Organic Cation Transporters in Psychiatric Disorders

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed medications for psychiatric disorders, yet they leave the majority of patients without full symptom relief. Therefore, a major research challenge is to identify novel targets for the improved treatment of these disorders. SSRIs act by blocking the serotonin transporter (SERT), the high-affinity, low-capacity, uptake-1 transporter for serotonin. Other classes of antidepressant work by blocking the norepinephrine or dopamine transporters (NET and DAT), the high-affinity, low-capacity uptake-1 transporters for norepinephrine and dopamine, or by blocking combinations of SERT, NET, and DAT. It has been proposed that uptake-2 transporters, which include organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), undermine the therapeutic utility of uptake-1 acting antidepressants. Uptake-2 transporters

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for monoamines have low affinity for these neurotransmitters, but a high capacity to transport them. Thus, activity of these transporters may limit the increase of extracellular monoamines thought to be essential for ultimate therapeutic benefit. Here preclinical evidence supporting a role for OCT2, OCT3, and PMAT in behaviors relevant to psychiatric disorders is presented. Importantly, preclinical evidence revealing these transporters as targets for the development of novel therapeutics for psychiatric disorders is discussed.

Keywords

Antidepressant · Anxiety · Autism spectrum disorder · Depression · Dopamine · Dopamine transporter · Norepinephrine · Norepinephrine transporter · Organic cation transporter · Plasma membrane monoamine transporter · Psychiatric disorder · Serotonin · Serotonin transporter

1 Introduction

A major research challenge is to identify and validate targets for the development of therapeutics with improved clinical effectiveness for the treatment of numerous psychiatric disorders, including depression. Globally, major depressive disorder is one of the most burdensome of psychiatric disorders, afflicting more than 300 million people and contributing to reduced worker productivity and unemployment (Donohue and Pincus 2007, World Health Organization 2017). Prevalence of depression and other psychiatric disorders, including post-traumatic stress disorder, obsessive compulsive disorder, generalized anxiety disorder, to name a few, is compounded by the relative lack of therapeutic benefit provided to patients by current medications, primary among these the commonly prescribed selective serotonin (5-HT) reuptake inhibitor (SSRI) class of antidepressant. Although reports vary, it is estimated that major depression is unsuccessfully treated in more than half of patients, underlining the urgent need to identify new targets for antidepressant medications. Moreover, in patients who do show a therapeutic response, significant depression can often persist or re-emerge, and relapse rates are much higher in these patients (Tranter et al. 2002; Baghai et al. 2006; Ruhé et al. 2006; Burcusa and Lacono 2007). In addition, antidepressant drugs tend to become less efficacious at alleviating depression over the course of prolonged treatment (Byrne and Rothschild 1998). What could account for these therapeutic shortcomings? We, and others, provide evidence that the answer may lie, at least in part, in the previously unknown role of organic cation transporters (OCTs), including three subtypes of organic cation transporters (OCT1, OCT2, and OCT3) and the plasma membrane monoamine transporter (PMAT) in central monoaminergic neurotransmission (for overview, see chapter "General Overview of Organic Cation Transporters in the Brain" in this volume, Koepsell 2021).

OCTs and PMAT are capable of low-affinity, but high-capacity uptake of biogenic amines, including 5-HT, norepinephrine (NE), and dopamine (DA). These have been coined "uptake-2" transporters, a term that can be applied to any

transporter with low affinity, but high capacity, to take up a particular substrate. The majority of currently prescribed antidepressant medications act by inhibiting one or more of the high-affinity ("uptake-1"), low-capacity transporters for these biogenic amines, the 5-HT transporter (SERT/5-HTT), the NE transporter (NET), and the DA transporter (DAT). It is the increase in extracellular levels of one or more of these neurotransmitters that is thought to initiate down-stream events needed for therapeutic benefit. Thus, the presence of low-affinity, but high-capacity OCTs/PMAT for 5-HT, NE, and DA in brain may prevent these neurotransmitters rising sufficiently to trigger the events required for optimal therapeutic benefit (Daws 2009; Daws et al. 2013). Because SSRIs, which act to block 5-HT uptake via the SERT, are currently the most commonly prescribed class of antidepressant, the remainder of the introduction focuses on 5-HT, but applies to NE and DA as well.

1.1 Role of Serotonin in Depression

Low extracellular levels of 5-HT have been traditionally associated with etiological underpinnings of depression and related psychiatric disorders, largely because SSRIs work by blocking SERT and presumably, elevating extracellular 5-HT. However, this dogma has come into question due to notable paradoxes. Perhaps, the best example is the finding that humans carrying a relatively common polymorphism of the SERT gene, the short (s) allele of the 5-HTT linked polymorphic region (5-HTTLPR), are more prone to depression than those without this polymorphism, particularly when exposed to stress (e.g., Caspi et al. 2003; Wilhelm et al. 2006; Cervilla et al. 2007; Caspi et al. 2010). Individuals harboring the s allele have less SERT mRNA and SERT binding, as well as reduced uptake of 5-HT into platelets and lymphocytes (Lesch et al. 1996; Greenberg et al. 1999; Daws and Gould 2011). It follows then that these individuals would have higher levels of extracellular 5-HT than those without the s allele. This idea is supported by reports that mice constitutively lacking SERT (knockout), or with reduced SERT expression (SERT heterozygous mice, which have half as many SERTs as wild-type mice, and serve as a useful murine model for human carriers of the s allele), have 9- and fivefold greater levels of extracellular 5-HT, respectively, than wild-type mice (Mathews et al. 2004; Shen et al. 2004). The expectation then is that humans carrying the s allele should have elevated extracellular 5-HT and ergo, be resistant to depression and related disorders, but clinical reports indicate this is not the case (e.g., Caspi et al. 2003, 2010; Wilhelm et al. 2006; Cervilla et al. 2007). Examples such as this led us to propose that it is the magnitude of the increment in extracellular 5-HT that follows the start of treatment that is important for therapeutic benefit, and not what basal or sustained levels of 5-HT may be (see Daws 2009, Daws et al. 2013).

Of course, the cause and treatment of depression, and related psychiatric disorders, is vastly more complex than simple changes in extracellular 5-HT, but what does seem apparent is that the 5-HT system is a crucial player. It might be argued that perhaps the glutamatergic system is more important, given the success of ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, in

producing rapid and long-lasting antidepressant effect, particularly in treatment resistant depression (Mathew et al. 2012; Mathews et al. 2012; Monteggia and Zarate 2015). However, it is important to recognize that ketamine also elevates extracellular 5-HT, and in recent studies from our lab, this appears to be due, at least in part, to ketamine's ability to inhibit SERT- and PMAT-dependent 5-HT clearance (Bowman et al. 2020b). Overall, it seems that dysregulation of 5-HT neurotransmission is fundamental to depression and related psychiatric disorders, regardless of the many other mechanisms at play. Importantly, it seems that increasing extracellular 5-HT is important for therapeutic effect. The following sections will discuss mechanisms controlling 5-HT and NE uptake in brain, how they may play into the therapeutic efficacy of currently available medications, and how OCTs and PMAT could be novel targets for the treatment of depression and related psychiatric disorders.

1.2 Uptake-2 Transporters

Credit must be given to the pioneers who first discovered a second, low-affinity, high-capacity uptake system for monoamines, including Bertler et al. (1964); Burgen and Iversen (1965); Lichtensteiger et al. (1967); Fuxe and Ungerstedt (1967); Shaskan and Snyder (1970); Butler et al. (1988). It is interesting, however, that their seminal observations did not become fully realized until relatively recently. A likely reason is that at that time, we recognized monoamine specific receptors and transporters to have nanomolar affinities for their respective endogenous neurotransmitter. Pioneering studies, which first revealed uptake-2 transporter mechanisms, used concentrations of substrate well in excess of nanomolar concentrations, with uptake-2 mechanisms only becoming apparent at concentrations in the micromolar range. This may explain the gap in uptake-2 research concerning clearance of extracellular monoamines, which, with the exception of one study (Butler et al. 1988), spanned approximately 30 years. It is likely that micromolar extracellular concentrations of monoamines were considered supraphysiological, and therefore, uptake-2 transporters would not play a role in monoamine clearance under normal physiological conditions (see Daws 2009). It wasn't until the late 1990s that synaptic concentrations of monoamines were realized to be in the millimolar range (Clements 1996; Bunin and Wightman 1998; Cragg and Rice 2004), meaning that the extrasynaptically located transporters for monoamines (both uptake-1 and uptake-2) would be exposed to micromolar concentrations of neurotransmitter diffusing from synapses. Research probing the role of uptake-2 transporters in central monoamine neurotransmission was rekindled soon thereafter.

Some of the first findings included work by Schmitt et al. (2003) who found that mRNA for OCT3 is increased in brains of SERT knockout mice, suggesting that OCT3 may compensate for constitutive loss of SERT. Baganz et al. (2008) went on to confirm and extend this finding, showing that OCT3, but not OCT1, mRNA and protein, was increased in hippocampus of SERT heterozygous and knockout mice compared with SERT wild-type mice. These investigators did not examine OCT2 or

PMAT expression in SERT mutant mice. At that time, OCT2 expression in hippocampus was reportedly very low (Gorboulev et al. 1997; Gründemann et al. 1997), and PMAT had only recently been identified as a transporter for monoamines (Engel et al. 2004). We now know that OCT2 and PMAT are expressed in limbic brain regions, including hippocampus (Amphoux et al. 2006; Bacq et al. 2012, Couroussé et al. 2015; Miura et al. 2017; and see chapter "Organic Cation Transporter Expression and Function in the CNS" in this volume, Sweet 2021), thus, it cannot be ruled out that OCT2 and/or PMAT might also be upregulated in SERT deficient mice. Regardless, a key implication for findings such as these is that human carriers of low expressing SERT gene variants may have increased expression and/or function of uptake-2 transporters to compensate. In turn, upregulation of such transporters could account for the relative lack of therapeutic efficacy of SSRIs in this population. By clearing 5-HT in the presence of SSRIs, uptake-2 transporters may dampen or prevent SSRIs from providing therapeutic benefit (Daws 2009; Daws et al. 2013; Horton et al. 2013).

In 2005, Feng and co-workers first investigated the effect of an OCT/PMAT blocker, decynium-22, on 5-HT levels in brain. They reported that perfusion of decynium via a dialysis probe in the medial hypothalamus of rats produced robust and dose-dependent increases in dialysate 5-HT. The magnitude of this effect (~200–650% increase, depending on dose) was remarkably similar to that reported for equivalent doses of the SSRI, fluoxetine, delivered by the same route to the hypothalamus (~400% increase in extracellular 5-HT) (Maswood et al. 1999). These data suggest that even when 5-HT levels are "normal" (i.e., not elevated due to genetic or pharmacological inactivation of SERT), blockade of uptake-2 transporters elevates extracellular 5-HT to levels that may be sufficient for antidepressant-like effects and/or to augment the antidepressant effect of SSRIs.

Leading the way in this line of thinking, Schildkraut and Mooney (2004) proposed the extraneuronal monoamine transporter (uptake-2) hypothesis as a strategy to reduce the time to onset of therapeutic action of antidepressants that act to increase NE. A consistent literature finding is that drugs that block NET lead to a gradual increase in normetanephrine, a metabolite of NE. Normetanephrine is a potent inhibitor of uptake-2 (Burgen and Iversen 1965; Männistö and Kaakkola 1999). Schildkraut and Mooney therefore posited that drugs that increase levels of normetanephrine or block uptake-2, given together with NET blockers, such as the tricyclic antidepressant, desipramine (DMI), would hasten the onset of therapeutic benefit. This hypothesis has not yet been tested in humans, however has garnered support from preclinical studies as discussed in subsequent sections.

Details of OCT1, OCT2, OCT3, and PMAT expression in brain and substrate affinity for monoamines and various psychoactive drugs are discussed throughout this volume of the Handbook of Experimental Pharmacology. The reader is referred to the following chapters in this volume for specific details: chapter "General Overview of Organic Cation Transporters in the Brain" (Koepsell 2021), chapter "Organic Cation Transporter Expression and Function in the CNS" (Sweet 2021), chapter "Genetic and Epigenetic Regulation of Organic Cation Transporters" (Kölz et al. 2021), chapter "Substrates and Inhibitors of Organic Cation Transporters and

Plasma Membrane Monoamine Transporter and Therapeutic Implications" (Bönisch 2021), chapter "The Interaction of Organic Cation Transporters 1-3 and PMAT with Psychoactive Substances" (Maier et al. 2021), and chapter "Brain Plasma Membrane Monoamine Transporter in Health and Disease" (Vieira and Wang 2021), so will not be discussed in depth here. Suffice to say that all are capable of monoamine transport in the micromolar to millimolar range, contingent upon species and substrate, with OCT2, OCT3, and PMAT being most densely expressed limbic brain regions important in controlling mood.

1.3 Organic Cation Transporter 1 (*SLC22A1*)

Although OCT1 is expressed in brain (Baganz et al. 2008; Koepsell 2020; see chapter "General Overview of Organic Cation Transporters in the Brain" (Koepsell 2021) and chapter "Organic Cation Transporter Expression and Function in the CNS" (Sweet 2021) in this volume), it is relatively scant compared to OCT2, OCT3, and PMAT. To date, OCT1 has not been identified in neurons, but mRNA for OCT1 has been detected in astrocytes (Inazu et al. 2005). In contrast, OCT1 is richly expressed in liver and kidney where it serves to transport a variety of endogenous cations, toxins, and drugs (Koepsell 2020). Constitutive OCT1 KO mice are viable, fertile, and normal in a range of physiological measures (Jonker et al. 2001), suggesting that other OCTs with overlapping expression can compensate for the loss of OCT1. Behavior of OCT1 knockout mice has yet to be characterized, making conclusions about possible involvement of OCT1 in emotion-relevant behavior difficult. However, given its relatively low expression in brain compared with other uptake-2 transporters, it seems unlikely to be a key driver of monoamine homeostasis.

1.4 Organic Cation Transporter 2 (*SLC22A2*)

OCT2 is widely expressed in brain, including limbic regions such as amygdala, cerebral cortex, hippocampus, and striatum (Amphoux et al. 2006; Bacq et al. 2012; Couroussé et al. 2015; Miura et al. 2017; and see chapter "Organic Cation Transporter Expression and Function in the CNS", Table 1 (Sweet 2021) in this volume) as well as kidney and other peripheral organs (see Koepsell 2020). OCT2 mRNA and protein have been found in neurons (Busch et al. 1998; Bacq et al. 2012, Couroussé et al. 2015), astrocytes (Inazu et al. 2005), and microglia (He et al. 2017).

Work from the Gautron group provides evidence for a role of OCT2 in depression and stress-related disorders. Their first approach was to make use of constitutive OCT2 knockout mice. Like OCT1 knockout mice, OCT2 knockouts are viable, fertile and have no apparent morphological abnormalities (Jonker et al. 2003). In keeping with a reduced ability to recapture released 5-HT and NE from the extracellular milieu, they found that tissue levels of these monoamines were decreased in several brain regions of OCT2 knockout mice, including hippocampus and striatum,

suggesting that OCT2 plays a role in 5-HT and NE clearance. Interestingly however, they found no difference in basal clearance of these monoamines between genotypes. It should be noted, however, that 50% recovery time (RT_{50}) of 5-HT- and NE-induced suppression of firing activity of CA3 pyramidal neurons in vivo was used as an indirect measure of clearance of these monoamines. Thus, it is possible that basal differences in clearance of these monoamines between genotypes could be detected via more direct methods (e.g., fast scan cyclic voltammetry, chronoamperometry). They did, however, reveal a role for OCT2 in 5-HT and NE clearance when mice were administered venlafaxine, a dual SERT and NET inhibitor. In the presence of venlafaxine, RT_{50} was significantly prolonged in OCT2 knockout compared to OCT2 wild-type mice, suggesting that OCT2 is important for the recovery of firing activity of CA3 neurons after stimulation by microiontophoretically applied 5-HT or NE.

Behaviorally, they found that male and female OCT2 knockout mice were less anxious than their wild-type counterpart in three different conflict paradigms (open field, elevated O-maze, and novelty suppressed feeding) (Bacq et al. 2012). OCT2 knockout mice also spent less time immobile in the forced swim test and tail suspension test, behavioral assays used to evaluate antidepressant-like activity of drugs. These findings suggest that OCT2 knockout mice show less behavioral despair compared with wild-type mice. Together, the behavioral phenotype of OCT2 knockout mice is consistent with a role for this transporter in emotion-relevant behaviors.

Investigations of the antidepressant-like response to acute administration of antidepressants in the forced swim test revealed that doses of venlafaxine (SERT/NET blocker) and reboxetine (NET blocker) that were inactive in wild-type mice produced antidepressant-like effects in OCT2 knockout mice (Bacq et al. 2012). These findings are consistent with OCT2 being important in clearance of NE and 5-HT, which may contribute to the relative lack of therapeutic benefit in patients taking these classes of antidepressants. Interestingly, OCT2 knockout mice showed a blunted antidepressant-like response to the SSRI citalopram compared with wild-type mice. Bacq et al. (2012) found no evidence that citalopram directly interacts with OCT2, discounting this as a possible explanation for the lack of antidepressant-like effect of citalopram in OCT2 knockout mice. Together these results suggest that perhaps OCT2 is more important for NE than 5-HT neurotransmission, though OCT2 knockout mice were found to have reduced 5-HT1A receptor density in a number of limbic brain regions, consistent with a role for OCT2 in serotonergic transmission (Bacq et al. 2012).

Bacq et al. (2012) went on to examine the long-term effects of venlafaxine in a corticosterone-induced (7 weeks, 35 μg/ml in drinking water) model of depression in male mice. This treatment led to a similar increase in circulating corticosterone between genotypes, as well as comparable manifestation of a depressed-like state (reduced sucrose consumption, time spent in open zone in elevated O-maze, grooming frequency after splash test and poor coat state). Three weeks of venlafaxine treatment reversed all of these corticosterone-induced behaviors in wild-type mice, but not in OCT2 knockout mice, suggesting that OCT2 is necessary

for the antidepressant-like effects of venlafaxine in this model. These intriguing findings are difficult to interpret given certain unknowns. For example, corticosterone reportedly interacts with OCT2 (Gründemann et al. 1998; Hayer-Zillgen et al. 2002; and see chapter "General Overview of Organic Cation Transporters in the Brain" of this volume, Koepsell 2021), so it is possible that results are, in part, due to loss of corticosterone's action at OCT2. It is also unknown if venlafaxine has any direct actions at OCT2, as has been reported for other antidepressants including doxepin (Hendrickx et al. 2013) and imipramine (Kido et al. 2011; Belzer et al. 2013; Hendrickx et al. 2013), which are also NET and SERT inhibitors.

Couroussé et al. (2015) examined a role for OCT2 in vulnerability to stress. After establishing that OCT2 is expressed in stress-related circuits in brain, they found that basal corticosterone levels were 87% greater in constitutive OCT2 knockout mice, compared with wild-type counterparts. When exposed to a 15 min swim stress, corticosterone levels in OCT2 knockout mice rose 56% beyond that of wild-type controls, which they showed was attributable to absence of OCT2 in brain versus adrenal glands (Couroussé et al. 2015). Based on these findings they tested the hypothesis that the increased stress response of OCT2 knockout mice confers increased vulnerability to repeated stressful conditions. Supporting this hypothesis they found that following 8 weeks of unpredictable chronic mild stress (UCMS) OCT2 knockout mice showed time-dependent (i.e., contingent upon how many weeks of UCMS) exaggerated depressive-like behaviors relative to wild-type controls including more severe coat deterioration, greater spatial memory deficits, decreased social interaction and nest building. Interestingly, swim stress-induced corticosterone release in OCT2 knockout mice following UCMS was less than following a single acute exposure to swim stress, whereas the corticosterone response in wild-type mice was similar in magnitude in both cases. This suggests that OCT2 knockout mice adapt to chronic stress, whereas OCT2 wild-type mice do not, at least in terms of corticosterone release. In this study, glycogen synthase kinase-3β (GSK3β) signaling, which is implicated in mood regulation (Kaidanovich-Beilin et al. 2004; O'Brien et al. 2004; Polter et al. 2010), was found to be aberrant in OCT2 knockout mice, although how this plays into the exacerbated stress response in these mice remains to be elucidated (Couroussé et al. 2015).

Using RT_{50} for 5-HT- and NE-induced suppression of CA3 pyramidal neuron firing in vivo as a proxy for clearance of these monoamines, Couroussé et al. (2015) found that corticosterone prolonged RT_{50} following microiontophoretically applied 5-HT or NE in both wild-type and OCT2 knockout mice. However, the magnitude of effect was greater in wild-type mice, consistent with the ability of corticosterone to prolong RT_{50} being in part OCT2-dependent. Moreover, corticosterone enhanced the ability of venlafaxine to increase RT_{50} in wild-type mice, but not in OCT2 knockout mice, again consistent with this action of corticosterone being OCT2-dependent. Interestingly, in wild-type mice enhancement of the venlafaxine-induced increase of RT_{50} was more robust following NE than 5-HT, again suggesting that OCT2 may be more important in modulating NE than 5-HT neurotransmission.

A major impediment to studies investigating OCTs and PMAT in central monoaminergic neurotransmission is a lack of ligands selective for each of the OCT

subtypes and PMAT. Decynium-22, the most potent blocker of these transporters, inhibits them all with similar IC50 values (see chapter "General Overview of Organic Cation Transporters in the Brain" in this volume, Koepsell 2021). Though decynium-22 has been a useful pharmacological tool, particularly when used in combination with knockout mice, selective ligands are much needed. To this end, using 3D homology modeling, Orrico-Sanchez et al. (2020) designed a putatively selective OCT2 blocker, H2-cyanome, with good brain penetrance. Currently it is unknown if H2-cyanome has activity at OCT1, OCT3, or PMAT. Regardless, daily injections of H2-cyanome to male mice were able to reverse depressed-like behaviors induced by 7 weeks of chronic corticosterone administration, including restoring sucrose preference, reducing anxiety, and improving social interaction, cognition and coat condition (Orrico-Sanchez et al. 2020). Moreover, these effects were similar in magnitude to the SSRI fluoxetine. Onset of reversal of depressed-like behavior was faster for some behaviors following H2-cyanome than fluoxetine. Together, these data suggest that blockade of OCT2, and putatively other OCTs or PMAT, is a promising strategy for improving treatment for depression and related psychiatric disorders.

As discussed in chapter "Genetic and Epigenetic Regulation of Organic Cation Transporters" of this volume (Kölz et al. 2021), gene variants of OCT2 exist (e.g., Bergen et al. 2014) however have not been extensively studied for their involvement in psychiatric disorders and their treatment. A recent study found that a gene variant of OCT2 (SLC22A2 808 C to A polymorphism) correlated with psychiatric symptoms in a cohort of human immunodeficiency virus (HIV) positive patients (Borghetti et al. 2019), encouraging further studies investigating the relation between OCT2 gene variants, psychiatric disorders and their treatment.

1.5 Organic Cation Transporter 3 (*SLC22A3*)

Like OCT2, OCT3 is widely expressed in brain, including limbic regions such as amygdala, cerebral cortex, hippocampus, and striatum (Wu et al. 1998; Amphoux et al. 2006; Baganz et al. 2008; Vialou et al. 2008; Gasser et al. 2009; Marcinkiewcz and Devine 2015; Miura et al. 2017; and see Table 1 in chapter "Organic Cation Transporter Expression and Function in the CNS" of this volume, Sweet 2021) as well as skeletal muscle, salivary gland, adrenal gland and a number of peripheral organs including heart and liver (see Koepsell 2020). OCT3 mRNA and protein have been found in neurons (see Table 2 in chapter "Organic Cation Transporter Expression and Function in the CNS" of this volume, Sweet 2021; Schmitt et al. 2003; Vialou et al. 2004, 2008; Cui et al. 2009; Gasser et al. 2009, 2017; André et al. 2012; Hill and Gasser 2013; Mayer et al. 2018), astrocytes (Inazu et al. 2003, 2005; Cui et al. 2009; Yoshikawa et al. 2013; Gasser et al. 2017), and ependymal cells (Vialou et al. 2004, 2008).

Work from numerous groups supports a role for OCT3 in psychiatric disorders and their treatment. Kitaichi et al. (2005) found that knocking down OCT3 in mouse brain by approximately 30% using antisense (0.25 μg/0.25 μl/h, continuous infusion

into third ventricle for 7 days) produced a robust antidepressant-like effect in the forced swim test, indexed by an approximately 75% decrease in immobility time compared to control mice that received scrambled antisense. A lower titer of antisense (0.075 μg/0.25 μl/h) was ineffective in reducing immobility time in the forced swim test, as was a low dose of the tricyclic antidepressant, imipramine (4 mg/kg), a blocker of NET and SERT. However, when given in combination, these treatments decreased immobility time by approximately 50%. Together, these data are consistent with a role for OCT3 in antidepressant-like behavior in mice.

Constitutive OCT3 knockout mice were first created by Zwart et al. (2001), and like OCT1 and OCT2 knockout mice, are viable, fertile and show no apparent anatomical abnormalities. Consistent with a reduced ability to recapture released monoamines, OCT3 knockout mice have brain region specific decreases in tissue levels of monoamines and their metabolites, including cortex and striatum (Vialou et al. 2008). However, studies to date provide no evidence for slower clearance of 5-HT (Horton et al. 2013) or DA (Mayer et al. 2018) in OCT3 knockout mice, relative to their wild-type counterpart, although DA clearance trended to be slower in OCT3 knockout mice. This is perhaps not surprising, since the concentration of 5-HT and DA being cleared was in a range consistent with that expected to engage mostly SERT and DAT, and not uptake-2 (Daws 2009). For example, using in vivo high-speed chronoamperometry, Baganz et al. (2008) found that clearance of 5-HT from hippocampus was SSRI-sensitive (SERT-sensitive) and decynium-22-insensitive (OCT/PMAT-insensitive) when 5-HT signal amplitudes were approximately 0.5 micromolar, but was SSRI-insensitive and decynium-22-sensitive when 5-HT signal amplitudes were approximately 2.5 micromolar. These findings are consistent with earlier work using synaptosomal preparations to show [^{3}H]5-HT uptake was SSRI-sensitive when the concentration of 5-HT ranged 0.01–0.5 micromolar, and SSRI-insensitive when the range was 0.1–2.0 micromolar (Butler et al. 1988). More recently, elegant studies using rotating disk voltammetry to measure 5-HT uptake into synaptosomes showed that SERT is the dominant transport mechanism only at relatively low 5-HT concentrations (less than 100 nM), with low-affinity 5-HT transporters playing the major role at higher concentrations (Hagen et al. 2011). It will be important in future studies to assess kinetics of monoamine clearance over a greater range of concentrations in OCT3 knockout mice, as well as OCT2 and PMAT deficient mice. For example, in vivo, SERT knockout mice clear 5-HT more slowly than wild-type mice at low (~ 0.5 micromolar) concentrations, but clear 5-HT as effectively as wild-type mice at higher concentrations, presumably due to clearance at these higher concentrations being driven predominantly by low-affinity, but high-capacity uptake-2 transporters (Daws and Toney 2007). Thus, the expectation might be for the opposite to occur in OCT3 knockout mice.

It was not until 2008 that emotion-linked behaviors were first studied in OCT3 knockout mice, where subtle increases in anxiety-like behavior were noted relative to control mice. In the open field test, OCT3 knockout mice spent modestly less time in the center than wild-type counterparts (Vialou et al. 2008), though overall locomotor activity in the open field did not differ between wild-type and OCT3 knockout mice (Vialou et al. 2008; Mayer et al. 2018). However, consistent with an

anxious phenotype, OCT3 knockout mice displayed less locomotor activity in the Y-maze, although time spent in each arm did not differ between genotypes (Vialou et al. 2008). In contrast, Wultsch et al. (2009) found OCT3 knockout mice to be less anxious. Using the prototypical test for anxiety, the elevated plus maze, they found OCT3 knockout mice spent significantly more time in the open arms than either wild-type or heterozygous counterparts. In the open field test, OCT3 knockout and heterozygous mice spent significantly more time in the center. While it is difficult to reconcile these different findings, possibilities include potential sex differences. Wultsch et al. (2009) used adult male mice. Likewise, Vialou et al. (2008) used adult mice, but sex of mice used is unclear. Since it was specified that male mice were used for immunohistochemistry studies in the Vialou et al. (2008) publication, and sex was not reported as a biological variable, it is reasonable to assume that adult male mice were used in all studies reported. Other possible reasons for these discrepant results include differences in the open field apparatus (white Plexiglass, 100 x 100 x 30 cm, Vialou et al. (2008) vs opaque gray plastic 82 x 82 x 25 cm, Wultsch et al. 2009), or even the sex of the experimenter, which has been reported to impact outcomes in behavioral tests in rodents (Sorge et al. 2014; Georgiou et al. 2018). Male OCT3 knockout mice were found to engage in less social interaction (Garbarino et al. 2019b), perhaps consistent with a more anxious phenotype. No differences between male OCT3 genotypes in memory performance, using the Morris water maze, and in aggressive behavior, using the resident intruder test (Wultsch et al. 2009) or in the tail suspension test (Horton et al. 2013) were reported. Thus, it appears that constitutive OCT3 knockout in male mice confers deficits in some emotion-relevant behaviors (though findings are inconsistent), but not others. Currently there are no data on emotion-relevant behaviors in female OCT3 knockout mice.

As mentioned previously, decynium-22 has been widely used to study the role of OCTs and PMAT in mood- and stress-related behaviors. It has high affinity for OCTs and PMAT, but lacks activity at DAT, NET, and SERT (Fraser-Spears et al. 2019). Decynium-22 at an intraperitoneal dose of 0.32 mg/kg or less does not impact behavior of wild-type mice in the tail suspension test, and at doses of 0.1 mg/kg or lower does not impact locomotor activity (Horton et al. 2013; Krause-Heuer et al. 2017). Decynium-22 at 1.0 mg/kg greatly suppresses locomotor activity (Krause-Heuer et al. 2017). Horton et al. (2013) found that decynium-22 (0.1 mg/kg) given with a sub-effective dose of the SSRI fluvoxamine (10 mg/kg) produced almost maximal antidepressant-like effects in the tail suspension test, with male C57BL/6 mice spending essentially no time immobile. Using high-speed chronoamperometry to measure 5-HT clearance from hippocampus in vivo in real time, they found the ability of fluvoxamine, decynium-22, and their combination to produce antidepressant-like effects in the tail suspension test positively correlated with their ability to inhibit 5-HT clearance. The greater the inhibition of 5-HT clearance, the greater the antidepressant-like response. In efforts to understand the mechanistic underpinnings, they turned to constitutive OCT3 knockout mice. Strikingly, they found the ability of decynium-22 (0.1 mg/kg) to enhance the antidepressant-like effect of the SSRI fluvoxamine was lost in OCT3 knockout mice, pointing to OCT3

as the major player in this action of decynium-22 (Horton et al. 2013). Interestingly, however, they found that the combination of fluvoxamine (10 mg/kg) and a higher dose of decynium-22 (0.32 mg/kg) did elicit an antidepressant-like response in OCT3 knockout mice, suggesting that other decynium-22-sensitive transporters, putatively OCT2 (see preceding section) or PMAT (following section) are involved in the antidepressant-like actions of higher doses of decynium-22. Moreover, Horton et al. (2013) found that the ability of decynium-22 to enhance the 5-HT clearance inhibiting effect of fluvoxamine in hippocampus persisted in OCT3 knockout mice. This finding suggests that inhibition of 5-HT clearance in hippocampus is not related to the antidepressant-like actions of drugs in the tail suspension test and also suggests that in this brain region, decynium-22 is acting at other of its targets, putatively OCT2 or PMAT, to enhance the ability of fluvoxamine to inhibit 5-HT clearance (Horton et al. 2013). In other studies, decynium-22 delivered to the medial hypothalamus produced robust, dose-dependent increases in dialysate 5-HT (Feng et al. 2005), equivalent to those produced by the SSRI fluoxetine (Maswood et al. 1999). Together, these studies underscore the potential therapeutic utility of targeting decynium-22-sensitive transporters to enhance 5-HT neurotransmission in the treatment depression and related disorders.

Regarding modulation of NE in antidepressant response, Schildkraut and Mooney (2004) proposed that the several weeks' delay to therapeutic onset of action following treatment with tricyclic antidepressants and other NET blocking antidepressants such as selective NET inhibitors and dual SERT/NET blockers could be attributed to uptake-2 transporters preventing extracellular NE increasing to levels needed for therapeutic benefit. In keeping with this idea, normetanephrine, a metabolite of NE and potent OCT3 blocker, accumulates after treatment with tricyclic antidepressants in a process that takes 2–6 weeks (Mooney et al. 2008). It was proposed that therapeutic benefit would emerge once normetanephrine levels had accumulated sufficiently to block OCT3, with NET blocking antidepressants on board. Studies in rodents support this idea. For example, Rahman et al. (2008) showed that normetanephrine enhanced the venlafaxine (NET/SERT blocker)-induced increase in extracellular NE in frontal cortex of rats and potentiated the antidepressant-like effect of desipramine in the tail suspension test in mice. Consistent with these findings, Bowman et al. (2020a) showed that decynium-22 enhanced the ability of desipramine to inhibit NE clearance in the dentate gyrus, as well as its antidepressant-like effects in the tail suspension test. In contrast to findings of Rahman et al. (2008), Bowman et al. (2020a) did not find decynium-22 to potentiate the neurochemical or behavioral actions of venlafaxine, nor could they recapitulate the finding that normetanephrine potentiated the antidepressant-like effect of desipramine. Use of different mouse strains and assay conditions may account for the different findings between the two studies. Regardless, taken together, these data support a role for OCT3, and putatively other decynium-22-sensitive transporters, in limiting the antidepressant actions of NET acting drugs.

As a corticosterone-sensitive transporter (e.g., chapter "Organic Cation Transporters and Nongenomic Glucocorticoid Actions" of this volume, Benton et al. 2021; Gasser et al. 2006; Gründemann et al. 1998; Hayer-Zillgen et al. 2002) OCT3, not surprisingly, has been the focus of numerous studies examining its role in

stress-related behaviors. It is well known that activation of the hypothalamic-pituitary-adrenal (HPA) axis increases extracellular 5-HT. Several studies provide support for corticosterone-induced inhibition of OCT3 as a prominent mechanism underlying stress-induced increases in 5-HT (Feng et al. 2009, 2010; Baganz et al. 2010; Hassell et al. 2019). Baganz and co-workers found that OCT3 protein expression was decreased in hippocampus of male mice after repeated swim stress (14 days), which led to modest, but persistent elevations in plasma corticosterone relative to non-stressed control mice. Interestingly, mice exposed to repeated swim spent less time immobile in the tail suspension test than control counterparts. This finding is consistent with literature showing that, dependent upon dose and duration of treatment, exogenously applied corticosterone can reduce immobility time (Stone and Lin 2008; Zhao et al. 2009). Similarly, environmental enrichment (another activator of the HPA axis), which evokes increases in plasma corticosterone in mice similar to those reported in the Baganz et al. (2010) study, also decreased immobility time in the tail suspension test (Xu et al. 2009). In the Xu et al. (2009) and Baganz et al. (2010) studies, outcomes corresponding with increased plasma corticosterone were lost in adrenalectomized mice, showing these outcomes to be dependent on corticosterone. Elevated endogenous corticosterone has also been dose-dependently associated with antidepressant- and anxiolytic-like effects in rats and C57BL/6J mice (Swiergiel et al. 2008; Mozhui et al. 2010). Although it is clear that corticosterone is not suitable for the treatment of depression and related disorders due to its actions at glucocorticoid and mineralocorticoid receptors and prodepressant outcomes under numerous conditions (for review, see McEwen 2008), studies such as these do add to a growing literature supporting selective blockade of corticosterone-sensitive uptake-2 transporters as viable targets for the development of novel therapeutics for the treatment of depression and related disorders, which lack unwanted off-target effects.

Further support for this contention comes from studies evaluating effects of acute and chronic stress in stress vulnerable Wistar-Kyoto rats and more stress resilient Long-Evans rats (Marcinkiewcz and Devine 2015). Hippocampal OCT3 mRNA expression was modulated as a function of stressor and rat strain. In naïve Long-Evans rats, OCT3 mRNA was upregulated after 2 h of restraint stress, but not in Long-Evans rats that had been previously exposed to repeated social defeat stress. In contrast, hippocampal OCT3 mRNA was not altered following 2 h of restraint stress in Wistar-Kyoto rats, but was markedly increased in Wistar-Kyoto rats that had been previously exposed to repeated social defeat stress and was accompanied by an increase in cytosolic OCT3 protein (Marcinkiewcz and Devine 2015). These findings provide a platform for understanding strain differences in physiological and behavioral responses to stress. Furthermore, these investigators found that decynium-22 produced antidepressant-like effects in the forced swim test in Wistar-Kyoto rats, but not in Long-Evans rats (Marcinkiewcz and Devine 2015). Given that Wistar-Kyoto rats are resistant to the antidepressant-like effects of SSRIs, these studies point to decynium-22-sensitive transporters, putatively OCT3, as a target for therapeutic intervention in individuals who are not effectively treated by SSRIs.

The work of Marcinkiewcz and Devine (2015) followed studies by Baganz et al. (2008) who showed that genetic deficiencies in SERT heterozygous and knockout mice were associated with increased OCT3 mRNA and protein in hippocampus, as described earlier in this chapter. These investigators found decynium-22 to produce antidepressant-like effects in male SERT heterozygous and knockout mice, but not wild-type counterparts. Given that SERT heterozygous mice provide an excellent murine model for human carriers of the short allele of the 5HTTLPR polymorphism, who express less SERT than non-carriers, it is possible that upregulation of OCT3 (and/or or OCT2 or PMAT, which are yet to be assessed) could account for the relative insensitivity of these individuals to SSRIs.

With growing evidence indicating that OCT3 (and OCT2) are novel targets for the development of more efficacious antidepressant drugs, several studies have assessed existing antidepressant and antipsychotic drugs for activity at these transporters. These studies are covered in detail in chapter "Substrates and Inhibitors of Organic Cation Transporters and Plasma Membrane Monoamine Transporter and Therapeutic Implications" of this volume (Bönisch 2021). Briefly, Bönisch and co-workers (Haenisch and Bönisch 2010; Haenisch et al. 2012) screened a large number of commonly prescribed drugs for the treatment of psychiatric disorders. They found that many inhibited OCT1, OCT2, OCT3 and PMAT, however only at supra therapeutic concentrations (i.e., concentrations that greatly exceeded the upper plasma concentrations of therapeutic doses of these drugs). They found only three of the compounds tested had the potential to interact with OCTs at therapeutic concentrations; bupropion (antidepressant and smoking cessation aid) at OCT2, nefazodone (antidepressant) at OCT3, and clozapine (antipsychotic) at OCT2 and OCT3 (Haenisch et al. 2012). None of the drugs tested had inhibitory actions at PMAT at therapeutically relevant concentrations (Haenisch and Bönisch 2010). Studies by Zhu et al. (2012) and Massmann et al. (2014) indicate potential actions of desipramine, sertraline (Zhu et al. 2012), ketamine, fluoxetine, and diazepam (Massmann et al. 2014) at OCT3. Although inhibition constants were in the micromolar range, and therefore likely beyond therapeutically relevant concentrations, in vitro studies such as these encourage further exploration of activity of these drugs at OCTs in vivo.

Efforts have been made to synthesize OCT3-selective ligands. Hu et al. (2016) synthesized 59 novel guanidine derivatives, seven of which had IC_{50} values to inhibit OCT3-dependent uptake of 1.9 to 24 micromolar. Lyer et al. (2019) characterized 2-amino-6-chloro-3,4-dihydroquinazoline (A6CDQ) and a positional isomer, A7CDQ. Both compounds produced antidepressant-like effects in mice. A6CDQ was originally thought to act as an antagonist at 5-HT3 receptors, but these investigators found that A6CDQ is also a 5-HT releasing agent, via SERT and a NET inhibitor. In contrast, A7CDQ was a weak SERT blocker, but a releaser at NET. Interestingly, both compounds were potent inhibitors of OCT3. These studies suggest that blockade of OCT3 contributes to the multimodal antidepressant-like effects of these compounds (Lyer et al. 2019). In 2017, Krause-Heuer and colleagues synthesized a small library of 7 decynium analogs, with the goal to develop OCT3-selective blockers, which unlike decynium lack activity at alpha-1 adrenoceptors and

produce standalone antidepressant-like effects – (in wild-type mice decynium does not have standalone antidepressant-like effects but augments the antidepressant-like effect of SSRIs (Horton et al. 2013) and NET blockers (Bowman et al. 2020a). Several of the compounds had the desired lower affinity for alpha-1 adrenoceptors and had less impact on locomotor activity than decynium. Importantly, some of the compounds produced standalone antidepressant-like effects in the tail suspension test (Krause-Heuer et al. 2017). Like decynium, all compounds had greater affinity for OCT3 than OCT2 or PMAT; however, none were superior to decynium in terms of distinguishing among OCT subtypes and PMAT (Fraser-Spears et al. 2019). Interestingly, some compounds had modest affinity for SERT and DAT, raising the possibility that their dual OCT/SERT, OCT/DAT blocking properties contribute to their standalone antidepressant-like effects (Fraser-Spears et al. 2019). Studies such as these encourage further development of OCT3-selective ligands, as well as compounds with dual uptake-1/uptake-2 inhibiting properties, which hold promise as improved therapeutics for the treatment of depression and related disorders.

As discussed in chapter "Genetic and Epigenetic Regulation of Organic Cation Transporters" of this volume, gene variants of OCT3 exist (Kölz et al. 2021) however, like OCT2, have not been extensively studied in terms of how they may be involved in psychiatric disorders and their treatment. Sakata et al. (2010) found three single nucleotide polymorphisms (SNPs) in the OCT3 gene (*SLC22A3*) that were associated with reduced uptake capacity of [^{3}H]histamine and [^{3}H]MPP$^+$. These data suggest that such polymorphisms may contribute to psychiatric disorders and their treatment by disrupting normal monoamine homeostasis. Consistent with this assertion, Lazar et al. (2008) found two OCT3 gene variants that were associated with early onset obsessive compulsive disorder. Another study evaluated seven OCT3 SNPs in a relatively small sample of depressed and non-depressed subjects and found no differences in OCT3 allele or genotype frequencies between groups (Hengen et al. 2011). Further studies investigating the relation of OCT3 gene variants and psychiatric disorders are warranted.

2 Plasma Membrane Monoamine Transporter (*SLC29A4*)

As for OCT2 and OCT3, PMAT is widely expressed in brain, including limbic regions such as amygdala, cerebral cortex, hippocampus, and striatum (Engel et al. 2004; Dahlin et al. 2007; Vialou et al. 2007; Miura et al. 2017; and see chapter "Organic Cation Transporter Expression and Function in the CNS", Table 1 (Sweet 2021) and chapter "Brain Plasma Membrane Monoamine Transporter in Health and Disease" (Vieira and Wang 2021) in this volume). PMAT is also expressed in heart, small intestine, pancreas, kidney, skeletal muscle, and liver, but its expression is highest in brain (see Wang 2016). PMAT mRNA and protein are expressed in neurons (Dahlin et al. 2007; Vialou et al. 2007; André et al. 2012), pericytes (Wu et al. 2015), primary brain vascular endothelial cells and microvessels (André et al. 2012, Wu et al. 2015; see chapter "Organic Cation Transporter Expression and

Function in the CNS" (Sweet 2021) and chapter "Brain Plasma Membrane Monoamine Transporter in Health and Disease" (Vieira and Wang 2021) in this volume).

PMAT knockout mice were first developed by Joanne Wang's group (Duan and Wang 2013). These mice are viable, fertile and have no apparent physiological defects. Gilman et al. (2018) examined anxiety-like and active-coping behaviors in PMAT mutant mice and found remarkably subtle consequences of genetic depletion or knockout. There was no effect of genotype on locomotor activity or compulsive/repetitive behaviors, assayed using the marble burying test. Male and female PMAT heterozygous mice displayed greater latency to enter the open arm in the elevated plus maze and tended to spend less time exploring the maze than wild-type or knockout counterparts (Gilman et al. 2018). That this phenotype was not evident in PMAT knockout mice suggests that perhaps compensation in other transporters/systems may occur in response to constitutive PMAT knockout, but not when PMAT is depleted by only 50%. Although mRNA for SERT, NET, DAT, and OCT3 does not differ among PMAT genotypes (Duan and Wang 2013), it is currently unknown if this translates to protein and/or the functional status of these transporters, thus the possibility of compensation in PMAT knockout mice remains. Interestingly, female PMAT knockout mice showed a modest increase in active-coping behavior, as indexed by increased time spent swimming in the forced swim test, suggesting that PMAT may be sex-dependently involved in certain behaviors (Gilman et al. 2018).

A recent study revealed a role for PMAT and SERT in the antidepressant-like effects of ketamine (Bowman et al. 2020b). As expected, ketamine produced robust antidepressant-like effects in wild-type mice in the forced swim test, effects that were lost both in PMAT and SERT knockout mice. Ketamine is well known to increase extracellular 5-HT; however, the mechanism through which this occurs is unclear. Using in vivo high-speed chronoamperometry, Bowman et al. (2020b) showed that ketamine robustly inhibited 5-HT clearance from hippocampus in wild-type mice. Consistent with their behavioral data, this effect was lost in mice lacking either PMAT or SERT. Although additional studies are needed to understand how constitutive loss of either PMAT or SERT is sufficient to void ketamine of its antidepressant-like effects and ability to inhibit 5-HT clearance, studies such as this add to a growing literature supporting PMAT as a target for the development of novel psychotherapeutic drugs.

Gene variants of PMAT have been associated with autism spectrum disorder (ASD) (Adamsen et al. 2014). These rare non-synonymous mutations result in reduced functionality of PMAT, which is hypothesized to disrupt serotonergic homeostasis. This could be particularly impactful during development when aberrant 5-HT signaling can lead to brain abnormalities, possibly related to the etiology of ASD (Garbarino et al. 2019a). Examining the relation between PMAT gene variants and other psychiatric disorders will be an important avenue for future investigations.

3 Conclusions and Future Perspective

This is an exciting time for research into uptake-2 transporters, in particular, OCT2, OCT3, and PMAT. As discussed in this chapter, the avenues for research are rich, with current data supporting a role for these transporters in controlling mood, anxiety, and social behavior. To date, few studies have included mice heterozygous for OCT2, OCT3, and PMAT. These mice will provide valuable murine models to investigate consequences of gene variants conferring reduced activity and/or expression of these transporters. Importantly, there is compelling evidence that these transporters are targets for development of novel therapeutic drugs, either as standalone treatments or as add-ons to currently available medications. Indeed, the important and previously unappreciated roles of these transporters in maintaining monoamine homeostasis make them ideal targets for therapeutic intervention, as well as provide a mechanistic basis for the relatively poor therapeutic outcomes afforded by psychotherapeutic drugs targeting uptake-1 transporters such as SERT, NET, and DAT. Of course, there is still much work to be done, including development of selective ligands for OCT2, OCT3, and PMAT, as well as investigating potential unwanted side effects due to their actions at these transporters located in organs other than the brain. However, preclinical studies to date encourage further study of the therapeutic potential of targeting these transporters for the treatment of psychiatric disorders.

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