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# MOLECULAR BIOLOGY PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

## SUCCINATE RECEPTORS (SUCNR1) AS A POTENTIAL TARGET FOR PHARMACOTHERAPY

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A considerable number of reports have been published in recent years on G protein-coupled receptors, their distribution in the body, mechanism of activation, and potential pathways for pharmacological actions. Intermediates in carbohydrate, fat, and protein metabolism and the tricarboxylic acid cycle, operating as endogenous ligands for a large group of ex-orphan receptors, have active roles in regulating metabolic processes, while their synthetic analogs, operating as both agonists and antagonists, may have potential for the development of new pharmaceuticals for a wide range of diseases (diabetes mellitus, obesity, metabolic syndrome, autoimmune disorders, hypertension, myocardial hypertrophy and ischemia, neurodegenerative processes, liver diseases, etc.). The present review addresses GPR91 (SUCNR1) receptors, which have been identified in fatty tissue, liver, kidneys, heart, brain, retinal neurons, dendritic cells, and platelets, and which are regarded as physiological regulators and cell sensors for stress-induced damage and hypoxia.

**Keywords**: GPR91 receptors, SUCNR1 receptors, succinate, reamberin.

G protein-coupled receptors (GPCR) were initially described as a family of receptors activated by hormones, neurotransmitters, and other mediators. Nonetheless, recent years have seen an increase in the number of GPCR known to be activated by endogenous metabolites. Many of these endogenous metabolites are substrates or intermediate products of energy metabolism.

As GPCR are ideal drug targets, receptors for endogenous metabolites have significant potential as targets for new pharmacotherapeutic strategies [1]. Synthetic ligands have been developed for most of these receptors, and clinical trials have started in some cases.

GPR91 (G protein-coupled receptor 91) is a member of the membrane purinergic P2Y receptor family. GPR91 has seven transmembrane regions and consists of 330 amino acids with an extracellular N terminus and an intracellular C terminus. In the new terminology based on identified endogenous ligands, this receptor is known as SUCNR1.

The endogenous ligand of SUCNR1 is succinate. The plasma succinate concentration measured in rodents varies from 6 to 20 μM, while human serum and plasma levels range from 2 – 3 to 2 – 20 μM respectively. The urinary succinate concentration in mice in physiological conditions is about 20 – 30 μM. Considering the $EC_{50}$ values for succinate in humans and mice (56 ± 8 and 28 ± 5 μM respectively), the levels of this endogenous ligand in plasma and urine in physiological conditions are too low to activate the receptor [4, 5]. However, as values in physiological conditions are only about two times below the $EC_{50}$, significant increases in plasma or urine succinate concentrations are needed for full activation of SUCNR1 [4].

Succinate-containing formulations generally produce transient increases in blood succinate levels, with rapid distribution to the tissues. Thus, studies of the pharmacokinetics of the N-methylglucamine salt of succinic acid (reamberin) showed that i.v. administration of a dose of 5 mg/kg was fol-

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lowed by a peak drug level (expressed as succinate) 1 min after administration, with a rapid decrease to 9 – 10 μg/ml. At 40 min post-administration, the blood succinate concentration returned to values close to baseline (1 – 6 μg/ml), such that dropwise intravenous infusion is required [6].

Testing of about 200 compounds, including those structurally similar to tricarboxylic acid cycle substrates, showed that apart from succinate, only malate and methylmalonate interacted with the receptor, though with activity levels 5 – 10 times below that of succinate [4].

Accumulation of extracellular succinate is primarily associated with leakage from mitochondria, which occurs in severe grades of cell damage, hypoxia, free-radical processes, mitochondrial dysfunction, and uncoupling of oxidative phosphorylation. Succinate accumulates extracellularly in ischemia, toxemia, and hyperglycemia, such that levels in chronic exposure to these pathological states can be greater than the level required for receptor sensitivity. As a result, SUCNR1 receptors can be regarded as cell sensors for stress-induced damage and hypoxia [7, 8].

Modulation of SUCNR1 activity via changes in succinate concentrations has been suggested as a means of controlling the secretion of metabolic hormones or regulating the metabolic activity of particular cells. Thus, in essence, the action of succinate can to some extent be termed hormone-like (in addition to its functions as an energy generation substrate) [9, 10].

The mechanisms of activation of SUCNR1 is linked to at least two signal pathways - $G_{i/o}$ and $G_q$ - depending on the tissue in which the receptors are located. The interaction of succinate with SUCNR1 leads to increases in calcium and inositol-3-phosphate (IP) levels and inhibition of cyclic adenosine monophosphate (cAMP) formation. On the other hand, succinate stimulates cAMP formation in cardiomyocytes and platelets, and also promotes calcium accumulation. Like other GPCR, constant stimulation of SUCNR1 leads to receptor internalization or desensitization [11]. Studies in cell cultures (HEK293 cells) have also shown that stimulation with succinate can lead to internalization of receptor into endosomes/lysosomes. At the same time, transient receptor desensitization in renal MDCK (Madin-Darby canine kidney) cells was seen, but was not followed by any significant receptor internalization [4].

SUCNR1 is expressed in white fatty tissue, liver, heart, retinal neurons, intestine, spleen, and immune system cells, including dendritic cells [5, 10]. The locations of succinate receptors are shown in Table 1.

Studies of SUCNR1-positive adipocytes from white fat have shown that succinate inhibits lipolysis and prevents fatty acid release. Considering the increase in the succinate level seen in rodents in models of diabetes mellitus and metabolic syndrome, succinate has been suggested to limit lipolysis in states in which glucose and free fatty acid molecules are present in excess [10, 11, 15].

**TABLE 1.** Locations of SUCNR1 Receptors and the Effects of their Activation

| Tissue/organ | Cells | Effect | References |
|---|---|---|---|
| Kidney | HEK293 (Human embryo kidney 293) | $Ca^{2+}$ accumulation, activation of ERK1/2 (extracellular signal-regulated kinases), PGE2 (prostaglandin E2) | [2, 4, 12, 13] |
| Kidney | Vascular endothelium, GEnC (glomerular endothelial cells) | $Ca^{2+}$ accumulation, NO- and PGE2-mediated renin release | [10, 2, 5, 12, 14, 3, 13] |
| Kidney | Macula densa cells, MMDD1 (macula densa-like cell line) | $Ca^{2+}$ accumulation, activation of ERK1/2 (extracellular signal-regulated kinases), PGE2 (prostaglandin E2) | [10, 11, 2, 5, 12, 14, 3, 15] |
| Kidney | Main collecting tubule cells, MDCK (Madin-Darby canine kidney) | $Ca^{2+}$ accumulation, activation of ERK1/2 (extracellular signal-regulated kinases), PGE2 (prostaglandin E2) | [2, 5, 14, 3] |
| Liver | Hepatic stellate cells | Increased α smooth muscle actin expression | [4, 10, 16] |
| Heart | Cardiomyocytes | Activation of protein kinase A, $Ca^{2+}$ accumulation, hypertrophy | [17, 10, 15, 18] |
| Brain, blood | $CD4^+$ precursor cells, megakaryocytes, erythroid precursor cells | Increased IP, activation of ERK1/2 (extracellular signal-regulated kinases), proliferation, antiapoptotic effect | [4, 10] |
| Blood | Platelets | Enhanced platelet aggregation via cAMP and IP | [4, 10, 19, 20] |
| Blood | Immature dendritic cells | $Ca^{2+}$ accumulation, chemotaxis, increased cytokine production | [4, 21, 22] |
| Retina | Retinal ganglion cells | VEGF (vascular endothelial growth factor) secretion | [4, 10, 23, 24, 25, 26, 15] |
| White fatty tissue | Adipocytes | Inhibition of lipolysis | [10, 19, 12] |
| Brain | Cortical neurons | Expression of major proangiogenic factors, regulation of NMDA (N-methyl-D-aspartate) receptor activity | [27, 23, 28, 29, 30] |
| Brain | Astrocytes | Changes in $Ca^{2+}$ levels | [27] |

In the liver, SUCNR1 is expressed exclusively in resting hepatic stellate cells (HCS), activation of which leads to rapid decreases in SUCNR1 expression. SUCNR1 has been suggested to serve as an early detector of hepatic stress or damage. The action of ischemia in a liver perfusion model leads to a 14-fold increase in the succinate concentration in the perfusate, to about 1 μM. Succinate-treated HSC show an increased level of expression of a myofibroblast marker (smooth muscle α actin), indicating that they independently stimulate HSC. Activation of HSC may both restore damaged tissue in the ischemic liver and, on prolonged exposure, promote the development of fibrosis. However, rapid down-regulation of SUCNR1 in conditions of increased HSC activity should limit this process. The signal pathways leading to HSC activation by succinate remain unknown. In contrast to adipocytes and kidney cells, succinate does not increase the cytosol $Ca^{2+}$ concentration in HCS, does not decrease the forskolin-induced cAMP level, and does not increase cAMP levels, suggesting that there is no coupling with $G_q$ or $G_i$ proteins respectively [2, 16].

Although SUCNR1 mRNA was not initially detected in the heart using real-time PCR (RT-PCR) [5], subsequent studies demonstrated that SUCNR1 mRNA and protein were present in fresh cardiomyocyte preparations from ventricles, where it is located in the sarcolemma and T-channel membranes [17]. Succinate increases cardiac output [15].

In acute or chronic myocardial ischemia, succinate activates SUCNR1 receptors to trigger phosphorylation of the extracellular domain of signal-regulated kinase (ERK1/2), elevation of intracellular calcium and cAMP contents, expression of calcium-calmodulin-dependent protein kinase IIδ (CaMKIIδ), and translocation of histone deacetylase 5 (HDAC5) into the cytoplasm, this being an intracellular signal triggering the processes of myocardial hypertrophy [17, 18]. This effect is linked with the signal function of nuclear protein kinases PI3k/Akt. An important discovery was that prolonged incubation of cardiomyocytes with high (10 mM) succinate concentrations induces apoptosis. SUCNR1 receptors were suggested to have a regulatory role in this process in the heart in ischemia and hypoxia [15].

SUCNR1 is expressed in hematopoietic precursor cells in several blood and immune cell types. In hematopoietic precursor cells, succinate-activated SUCNR1 receptors induce cell proliferation and prevent cell apoptosis, which in mouse models of chemotherapy-induced myelosuppression lead to increases in hemoglobin, platelet, and neutrophil levels. This effect may be useful in patients receiving antitumor chemotherapy and during recovery from this treatment [2]. In platelets, the action of succinate on receptors leads to platelet aggregation via a decrease in the activity of cAMP-dependent pathways and activation of the phosphoinositol-3-β-kinase pathway and the resulting increase in the activity of IIb/IIIa receptors, which can occur in atherothrombosis, when the local succinate concentration increases because of local hypoxia [20].

SUCNR1 is not present in monocytes or T- or B-lymphocytes, but is present in immature dendritic cells (DC). It has been suggested that SUCNR1 expression is induced when monocytes develop into immature DC, though maturation is associated with significant decreases in receptor density [22]. In immature DC, succinate produces dose-dependent stimulation of cell migration, thus mediating chemotaxis. In addition, synergistic release of tumor necrosis factor α and interleukin-1-β occurs via SUCNR1 and Toll-like receptor. In mice given tetanus toxin, the lymph nodes accumulate higher levels of mature DC than mice lacking SUCNR1 receptors ($SUCNR1^{-/-}$ mice). Skin transplants obtained from $SUCNR1^{-/-}$ mice had lower levels of post-transplant rejection, suggesting potential for the use of SUCNR1 receptor antagonists in organ transplant patients [22].

In the retina, SUCNR1 expression occurs mainly in retinal ganglionic cells and has an important role in vascularization. It has been suggested that the SUCNR1-regulated increase in vessel growth occurs as a result of the secretion of vascular endothelial growth factor (VEGF) and is significantly activated in diabetes mellitus and retinal ischemia [4]. Quite recent studies demonstrated SUCNR1 expression in the pigmented epithelium of the apical membrane of the retina and showed that deficit of this receptor might be among the pathogenetic mechanisms underlying the development of age-related macular dystrophy [25].

In the kidneys, SUCNR1 is located in the efferent arterioles and the vascular network of the glomeruli. In addition, SUCNR1 is detected in cell membranes in the cortical layer of the ascending part of the loop of Henle, macula densa, and the cortical and medullary layers of the collecting tubules [3, 10, 15]. Succinate is a regulator of renin release from the juxtaglomerular apparatus (JGA) via SUCNR1 in the cell membranes of the macula densa, with an involvement in the functioning of the renin-angiotensin-aldosterone cascade [3, 12 – 14, 31]. Microperfusion studies combined with vital visualization of isolated glomeruli showed that perfusion with succinate-containing buffer induces renin release from JGA cells and induces vasodilation of afferent arterioles [25]. Stimulation of SUCNR1 receptors in the kidneys leads to triggering of the mechanism of intracellular calcium mobilization, phosphorylation of extracellularly regulated kinase (ERK) 1/2, and activation of the arachidonic acid cascade with formation of prostacyclin and prostaglandin PGE2. Activation of renal receptors by succinate increased phosphate and glucose reabsorption and stimulated gluconeogenesis. SUCNR1-mediated release of renin from the JGA is mediated by nitric oxide and PGE2, with transactivation of prostaglandin EP2 and/or EP4 receptors on JGA granular cells. Experimental studies have demonstrated that administration of exogenous succinate can increase arterial pressure, which is normalized by angiotensin receptor blockers [13, 32].

The locations and functions of SUCNR1 receptors in the CNS have received insufficient study. Receptors have been detected in neurons in the mouse cerebral cortex and, in

smaller quantities, in astrocytes [23, 27, 28, 30, 33]. Succinate has been suggested to operate as a central trigger controlling the release of proangiogenic factors, for example after neonatal hypoxia/ischemia, allowing infarct size to be limited. Intraventricular administration of succinate in mice leads to expression of one of the main proangiogenic factors, VEGF, with a peak at 24 h [27]. There are simultaneous increases in the formation of angiopoietins 1 and 2 (Ang1, Ang2) and angiogenic mediators of inflammation – interleukins 1β and 6 [24]. It has also been established that this angiogenic gene regulation depends on succinate-induced PGE2 production, acting via specific prostaglandin EP4 receptors [27]. Studies in a model of hypoxic/ischemic encephalopathy in animals have shown that activation of PGE2 EP4 receptors induces short- and long-term cerebroprotection, via increased perfusion of both the ipsi- and contralateral hemispheres. These dynamics of changes in blood flow provide evidence that there is no "robbing" phenomenon and that blood flow may be redistributed towards the injured hemisphere [30].

In $SUCNR1^{-/-}$ mice, there was no increase in vessel density 96 h after hypoxia/ischemia, and the ischemic zone was three times larger than that in controls. Intraventricular administration of succinate at a concentration equivalent to that seen in brain tissues in hypoxia/ischemia decreased the sizes of the penumbral zone and the main infarct by about 50% 96 h after hypoxia/ischemia [23, 28, 34].

Apart from SUCNR1, the CNS (astrocytes in the rat forebrain and the human nucleus accumbens) was proposed to contain a special subtype of succinate receptor, which recognizes not only succinate, but also γ-hydroxybutyrate. By analogy with SUCNR1, it was named SUCBR1 [33, 35 – 38]. Activation of the proposed receptor in the brain by its agonists induced repeating changes in $Ca^{2+}$ levels in astrocytes independently of neuronal signaling [33, 35].

Overall, these data provide evidence that succinate, acting via specific receptors, has a role in energy and revascularization processes in the recovery of the brain after hypoxia/ischemia.

In addition, succinate receptors in the CNS disinhibit NMDA-mediated mechanisms of behavior and convulsive activity. Thus, intraventricular administration of high doses of succinate (7.5 – 0.8 μmol) induces dose-dependent convulsive behavior in mice. Combined administration with dizocilpine – an antagonist of excitatory amino acid receptors – completely prevented succinate-induced convulsions, suggesting a role for NMDA receptors in the convulsive activity of succinate [29].

Lack of the enzyme succinate semialdehyde dehydrogenase in the CNS, leading to accumulation of γ-hydroxybutyrate (and, hence, γ-hydroxybutyric acid), and succinate semialdehyde with a parallel decrease in the succinate level in children can lead to delayed acquisition of motor and language skills, convulsive seizures, mental developmental delay, sleep disorders, ataxia, muscle hypotonia, and behavioral disorders [39].

Recent studies have shown that succinate (and also fumarate) can induce the synthesis of anti-inflammatory proteins, stress-adapting hormones, and expression of the gonadotropin-releasing hormone (GnRH) gene, and can also suppress feeding behavior [11].

Thus, considering the important regulatory roles played by succinate in lipid metabolism, blood cell and vessel formation, the regulation of blood pressure and the cardiovascular system, and immune responses, there is potential for seeking new pharmacological approaches to succinate-mediated regulation. There is significant interest in finding agonists and antagonists of SUCNR1 receptors as potential substances for the pharmacotherapy of hypoxic disorders, renal hypertension, diabetic lesions, metabolic syndrome, autoimmune diseases, etc. Better understanding of the mechanisms controlling and regulating metabolic functions in health and pathology will allow the development of fundamentally new pharmacological strategies for the prevention and treatment of these disorders.

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