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Targeting solute carriers to modulate receptor–ligand interactions

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Solute carrier transporters (SLCs) limit receptor activation via uptake of extracellular ligands. Novel concepts are emerging that describe the modulation of intracellular and plasma membrane receptors by ligand influx and efflux via SLCs, respectively. Here, we evaluate recent insights and provide an outlook for developing potential therapeutic strategies.

Solute carrier transporters

SLCs comprise a large superfamily of over 450 proteins with heterogeneous functions, structures, and expression patterns. As such, the array of physiological roles that are fulfilled by SLCs make this protein class elusive, illustrated by the high publication asymmetry and relatively low number of drug discovery efforts compared with other protein superfamilies [1]. Nevertheless, historically, there has been a handful of SLCs that are therapeutically relevant, including the monoamine transporters as targets for a range of antidepressants. In essence, these transport proteins facilitate the removal (i.e., uptake) of the endogenous neurotransmitter (e.g., dopamine, norepinephrine, or serotonin) from the target compartments, which contain cell surface receptors, such as G protein-coupled receptors (GPCRs) and ligand-gated ion channels. Pharmacological inhibition of monoamine transporters modulates the ligand availability for the target receptor, thereby indirectly altering secondary signaling events that contribute to a therapeutic outcome. While these have become trivial concepts in the understanding of cell signaling and drug action, the number of SLCs that modulate ligand availability is not limited to the status quo of the few established SLC drug targets. Over the past few years, light has been shed on novel physiological mechanisms through which SLCs mediate ligand access to receptors localized at the plasma membrane and intracellular compartments (Figure 1). Here, we use five recent examples of SLC–GPCR pairings to discuss potential therapeutic implications that lie ahead.

Modulation of ligand availability at the plasma membrane

Most GPCRs are expressed at the plasma membrane, where they are receptive to extracellular ligands that, upon binding to the receptor, result in distinct secondary messenger responses. In the traditional dogma, ligand transporters (e.g., neurotransmitter transporters) act to 'limit' receptor activation via substrate influx (Figure 1A), whereas some transporters are now recognized to 'permit' receptor activation by ligand efflux, which adds another layer of signaling regulation by SLCs.

Succinate

During ischemia/reperfusion injury, the tricarboxylic acid cycle intermediate succinate is oxidized in the mitochondria to form reactive oxygen species, driving the injury. At the same time, a significant portion of succinate was found to be effluxed into the circulation via the proton-coupled monocarboxylate transporter 1 (MCT1, SLC16A1) [2]. Subsequent activation of the succinate receptor (SUCNR1) on immune cells by excreted succinate induced proinflammatory responses that exacerbated the reperfusion injury (Figure 1B), although the exact contributions of SUCNR1 to this process remain a subject of investigation. Notably, MCT1 inhibition appeared to reduce infarct size in mice, likely due to limited SUCNR1 activation [2], which implicates MCT1 as an important mediator of reperfusion injury. Whether targeting succinate transport is an attractive therapeutic venue remains to be seen, although recently renewed interest in succinate as a signaling metabolite could provide resolution in the near future [3].

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P) is a potent polar signaling lipid that invokes prosurvival effects via activation of S1P receptors (S1PRs) in an autocrine or paracrine manner (Figure 1C). Upon intracellular biosynthesis, S1P is transported out of the cell mainly via spinster homolog 2 (SPNS2, SLC63A2) in lymphatic endothelial cells and through major facilitator superfamily domain-containing protein 2 (MFSD2A/B, SLC59A1/2) in vascular endothelial cells. Increasing evidence suggests that SPNS2 has a regulatory role in metastasis, lymphocyte trafficking, and angiogenesis [4]. Mice studies indicated that the absence of SPNS2 reduces metastatic burden, likely as a result of reduced S1PR engagement, which suggests SPNS2 as a target to combat metastasis after surgical tumor resection. Our understanding of SPNS2 involvement in disease would be aided by the development of selective inhibitors of the protein, of which none are publicly available, denoting a gap in current progress.

Modulation of ligand availability to intracellular receptors

An increasing number of GPCRs have been found to localize preferably or exclusively to membranes of intracellular compartments, such as the endoplasmic reticulum, Golgi, or nucleus [5]. Moreover, it is suggested that spatially distinct localizations of the same GPCRs contribute to distinct signaling responses, which may contribute in unexpected ways to disease development [5]. As such, the question arises how these intracellular receptors gain access to their

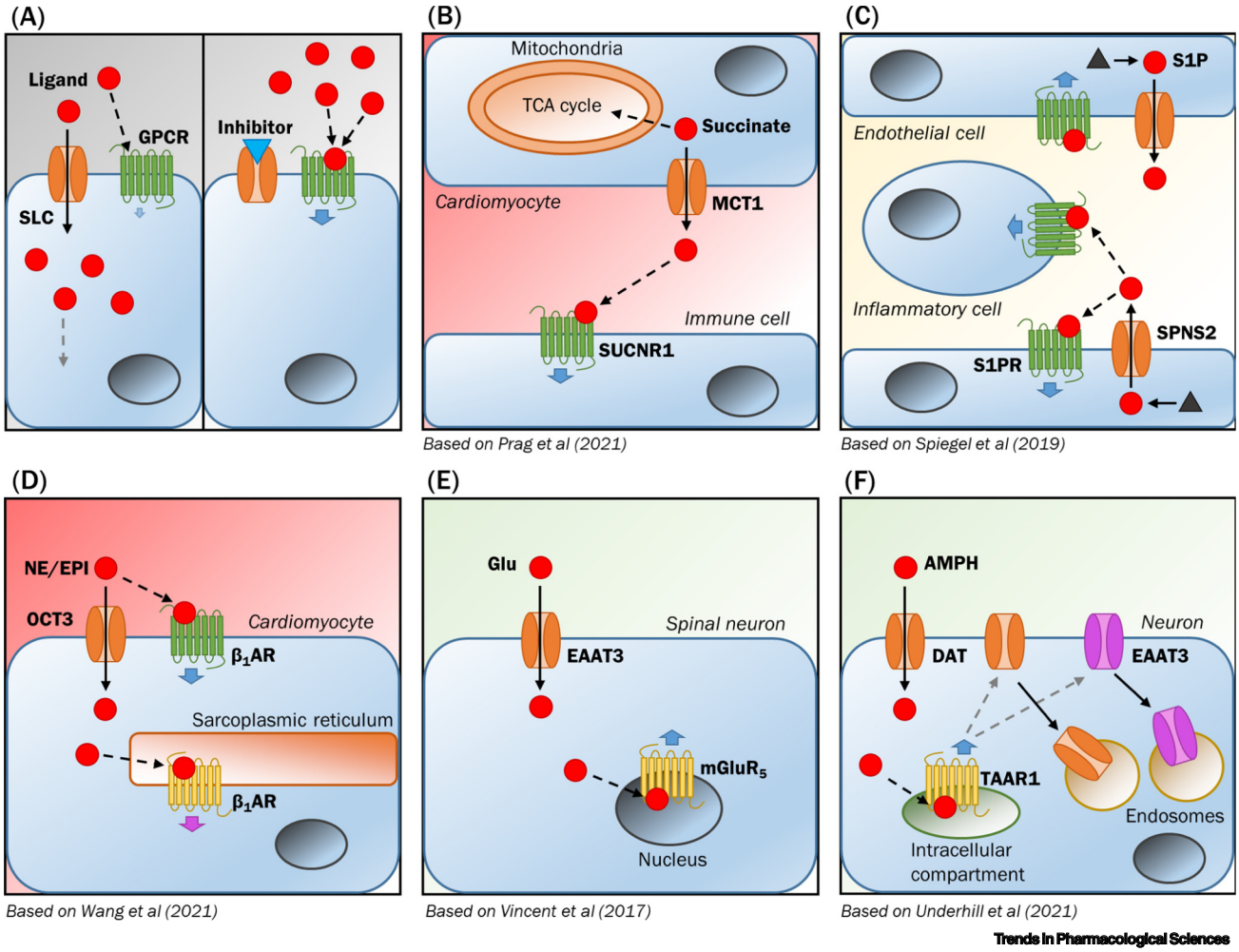

Figure 1. Mechanisms of transporter-mediated modulation of receptor–ligand interactions. (A) Traditional dogma of a solute carrier transporter (SLC) that limits activation of a G protein-coupled receptor (GPCR) on the plasma membrane through ligand influx (left panel). SLC inhibition elevates the extracellular ligand concentration, enhancing GPCR activation (right panel). (B) Succinate is oxidized in the mitochondria and effluxed by monocarboxylate transporter 1 (MCT1), after which it is able to activate the succinate receptor (SUCNR1) on immune cells. (C) Sphingosine-1-phosphate (S1P) is synthesized in endothelial cells and effluxed into the (lymphatic) circulation via spinster homolog 2 (SPNS2). S1P activates S1P receptors (S1PRs) on circulating immune cells or endothelial cells. (D) Norepinephrine (NE) and epinephrine (EPI) are transported into cardiomyocytes via organic cation transporter 3 (OCT3), where both ligands activate beta-1 adrenergic receptors (β_1ARs) in the sarcoplasmic reticulum. Intracellular responses are distinct from β_1AR on the plasma membrane. (E) Glutamate enters neuronal cells via excitatory amino acid transporter 3 (EAAT3), where it activates metabotropic glutamate receptor 5 ($mGluR_5$) on the nuclear membrane. (F) Amphetamine (AMPH) is transported into the cell via the dopamine transporter (DAT), where it activates the trace amine-associated receptor 1 (TAAR1), which leads to internalization of DAT and EAAT3. See [2,4,6,8,9]. Abbreviation: TCA, tricarboxylic acid.

cognate ligands, with possible answers being via on-demand synthesis, passive diffusion, or ligand influx via SLCs.

(Nor)epinephrine

Adrenergic signaling is mainly concerted on the plasma membrane via alpha- and beta-adrenergic receptors, although intracellular localization of these receptors has been reported. It was recently found that organic cation transporter 3 (OCT3, SLC22A3) is required for the influx of (nor)epinephrine to activate beta-1 adrenergic receptor (β_1AR) at the sarcoplasmic reticulum (SR) of cardiomyocytes (Figure 1D) [6]. Knock-out or inhibition of OCT3 in mice blunted β_1AR-mediated cardiac function, indicating that OCT3 is an essential component of (nor)epinephrine-induced myocardial contractility. Thus, regulation of catecholamine uptake could be a therapeutic strategy for cardiovascular conditions.

Glutamate

Glutamate transporters rapidly bind, and eventually take up, glutamate upon release in the synaptic cleft, thereby buffering the

extracellular glutamate concentrations and shaping the activation kinetics of synaptic glutamate receptors [7]. Recently, the uptake of glutamate via excitatory amino acid transporter 3 (EAAT3/SLC1A1) in neuronal cells of the spinal cord was found to be crucial for activation of metabotropic glutamate receptor 5 ($mGluR_5$) on the nuclear membrane (Figure 1E). In an inflammatory pain model in rats, elevated intracellular levels of glutamate were linked to $mGluR_5$-mediated pain responses. Indeed, selective inhibition of neuronal EAAT3, but not glial EAAT1 or EAAT2, produced an analgesic effect in rats. This suggests a substantial contribution of intracellular $mGluR_5$ to pain development, which defies the notion that only glutamate receptors at the plasma membrane are involved. As such, inhibition of neuronal EAAT or intracellular $mGluR_5$ is among the future treatment possibilities for pain disorders [8].

Amphetamines

The interplay between SLCs and GPCRs extends beyond the modulation of ligand availability, because receptor activation can, by itself, affect transporter function and localization, indirectly influencing GPCR activation. For example, trace amine-associated receptor 1 (TAAR1) is expressed on intracellular compartments of monoaminergic neurons, and its activation by trace amines and monoamines depends on transporter-mediated influx. Amphetamines, which are used in the treatment of attention-deficit hyperactivity disorder, enter the cell via the dopamine transporter (DAT/SLC6A3) and activate TAAR1. This leads to endocytosis of DAT and glutamate transporter EAAT3 and subsequent potentiation of excitatory responses in dopaminergic neurons by facilitating cognate receptor–ligand interactions (Figure 1F) [9]. This identifies TAAR1 as a critical component of psychostimulant action and underlines the intricacy of transporter function and regulation in monoaminergic disorders.

Concluding remarks and future perspectives

The relationship between SLCs and other membrane proteins, such as GPCRs, is becoming increasingly appreciated, as illustrated by the examples in this article and by the development of *in vitro* techniques that use receptors as 'tools' to study transporter function [10]. While the main focus here is on the translocation of receptor ligands, many SLCs (EAAT3, DAT, and MCT1 in this article) harness the electrochemical gradient of ions to facilitate transport and thereby alter the levels of these ions in the cytosol and the vicinity of the membrane. Several ions, most prominently Na^+, act as allosteric modulators of many GPCRs via distinct and conserved binding sites [11], which would imply that ion-coupled SLCs beyond the examples in this article can act as indirect receptor modulators.

A note of caution is warranted when SLCs, such as those described in this article, are to be considered as therapeutic targets. Given that substrates may engage with other proteins or serve as metabolic intermediates, preventing their translocation could disrupt key cellular processes. If such secondary effects are detrimental and cannot be mitigated despite the specificity of the intervention, it might be more beneficial to target specific downstream proteins. In line with this, modulation of polyspecific transporters (e.g., MCT1 also transports lactate) might affect disease-unrelated pathways vital for other substrates. Moreover, ubiquitous expression of an SLC in various tissues could compromise the selectivity of the treatment. As such, the cell-specific expression and localization of both the SLC and GPCR should be evident to ensure selective targeting and prevent adverse effects.

The cases that we discuss here highlight the efforts made to connect pairs of previously characterized SLCs and GPCRs with known substrate specificities and affinities. However, based on current knowledge, we estimate that at least 100 unique SLC–GPCR pairs are conceivable. Nevertheless, ~30% of SLCs and 15% of GPCRs have an orphan status, meaning that their function and substrate(s)/ligand(s) are unknown [12,13]. Ongoing efforts to deorphanize these proteins could unveil novel SLC–GPCR pairs, which could spark novel hypotheses with physiological and therapeutic implications [13]. International collaborations and consortia, such as RESOLUTE [14], which aim at deorphanization, reagent generation, and function elucidation of all SLCs, substantiate the expected relevance of transporters in physiology and disease and contribute to the overall progress of putting forward SLCs as potential drug targets.

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Declaration of interests

None declared by authors.

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References

1. César-Razquin, A. *et al.* (2015) A call for systematic research on solute carriers. *Cell* 162, 478–487
2. Prag, H.A. *et al.* (2021) Mechanism of succinate efflux upon reperfusion of the ischaemic heart. *Cardiovasc. Res.* 117, 1188–1201
3. Fernández-Veledo, S. *et al.* (2021) Rethinking succinate: an unexpected hormone-like metabolite in energy homeostasis. *Trends Endocrinol. Metab.* 32, 680–692

4. Spiegel, S. *et al.* (2019) New insights into functions of the sphingosine-1-phosphate transporter SPNS2. *J. Lipid Res.* 60, 484–489
5. Jong, Y.J.I. *et al.* (2018) GPCR signalling from within the cell. *Br. J. Pharmacol.* 175, 4026–4035
6. Wang, Y. *et al.* (2021) Intracellular β1-adrenergic receptors and organic cation transporter 3 mediate phospholamban phosphorylation to enhance cardiac contractility. *Circ. Res.* 128, 246–261
7. Rose, C.R. *et al.* (2018) Astroglial glutamate signaling and uptake in the hippocampus. *Front. Mol. Neurosci.* 10, 1–20
8. Vincent, K. *et al.* (2017) Spinal intracellular metabotropic glutamate receptor 5 (mGluR5) contributes to pain and c-fos expression in a rat model of inflammatory pain. *Pain* 158, 705–716
9. Underhill, S.M. *et al.* (2021) Amphetamines signal through intracellular TAAR1 receptors coupled to Gα13 and GαS in discrete subcellular domains. *Mol. Psychiatry* 26, 1208–1223
10. Sijben, H.J. *et al.* (2021) A study of the dopamine transporter using the TRACT assay, a novel in vitro tool for solute carrier drug discovery. *Sci. Rep.* 11, 1312
11. Zarzycka, B. *et al.* (2019) Harnessing ion-binding sites for GPCR pharmacology. *Pharmacol. Rev.* 71, 571–595
12. Alexander, S.P.H. *et al.* (2019) The concise guide to pharmacology 2019/20: G protein-coupled receptors. *Br. J. Pharmacol.* 176, S21–S141
13. Meixner, E. *et al.* (2020) A substrate-based ontology for human solute carriers. *Mol. Syst. Biol.* 16, 1–9
14. Superti-Furga, G. *et al.* (2020) The RESOLUTE consortium: unlocking SLC transporters for drug discovery. *Nat. Rev. Drug Discov.* 19, 429–430