Review

Succinate Receptor 1: An Emerging Regulator of Myeloid Cell Function in Inflammation

Grzegorz Krzak,1,3 Cory M. Willis,1,3 Jayden A. Smith,2 Stefano Pluchino,1,* and Luca Peruzzotti-Jametti1,*

The rapidly evolving area of immunometabolism has shed new light on the fundamental properties of products and intermediates of cellular metabolism (metabolites), highlighting their key signaling roles in cell-to-cell communication. Recent evidence identifies the succinate–succinate receptor 1 (SUCNR1) axis as an essential regulator of tissue homeostasis. Succinate signaling via SUCNR1 guides divergent responses in immune cells, which are tissue and context dependent. Herein, we explore the main cellular pathways regulated by the succinate–SUCNR1 axis and focus on the biology of SUCNR1 and its roles influencing the function of myeloid cells. Hence, we identify new therapeutic targets and putative therapeutic approaches aimed at resolving detrimental myeloid cell responses in tissues, including those occurring in the persistently inflamed central nervous system (CNS).

The Succinate–SUCNR1 Axis in Inflammation

The past decade has seen an impressive increase in our understanding of the wider role of metabolites in the regulation of cellular functions [1]. In innate immune cells, extensive intracellular metabolic reprogramming controls cell behavior, phenotype, and differential states of activation in response to external stimuli [2]. This is a fine-tuned process that requires the coordination of several metabolic networks that ultimately modulate intracellular and extracellular signaling pathways.

The metabolite succinate, an intermediate of the tricarboxylic acid (TCA) cycle, has emerged as a key modulator of innate immune responses in mammals (Box 1). Proinflammatory macrophages accumulate succinate intracellularly as a consequence of TCA cycle breaks [3]. Intracellular succinate acts as an immunometabolite (see Glossary) to guide macrophage effector functions via the transcription of proinflammatory cytokines [3] and the production of reactive oxygen species (ROS) [4]. In chronic inflammation, such as in rheumatoid arthritis (RA), succinate is released into the extracellular compartment, where it regulates cell-to-cell communication. In mouse disease models, extracellular succinate acts as both a metabolokine and an alarmin to modulate immune cell function by binding to its cognate receptor SUCNR1 in vitro and in vivo, thus eliciting complex responses that are tissue and context dependent [5,6].

Herein, we explore the main cellular pathways regulated by the succinate–SUCNR1 axis and focus on the biology of SUCNR1 and its roles in modulating adaptive and innate immune responses. We then focus on myeloid cells, describing evidence of the dual role of SUCNR1 in these cells.

We anticipate that unraveling the complex mechanisms regulating this novel extracellular signaling pathway may be crucial to understanding the mechanisms sustaining innate immune responses during persistent inflammation. This understanding should be instrumental to developing new

Highlights

The signaling properties of the G-protein-coupled SUCNR1 depend on cell and tissue specificity, as well as pathophysiological context.

SUCNR1 activation can not only drive proinflammatory responses, but also directly participate in the resolution of inflammation.

Succinate signaling via SUCNR1 has a role in the progression of tissue damage and resolution of inflammation in the CNS.

Careful consideration of these aspects must be adopted when targeting SUCNR1 signaling to develop new candidate therapies aimed at mitigating the effects of persistent CNS inflammation.

1Department of Clinical Neurosciences and NIHR Biomedical Research Centre, University of Cambridge, Cambridge, UK
2Cambridge Innovation Technologies Consulting (CITC) Ltd, Cambridge, UK
3These authors contributed equally

*Correspondence: spp24@cam.ac.uk (S. Pluchino) and lp429@cam.ac.uk (L. Peruzzotti-Jametti).
Intracellular signaling on human kidney macula densa cells [13], quiescent hepatic stellate cells of the rat liver [17], and cardiomyocytes of the rat heart [18]. In these cells, SUCNR1 regulates blood pressure and contributes to the pathophysiology of liver injury and cardiomyocytes viability [13,17,18]. SUCNR1 is also expressed in the musculoskeletal system [19] and in certain cancer and stem cells where it promotes proliferation, migration, and tissue remodeling [20,21].
SUCNR1 activation of the G_{qγ} subunit leads to inhibition of adenylyl cyclase (AC), which results in decreased cAMP concentrations, decreased activity of protein kinase A (PKA), and, ultimately, the release of proinflammatory cytokines [14,15]. By contrast, SUCNR1 activation of the G_{qα} subunit has opposite effects, leading to increased PKA activity and regulation of transcription of anti-inflammatory mediators via cAMP response element binding protein (CREB) activity [16]. Finally, SUCNR1 activity via the G_{qγ} subunit instead enhances the activity of phospholipase C (PLC), which produces the secondary messengers inositol trisphosphate (IP_{3}) and diacylglycerol (DAG). While IP_{3} increases Ca^{2+} in the cytosol leading to the activation of Ca^{2+}-dependent mitogen-activated protein kinase-extracellular signal-regulated kinases 1/2 (MAPK-ERK1/2) pathways, DAG activates protein kinase C (PKC). Both of these pathways lead to proinflammatory cascades [such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), MAPK-ERK1/2, and p38], nitric oxide (NO) production, and prostaglandin E_{2} (PGE_{2}) secretion [40,69]. Of note, recent evidence suggests that PLC activation in human cells may be mediated by the βγ subunits of SUCNR1 [70]. Red and green arrows indicate downregulation and upregulation, respectively. Abbreviation: KLF4, Krüppel-like factor 4.

Despite the brain being the most metabolically active organ of the body, basal SUCNR1 expression is nearly undetectable in the healthy mouse CNS [22]. Publicly available transcriptomics databases of healthy human and mouse samples confirmed that basal SUCNR1/Sucnr1 expression is low throughout the CNS, with the highest expression observed in the olfactory bulbs, hippocampus, lateral ventricles, and cerebellum (Table 1) [23,24].

Regardless of its low basal expression in the healthy CNS, it appears that SUCNR1 activation is key in the response of CNS cells to hypoxic-ischemic injury and inflammatory damage (Figure 2). For example, in rodent retinal ganglion cells (RGCs), the activation of the succinate–SUCNR1 axis leads to increased release of vascular endothelial growth factor (VEGF) and prostaglandin E_{2} (PGE_{2}) in vitro, thus favoring vascularization via the activation of the mitogen-activated protein kinase–extracellular signal-regulated kinases 1/2 (MAPK-ERK1/2) signaling pathway [25,26]. In fact, the small interfering (si)RNA-mediated retinal downregulation of Sucnr1 in wild-type (WT) rats abolished neovascularization in the presence of succinate, indicating that SUCNR1 expression in RGCs is a key regulator of the rodent retinal vascular network [27]. This could be of particular relevance in pathological settings, such as in hypoxic-ischemic injury [28]. While on the one hand, succinate–SUCNR1 signaling is beneficial, because it promotes neovascularization through PGE_{2}-dependent release of angiogenic factors [29], on the other hand, succinate signaling through SUCNR1 can...
Table 1. SUCNR1 Expression and Signaling Pathways in Rodent and Human Neural and Immune Cells

<table>
<thead>
<tr>
<th>Cell type (SUCNR1 expression)</th>
<th>Function of SUCNR1</th>
<th>Activated G-protein</th>
<th>Activated pathway</th>
<th>Studied model</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal ganglion cells</td>
<td>Increased expression of proangiogenic factors (Vegf, Ang1, Ang2) and proinflammatory cytokines (IL1b, IL6); increased secretion of PGE2 and VEGF</td>
<td>Unknown</td>
<td>MAPK-ERK1/2; PGE2-EP4-dependent mechanism</td>
<td>Ex vivo Sucnr1^{+/+} mice; in vitro rat RGC-5 cells</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Vascularization in diabetic retinopathy mediated by VEGF induction and secretion</td>
<td>Unknown</td>
<td>MAPK-ERK1/2/C/EBPβ; (c-Fos, HIF-1α); MAPK-ERK1/2-GOX2–PGE2</td>
<td>In vitro rat RGC-5 cells; in vitro rat primary retinal ganglion cells</td>
<td>[25,26,59]</td>
</tr>
<tr>
<td>Retinal pigment epithelium</td>
<td>Iron homeostasis; VEGF secretion; increased expression in juvenile hemochromatosis</td>
<td>Possibly Gαo</td>
<td>BMP6-Smad1/5/8-pSmad5</td>
<td>In vitro primary mouse RPE cells; in vitro human RPE cell lines ARPE-19 and HiRPE</td>
<td>[30,31]</td>
</tr>
<tr>
<td>(RPE)</td>
<td>astrocytes</td>
<td>Increased expression of proangiogenic factors (Vegf, Ang1, Ang2) and proinflammatory cytokines (IL1b, IL6); increased secretion of PGE2</td>
<td>Unknown</td>
<td>PGE2-EP4-dependent mechanism</td>
<td>In vitro rodent brain cortex astrocytes</td>
</tr>
<tr>
<td>Neural stem cells (NSCs)</td>
<td>Increased succinate uptake; increased Slc13a3/5 and Ptgs2; release of PGE2</td>
<td>Unknown</td>
<td>P-p38 MAPK</td>
<td>In vivo mouse EAE model; in vitro mouse NSCs/iNSCs</td>
<td>[32]</td>
</tr>
<tr>
<td>Erythroblasts (EBs) (mRNA, protein)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>In vitro human EBs from hematopoietic CD34^{+} cells</td>
<td>[37]</td>
</tr>
<tr>
<td>Platelets (mRNA, protein)</td>
<td>Stimulates aggregation, enhances platelets-derived eicosanoid release</td>
<td>Gαi, Gβγ</td>
<td>cAMP–PKA pathway; Src kinase activation and PI3K/Akt1</td>
<td>In vitro human platelets</td>
<td>[14,70]</td>
</tr>
<tr>
<td>T cells (CD4^{+}) (protein)</td>
<td>Possibly regulate T cell-dependent B cell activation</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Ex vivo human umbilical cord blood</td>
<td>[37]</td>
</tr>
<tr>
<td>B cells (CD8^{+}) (protein); B cells (CD19^{+}, IgD “CD27”, IgD “CD27”, and IgD “CD27”) (protein)</td>
<td>IgG and IgM secretion in synergy with IL-10 from naïve B cells</td>
<td>Unknown</td>
<td>MAPK-ERK1/2</td>
<td>Ex vivo human blood from patients with systemic lupus erythematosus</td>
<td>[38]</td>
</tr>
<tr>
<td>Immature monocyte-derived dendritic cells (MoDCs) (mRNA)</td>
<td>Promotes chemotaxis, proinflammatory augmentation, T cell activation enhancement</td>
<td>Gαi</td>
<td>MAPK-ERK1/2</td>
<td>Ex vivo human MoDCs; ex vivo Sucnr1^{+/+} mouse DCs</td>
<td>[40]</td>
</tr>
<tr>
<td>Bone marrow-derived DCs (mMDCs) (mRNA)</td>
<td>Promotes proinflammatory phenotype and chemotaxis</td>
<td>Unknown</td>
<td>Unknown</td>
<td>In vitro mouse BMDCs; in vivo Sucnr1^{−/−} mouse antigen-induced arthritis (AIA) model</td>
<td>[41]</td>
</tr>
<tr>
<td>Bone marrow-derived macrophages (BMDCs)</td>
<td>Modulates inflammatory response</td>
<td>Unknown</td>
<td>Unknown</td>
<td>In vivo Sucnr1^{−/−} mouse model; in vitro mouse BMDCs</td>
<td>[39,46]</td>
</tr>
<tr>
<td>Monocyte-derived macrophages (M2 (IL-4), M1 (IFNγ + LPS)) (mRNA)</td>
<td>Enhances chemotaxis in white adipose tissue</td>
<td>Unknown</td>
<td>Unknown</td>
<td>In vitro human blood monocytes</td>
<td>[42]</td>
</tr>
<tr>
<td>Peripheral blood mononuclear cells (anti-inflammatory (IL-4, IL-13) (mRNA)</td>
<td>Decreases expression of IL-10, TLR4 and TLR5 while upregulating TNF-1α</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Ex vivo human blood mononuclear cells</td>
<td>[45]</td>
</tr>
<tr>
<td>Adipose tissue macrophages (ATMs) (mRNA, protein)</td>
<td>Promotes anti-inflammatory phenotype via IL-4</td>
<td>Gαi</td>
<td>PKA–CREB–KLF4 pathway</td>
<td>In vivo LysoM-Cre Sucnr1^{+/−} mice; in vitro human monocyte THP1 cell line</td>
<td>[16]</td>
</tr>
</tbody>
</table>
also be detrimental by leading to vessel hyperproliferation, such as in the context of proliferative ischemic retinopathy [27].

The apical membrane of the retinal pigment epithelium (RPE) also expresses Sucnr1 under homeostatic conditions [30,31]. Here, SUCNR1 has a putative role in iron homeostasis, because both the in vivo genetic disruption of the iron regulatory genes Hfe (Hfe−/−) or Hfe2 (Hjv−/−) in mice, and the in vitro exposure of primary RPE cells to soluble iron, led to increased SUCNR1 expression [30,31]. This evidence is clinically relevant to hemochromatosis, an iron storage disorder associated with excessive iron accumulation. In fact, when human RPE cells are exposed in vitro to succinate and soluble iron, SUCNR1-dependent increased VEGF expression is observed [30]. Moreover, in the mouse model of juvenile hemochromatosis (Hjv−/−), enhanced bone-morphogenetic (BMP)-6 signaling in combination with succinate, facilitated the interaction of pSmad4 with the Sucnr1 promoter sequence, promoting angioma formation through a Sucnr1- and Vegf-dependent mechanism [31].

Active SUCNR1 expression and signaling is also present in both mouse and human induced neural stem cells (iNSCs) in vitro [32]. However, its precise roles in regulating endogenous NSC function, differentiation, and how it could interact with their metabolism in conditions of inflammation [33] are still poorly understood. Similarly, rat astrocytes express Sucnr1 [29] and, when exposed to succinate in vitro, there is increased expression of proangiogenic factors (Vegf, Ang1, and Ang2) and proinflammatory cytokines [interleukin (IL)1β and IL6] [29]. Succinate also increases the secretion of PGE2, but not of VEGF, from RCA cells [29].

These data, together with the recent finding of genome-wide variation in SUCNR1 function in humans [34], suggest that SUCNR1 activity is a predisposing factor for the development of CNS-intrinsic dysfunctions, although this remains to be thoroughly investigated.

**SUCNR1 Signaling in Adaptive and Innate Immune Cells**

Human hematopoietic progenitor cells (HPCs) are known to express SUCNR1 [35]. SUCNR1 has been linked with the maintenance of the self-renewing pool of HPCs, mostly via the activation of the MAPK signaling pathway [35,36]. Megakaryocytes and erythroblasts also express SUCNR1 [37], as do many cells of the adaptive and innate immune systems across species (Table 1). The conserved expression of SUCNR1 in immune cells suggests a key role in immune system function, acting as a regulator of both homeostatic and inflammatory cellular responses following exposure to pathogens or after tissue damage.

Within the adaptive immune system, SUCNR1 is detected on human T lymphocytes (both CD4+ and CD8+ subsets) and all major human B lymphocyte subsets [37,38]. Although the exact

<table>
<thead>
<tr>
<th>Cell type (SUCNR1 expression)</th>
<th>Function of SUCNR1</th>
<th>Activated G-protein</th>
<th>Activated pathway</th>
<th>Studied model</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-associated macrophages (TAMs) (mRNA, protein)</td>
<td>Promotes tumor metastasis via IL-6</td>
<td>Unknown</td>
<td>PI3K-HIF-1α pathway</td>
<td>In vivo mouse cancer models; in vitro mouse peritoneal macrophages; in vitro mouse Sucnr1−/− peritoneal macrophages</td>
<td>[21]</td>
</tr>
<tr>
<td>Mast cells (MCs) (mRNA)</td>
<td>Hyper-reactive phenotype of Sucnr1−/− MCs</td>
<td>Unknown</td>
<td>Unknown</td>
<td>In vitro human MCs; in vitro mouse bone marrow derived MCs; in vitro mouse Sucnr1−/− dermatitis model</td>
<td>[42]</td>
</tr>
</tbody>
</table>

**Table 1. (continued)**
The functional effects of SUCNR1 activation in innate immune cells are not always straightforward, because they are both cell and context dependent [16,39]. Within the innate immune system, SUCNR1 is expressed on DCs, mast cells, and mononuclear phagocytes (MPs) [such as bone marrow-derived macrophages (BMDMs), tumor-associated macrophages (TAMs), adipose tissue macrophages, and microglia] (details on species and cells are given in Table 1).

Among the innate immune cells, human immature DCs show maximal expression, and SUCNR1 activity controls their chemotaxis in a succinate concentration-dependent manner [40]. Here, SUCNR1 acts synergistically with toll-like receptor (TLR)-3 and 7, but not TLR-2 or TLR-4, to increase the expression of the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-1β.
Overall, this leads to enhanced antigen-specific presentation and subsequent CD4+ T cell activation [40]. Of relevance, this response appears to be time limited, because SUCNR1 is swiftly downregulated once DCs become fully activated [40]. Also in mouse DCs, SUCNR1 enhances lipopolysaccharide (LPS)-induced (i.e., TLR-4-dependent) production of proinflammatory cytokines [41]. Indeed, in mice with experimental antigen-induced arthritis, SUCNR1-mediated chemotaxis guided mouse DCs into lymph nodes in vivo, leading to the expansion of pathogenic T helper (Th)17 cells, which in turn contributed to autoimmunity [41].

The activity of mast cells also relies on the presence of functional SUCNR1. In Sucnr1−/− mice, mast cells displayed a hyperreactive phenotype, as evidenced from increased TNF-α production in response to oxazolone-induced allergic contact dermatitis, relative to controls [42]. However, the hyper-reactive response of mast cells within this context does not correlate with an equally hyper-reactive adaptive immune response, as measured by either T cell infiltrates or the expression of the Th2-like (anti-inflammatory) cytokines IL-4 and IL-13 [42]. These findings suggest a key physiological role for succinate–SUCNR1 signaling in mast cells that impacts their differentiation and maturation capacity, although this warrants further studies.

Overall, these data suggest that coordinated expression and activation of SUCNR1 among multiple cell types of the adaptive and innate immune systems is necessary for modulating homeostatic and inflammatory responses via this newly recognized metabolic signaling pathway.

**Context-Dependent Role of SUCNR1 in MPs**

While the succinate–SUCNR1 axis is rather defined in DCs and mast cells [40–42], its role in the chemotaxis and activation of MPs remains controversial.

In Sucnr1−/− mice, macrophage infiltration into succinate-producing adipose tissue is significantly reduced in vivo relative to controls [43]. However, the lack of a clear chemoattractant effect for succinate alone on macrophages in vitro questions the specificity of these findings. In fact, Sucnr1−/− macrophages show reduced migration only in response to tissue culture media derived from apoptotic/hypoxic adipocytes, which suggests that the presence of receptors other than SUCNR1 [e.g., reduced C-C chemokine receptor type 1 (CCR1) expression] has a major role in MP chemotaxis [43]. Nevertheless, recent data demonstrated that succinate alone can promote the chemotaxis of SUCNR1-expressing TAMs in both syngeneic and xenogeneic lung cancer grafts models in mice, because this effect was abrogated by pretreatment with anti-SUCNR1 antibodies in vitro, suggesting a direct role of SUCRN1 in TAM chemotaxis [21].

Engagement of SUCNR1 has also been linked to microglial chemotaxis, as suggested by the increased accumulation of microglia in the retina of Sucnr1−/− mice relative to controls [44]. However, this phenotype only occurs when the hosts are globally deficient in Sucnr1, suggesting that the SUCNR1-mediated control of retinal microglia chemotaxis is in fact dispensable [44].

Besides chemotaxis, the effects of the succinate–SUCNR1 axis on inflammatory responses of MPs have also led to conflicting results (Figure 3, Key Figure). For example, a proinflammatory effect of SUCNR1 was observed in alternatively activated (anti-inflammatory) macrophages derived from human peripheral blood mononuclear cells (PBMCs) [45]. Here, when macrophages were challenged ex vivo with IL-4 and IL-13, they significantly upregulated SUCNR1. However, subsequent stimulation with succinate, or the SUCNR1 agonist compound 131, decreased the expression of the anti-inflammatory cytokine IL-10, while upregulating the proinflammatory cytokine TNF-α, thus enhancing proinflammatory responses [45]. These findings suggest a prominent role for the succinate–SUCNR1 axis in maintaining inflammation by dampening anti-
inflammatory responses. In addition, while previous evidence has shown that the absence of SUCNR1 on mouse peritoneal macrophages has no effect on intracellular IL-1β concentrations, or on the secretion of TNF-α and IL-6 (in response to 24-h stimulation with 10 ng/ml LPS [43]), recent data suggested that, when BMDMs are stimulated for 24 h with higher doses (100 ng/ml) of LPS, or with a combination of LPS (1 ng/ml) and monosodium urate (180 ug/ml) in vitro, the absence of SUCNR1 led to significantly lower IL-1β production [39]. In addition, IL-1β treatment per se increased Sucnr1 mRNA expression in mouse BMDMs in vitro [39]. This finding anticipates a possible bidirectional crosstalk between proinflammatory mediators and the activity of SUCNR1, and the existence of a positive feedback mechanism in inflammation. In this model, IL-1β would trigger the production and release of succinate from macrophages, while succinate in
turn would stimulate SUCNR1-expressing MPs to increase IL-1β, thus maintaining a chronic inflammatory state via an autocrine and paracrine loop [39]. Accordingly, in vivo data suggest that Sucnr1−/− bone marrow chimeric mice are protected from antigen-induced experimental arthritis as early as 2 days after challenge, relative to controls [39].

However, recent work has challenged the prevalent proinflammatory role of SUCNR1 by showing that mouse BMDMs lacking SUCNR1 (Sucnr1−/−) exhibit an increased proinflammatory phenotype relative to controls, characterized by increased release of IL-6, TNF-α, and nitric oxide (NO), in response to LPS (100 ng/ml) or LPS + interferon (IFN)-γ (10 ng/ml + 10 U/ml, respectively) for 24 h in vitro [46]. Since the Sucnr1−/− mice used to derive BMDMs were identical in both of the aforementioned studies [39,46], discrepancies in published results might be related to the use of different BMDM maturation protocols (i.e., recombinant M-CSF + IFN-γ pulse [39] versus L-929 conditioned media [46]), as well as the age of the animals (age-matched [39] versus an average of 28 weeks difference between WT and Sucnr1−/− mice [46]). However, the exact mechanisms behind these apparent discrepancies are yet to be fully addressed.

Further data supporting the anti-inflammatory effect of SUCNR1 come from a study of an inflamed CNS, where SUCNR1 stimulation can directly participate in the resolution of inflammation in vivo [32]. Specifically, delayed accumulation of succinate occurred in the cerebrospinal fluid (CSF), but not in peripheral blood of mice with experimental autoimmune encephalomyelitis (EAE), a model of chronic multiple sclerosis (MS) [32]. In this study, CSF succinate, most likely released by proinflammatory macrophages and microglia, signaled to grafted NSCs via SUCNR1; NSCs, in response, initiated the secretion of PGE2 and the scavenging of extracellular succinate, both contributing to the resolution of neuroinflammation [32].

Subsequent work confirmed the anti-inflammatory role of SUCNR1 in vivo, showing that SUCNR1 in mouse macrophages induced a prevalent anti-inflammatory phenotype [16]. Specifically, in a LysMCreSucnr1fl/fl myeloid cell-specific Sucnr1 conditional knockout mouse line, a significant increase in CD11b+/CD11c+/CD206+ proinflammatory macrophage numbers and proinflammatory genes (If12b, Tnf, and Nos2) was reported in white adipose tissue (WAT) in vivo relative to controls [16]. In vitro, treatment with IL-4 (30 ng/ml) induced Sucnr1 in mouse peritoneal macrophages [while treatment with LPS (250 ng/ml) had the opposite effect (decreased SUCNR1 expression) in macrophages derived from human PBMCs] [16]. Of note, the SUCNR1-dependent induction of an anti-inflammatory phenotype in macrophages occurred in response to IL-4 via an alternative cAMP-dependent protein kinase A (PKA) phosphorylation signaling cascade, subsequent to Gαs stimulation [16].

Finally, recent evidence in syngeneic and xenogeneic lung cancer grafts models in mice provides further confirmation of the role of succinate–SUCNR1 signaling in skewing macrophages into anti-inflammatory TAMs [21]. In this setting, peritoneal macrophages treated with succinate (1 mM) for 24 h showed a significant increase in the expression of anti-inflammatory mRNAs Arg1, Fizz1, Mgl1, and Mgl2 in vitro relative to controls, which was completely abolished via siRNA-mediated SUCNR1 downregulation. Furthermore, succinate released by the tumor induced a significant increase in the number of VCAM1+ /CD11c+/CD11blow TAMs in vivo, suggesting that the succinate–SUCNR1 axis was indispensable for inducing the expansion of anti-inflammatory TAMs [21]. Altogether, these findings suggest that succinate, produced (and released) by proinflammatory MPs, can act on SUCNR1 via competing feedback loops regulating both proinflammatory and anti-inflammatory MPs. However, the exact circumstances and mechanisms guiding the prevalence of either response remain to be fully elucidated.
SUCNR1 as a Potential Pharmacological Target for Chronic Neuroinflammation

While the role of acute inflammation is well known, the transition towards a chronic state of inflammation has now become a therapeutic focus, particularly in the context of inflammatory and degenerative neurological diseases [47,48]. Under persistent neuroinflammation, the activation of innate immune cells leads to a state of ‘sterile’ inflammation, wherein the proinflammatory phenotype of MPs continues in the absence of triggering stimuli, thus leading to constant damage of neurons and oligodendrocytes [5].

As described earlier, there is evidence supporting a key role for the succinate–SUCNR1 axis in regulating the phenotype and function of innate immune cells, and the response of CNS cells to damage; thus, pharmacological targeting of this pathway may be therapeutically relevant for certain conditions associated with persistent (detrimental) innate immune responses in the CNS, such as progressive MS. Unfortunately, the expression of SUCNR1 on a range of CNS and immune cells, as well as the seemingly disparate functional effects that can result from activating this pathway, complicate the study and putative development of therapeutics targeting SUCNR1. Major considerations regarding the cell- and context-specific effects of SUCNR1 signaling must be taken into account when designing and interpreting experimental results, as should the CNS permeability of any prospective succinate analogs [46,49]. Ideally, attention to these key points might not only lead to a better understanding of the succinate–SUCNR1 axis, but also inform the development of potential therapies that might target chronic/persistent neuroinflammation.

Most work in this area has been related to the identification of SUCNR1 agonists. By using a structural model based on the crystal structure of the closely related P2Y1 receptor (27% homology to SUCNR1), the binding site of SUCNR1 was characterized and virtual screening was used to identify its putative ligands [45]. Several non-metabolite agonists were identified, with the most potent candidates having structures based on the succinate backbone with an amide-linked hydrophobic moiety capable of occupying a side pocket at the SUCNR1 binding site [45]. Certain agonists were determined to have 10–100 times higher potency than succinate, exhibiting specificity for either human or mouse SUCNR1, and displaying similar signaling effects to succinate in human ex vivo macrophages [45]. Further structure–function studies of this agonist series identified nanomolar potency ligand candidates with excellent in vitro stability, but high hydrophilicity [50]. The most potent succinate analog is cis-epoxysuccinic acid, with a half maximal effective concentration (EC50) of 2.7 μM; this compound is not succinate dehydrogenase active but has the same effect as the canonical SUCNR1 ligand, because it increased blood pressure in rats upon intravenous administration [51]. For reference, EC50 for succinate for the activation of SUCNR1 has been reported in the range of 17–56 μM [9,45], while typical plasma concentrations of succinate in humans are on the order of 2–30 μM (see Clinician’s Corner) [45]. Of note, the xanthone natural products vinaxanthone and xanthofulvin have also been found to be positive allosteric modulators of SUCNR1, enhancing the affinity of the receptor for succinate [52].

Based on the predominant role of SUCNR1 as a positive regulator of innate immune cell chemotaxis and chronic inflammation, it is not a stretch to consider that SUCNR1 antagonists might be protective in environments where inflammation is exacerbated, such as in certain autoimmune or neuroinflammatory conditions, including progressive MS. Several small-molecule inhibitors of human SUCNR1 were developed from a single hit identified in a high-throughput in vitro screen of a commercial compound library [53]. Through structure–activity relationship-guided systematic modification of the naphthyridine-based hit, potent and selective antagonists were identified, most notably those labeled ‘2c’ and ‘4c’. These compounds exhibited promising half maximal inhibitor concentration (IC50) values of 30 nM and 7 nM, respectively, and were >1000 times
more selective for human SUCNR1 over hGPR99 (which shares 33% protein sequence homology) [53]. While both 2c and 4c exhibit poor oral bioavailability, intraperitoneal administration into succinate-treated Wistar rats of either compound readily ameliorated hypertension relative to controls [53]. Other compounds synthesized in the study traded improved oral bioavailability for decreased affinity/selectivity [53]. Assays using another compound in the series, 2d, demonstrated the ability of the SUCNR1 inhibitor to suppress type I collagen induction in rat hepatic stellate cells subjected to activation by high glucose (700 mg/dl) or high succinate (3 mM) conditions modeling nonalcoholic steatohepatitis (NASH) in vitro [54]. An alternative series of SUCNR1 inhibitors was identified through high-throughput screening with one compound, NF-56-EJ40, demonstrating an impressive selectivity for human SUCNR1 (IC50 = 25 nM), over rat SUCNR1 (IC50 = 100 μM) [12]. The structural basis of this species selectivity was elaborated through crystallography and molecular modeling, with comparisons to the structurally similar P2Y1 receptor providing leads for prospective allosteric inhibitors. While this series of antagonists demonstrated poor permeability due to their highly polar zwitterionic nature, systematic optimization of the structural scaffold, including incorporation of an internal salt bridge, yielded potent inhibitors with good bioavailability and oral exposure [55].

Follow-up studies of the therapeutic utility of SUCNR1 antagonists have yet to be reported in the scientific literature, but several of the co-authors of the naphthyridine-based antagonist study are listed as inventors on various patents describing a complementary series of SUCNR1 inhibitors with prospective applications in treating NASH and related conditions. Indeed, both fibroblast growth factor 21 and its recombinant peptide analog have lowered α-smooth muscle actin production through inhibition of the succinate–SUCNR1 signaling axis, thus reducing liver fibrosis in mouse models [56]. The mitochondrial fission-activating type 2 diabetic drug metformin has likewise been ascribed an inhibitory role in succinate–SUCNR1 signaling, ameliorating inflammation and fibrosis in a mouse model of NASH [57].

RNAi-mediated downregulation of SUCNR1 has also been used to explore the physiological ramifications of succinate–SUCNR1 signaling (and, thus, its potential therapeutic modulation). SUCNR1 is alleged to be a key target of miR-758, with oxidized low-density lipoprotein-induced overexpression of this miRNA associated with suppression of SUCNR1 and its downstream signaling pathways, resulting in damage to human vascular endothelial cells in vitro [58]. Alternatively, short hairpin RNA-mediated knockdown of Sucnr1 expression in a rat model of retinopathy attenuated the avascular area, abnormal neovascularization, and loss of pericytes through regulation of VEGF relative to controls [59]. One novel approach envisions an enhanced-affinity recombinant SUCNR1 mutant able to sequester elevated concentrations of succinate in the gut, such as that associated with inflammatory bowel disease.

There may be a significant opportunity to therapeutically intervene in conditions of chronic inflammation through amelioration of SUCNR1 overactivity in the innate immune system. The benefits of such an approach are perhaps most immediately achievable in metabolic conditions, such as NASH, obesity, and type 2 diabetes mellitus, which are marked by a strong inflammatory basis to their pathology and in which GPCR-targeting drugs have shown significant potential [60,61]. Nonetheless, the need remains to elucidate the pathological relevance of the succinate–SUCNR1 axis in human disease (given that preclinical animal models of GPCR signaling modulation have not always been successfully translated) [60,62].

Beyond systemic metabolic conditions, applications for SUCNR1 inhibitors in the treatment of neuroinflammatory diseases are feasible and worth pursuing. In conditions of elevated CNS succinate, potent and selective antagonists offer the potential to restore homeostasis to the
Box 2. Constitutive SUCNR1-Knockout Mice

Constitutive knockout technology provides a powerful in vivo tool to understand the functional and behavioral phenotype of gene deletion. To this extent, Sucnr1−/− mice have been useful because they display normal development and maturation, despite a significantly lower body weight, compared with their WT littermates [69]. Sucnr1−/− mice are fertile, show no overt blood pressure abnormalities, no abnormal brain vascularization [39,67], no alteration of food intake, and no deposition of fat or lean mass compared with WT littermates [68]. However, Sucnr1−/− mice do show defects in conditions of stress and disease. Their endurance on a forced treadmill running test is significantly impaired, as indicated by overall lower average running speed and increased muscle strength relative to WT mice [19]. When fed a high-fat diet (HFD), Sucnr1−/− mice showed increased fat deposition, hyperglycemia, reduced insulin secretion, and augmented hepatocyte damage compared with WT littermates [69]. Moreover, Sucnr1−/− mice intravenously supplemented with succinate showed abnormal cardiac parameters related to the function of the left ventricle, such as decreased systolic volume and ejection fraction [69]. In the future, to circumvent the well-known limitations of global gene knockout (such as compensatory mechanisms and off-target cellular effects), cell-specific gene knockout-targeting strategies should be used to provide more accurate insights into the function of SUCNR1 [16].

Outstanding Questions

Cellular cues, such as hypoxia, inflammation, and necrosis, stimulate succinate release. Mechanistically, accumulated succinate in the mitochondrial matrix is transported to the mitochondrial inner space by SLC25A10 mitochondrial dicarboxylate carrier (DIC). Succinate transport from mitochondria to the cytosol is controlled by voltage-dependent anion channel (VDAC). The extracellular release of succinate is mediated by solute carrier family 13 group (SLC13) transporters, expressed by neurons, astrocytes (SLC13A1), and NSCs (SLC13A3/5), or the proton-coupled monocarboxylate transporter (MCT) 1, expressed by muscle cells. How is this mechanism of succinate release (and uptake) modulated in different cells and pathological conditions?

SUCNR1 activation leads to receptor internalization and desensitization via β-arrestin signaling [e.g., after either succinate (200 µM for 2 h) or LPS (250 ng/ml for 6 h) treatment]. In kidney cells, desensitization with 200 µM succinate lasted for 15 min, after which rapid resensitization occurred within 15 min. How stable is SUCNR1 activity and how does the cycling of SUCNR1 influence the pro- and anti-inflammatory phenotype of myeloid cells?

In the healthy CNS, basal SUCNR1 expression is nearly undetectable. However, during neuroinflammation, blood-borne and tissue-resident macrophage activation is linked to the upregulation of SUCNR1. Similarly, neural cells upregulate SUCNR1 in response to tissue damage. How are the expression dynamics of SUCNR1 in immune cells and neural cells regulated, and what is the overall contribution of these dynamics to the maintenance of chronic inflammation in the CNS?

Notably, while the apparent limited activity of SUCNR1 in a nonpathological context suggests that such a compound would have a high therapeutic index, a greater understanding of the systemic effects of pharmaceutical modulation of the succinate–SUCNR1 axis is essential for ruling out any unanticipated off-target effects.

Concluding Remarks

Overall, SUCNR1 has thus far been largely overlooked as a putative therapeutic target for certain inflammatory pathologies, perhaps due to its complex and tissue/context-dependent functions. While large pharmaceutical companies have previously filed patents on methods for screening SUCNR1 modulators or the use of SUCNR1 as a marker/target for immune cells, these have been seemingly neglected. Several research groups and small companies are furthering the development of more bioavailable agonists and antagonists; however, future therapeutic exploitation of the succinate–SUCNR1 axis will necessitate greater elucidation of its complexities.

The succinate–SUCNR1 axis appears to be relevant in conditions of stress and damage, while its role in homeostatic conditions is negligible (Box 2). This might be advantageous in developing candidate therapeutics to treat certain pathologies, given that the off-target effects of SUCNR1 modulation are likely to have minimal effects on nonactivated SUCNR1 cells in the host, although this remains to be rigorously tested (see Outstanding Questions). In our view, understanding the complex role of SUCNR1 in regulating neuro-immune interactions in health and disease can uncover the right window of opportunity to modulate this axis, where its targeting could contribute to anti-inflammatory and proreregenerative responses that might resolve chronic neuroinflammation.

Acknowledgments

The authors wish to acknowledge the contribution of past and present members of the Pichurino laboratory, who have contributed to (or inspired) this review. We are grateful to Giovanni Pichurino for critical insights into this manuscript. This work received support from the National MS Society (USA; grant RG-1802-30200 to S.P.), the Italian Multiple Sclerosis Association (AISM; grant 2018/R/14 to S.P.), the US Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) (grant MS-140019 to S.P.), and the Bascule Charitable Trust (RG 75149 and RG 98181 to S.P.). L.P.J. was supported by a senior research fellowship Fondazione Italiana Sclerosi Multipla (FISM cod. 2017/B/5 and financed or co-financed by the ‘5 per mile’ public funding), a clinical research career development fellowship from the Wellcome Trust (220711/Z/20/Z), and the Addenbrooke’s Charitable Trust (RG 97519).
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