forskolin-induced cAMP levels or increase cAMP levels by itself.\textsuperscript{12} For comparison, the downstream effectors of SUCNR1 signaling in different tissues and cell types are summarized in Table 1.

**Apopotosis of Cardiomyocytes**

A recent study by Aguiar et al.\textsuperscript{14} 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 transactivation of prostaglandin receptors.\textsuperscript{14} As such, SUCNR1 regulates apoptosis in the heart in states of ischemia and hypoxia.

**Maturation and Sensitizing of Blood and Immune Cells**

Although not initially identified in tissue panels,\textsuperscript{3,4} it is now evident that SUCNR1 is also expressed in hematopoietic precursor cells and multiple subtypes of blood and immune cells,\textsuperscript{15,16} as summarized in Table 1. When administered to platelets, succinate potentiates aggregation in a dose-dependent manner, increasing maximum aggregation compared with controls.\textsuperscript{16} This suggests a role for succinate in atherothrombosis, in which succinate levels may increase because of local hypoxia.

In hematopoietic progenitor cells, activated SUCNR1 signals through $G_{i/o}$ proteins to induce cell proliferation via extracellular regulated kinases (ERK)1 and 2. Also, SUCNR1 activation protects the erythroleukemic cell line, TF1, from serum starvation-induced apoptosis. Together, this explains how administration of succinate in a mouse model of chemotherapy-induced myelosuppression leads to increased levels of hemoglobin, platelets, and neutrophils;\textsuperscript{11} succinate therefore may be beneficial to patients recovering from chemotherapy.

In contrast, Rubic et al.\textsuperscript{17} 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.\textsuperscript{17} 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.\textsuperscript{17} 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.\textsuperscript{18} 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 succi-

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**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>Ca$^{2+}$, ERK1/2, prostaglandins</td>
<td>3</td>
</tr>
<tr>
<td>Kidney</td>
<td>Vascular endothelium, GENC</td>
<td>Ca$^{2+}$, NO, PGE$_2$, renin release</td>
<td>11</td>
</tr>
<tr>
<td>Kidney</td>
<td>Macula densa, MMDD1</td>
<td>p38, ERK1/2, COX-2, PGE$_2$, renin release</td>
<td>20</td>
</tr>
<tr>
<td>Kidney</td>
<td>MDCK, collecting duct principal cells</td>
<td>Ca$^{2+}$, ERK1/2, PGE$_2$, PGI$_2$</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, Ca$^{2+}$</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>Ca$^{2+}$, 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>

ERK, extracellularly regulated kinase; PGE$_2$, prostaglandin E$_2$; COX-2, cyclooxygenase 2; PGI$_2$, prostaglandin I$_2$; α-SMA, α-smooth-muscle actin; IP, inositol phosphate; VEGF, vascular endothelial growth factor.
nate induce high rates of neovascularization, leading to retinopathy.\textsuperscript{17} In this 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 \textit{et al.}\textsuperscript{3} 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).\textsuperscript{11,19,20} 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\textsuperscript{11} or along the apical membrane of MD cells.\textsuperscript{19–21} 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 E\textsubscript{2} that subsequently trans-activates EP2 and/or EP4 receptors on granular cells.\textsuperscript{11} 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.\textsuperscript{20} The SUCNR1-mediated release of renin described above is shown in Figure 3B.

Analogous to the development of hypertension on administration of succi-
nate 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 succinate 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 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−/− mice. The JGA and whole kidney renin content of diabetic mice are elevated compared with nondiabetic 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 dilation 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−/− 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 CD19 leads to increased ERK1/2 phosphorylation, whereas this effect is absent in SUCNR1−/− 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.

Table 2. SUCNR1 in pathophysiology

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Model System</th>
<th>SUCRN1 KO/ No SUCRN1</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 vascularityization</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.

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 in-
creased 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 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 immunity. P.M.T.D. is a recipient of VICI Grant 865.07.002 of the Netherlands Organization for Scientific Research (NWO). P.M.T.D. is supported by grants from the Dutch Kidney Foundation (C03-2060), NWO (865.07.002), and Coordination Theme 1 (Health) of the European Community’s 7th Framework Program (HEALTH-F2-2007-201590, entitled EUFERROR). J.H.R. is supported by the Dutch Kidney Foundation (KJPB 09.012).

DISCLOSURES
None.

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