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Lynette C. Daws *Editor*

Organic Cation Transporters in the Central Nervous System

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Preface

Organic cation transporters (OCTs) have long been known to play fundamental roles in the transport of nutrients, drugs, toxins, and processing of metabolic waste products, largely through their presence in organs including kidney, liver, intestine, and placenta. In contrast, an important role for OCTs in central nervous system (CNS) function has only been realized in the relatively recent past. OCTs, including OCT2 and OCT3, as well as the more recently discovered plasma membrane monoamine transporter (PMAT), have a low affinity to transport monoamine neurotransmitters (e.g., dopamine, histamine, norepinephrine, and serotonin); however, they have a high capacity to do so. They are also located on neurons and glia in brain regions important for reward, mood, stress, and processing of physiological information, such as osmolality. Thus, these transporters are anatomically located in brain to exert control over monoamine homeostasis, and especially so when extracellular monoamine concentrations are high, as may occur under pathophysiological conditions and/or when high-affinity, low-capacity monoamine transporters (e.g., dopamine, norepinephrine and serotonin transporters, DAT, NET, SERT, respectively) are pharmacologically or genetically inactivated.

Because OCTs and PMAT are low-affinity/high-capacity transporters for monoamines, they have been labeled uptake-2 transporters, whereas high-affinity/low-capacity monoamine transporters (e.g., DAT, NET, SERT) are known as uptake-1 transporters for monoamines. Of note, the concept of uptake-2 transporters has existed since the 1960s (see the chapter "General Overview of Organic Cation Transporters in Brain"); however, their functional identity as OCTs and PMAT in brain only emerged in the 2000s.

Pictured in Figs. 1 and 2 are some of the pioneers of this field. Figure 1 shows Professor Hermann Koepsell with his PhD students (from India, Russia, and Germany) at the University of Würzburg in 2009, as well as a portrait of Professor Koepsell taken the same year. Among Professor Koepsell's many scientific discoveries and accomplishments, he made seminal contributions to the discovery of OCTs as a family of transporters responsible for absorption, excretion, and tissue distribution of many drugs. His pioneering research helped to elucidate the structure and function of these transporters, as well as identify polymorphisms in the genes coding them. In 1994, Professor Koepsell's group was the first to clone and characterize OCT1 (SLC22A1), the first member of the SLC22 transporter family

Fig. 1 Professor Hermann Koepsell, pictured with his PhD students in 2009 at the University of Würzburg, Germany. Photos courtesy of Professor Hermann Koepsell, PhD

Fig. 2 Professor Leslie Iversen (right), visiting the Institute of Pharmacology and Toxicology at the University of Würzburg, June 14, 1978. Professor Iversen made the seminal discovery of "uptake-2" transporters in heart, in 1965. Pictured from left to right, Ullrich Trendelenburg, MD, (supervisor of Heinz Bönisch when he was a PhD student), Karl-Heinz Graefe, MD, Heinz Bönisch, PhD (as a postdoctoral fellow) and Leslie Iversen, PhD. Photo courtesy of Professor Heinz Bönisch, PhD

identified. In the chapter "General Overview of Organic Cation Transporters in Brain" of this volume, Professor Koepsell provides an authoritative overview of OCTs in brain. Figure 2 shows Professor Heinz Bönisch, author of the chapter "Substrates and Inhibitors of OCTs and PMAT and Therapeutic Implications," as a postdoctoral fellow in the laboratory of Professor Ullrich Trendelenburg at the

University of Würzburg in 1978. Professor Bönisch has made important contributions to our understanding of uptake-2 transporters during his highly successful and productive scientific career. His particular focus in this volume is on research investigating substrates and inhibitors of OCTs and PMAT, and their therapeutic implications.

This volume of the *Handbook of Experimental Pharmacology (HEP)* provides the current state of knowledge of the role OCTs and PMAT play in various aspects of CNS function. In addition to the contributions from Professors Koepsell (chapter "General Overview of Organic Cation Transporters in Brain") and Bönisch (chapter "Substrates and Inhibitors of OCTs and PMAT and Therapeutic Implications"), this volume provides a comprehensive overview of the expression and function of OCTs and PMAT in brain (chapter "Organic Cation Transporter Expression and Function in the CNS" by Douglas Sweet), as well as genetic and epigenetic regulation of these transporters and consequences for CNS function (chapter "Genetic and Epigenetic Regulation of Organic Cation Transporters," by Charlotte Költz, Elke Schaeffeler, Mattias Schwab, and Anne Nies). Chapter "Experimental Methods for Investigating Uptake 2 Processes In Vivo," by Anna Marie Buchanan, Brenna Parke, and Parastoo Hashemi, describes experimental approaches to studying uptake-2 processes in brain, including scintillation microspectrophotometry, microdialysis, chronoamperometry, and voltammetry. The role of OCTs and PMAT in brain histamine and catecholamine homeostasis, and implications for regulation of mood and reward are described, respectively, in the chapter "Organic Cation Transporters in Brain Histamine Clearance: Physiological and Psychiatric Implications" (by Fumito Naganuma and Takeo Yoshikawa) and the chapter "Organic Cation Transporters in Brain Catecholamine Homeostasis" (by Paul Gasser). Chapter "The Interaction of Organic Cation Transporters 1-3 and PMAT with Psychoactive Substances," by Julian Maier, Marco Niello, Deborah Rudin, Lynette Daws, and Harald Sitte, brings us up to date with current knowledge of interactions of psychoactive substances, including amphetamine and its congeners, as well as synthetic cathinones, with OCTs and PMAT and behavioral consequences. Indeed, this was the subject that caught the attention of the National Institute on Drug Abuse, ultimately leading to the birth of this HEP volume. Chapter "Organic Cation Transporters in Psychiatric Disorders," by Lynette Daws, provides perspectives on OCTs and PMAT as players in psychiatric disorders and their treatment, with an emphasis on depression. In the chapter "Organic Cation Transporters and Nongenomic Glucocorticoid Action," Kelsey Benton, Christopher Lowry, and Paul Gasser discuss the role of OCTs in the nongenomic actions of glucocorticoids in brain, and implications for stress-related modulation of physiology and behavior. PMAT was discovered and first cloned by Joanne Wang and colleagues. In the chapter "Brain Plasma Membrane Monoamine Transporter in Health and Disease," Letícia Vieira and Joanne Wang provide a comprehensive history of PMAT and bring us up-to-date in the state of knowledge of its role in CNS health and disease. Chapter "Regulation of Neurogenesis by Organic Cation Transporters: Potential Therapeutic Implications," by Takahiro Ishimoto and Yukio Kato, discusses the novel concept and newly emerging data supporting a role for OCTs in neurogenesis

and therapeutic implications. Finally, in the chapter "Organic Cation Transporter (OCT/OCTN) Expression at Brain Barrier Sites: Focus on CNS Drug Delivery," Robert Betterton, Thomas Davis, and Patrick Ronaldson provide a focused review on OCTs located at brain barrier sites and their importance in CNS drug delivery.

In closing, it has been an absolute delight, and honor, to work with all the contributors to this volume of HEP. Given the relatively early stage of research into OCTs and PMAT in CNS function, reaching back less than two decades, this volume is particularly timely. My hope is that the exciting discoveries and future directions described within these chapters will serve as a catalyst for increased research into the important role of OCTs and PMAT in CNS homeostasis, a role that is critical in human CNS health and disease.

San Antonio, TX, USA Lynette C. Daws

Acknowledgments

First and foremost, I thank all the authors who contributed to the thirteen excellent chapters in this volume of the Handbook of Experimental Pharmacology focusing on organic cation transporters in the central nervous system. I express my deepest appreciation for their efforts. I would like to pay special thanks to Professor James E. Barrett, PhD, Editor-in-Chief of HEP, for suggesting this exciting project to me, and to the National Institute on Drug Abuse, for inviting us both to participate in a special workshop on "New Targets for Stimulant Use Disorders." It was at this workshop where the idea for this HEP volume was born. Finally, I thank Alamelu Damodharan, Susanne Dathe and all staff at Springer Nature for their outstanding execution in bringing this volume to life.

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General Overview of Organic Cation Transporters in Brain

Hermann Koepsell

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Abstract

Inhibitors of Na^+/Cl^- dependent high affinity transporters for norepinephrine (NE), serotonin (5-HT), and/or dopamine (DA) represent frequently used drugs for treatment of psychological disorders such as depression, anxiety, obsessive-compulsive disorder, attention deficit hyperactivity disorder, and addiction. These transporters remove NE, 5-HT, and/or DA after neuronal excitation from the interstitial space close to the synapses. Thereby they terminate transmission and modulate neuronal behavioral circuits. Therapeutic failure and undesired central nervous system side effects of these drugs have been partially assigned to neurotransmitter removal by low affinity transport. Cloning and functional characterization of the polyspecific organic cation transporters OCT1 (*SLC22A1*), OCT2 (*SLC22A2*), OCT3 (*SLC22A3*) and the plasma membrane monoamine transporter PMAT (*SLC29A4*) revealed that every single transporter mediates low affinity uptake of NE, 5-HT, and DA. Whereas the organic transporters are all located in the blood brain barrier, OCT2, OCT3, and PMAT are expressed in neurons or in neurons and astrocytes within brain areas that are involved in behavioral regulation. Areas of expression include the dorsal raphe, medullary motoric nuclei, hypothalamic nuclei, and/or the nucleus accumbens. Current knowledge of the transport of monoamine neurotransmitters by the organic cation transporters, their interactions with psychotropic drugs, and their locations in the brain is reported in detail. In addition, animal experiments including behavior tests in wildtype and knockout animals are reported in which the impact of OCT2, OCT3, and/or PMAT on regulation of salt intake, depression, mood control, locomotion, and/or stress effect on addiction is suggested.

Keywords

Antidepressants · Monoamine neurotransmitters · Neurotransmitter reuptake inhibitors · OCT1 · OCT2 · OCT3 · Organic cation transporters · PMAT · Psychotropic drugs

Abbreviations

5-HT	5-Hydroxytryptamine (serotonin)
ADHD	Attention deficit hyperactivity disorder
ASD	Autism spectrum disorders
BBB	Blood–brain barrier
BLA	Basolateral amygdala complex

CP	Choroid plexus
CSF	Cerebrospinal fluid
CSFBB	CSF–blood barrier
DA	Dopamine
DAT	Dopamine transporter
DMH	Dorsomedial hypothalamus
FST	Forced swim test
GABA	γ-Aminobutyric acid
HPA	Hypothalamic-pituitary-adrenal
KO	Knockout
METH	Methamphetamine
MPP	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAC	Nucleus accumbens
NE	Norepinephrine
NET	Norepinephrine transporter
NTS	Nucleus tractus solitarii
OCD	Obsessive-compulsive disorder
OCT	Organic cation transporter
PD	Parkinson's disease
PMAT	Plasma membrane monoamine transporter
RTI-55	3β-(4-iodophenyl)-tropane-2-carboxylic acid
SERT	Serotonin transporter
SN	Substantia nigra
SSRI	Selective serotonin reuptake inhibitor
TEA	Tetraethylammonium
UCMS	Unpredictable chronic mild stress
VMH	Ventromedial hypothalamic nucleus
WT	Wildtype

1 Introduction

Neuronal networks in the brain control complex cerebral functions such as learning, reasoning, mood control, motivation, and motoric coordination. These networks consist of neurons that release specific neurotransmitters upon activation at their synapses that are detected by receptors in postsynaptic membranes. The secreted neurotransmitters are taken up by cognate transporters located extrasynaptically in neuronal terminals where they are packed into intracellular vesicles for future neuronal release. This synaptic, "wiring-type" activation is accompanied by activation of receptors on more distant neurons occurring via neurotransmitters that diffuse out of the synaptic cleft. This activation of remote receptors by "volume transmission" imposes a much higher degree of complexity and regulatory options. In addition to other mechanisms including cerebral effects of hormones, volume transmission activation of neighboring neurons contributes to overarching psycho-

emotional or psycho-behavioral effects, such as increased severity of depression or increased remission in addicts during prolonged stress. Volume transmission mediated activation is shaped by neurotransmitter uptake via local specific and nonspecific neurotransmitter uptake systems.

This present article is an introductory overview of biomedical functions of polyspecific low affinity uptake systems supplementing neurotransmitter reuptake by specific monoamine neurotransmitter transporters. The properties and biomedical impact of high affinity Na^+/Cl^- dependent transporters for noradrenaline/norepinephrine (NE), dopamine (DA), and serotonin (5-HT) are shortly recalled. Thereafter, functional properties, sites of expression, and selectivity of neurotransmitters and psychotropic drugs of the low affinity organic cation transporters OCT1-3 and PMAT that are expressed in brain are compiled. *In vivo* experiments in rodents are listed that shed light on the impact of these transporters during psychiatric disorders. Discussing these data, the limitations of the employed methodological tools are outlined. This overview intends to serve as an introductory guide for the more specialized presentations in this volume. In this overview, detailed data concerning sites of cerebral expression, neurotransmitter substrates, interacting psychotropic drugs, and partially selective inhibitors of organic cation transporters are compiled. It is apparent that the current data are rather fragmentary and that more investigations are warranted to obtain a better understanding of the roles of organic cation transporters in the brain during health and psychiatric disorders.

2 High Affinity Na^+/Cl^- Dependent Monoamine Neurotransmitter Transporters

2.1 Locations in the Brain and Transport Properties

The high affinity monoamine neurotransmitter transporters NET (*SLC6A2*), DAT (*SLC6A3*), and SERT (*SLC6A4*) are expressed in noradrenergic, dopaminergic, and serotoninergic neurons, respectively (Kristensen et al. 2011; Torres et al. 2003). They are essential for synaptic neurotransmission and influence neuronal crosstalk. NET, DAT, and SERT are localized to cell bodies, dendrites, and neurites of their cognate neurons in many brain areas (Table 1). Noradrenergic, dopaminergic, and serotoninergic neurons may lie close together and may be functionally connected (Hoffman et al. 1998; Liprando et al. 2004). In the neurites, the monoamine neurotransmitter transporters are mainly localized in presynaptic plasma membranes close to synapses (Hoffman et al. 1998; Nirenberg et al. 1997a; Pickel and Chan 1999; Tao-Cheng and Zhou 1999; Zhou et al. 1998) (Fig. 1). NET, DAT, and SERT are Na^+/Cl^- cotransporters of NE, DA, and 5-HT that show some overlapping neurotransmitter selectivity (Table 2). For example, NE and DA are transported by human NET (hNET) and human DAT (hDAT) (Carboni et al. 1990; Giros et al. 1994; Moron et al. 2002). The monoamine neurotransmitter transporters exhibit transmitter/Na^+/Cl^- stoichiometries of 1:1:1 (NET), 1:2:1 (DAT), and 1:1:1 (SERT) (Kristensen et al. 2011; Torres et al. 2003). In SERT cotransport of 5-HT with Na^+

Table 1 Brain areas where monoamine neurotransmitter transporters and organic cation transporters have been detected in rats and/or mice

Transporter	Cellular location	Location in the brain
Net (*Slc6a2*)	Noradrenergic neurons	Nuclei of medulla oblongata, raphe nuclei, striatum, locus coeruleus, thalamus, hypothalamus, hippocampus, amygdala, cerebral cortex
Dat (*Slc6a3*)	Dopaminergic neurons	Ventral tegmental area, basal midbrain ganglia, striatum, SN, pallidum, claustrum, medial forebrain bundle, nigrostriatal bundle, lateral habenula, zona incerta, hypothalamic dorsomedial arcuate nucleus, median eminence, amygdala, NAC, olfactory bulb, olfactory tubercle, cingulate cortex, prefrontal cortex
Sert (*Slc6a4*)	Endothelial cells of BBB, serotonergic neurons	BBB, nuclei of medulla oblongata, raphe nuclei, locus coeruleus, tegmental nuclei, cochlear and olivary nuclei, cerebellum, SN, nucleus ruber, pallidum, caudate nucleus, putamen, hypothalamus (dorsomedial nucleus, septal nuclei), olfactory bulb, olfactory nuclei, forebrain cortex, hippocampus, NAC, amygdala
Oct1 (*Slc22a1*)	Endothelial cells of BBB	BBB, CP, hippocampus
Oct2 (*Slc22a2*)	Endothelial cells of BBB, neurons, epithelial cells of CP, ependymal cells	BBB, CP, raphe nuclei, locus coeruleus, cerebellum, thalamus, hypothalamus, median eminence, cerebral cortex, hippocampus, amygdala
Oct3 (*Slc22a3*)	Endothelial cells of BBB, neurons, astrocytes, non-astrocyte glial cells, ependymal cells	BBB, CP, nuclei of medulla oblongata, pontine nuclei, NTS, raphe nuclei, locus coeruleus, tegmentum, cerebellum, striatum, SN, circumventricular organs including the area postrema, subfornical organ, subcommissural organ, pineal gland, thalamus, hypothalamus, lateral septum, olfactory bulb, cerebral cortex, hippocampus, subiculum, NAC, amygdala
Pmat (Slc29a4)	Endothelial cells of BBB, pericytes, astrocytes, neurons	BBB, nuclei of medulla oblongata and mesencephalon, nuclei of rhombencephalon, inferior olivary complex, raphe nuclei, pontine nuclei, cerebellum, striatum, pallidum, SN, nucleus ruber, putamen, septum, thalamus, hypothalamus, olfactory bulb,

(continued)

Table 1 (continued)

Transporter	Cellular location	Location in the brain
		olfactory tubercle, cerebral cortex, hippocampus, amygdala

Net: (Lorang et al. 1994; Pacholczyk et al. 1991; Schroeter et al. 2000; Torres et al. 2003), **Dat**: (Cerruti et al. 1993; Ciliax et al. 1995; Freed et al. 1995; Hersch et al. 1997; Hoffman et al. 1998; Holleran et al. 2020; Lorang et al. 1994; Nirenberg et al. 1996, 1997a, b; Revay et al. 1996; Torres et al. 2003), **Sert**: (Bengel et al. 1998; Brust et al. 2000; Hoffman et al. 1998; Pickel and Chan 1999; Qian et al. 1995; Sur et al. 1996; Tao-Cheng and Zhou 1999; Zhou et al. 1998) (Bengel et al. 1998; Brust et al. 2000; Cerruti et al. 1993; Chang et al. 1996; Hoffman et al. 1998; Pickel and Chan 1999; Qian et al. 1995; Sur et al. 1996; Tao-Cheng and Zhou 1999; Torres et al. 2003; Zhou et al. 1998), **Oct1**: (Baganz et al. 2008; Choudhuri et al. 2003; Duan and Wang 2013; Lin et al. 2010; Sekhar et al. 2019; Wu et al. 2015b),**Oct2**: (Amphoux et al. 2006; Bacq et al. 2012; Choudhuri et al. 2003; Courousse et al. 2014; Lin et al. 2010; Sweet et al. 2001; Wu et al. 2015b)**, Oct3**: (Amphoux et al. 2006; Baganz et al. 2008; Chaves et al. 2020; Cui et al. 2009; Gasser et al. 2006, 2009, 2017; Graf et al. 2013; Haag et al. 2004; Hill and Gasser 2013; Holleran et al. 2020; Mayer et al. 2018; Miura et al. 2017; Nakayama et al. 2007; Schmitt et al. 2003; Sweet et al. 2001; Vialou et al. 2004, 2008; Wu et al. 1998a; Wyler et al. 2015) **Pmat**: (Dahlin et al. 2007, 2009; Sekhar et al. 2019; Vialou et al. 2007; Wu et al. 2015a)

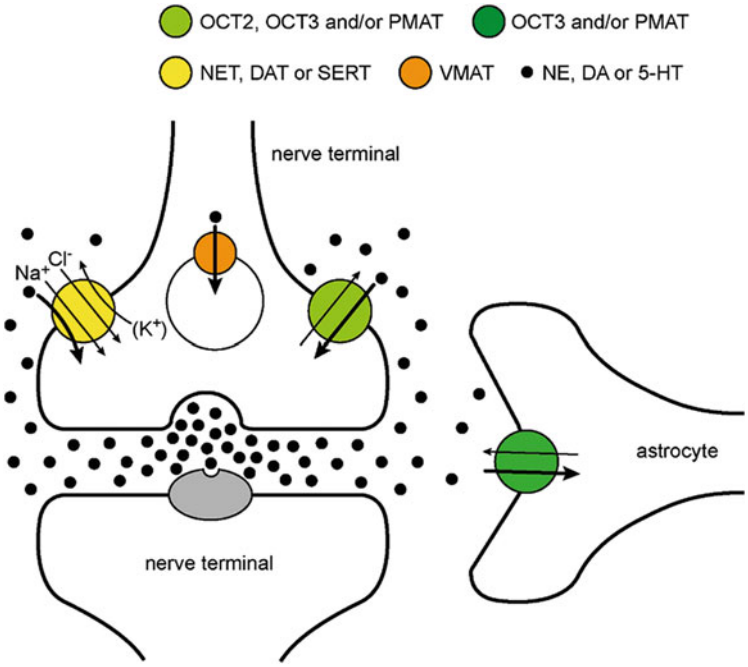

Fig. 1 Schematic depiction of locations of monoamine neurotransmitter transporters in cerebral neurons and glial cells. The indicated high affinity transporters NET, DAT, and SERT are expressed in their different cognate neurons. OCT2, OCT3, and PMAT or OCT3 and PMAT may be expressed in identical or different neurons and astrocytes. The indicated neurotransmitter concentrations simulate a post-excitatory situation

Table 2 K_m values of cationic neurotransmitters transported by high affinity neurotransmitter transporters and organic cation transporters from humans and rodents

Compound		K_m [μM] NET/Net	DAT/Dat	SERT/Sert	OCT1/Oct1	OCT2/Oct2	OCT3/Oct3	PMAT/Pmat
Norepinephrine	Human Rat (Mouse)	0.46–2.6 1.4 (4)	20		t 800	1,500–5,450 2,100, 4,400 (t)	182–2,630 1,900 (336, 566)	1,078, 2,606 (515)
Epinephrine	Human Rat (Mouse)				t 1,100	420 1,370, 1,900	240, 458 1,500	951
Dopamine	Human Rat (Mouse)	0.67	1.2–2.5 0.3–1.2 (2.0)		t 19–600	390–1,400 2,100 (n.t.d.)	800, 1,033 1,500 (785)	201–406 271 (160, 466)
Serotonin	Human Rat (Mouse)		t	0.2–1 0.32 (0.40)	197 38, 900	80, 290 760, 3,600 (313)	900, 988 500 (430)	114–283 93, 231 (120)
Histamine	Human Rat (Mouse)				t 99	520–1,300 278, 890 (111)	180–641 540 (1,670)	4,379 3,620 (1,520)

t transported, *n.t.d.* no transport detected

Norepinephrine: (Amphoux et al. 2006; Bacq et al. 2012, Busch et al. 1998; Chen et al. 2014; Daws 2009; Duan and Wang 2010; Engel et al. 2004; Giros et al. 1994; Gründemann et al. 1998a, b; Miura et al. 2017; Muck et al. 2007; Pacholczyk et al. 1991; Paczkowski et al. 1999; Song et al. 2019), **epinephrine**: (Amphoux et al. 2006; Duan and Wang 2010; Gründemann et al. 1998a, b), **dopamine**: (Amphoux et al. 2006; Daws 2009; Bednarczyk et al. 2003; Busch et al. 1996, 1998; Campbell et al. 2019; Duan and Wang 2010; Engel et al. 2004; Giros et al. 1991, 1992, 1994; Gründemann et al. 1998a; Kilty et al. 1991; Miura et al. 2017; Shimada et al. 1991; Shirasaka et al. 2017; Sitte et al. 1998; Wu and Gu 1999; Zolk et al. 2009a), **serotonin**: (Amphoux et al. 2006; Daws 2009; Blakely et al. 1991; Boxberger et al. 2014; Busch et al. 1996, 1998; Chang et al. 1996; Chen et al. 2014; Duan and Wang 2010; Engel et al. 2004; Gründemann et al. 1998a; Kristensen et al. 2011; Miura et al. 2017; Shirasaka et al. 2017; Zhou et al. 2002), **histamine**: (Amphoux et al. 2006; Arndt et al. 2001; Bednarczyk et al. 2003; Busch et al. 1996, 1998; Duan and Wang 2010; Miura et al. 2017; Usui et al. 2016; Yoshikawa and Yanai 2017)

and Cl^- is additionally coupled with antiport of K^+. The inwardly directed concentration gradients of Na^+ and Cl^- and the outwardly directed K^+ gradient provide large driving forces for transmitter uptake allowing a dramatic intracellular accumulation of NE, DA, and 5-HT at low extracellular concentrations of neurotransmitters.

2.2 Physiological Roles

The central role of the Na^+/Cl^- dependent monoamine neurotransmitter transporters is the rapid removal of neurotransmitters that are released during neurotransmission (Kristensen et al. 2011; Torres et al. 2003). Thereby, neurotransmission is terminated, and presynaptic nerve terminals are supplied with neurotransmitters for replenishment of presynaptic vesicles via vesicular neurotransmitter transporters. This is essential for maintenance and fine-tuning of neurotransmission. In addition, the monoamine neurotransmitter transporters participate in the control of ambient concentrations of NE, DA, and 5-HT which modulate ontogeny of neurons in the embryo, regulation of neuronal properties in adults, and crosstalk between neurons (Daws and Gould 2011). Based on these features, it is not surprising that monoamine neurotransmitter transporters are involved in diverse central nervous system functions. For example, NET regulates learning, memory, attention, mood control, motoric functions, and responses to stress. Mice in which NET was removed showed decreased NE tissue concentrations in various brain areas, decelerated NE clearance after activation of noradrenergic neurons, and an increased response to behavioral despair (Xu et al. 2000). DAT participates in the regulation of DA neurotransmission that is involved in motor activity, cognition, emotion, motivation, and reward behavior. Functional failure and dysregulation of DAT have been associated with neurological and psychiatric disorders including schizophrenia, depression, Parkinson´s disease (PD), attention deficit hyperactivity disorder (ADHD), bipolar disease, autism spectrum disorders (ASD), and drug addiction (Bowton et al. 2014; Dreher et al. 2009; Hamilton et al. 2013; Mazei-Robinson and Blakely 2006; Mazei-Robison et al. 2005). DAT knockout (KO) mice exhibited slowed removal of DA from the extracellular space surrounding dopaminergic neurons and increased hyperlocomotion (Giros et al. 1996). Also, the 5-HT releasing serotonergic system that is modulated by SERT is involved in various brain functions (Deneris and Wyler 2012; Lesch et al. 2012; Lesch and Waider 2012), including cognition, motor activity, mood control, aggression, appetite, and sleep. Mice in which SERT was removed showed decreased 5-HT concentrations in several brain areas and exhibited improved learning during a reversal task (Bengel et al. 1998; Brigman et al. 2010). Noteworthy, NET, DAT, and SERT collaborate to regulate central nervous system circuits controlling mood, attention, psychological drive, and reward behavior. This is, between others, due to overlapping neurotransmitter selectivity of the monoamine neurotransmitter transporters, closely associated noradrenergic, dopaminergic, and serotonergic neurons, and neuronal volume transmission (Sulzer and Edwards 2005; Torres et al. 2003; Zhou et al. 2002, 2005; Zoli et al. 1998).

2.3 Psychoactive Drugs That Interact with Na^+/Cl^- Dependent Monoamine Neurotransmitter Transporters

Compounds interacting with NET, DAT, and/or SERT have psychoactive effects (Mika et al. 2013; Torres et al. 2003). They are employed for treatment of ADHD, depression, addiction, pain, and obsessive-compulsive disorder (OCD). Many of these compounds are also substrates and/or inhibitors of organic cation transporters. These drugs are depicted in Table 3. Amphetamine is a stimulant that induces excitement, euphoria, and the feeling of wakefulness but may create dependence. It increases the synaptic concentrations of DA, NE, and 5-HT at cognate synapses by different mechanisms including stimulation of monoamine transporter mediated neurotransmitter release and inhibition of neurotransmitter reuptake (Jones et al. 1998; Mazei-Robison et al. 2008; Sitte and Freissmuth 2015; Sulzer et al. 1995; Torres et al. 2003). The selective NE reuptake inhibitor, atomoxetine, exhibits psychomotor effects and is approved for treatment of ADHD. Selective SERT inhibitors (SSRIs) like citalopram and fluoxetine and compounds that inhibit SERT and NET (amitriptyline, clomipramine, doxepin, imipramine, and desipramine) have complex psychotropic effects such as mood enhancement, sedation, and anxiolysis. They are used in the treatment of depression, anxiety, and related disorders. SSRIs and clomipramine are recommended for treatment of OCDs (Pittenger and Bloch 2014). Fluoxetine, amitriptyline, doxepin, imipramine, and desipramine are also used for treatment of chronic pain (Mika et al. 2013).

3 Emergence of the Concept That Low Affinity Monoamine Neurotransmitter Transporters Operate in Brain

3.1 Pioneering Demonstration of Low Affinity Noradrenaline Transport in the Heart

In 1965 Iversen described transporter mediated low affinity uptake of epinephrine and NE in addition to high affinity uptake in rat hearts (Iversen 1965). He named the low affinity uptake "uptake 2" in contrast to high affinity "uptake 1". In addition to much higher K_m values determined for uptake 2, differences in inhibitor selectivity were observed. For example, in contrast to uptake 1, uptake 2 did not discriminate between stereoisomers of epinephrine and NE. Moreover, evidence was provided for different transport properties of both systems. Whereas uptake but no efflux of epinephrine and NE was observed for uptake system 1, bidirectional transport was demonstrated for uptake system 2. Employing fluorescence microscopy, uptake 2 mediated transport was localized to heart muscle cells (Clarke et al. 1969; Iversen 1971).

Table 3 Apparent K_m values for drug uptake, IC_{50} or K_i values for drug inhibition of monoamine neurotransmitter uptake by human sodium-dependent monoamine neurotransmitter and/or organic cation transporters or K_D values for binding

	***K*m** (*IC*50 /*K*i) $\underline{K_D}$ [μM]						
Drug, therapeutic effect, *mechanism*	NET	DAT	SERT	OCT1	OCT2	OCT3	PMAT
Amphetamine, stimulant, *stimulation of DA release and inhibition of DA uptake via DAT, inhibition of NET*	**t** (0.06–0.20)	**0.7, 0.8** (0.39–2.3)	(38)	(97,202)	**0.8, 534** (0.7–145)	(24–460)	
Metamphetamine, stimulant, *stimulation of NE and DA release*		(0.2)		(0.3, 400)	**2.1** (1.2, 58)	(247, 300)	
Atomoxetine, treatment of ADHD, *inhibition of NET, NMDA receptor antagonist*	(0.005)	(0.69)	(0.15)		(3.5-20)		
Citalopram, antidepressant, analgetic, *inhibition of SERT*	(30) $\underline{4.1}$	(10) $\underline{28}$	**0.009** (0.001, 0.005) $\underline{0.001}$	(3, 19)	(12)	(145)	(117)
Fluoxetine, antidepressant, analgetic, *inhibition of SERT*	(1, 2) $\underline{0.24}$	(19.5) $\underline{3.6}$	(0.007, 0.020) $\underline{0.0008}$	**t** (3–6)	(17–57)		(11-28)
Sertraline, antidepressant, *inhibition of SERT*	$\underline{0.42}$	$\underline{0.025}$	$\underline{0.0003}$			(7.4)	(5.1, 14)
Amitriptyline, antidepressant, analgetic, *inhibition of SERT and NET*	(0.1) $\underline{0.035}$	(3.0) $\underline{3.3}$	(0.015, 0.036) $\underline{0.004}$	(3.5–17)	(0.5–14)	(>100)	(23)
Clomipramine, antidepressant, treatment of obsessive, compulsive disorders, *inhibition of SERT and NET*	(0.054) $\underline{0.038}$	(3.0) $\underline{2.2}$	(0.00004 0.00014) $\underline{0.0003}$	(5, 19)			
Doxepin, antidepressant, analgetic, *inhibition of SERT and NET*	$\underline{0.030}$	$\underline{12}$	$\underline{0.068}$	(1.5, 12)	(0.25–13)		
Imipramine, antidepressant, analgetic, *inhibition of SERT and NET*	(0.07–0.14) $\underline{0.037}$	(25) $\underline{8.5}$	(0.005-0.020) $\underline{0.001}$	(6–37)	(0.4–6)	(11, 42)	(21)

Desipramine, active metabolite of imipramine, antidepressant, analgetic, *inhibition of NET and SERT*	(0.004–0.01) 0.0008	(13–79) 3.2	(0.04–0.16) 0.018	(5–57)	(16)	(0.7–68)	(15, 33)
Sulpiride, antidepressant, antipsychotic, *dopamine receptor antagonist*				**260**	**26, 187**	**160**	
Berberine, antidepressant, *inhibitor of monoamine oxidase*				**15**	**1.0, 4.4** (0.4–0.9)	**2.2** (0.4–10)	
Trimipramine, anxiolytic, sedative, *antagonist of neurotransmitter receptors*				(28)	(0.44)		(12)
Buspirone, anxiolytic, *serotonin receptor agonist and dopamine receptor antagonist*					(8.6-69)		
Apomorphine, anti-Parkinson, *DA receptor agonist*				(21)	**t**		
Benztropine, anti-Parkinson, a*cetylcholine receptor antagonist, inhibition of uptake by NET and DAT*	(0.3, 0.8)	(0.04, 0.06)	(47)		(0.3–13)		
Memantine, anti-Parkinson, treatment of AD, *NMDA receptor antagonist*				(4, 27)	**34** (7.3)	(236)	
Procyclidine, anti-Parkinson, *acetylcholine receptor antagonist*					(<0.1)		
Selegiline, anti-Parkinson, *inhibition of monoamine oxidase*					**t**		
Amisulpride, antipsychotic, *dopamine receptor antagonist*				**31**	**168**	**192**	
Chlorpromazine, antipsychotic, *antagonist of various neurotransmitter receptors*				(2.6–52)	(2.6, 14)		
Chlorprothixene, antipsychotic, *antagonist of various neurotransmitter receptors*					**t**		
Clozapine, antipsychotic, *antagonist of various neurotransmitter receptors*							(13)
Haloperidol, antipyschotic, *antagonist of various neurotransmitter receptors*				(142)			(11)
Olanzapine, antipsychotic, *antagonist of various neurotransmitter receptors*					(<1)		(149)
Perphenazine, antipsychotic, *dopamine receptor antagonist*				**t**			

(continued)

Table 3 (continued)

Drug, therapeutic effect, *mechanism*	K_m (IC_{50} /K_i) K_D [μM]						
	NET	DAT	SERT	OCT1	OCT2	OCT3	PMAT
Promazine, antipsychotic, *antagonist of various neurotransmitter receptors*				(17)			
Risperidone, antipsychotic, *antagonist of various neurotransmitter receptors*							(7.0)
Phenytoin, anticonvulsant, *sodium channel blocker*						(0.75)	
Clomacran, hypnotic, *GABA receptor antagonist*					(<1)		
Zolpidem, hypnotic, *allosteric activator of GABA receptor*					(0.15)		
Ketamine, anesthetic, hypnotic, *NMDA receptor blocker*				**74**[a]	**34**[a]	**53**[a], **365**	
Morphine, analgetic, *opioid receptor agonist*				**3.4** (4.2, 28)		(538)	
Diphenhydramine, antiemetic, sedative, *histamine receptor antagonist, inhibitor of SERT*				**t** (3.4, 4.1)	(5.8–21)		
Domperidone, antiemetic, *dopamine receptor antagonist*				(33)	(7.9)		
Granisetron, antiemetic, *serotonin receptor antagonist*					(4.3)		
Metoclopramide, antiemetic, *dopamine receptor antagonist*				**t** (16, 95)	**t**		
Ondansetron, antiemetic, *serotonin receptor antagonist*				(1.2–64)	(0.9–16)	(1.7, 17)	
Scopolamine, antiemetic, *acetylcholine receptor antagonist*				(6.7)	(541)	(218)	

[a]Measured in presence of an inwardly directed proton gradient

Drugs which have not been tested for transport or could not be identified as substrates are only indicated if an IC_{50} or K_i value <20 μM was determined for at least one organic cation transporter. For determination of IC_{50} values substrate concentrations far below their K_m values were employed

t transported, K_D values for replacement of binding of [^{3}H]imipramine to SERT, [^{3}H]nisoxetine to NET, and [^{3}H]WIN3428 to DAT were measured (Tatsumi et al. 1997)

Amphetamine: (Amphoux et al. 2006; Eshleman et al. 1994; Giros et al. 1992, 1994; Kristensen et al. 2011; Pacholczyk et al. 1991; Sitte et al. 1998; Wagner et al. 2017; Wu and Gu 1999; Wu et al. 1998a; Zhu et al. 2010), **Metamphetamine:** (Eshleman et al. 1994; Wagner et al. 2017; Wu et al. 1998a)**, Atomoxetine:** (Kristensen et al. 2011; Sandoval et al. 2018), **Citalopram**: (Ahlin et al. 2008; Apparsundaram et al. 2008; Nies et al. 2011; Owens et al. 1997; Pacholczyk et al. 1991; Tatsumi et al. 1997; Torres et al. 2003; Zhou et al. 2007), **Fluoxetine**: (Boxberger et al. 2014, 2018; Engel et al. 2004; Haenisch and Bönisch 2010;

Kristensen et al. 2011; Owens et al. 1997; Sandoval et al. 2018; Tatsumi et al. 1997; Tzvetkov et al. 2018; Zhou et al. 2007; Zhu et al. 2012, 2018), **Sertraline**: (Haenisch and Bönisch 2010; Tatsumi et al. 1997; Zhou et al. 2007; Zhu et al. 2012), **Amitriptyline**: (Ahlin et al. 2008; Belzer et al. 2013; Giros et al. 1992; Hacker et al. 2015; Haenisch and Bönisch 2010; Matthaei et al. 2016; Owens et al. 1997; Pacholczyk et al. 1991; Sandoval et al. 2018; Sata et al. 2005; Tatsumi et al. 1997; Torres et al. 2003; Tzvetkov et al. 2013; Zolk et al. 2009a), **Clomipramine**: (Ahlin et al. 2008; Apparsundaram et al. 2008; Hendrickx et al. 2013; Millan et al. 2001; Tatsumi et al. 1997), **Doxepin**: (Belzer et al. 2013; Chen et al. 2017a; Hacker et al. 2015; Mika et al. 2013; Tatsumi et al. 1997; Zolk et al. 2009a), **Imipramine**: (Ahlin et al. 2008; Belzer et al. 2013; Haenisch and Bönisch 2010; Hendrickx et al. 2013; Kido et al. 2011; Owens et al. 1997; Pacholczyk et al. 1991; Paczkowski et al. 1999; Sandoval et al. 2018; Tatsumi et al. 1997; Torres et al. 2003; Tzvetkov et al. 2013; Wu et al. 2000; Zhu et al. 2012, 2018; Zolk et al. 2009a), **Desipramine**: (Ahlin et al. 2011; Ahlin et al. 2008; Chen et al. 2017a; Engel et al. 2004; Giros et al. 1992, 1994; Gorboulev et al. 1997; Haenisch and Bönisch 2010; Owens et al. 1997; Pacholczyk et al. 1991; Paczkowski et al. 1999; Tatsumi et al. 1997; Torres et al. 2003; Wu et al. 1998a, 2000; Zhang et al. 1998; Zhu et al. 2012; Zolk et al. 2009a), **Sulpiride**: (Bai et al. 2017; dos Santos Pereira et al. 2014; Li et al. 2017; Takano et al. 2017), **Berberin**: (Nies et al. 2008; Sun et al. 2014), **Trimipramine**: (Ahlin et al. 2008; Hacker et al. 2015; Haenisch and Bönisch 2010), **Buspirone**: (Sandoval et al. 2018), **Apomorphine**: (Ahlin et al. 2008; Hendrickx et al. 2013), **Benztropine**: (Giros et al. 1992; Kristensen et al. 2011; Pacholczyk et al. 1991; Sandoval et al. 2018), **Memantine**: (Ahlin et al. 2008; Amphoux et al. 2006; Busch et al. 1998; Hendrickx et al. 2013), **Procyclidine**: (Kido et al. 2011), **Selegiline**: (Hendrickx et al. 2013), **Amisulpride**: (dos Santos Pereira et al. 2014), **Chlorpromazine**: (Ahlin et al. 2011; Ahlin et al. 2008; Bednarczyk et al. 2003; Belzer et al. 2013; Zolk et al. 2009b)**, Chlorprothixene**: (Hendrickx et al. 2013), **Clozapine**: (Haenisch and Bönisch 2010), **Haloperidol**: (Ahlin et al. 2008; Haenisch and Bönisch 2010) **Olanzapine**: (Haenisch and Bönisch 2010; Kido et al. 2011), **Perphenazine**: (Hendrickx et al. 2013), **Promazine**: (Ahlin et al. 2008), **Risperidone**: (Haenisch and Bönisch 2010), **Phenytoin**: (Hasannejad et al. 2004), **Clomacran**: (Kido et al. 2011), **Zolpidem**: (Hacker et al. 2015), **Ketamine**: (Hendrickx et al. 2013; Keiser et al. 2018), **Morphine**: (Ahlin et al. 2008; Tzvetkov et al. 2013; Zhu et al. 2018), **Diphenylhydramine**: (Belzer et al. 2013; Boxberger et al. 2014, 2018; Müller et al. 2005; Zolk et al. 2009b), **Domperidone**: (Wittwer et al. 2013), **Granisetron**: (Wittwer et al. 2013), **Metoclopramide**: (Ahlin et al. 2008; Hendrickx et al. 2013; Matthaei et al. 2016), **Ondansetron**: (Ahlin et al. 2008; Kido et al. 2011; Tzvetkov et al. 2013; Tzvetkov et al. 2012; Wittwer et al. 2013; Zhu et al. 2018), **Scopolamine**: (Chen et al. 2017b; Hendrickx et al. 2013)

3.2 Reasons for the Need of Low Affinity Monoamine Neurotransmitter Transporters in Brain

Monoamine neurotransmitters released at synapses do activate not only postsynaptic receptors but also neuronal auto-receptors and receptors on nearby neurons. The synaptic "wiring transmission" allows rapid activation of neuronal circuits whereas the activation of remote neurons promoted by the so-called volume transmission occurs much more slowly and serves complex integrative and regulatory functions (Bunin and Wightman 1999; Smiley et al. 1994; Zoli et al. 1998). The concentration of monoamine neurotransmitters within and around synaptic clefts varies considerably (Bunin and Wightman 1998; Clements 1996). In the resting state, concentrations are in the low nanomolar range and are similar within and around synaptic clefts (Mathews et al. 2004). After neuronal activation, neurotransmitter concentrations within synaptic clefts and in remote tissue regions may be largely different. They depend on various factors that include firing rate, time after firing, anatomy of the synaptic cleft, density of cognate neurons as well as densities, properties, and locations of related neurotransmitter transporters (Bunin and Wightman 1998; Clements 1996; Garris and Wightman 1994). The concentration of DA and NE in synaptic clefts of cognate neurons after neuronal activation has been estimated to be in the millimolar range (Bunin and Wightman 1998; Garris et al. 1994). Outside synaptic clefts, the monoamine neurotransmitter concentration ranges from nanomolar to the low millimolar range (Williams and Millar 1990; Zoli et al. 1998). Because monoamine neurotransmitter concentrations close to the synapses are frequently orders of magnitude higher compared to the K_m values of the ambient high affinity neurotransmitter transporters (see Table 2), low affinity monoamine neurotransmitter transporters are required to enable an effective removal of neurotransmitters after nerve excitation. The coexistence of the low and high affinity neurotransmitter transporters enables a rapid location- and function-dependent adjustment of monoamine neurotransmitter concentrations. In the presence of drugs that inhibit high affinity monoamine neurotransmitter transporters, the neurotransmitter concentrations are increased, and their reuptake by low affinity transporters gains special importance (Daws 2009).

3.3 First Data Indicating That OCTs Translocate Monoamine Neurotransmitters and Are Expressed in Brain

Two years after the first cloning of the polyspecific organic cation transporter OCT1 from the rat (rOCT1) (Gründemann et al. 1994), it was observed that rOCT1 also mediates low affinity transport of monoamine neurotransmitters (Busch et al. 1996). Whereas early publications reported no expression of rOCT1 in the brain, cerebral expression of OCT2 in rats (rOCT2) was demonstrated by RT-PCR (Gründemann et al. 1997). In 1998, it was reported that human organic cation transporter 2 (hOCT2) is expressed in neurons of several brain areas and mediates low affinity transport of NE, 5-HT, DA, and histamine (Busch et al. 1998). In the same year,

cloning and broad expression of OCT3 from rats (rOCT3) and humans (hOCT3), including expression in brains and hearts, was reported (Gründemann et al. 1998b; Kekuda et al. 1998; Wu et al. 1998b). It was observed that hOCT3 transported epinephrine and NE in addition to other organic cations and that tetraethylammonium (TEA) transport by rOCT3 was inhibited by DA and 5-HT (Gründemann et al. 1998b; Wu et al. 1998a). Since rOCT3 and hOCT3 are also expressed in the heart, it was concluded that OCT3 is responsible for "uptake 2" as described by Iversen (1965). Because plasma membrane monoamine transporter PMAT that transports epinephrine and NE is also expressed in the heart (Engel et al. 2004), PMAT may also contribute to "uptake 2" in the heart.

4 Organic Cation Transporters Expressed in Brain

4.1 Basic Functional Properties of OCT1–3 (*SLC22A1–3*) and PMAT (*SLC29A4*)

The organic cation transporters OCT1, OCT2, OCT3, and PMAT are facilitated diffusion systems that translocate structurally different organic cations in both directions across the plasma membrane (Koepsell 2020; Wang 2016). The driving forces for cellular uptake by these transporters are the transmembrane concentration gradient of transported cation and the inside negative membrane potential. Extracellular protons also stimulate transport of organic cations by PMAT. OCT1, OCT2, OCT3, and PMAT accept a variety of structurally different organic cations as substrates, including monoamine neurotransmitters. In addition, they are inhibited by various organic cations and neutral compounds that are not transported. The substrate and inhibitor specificities of the four organic cation transporters overlap. Mutagenesis experiments and tertiary structure homology modeling of OCT1 and OCT2 indicate that these transporters, and probably also the highly homologous OCT3, contain substrate binding regions with partially overlapping cation binding sites within a large binding cleft (Koepsell 2019, 2020). After binding of one or two cationic substrates to one or two transport relevant binding sites within the inner part of the outward-open cleft, the transporter undergoes conformational changes including a state in which the substrates are occluded, triggering an inward-open state that allows the intracellular release of the substrates. The OCTs also contain high affinity cation binding sites that are accessible extracellularly and may modulate organic cation transport and sensitivity of inhibitors. The complex tertiary structure of the cation binding region containing interacting binding sites for structurally different cations and the existence of high affinity cation binding sites promoting structural changes within the substrate binding region provide a rationale why the sensitivity of inhibitors is often influenced drastically by the molecular structure and the concentration of transported cations (Koepsell 2019, 2020; Nies et al. 2011).

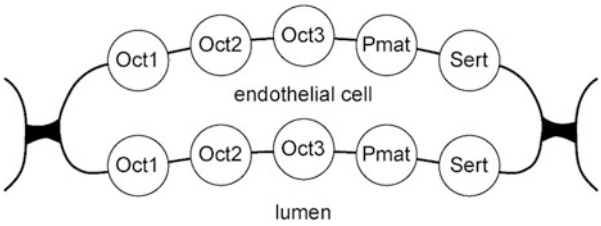

Fig. 2 Expression of Oct1, Oct2, Oct3, and Pmat in endothelial cells of the BBB identified in rodents. The indicated membrane locations of Oct1-3 and Pmat are suggested by immunohistochemical data (Oct1, Oct2, Oct3, Pmat) or presumed (Sert)

4.2 OCT1 (*SLC22A1*) in Brain

In the human brain, mRNA of OCT1 was detected by RT-PCR (Duan and Wang 2013; Gorboulev et al. 1997). OCT1 mRNA was also detected in the hippocampus of mice and in the choroid plexus (CP) of mice and rats (Baganz et al. 2008; Choudhuri et al. 2003; Duan and Wang 2013). A relatively low expression of OCT1 was observed in small blood vessels isolated from brains of humans, rats, and mice (Chaves et al. 2020; Geier et al. 2013; Lin et al. 2010; Sekhar et al. 2019; Wu et al. 2015b). In cultured rat brain endothelial cells, rOct1 was located to the luminal and abluminal membranes with higher luminal expression (Lin et al. 2010). The monoamine neurotransmitter transporters expressed in endothelial cells of rodents including their presumed plasma membrane location are depicted in Fig. 2.

Like the other organic cation transporters that are also expressed in the blood–brain barrier (BBB) (Table 1) including the novel organic cation transporter OCTN2 (*SLC22A5*), multidrug and toxin exclusion proteins MATE1 (*SLC47A1*) and MATE2-K (*SLC47A2*), which transport 5-HT and/or sulpiride (Koepsell 2020), OCT1 may be involved in translocation of organic cations across the BBB in both directions. It has been demonstrated that OCT1/Oct1 transports NE, 5-HT, DA, acetylcholine, and histamine like OCT2, OCT3, and PMAT (Breidert et al. 1998; Busch et al. 1996) (Table 2). The biomedical relevance of OCT1 and the other organic cation transporters in the BBB has not been resolved. Considering the overlapping specificity of the transporters, the biomedical relevance may be limited to individual drugs preferred by OCT1 and specific therapeutic situations, meaning situations in which other transporters are inhibited by drugs. Because blood concentrations of monoamine neurotransmitters and histamine are generally lower compared to their respective concentrations in the brain, their uptake into the brain has probably no biomedical relevance. In contrast, efflux of 5-HT across the BBB by the organic cation transporters may play a role in neuronal serotonergic regulation of microvascular blood flow and BBB permeability (Cohen et al. 1996; Leybaert 2005). Brain capillaries are associated with serotonergic nerve terminals that often originate from neurons in raphe nuclei and form tripartite neurovascular units together with astrocytes (Cohen et al. 1996; Reinhard et al. 1979). During serotonergic regulation of blood flow, 5-HT is released at the capillaries and activates 5-HT receptors at the endothelial cells (Parsons 1991). The activation is terminated by 5-HT uptake into

the endothelial cells where it may be degraded by monoamine oxidase (Kalaria and Harik 1987; Maruki et al. 1984). 5-HT uptake into the endothelial cells is mediated by SERT (Brust et al. 2000) and probably also by the low affinity organic transporters enabling efficient 5-HT uptake within a large concentration range.

Psychoactive, antiemetic, and analgetic drugs have been identified as substrates and/or inhibitors of human OCT1 and the other organic cation transporters (Table 3). Human OCT1 may be involved in cerebral uptake of fluoxetine, amisulpride, perphenazine, and morphine but probably not in uptake of amisulpride since the plasma concentrations of amisulpride in patients are much lower compared to the K_m value for uptake by hOCT1 (Table 3). Drug–drug interactions at the levels of OCT1 and/or the other organic cation transporters in the BBB may decrease transporter mediated drug uptake into the brain. Of note, the IC_{50} values presented in Table 3 have been determined with relatively high concentrations of model substrates such as tetraethylammonium (TEA) or 1-methyl-4-phenylpyridinium (MPP). Hence, these IC_{50} values may be orders of magnitude higher than the IC_{50} values for inhibition of transported drugs. As outlined above, the affinity for inhibition of OCTs is highly dependent on the molecular structure and concentration of the transported drug, and high affinity inhibition may occur when the concentration of the transported drug is far below its respective K_m (Koepsell 2019, 2020).

4.3 OCT2 (*SLC22A2*) in Brain

4.3.1 Locations in Brain

In the human brain hOCT2 was detected in pyramidal cells of hippocampus and occipital cortex and in the caudate nucleus (Busch et al. 1998). In microvessels isolated from human, rat, and mouse brain OCT2 was identified and located to luminal and abluminal membranes of the endothelial cells (Geier et al. 2013; Lin et al. 2010; Wu et al. 2015b). In rats and mice, expression of OCT2 was also observed in choroidal epithelial cells and localized to their apical membrane (Amphoux et al. 2006; Choudhuri et al. 2003; Duan and Wang 2013; Sweet et al. 2001). *In situ* hybridization in rats revealed rOct2 related staining at the borders between brain ventricles and parenchyma suggesting expression in ependymal cells (Amphoux et al. 2006). In the rat, rOct2 mRNA was also observed in granular cells of cerebellum and in granular and pyramidal cells of the hippocampal CA1, CA2, and CA3 regions (Amphoux et al. 2006). In mice, cerebral location of Oct2 protein was investigated in detail by immunohistochemistry employing Oct2-KO mice as negative controls (Bacq et al. 2012; Courousse et al. 2014). Expression of mouse Oct2 (mOct2) was observed in raphe nuclei, locus coeruleus, thalamic paraventricular nucleus, and hypothalamic dorsomedial, ventromedial, and arcuate nuclei. As in rats, mOct2 protein was also detected in the median eminence, in CA1, CA2, and CA3 regions of hippocampus, as well as in amygdala, and in various cortical regions such as precentral and cingulate gyri, prelimbic cortex, and infralimbic regions of prefrontal cortex. Co-labeling revealed that mOct2 was expressed in most noradrenergic neurons of the locus coeruleus and in a fraction of the serotonergic neurons of the dorsal raphe nucleus (Bacq et al. 2012).

4.3.2 Specificity for Transport of Monoamine Neurotransmitters and Interaction with Psychiatric Drugs

Like OCT1, OCT3, and PMAT, OCT2 accepts NA, epinephrine, DA, 5-HT, and histamine as substrates (Table 2). For uptake of NE by both hOCT2 and rOct2, diverging K_m values were reported. They range between 1.5 and 5.5 mM offering no indication of species related differences. These K_m values are at least 500 times higher than the K_m values reported for NE uptake by hNET. For DA uptake by hOCT2 or rOct2 diverging and overlapping K_m values between 0.39 and 2.1 mM were reported. These values are at least 150 times higher than the K_m values measured for hDAT and at least 1,000 times higher compared to the K_m values reported for rat Dat (rDat). K_m values of 0.08 and 0.29 mM were reported for uptake of 5-HT by hOCT2 while K_m values of 0.76 and 3.6 mM were reported for uptake of 5-HT by rOct2, suggesting a lower affinity of the rat transporter. The low K_m value of 0.08 mM reported for hOCT2 is 80 times higher than the K_m value reported for 5-HT uptake by human SERT (hSERT). For uptake of epinephrine by hOCT2, a K_m value of 0.4 mM has been reported that is about three times lower than the lower value reported for rOct2. Similar K_m values ranging between 0.28 and 1.3 mM were determined for histamine uptake by hOCT2 and rOct2. The data suggest complementary functions of OCT2 and coexpressed high affinity monoamine neurotransmitter transporters.

Transport by hOCT2 has been demonstrated for various psychotropic drugs including the stimulants amphetamine and metamphetamine, the antidepressant sulpiride, the antipsychotic amisulpride, and the anesthetic ketamine (Table 3). Considering the low plasma concentrations of psychotropic drugs in patients in relation to the determined K_m values for OCT2 (Table 3), OCT2 probably only participates significantly in translocation of amphetamine, metamphetamine, and ketamine across the BBB. Psychoactive drugs that have been shown to inhibit OCT2 (Table 3) may be also transported and OCT2 could be relevant for their uptake into brain. If OCT2 mediated drug uptake across the BBB turns out to be significant, the possibility of drug–drug interactions at OCT2 should be considered. As discussed for OCT1, OCT2 mediated drug uptake across the BBB may be inhibited by very low concentrations of coadministered drugs that also interact with OCT2. Psychoactive drugs in the brain interstitium may also inhibit OCT2 mediated uptake of monoamine neurotransmitters into neurons, because the relatively high IC_{50} values for hOCT2 shown in Table 3 do not exclude high affinity inhibition of neurotransmitter *in vivo* (Koepsell 2019, 2020).

4.3.3 Presumed Biomedical Functions

Employing Oct2 knockout (KO) mice, cerebral functions of mOct2 were investigated (Bacq et al. 2012; Courousse and Gautron 2015). In the hippocampus of Oct2-KO mice the *in vivo* clearance of iontophoretically applied NE and 5-HT was observed to be reduced compared to wildtype (WT) mice if uptake by NET and SERT was blocked (Bacq et al. 2012; David et al. 2003). This indicates the capability of mOct2 to participate in the adjustment of interstitial concentrations of 5-HT and NE. Using animal models for psychiatric disorders, data were obtained

suggesting that OCT2 is relevant for the performance of serotonergic circuits that are related to anxiety and depression.

After removal of Oct2, mice showed signs for decreased anxiety and increased depression-related behaviors. Decreased anxiety was suggested using the open field test, the O-maze test, and a test on feeding behavior after food application in a novel adverse environment. Compared to WT, Oct2-KO mice stayed in an open field longer, spent more time in the elevated open arms area in the O-maze test, and showed a reduced latency to feed in a novel environment. As paradigms for depression, the time periods of immobility after exposure to unescapable stress were tested in forced swim test (FST) and tail suspension tests. Whereas inhibitors of SERT and NET had no effects in Oct2-KO mice compared to WT mice, the time periods of immobility, indicating despair, were increased. These data indicate a complex, not yet understood impact of OCT2 mediated modulation of neurotransmitter homoeostasis on depression.

Chronic stress leading to increased levels of corticosterone is considered a risk factor for the onset of depression. Since OCT2 is expressed in neuronal circuits that trigger corticosterone secretion via activation of the hypothalamic-pituitary-adrenocortical axis, the effect of mOct2 removal on corticosterone secretion in response to acute stress was tested (Courousse and Gautron 2015; Krishnan and Nestler 2008). After acute stress, the brain induced increase of corticosterone secretion observed in Oct2-KO mice was higher compared to WT. This indicates an attenuating effect of Oct2 mediated neurotransmitter transport on stress induced corticosterone secretion. The effect of mOct2 removal on the vulnerability of mice for depression in response to unpredictable chronic mild stress (UCMS) was studied by analyzing the deterioration of coat state, emergence of spatial memory deficits, and decline of social behavior (Courousse and Gautron 2015). After UMCS conditions, an accelerated deterioration of the coat state, a higher deficit in spatial memory, and enhanced social deficits were observed in Oct2-KO mice compared to WT mice. These data suggest that depression-like behavior induced by chronic stress is blunted by OCT2 mediated neurotransmitter transport via decrease of corticosterone secretion.

4.4 OCT3 (*SLC22A3*) in Brain

4.4.1 Locations in Brain

When successful cloning of OCT3 from humans (hOCT3) and rats (rOct3) was reported, expression of OCT3 in brain and placenta was emphasized (Gründemann et al. 1998b; Kekuda et al. 1998). In the human brain, expression of OCT3 was detected in microvessels, CP, neurons of the substantia nigra (SN) and cerebellum, and in astrocytes (Cui et al. 2009; Duan and Wang 2013; Geier et al. 2013; Yoshikawa et al. 2013). In rodents, Oct3 expression was observed in many brain areas (Table 1) (Amphoux et al. 2006; Cui et al. 2009; Gasser et al. 2006, 2009, 2017; Graf et al. 2013; Haag et al. 2004; Hill and Gasser 2013; Nakayama et al. 2007; Sweet et al. 2001; Vialou et al. 2004; Wu et al. 1998a). Rat Oct3 was identified in pontine nuclei, nucleus tractus solitarii (NTS), raphe nuclei, locus coeruleus, colliculi of tectum, SN, circumventricular organs, and pineal gland. In rats, Oct3

was also detected in cerebellum, striatum, thalamus (anterodorsal, posterior paraventricular, lateral geniculate, arcuate nuclei), hypothalamus (dorsomedial and ventromedial nuclei), lateral septum, various regions of cerebral cortex, olfactory bulb, hippocampus, subiculum, nucleus accumbens (NAC), amygdala including the basolateral amygdala complex (BLA), and CP. ROct3 was observed in granular and Purkinje cells in the cerebellum, and in granular and pyramidal cells in the hippocampus. Employing immunohistochemical double labeling in area postrema and subfornical organ, expression of rOct3 was observed in neurons but not in astrocytes (Vialou et al. 2004). In contrast, in the dorsomedial hypothalamus (DMH) expression of rOct3 was detected in glial-like cells (Gasser et al. 2006). In the pineal gland, expression of rOct3 was observed in pinealocytes (Vialou et al. 2004). Moreover, rOct3 was located in ependymal cells (Gasser et al. 2006, 2009; Nakayama et al. 2007; Vialou et al. 2004) and in brain microvessels (Chaves et al. 2020). In mice, Oct3 (mOct3) was demonstrated in the BBB, in ependymal cells, and in many of the brain areas where expression of rOct3 was also observed in rats (Cui et al. 2009; Gasser et al. 2017; Vialou et al. 2008). Like rOct3, mOct3 was mainly located in neurons. In pars compacta of SN, expression of mOct3 was demonstrated in dopaminergic neurons (Vialou et al. 2008) whereas in the dorsal raphe, mOct3 was expressed in serotonergic neurons (Wyler et al. 2015). In various brain areas such as neostriatum, SN, and hypothalamus, expression of mOct3 was detected in astrocytes (Cui et al. 2009; Vialou et al. 2008). The expression of rOct3 and mOct3 in neurons, astrocytes, non-astrocyte glial cells, and capillary endothelial cells was confirmed with electron microscopic immunostaining in BLA of rat and mice (Gasser et al. 2017). In the endothelial cells, rOct3 and mOct3 were located to the luminal and abluminal plasma membrane (Fig. 2). In neurons, rOct3/mOct3 related immunoreactivity was observed at somatic plasma membranes, intracellular membranes, neurites and dendrites whereas the staining in glial cells was associated with somatic plasma membranes and cell processes (Gasser et al. 2017).

4.4.2 Specificity for Transport of Monoamine Neurotransmitters and Interaction with Psychiatric Drugs

Like the other organic cation transporters, OCT3 of humans and rodents transports NE, epinephrine, DA, 5-HT, and histamine (Table 2). For uptake of NE, epinephrine, DA, and histamine by hOCT3 similar K_m values were reported as for hOCT2. Similar K_m values were determined for uptake of NE by hOCT3 compared to human PMAT (hPMAT), higher K_m values for uptake of DA and 5-HT by hOCT3 versus hPMAT, and lower K_m values for uptake of histamine by hOCT3 versus hPMAT. Comparing OCT3 from humans and rats, the K_m values for NE, 5-HT, and DA were similar whereas an approximately threefold higher K_m value for epinephrine uptake by rOct3 compared to hOCT3 was determined. Like hOCT2, the K_m values for uptake of monoamine neurotransmitters by hOCT3 are orders of magnitude higher compared to hNET, hDAT, and hSERT. OCT3 extends the capacity for neurotransmitter reuptake in brain. Regional differences in expression compared to OCT1, OCT2, and PMAT and differences in regulation including the high sensitivity of OCT3 to corticosterone (Table 4) effectuate the diversification of neurotransmitter reuptake in brain.

Table 4 IC_{50} values for inhibition of cation uptake by organic cation transporters of humans and rodents by partial selective inhibitors

Compound		IC_{50} [μM]						
		NET/Net	DAT/Dat	SERT/Sert	OCT1/Oct1	OCT2/Oct2	OCT3/Oct3	PMAT/Pmat
Decynium 22	Human				0.98–12	0.1–10	0.09, 0.2	0.1–1.1
	Rat				0.36, 11			0.31
	(Mouse)	(35)	(13)	(7.9)	(5.3)	(0.43)		(0.48, 0.59)
Corticosterone	Human				7.0–22	5.4–80	0.12–0.62	430–1,059
	Rat				72, 151	4, 4.2	4.9, >10	
	(Mouse)	(840)	(364)	(445)		(8.7)	(11)	(n.i.d.)
β-Estradiol	Rat				>100	85	1.1	
Lopinavir	Human	n.i.d.	n.i.d.	36	174	n.i.d.	n.i.d.	1.4
	Rat							1.0
Mepiperhenidol	Rat				7.1	474		
Methylisoprenaline	Human							26
	Rat				37	2,600		
	(Mouse)			(8.4)	(8.4)	(>100)		
Amantadine	Human				10, 236	27, 28	>1,000	
	Rat				12	82	>1,000	
Phencyclidine	Human				4.4	25	333	
	Rat				0.16	16	3	
Memantine	Human				3.7, 27	7.3, 34	236	
	Rat				1.7	73	295	
RTI-55	Human	0.003, 0.023	0.003, 0.030	0.019, 0.0005				>100

n.i.d. no inhibition detected

Decynium 22: (Engel and Wang 2005; Engel et al. 2004; Floerl et al. 2020; Fraser-Spears et al. 2019; Gorboulev et al. 1997; Gründemann et al. 1994; Haenisch and Bönisch 2010; Hayer-Zillgen et al. 2002; Miura et al. 2017; Shirasaka et al. 2017; Zhang et al. 1998), **corticosterone**: (Arndt et al. 2001; Engel and Wang 2005; Engel et al. 2004; Fraser-Spears et al. 2019; Gründemann et al. 1998b, 2002; Hayer-Zillgen et al. 2002; Minematsu et al. 2010; Miura et al. 2017; Wu et al. 1998a; Zhang et al. 1998), **β-estradiol**: (Wu et al. 1998a), **lopinavir**: (Duan et al. 2015; Usui et al. 2016), **mepiperhenidol**: (Arndt et al. 2001), **O-methylisoprenaline**: (Arndt et al. 2001; Koepsell et al. 2007), **amantadine**: (Amphoux et al. 2006; Bednarczyk et al. 2003; Busch et al. 1998), **phencyclidine**: (Amphoux et al. 2006), **memantine**: (Ahlin et al. 2008; Amphoux et al. 2006; Busch et al. 1998), **RTI-55 (3β-(4-iodophenyl)-tropane-2-carboxylic acid)**: (Duan and Wang 2013; Torres et al. 2003)

Concerning OCT3 mediated transport of psychoactive drugs, transport has only been reported for sulpiride, berberine, amisulpride, and ketamine. The K_m values for transport of these drugs by hOCT3 are similar to those for transport by hOCT2 (Table 3). Twelve additional psychoactive drugs (amphetamine, metamphetamine, citalopram, sertraline, amitriptyline, imipramine, desipramine, memantine, phenytoin, morphine, ondansetron, and scopolamine) have been identified as inhibitors of hOCT3 but have not been tested for transport.

4.4.3 Presumed Biomedical Functions

Physiological and biomedical functions of OCT3 in brain were investigated employing rodents in which Oct3 was removed by genetic knockout or downregulated by antisense RNA technology. Some experiments were performed in combinations with inhibitors. Effects on physiological and psychological behaviors were measured using behavioral tests. In addition, effects of stress and treatment with stimulants were investigated. Moreover, the role of Oct3 in toxic degeneration of dopaminergic neurons was explored.

After Oct3 removal in mice, the concentrations of DA and NE in the SN and the ventral tegmental area and the concentration of histamine in thalamus and hypothalamus were decreased (Vialou et al. 2008). Employing cocaine and corticosterone as inhibitors the involvement of Oct3 in the clearance of catecholamines (DA and NE) after neuronal activation was investigated in rats (Holleran et al. 2020). Cocaine is a high affinity inhibitor of DAT whereas corticosterone inhibits rOct3 and rOct2 with higher affinity compared to rPmat (Table 4). After electric stimulation, the clearance rate of catecholamines in NAC and BLA was decreased by both cocaine and corticosterone. Since Oct3 but not Oct2 was detected in NAC (Table 1) these data suggest that Oct3 is critically involved in clearance of catecholamines.

In Oct3-KO mice the spontaneous ingestion of hypertonic saline in response to withdrawal of water and application of the sodium-wasting diuretic furosemide was 40% higher compared to WT mice (Vialou et al. 2004). These data suggest an impact of Oct3 on the regulation of osmolarity in neuronal circuits that involve neurons that are located in the subfornical organ, area postrema, hypothalamic paraventricular nuclei, and the NTS where rOct3 is expressed (Table 1).

The forced swim test (FST) was used on mice to elucidate whether Oct3 has an impact on depression. In the FST the time of immobility indicating behavioral despair was decreased when cerebral expression of mOct3 was decreased by injection of mOct3 antisense RNA into the third ventricle (Kitaichi et al. 2005). This suggests an anti-depressive effect due to impaired mOct3 mediated neurotransmitter removal from interstitial space.

Moreover, complementary functions of OCT3 and SERT were investigated. In Sert-KO mice, the expression of mOct3 was increased in hippocampus but not altered in cerebral cortex, striatum, brainstem, and cerebellum (Baganz et al. 2008; Schmitt et al. 2003). In contrast to WT, decynium 22 decreased the clearance rate of 5-HT injected into the CA3 region of the hippocampus in Sert-KO mice (Baganz et al. 2008). This is consistent with an inhibition of Oct3 mediated 5-HT uptake after elimination of Sert but could also be due to inhibition of 5-HT uptake by Oct2

(Table 4). The clinical impact of 5-HT transport by OCT3 and/or OCT2 during compromised function of SERT was suggested by the observation that decynium 22 decreased depressive-like behavior in Sert-KO mice measured in the tail suspension test (Baganz et al. 2008).

The impact of OCT3 on effects of the stimulants metamphetamine (METH) and amphetamine was explored. In mice and rats, the stimulative effect of METH on locomotion with and without downregulation of Oct3 was investigated (Kitaichi et al. 2005; Nakayama et al. 2007). It was observed that the METH induced stimulation of locomotion was amplified when the expression of mOct3/rOct3 was reduced by injection of Oct3 antisense RNA into the third ventricle. The application of METH to rats caused an increase of the interstitial DA concentration in prefrontal cortex and NAC, and this DA increase was reinforced when the expression of rOct3 was downregulated suggesting reuptake of DA by rOCT3 (Nakayama et al. 2007). After treating mice with amphetamine, an increase of neuronal activity of dopaminergic neurons was observed that was associated with an increase of DA in the vicinity of the dopaminergic neurons (Mayer et al. 2018). This increase of DA was blunted by decynium 22. The effect of decynium 22 was apparently due to inhibition of Oct3 because it was not observed in mOct3-KO mice. Supported by the observation that amphetamine stimulated cyanine 22-inhibitable MPP^+ efflux from MPP^+-preloaded neurons of the superior ganglion, the hypothesis was raised that amphetamine stimulates OCT3-mediated efflux of DA from dopaminergic neurons (Mayer et al. 2018).

Furthermore, the impact of OCT3 on cocaine addiction was investigated in rats in which expression of rOct3 was demonstrated in neurons of the NAC (Gasser et al. 2009). Glutaminergic and dopaminergic neuronal circuits in NAC have been shown to be involved in relapse of recovering cocaine addicts (Bachtell et al. 2005; Madayag et al. 2010). In addicts, the probability of relapse is increased by environmental factors associated with stress that activates the hypothalamic-pituitary-adrenal (HPA) axis and leads to an increase of plasma cortisol in humans and plasma corticosterone in rodents (Sinha et al. 2003). In rats, the impact of corticosterone sensitive Oct3 in NAC on stress related cocaine reward was investigated by measuring drug-seeking behavior after a period of cocaine self-administration that was followed by extinction and reinstatement (Graf et al. 2013). The reinstatement was primed with one administration of cocaine with and without preceding stress that had been induced by electrical foot-shock treatment. It turned out that dopamine receptor blockers blunted stress-increased reinstatement behavior. A stress-like effect was observed when corticosterone was applied systemically or injected into the NAC. In addition, it was observed that corticosterone decreased the dopamine clearance in the NAC. Evidence for the involvement of mOct3 was provided by the demonstration that cocaine-primed corticosterone-induced reinstatement of reward-like behavior observed in WT mice could not be detected in Oct3-KO mice.

Finally it was investigated in mice whether Oct3 in SN and striatum contributes to death of dopaminergic neurons after ingestion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Cui et al. 2009). MPTP is a protoxin that is metabolized to toxic MPP^+ (Javitch et al. 1985). In nigrostriatum MPP^+ is taken up by

dopaminergic neurons via Dat where it induces neuronal death causing Parkinson's disease (Dauer and Przedborski 2003; Javitch et al. 1985). Cui and coworkers observed that mOct3 is expressed in astrocytes in the vicinity of dopaminergic neurons (Cui et al. 2009). MPTP is transported into astrocytes and metabolized to MPP^+, and MPP^+ is released into the interstitial space via mOct3. The astrocytes tolerate the emerging intracellular MPP^+ concentrations. After removal of mOct3, the decay of dopaminergic neurons in nigrostriatum after application of MPTP was reduced indicating a critical involvement of mOct3 in neuronal poisoning (Cui et al. 2009).

4.5 PMAT (*SLC29A4*) in Brain

4.5.1 Locations in Brain

PMAT is broadly expressed in brain. In humans, PMAT was detected in the BBB, CP, medulla oblongata, pons, cerebellum, SN, putamen, caudate nucleus, cerebral cortex, hippocampus, and NAC (Dahlin et al. 2009; Duan and Wang 2010, 2013; Engel et al. 2004; Geier et al. 2013; Sekhar et al. 2019). In all regions except the caudate nucleus, the mRNA abundance of human PMAT (hPMAT) was higher compared to hOCT3 (Duan and Wang 2010). Human PMAT was located to the endothelial cells in the BBB (Sekhar et al. 2019), to the luminal membrane of choroid epithelial cells (Duan and Wang 2013), and to neurons (Dahlin et al. 2007; Engel et al. 2004). Expression of hPMAT was also observed in primary cultured human astrocytes (Yoshikawa et al. 2013). Also in rodents, Pmat was detected in the BBB, the CP, and various brain regions (Dahlin et al. 2007; Sekhar et al. 2019; Vialou et al. 2007; Wu et al. 2015a). In rodents, Pmat was observed in brain areas that contain diverse neuron populations, particularly cholinergic, glutamatergic, GABAergic, and noradrenergic neurons. In rodents, expression of Pmat was assigned to many specific locations (Dahlin et al. 2007; Vialou et al. 2007). Between others, Pmat was detected in several motor nuclei of medulla oblongata and mesencephalon including NTS, in specific areas of rhombencephalon (dorsal raphe, motor nuclei, medial vestibular nuclei), in the inferior olivary complex, in pontine nuclei, in cerebellar cortex, in ventral striatum, in lateral and ventral pallidum, in pars compacta of SN, in nucleus ruber, in ventral tegmental area, caudate putamen, septum (indusium griseum, diagonal band of Broca, and triangular nucleus), in thalamic nuclei (anteromedial, anterodorsal, and reticular nuclei), in hypothalamic nuclei (dorsomedial, lateral mammillary, tuberomammillary, arcuate, circular, and supraoptic nuclei), in the olfactory system (bulbus and olfactory tubercle), in various areas of cerebral cortex (amongst others telencephalon, piriform, lateral entorhinal, ectorhinal, insular, and temporal cortex), in hippocampus (regions CA1–CA3, dentate gyrus), and in amygdala (for example, in lateral amygdaloid nucleus). The immunostaining of Pmat was observed in neurons and astrocytes (Wu et al. 2015a). Immunohistochemical colocalization of mouse Pmat (mPmat) in striatum indicated mPmat in somata and fibers of most neurons (Dahlin et al. 2007). Co-labeling of rat brain using immunohistochemistry and *in situ*

hybridization revealed that rat Pmat (rPmat) was observed in many cholinergic but only few dopaminergic neurons (Vialou et al. 2007). In microvessels isolated from the brains of rats and mice Pmat was located to the luminal and abluminal membrane of endothelial cells (Wu et al. 2015a).

4.5.2 Specificity for Transport of Monoamine Neurotransmitters and Interaction with Psychiatric Drugs

Like OCT1-3, PMAT transports NE, DA, 5-HT, epinephrine, and histamine (Table 2). The K_m values are similar for NE uptake by hPMAT, hOCT2, and hOCT3. They are higher for histamine uptake by hPMAT compared to hOCT2 and hOCT3 but lower for uptake of DA and 5-HT by hPMAT compared to hOCT3. Psychotropic drugs have not been tested for transport by PMAT, however, citalopram, fluoxetine, sertraline, amitriptyline, imipramine, desipramine, trimipramine, clozapine, haloperidol, olanzapine, and risperidone were identified as inhibitors (Table 3). Additional experiments are required to elucidate whether PMAT is involved in drug uptake across the BBB, across the CSF–blood barrier (CSFBB) and/or into neurons, and whether clinically relevant drug–drug interactions exist in the brain.

4.5.3 Presumed Biomedical Functions

The strong expression of PMAT in various brain areas, the BBB, and the CSFBB implicates relevant biomedical functions. In the BBB, PMAT may be critically involved in removal of toxic compounds such as MPP^+ and MTPT from the brain (Fig. 2). PMAT may mediate uptake into the endothelial cells across the abluminal membrane and – at low intracellular pH – also efflux across the luminal membrane into the blood (Itagaki et al. 2012; Okura et al. 2011). In the luminal membrane of choroid epithelial cells PMAT is supposed to play a dominant role for cerebral clearance of toxic compounds and of histamine across the CSFBB (Duan and Wang 2013; Usui et al. 2016). Being expressed in astrocytes, PMAT may be also engaged in the clearance of histamine from the extracellular space close to histaminergic neurons (Yoshikawa et al. 2013).

Hosford and coworkers provided data from rats suggesting that 5-HT uptake by rPmat in the NTS is critically involved in cardiovascular regulation (Hosford et al. 2015). A stimulation of vagal afferents that slowed heartbeat and decreased blood pressure promoted release of 5-HT from serotonergic neurons in the NTS leading to a transient increase of 5-HT in the interstitial space. The 5-HT increase was unaffected by the SSRI citalopram but was reinforced by decynium 22. The slowdown of heartbeat after vagal stimulation was not influenced by citalopram but amplified by decynium 22. Since the clearance of locally applied 5-HT in NTS was slowed down by decynium 22 but not altered by corticosterone that inhibits rOct3 but probably not rPmat (Table 4), the effects of decynium 22 after vagal stimulation were assigned to Pmat.

Gilman and coworkers compared locomotor activity, anxiety-like behavior, and stress coping behaviors between Pmat-KO mice and WT mice using various behavioral tests (Gilman et al. 2018). With exception of a small gender independent

increase of anxiety-related behavior in heterozygous Pmat-KO mice and a mild increase in active coping behavior in female homozygous KO mice, no significant effects of Pmat removal were observed. Additional studies are required to elucidate whether the observed effects are due to compensatory upregulations in the KO mice or indicate a minor impact of Pmat on these behavior disorders when the other monoamine transporters are functional.

Changed functions of PMAT in the brain may also contribute to the emergence of ASD (Garbarino et al. 2019). ASD is a complex disorder comprising social behavior deficits and restrictive repetitive behavior that may be associated with anxiety, mood disorders, attention deficit, and hyperactivity. ASD is thought to be caused by changed 5-HT signaling in the brain during embryonic development that affects the maturation of serotonergic circuits. Amongst others, disturbed 5-HT signaling may be a consequence of dysregulation of cerebral 5-HT concentrations that may be caused by changed 5-HT reuptake. In eight of 284 patients with ASD three inherited non-synonymous mutations were identified in hPMAT that are extremely rare in non-diseased control individuals (Adamsen et al. 2014). Two mutations induced an impairment of hPMAT mediated 5-HT transport. The data suggest that changed function and expression of hPMAT may contribute to the emergence of ASD.

4.6 Potential of Inhibitors to Distinguish Between Transport by Different Organic Cation Transporters

The overlapping expression and selectivity of monoamine neurotransmitter transporters represent serious obstacles in determining the contributions of individual transporters to cerebral functions. This can be achieved by *in vivo* experiments in combination with genetic transporter knockout, decrease of transporter expression by antisense technology, and/or inhibition of transporters with selective inhibitors. Each of these methods has limitations. Specifically, these limitations are compensatory expression of transporters with overlapping selectivity in knockout animals, incomplete decrease of transporter expression by antisense technology, and usage of inhibitors with inadequate selectivity. In Table 4, IC_{50} values are compiled that have been reported to inhibit cation uptake by human and rodent monoamine neurotransmitter transporters. Conspicuously, none of the inhibitors have been tested for all relevant transporters in humans, rats, and mice. Comparing human transporters, it is noteworthy that the reported IC_{50} values vary considerably. The differences are thought to be due to usage of different substrates and substrate concentrations for inhibition experiments as discussed earlier (Koepsell 2019, 2020). Species differences between humans and rodents also exist. For example, whereas corticosterone has an at least 8.7-fold lower IC_{50} value for hOCT3 compared to hOCT2, the IC_{50} values for rOct3 and rOct2 are not different. Unfortunately, for mouse transporters, only few IC_{50} values have been reported. In summary, for a valid distinction between individual monoamine transporters with inhibitors, the functionally relevant IC_{50} values should be known. Strictly spoken, these should be the IC_{50} values for inhibition of transport by the transporter of the investigated species using

the substrate of interest at its biomedically relevant concentration. On the basis of existing data, it may be argued that it is possible to distinguish between transport by PMAT/Pmat and OCT3/Oct3 in humans and rodents using corticosterone, between hPMAT and the other transporters in humans using lopinavir, between Oct1 and Oct2 in rodents using methylisoprenaline, between OCT3/Oct3 and OCT2/Oct2 in humans and rats using amantadine, and between hPMAT and the high affinity Na^+/Cl^- dependent monoamine neurotransmitter transporters in human using RTI-55.

5 Conclusions

Sophisticated experiments in rodents have indicated the impact of organic cation transporters on complex cerebral functions like anxiety, mood control, and motor activity and on their modulatory functions during medical treatment of psychiatric disorders. These data implicate the possibility of developing a new generation of more effective psychoactive drugs that inhibit monoamine neurotransmitter reuptake by high and low affinity neurotransmitter transporters (Orrico-Sanchez et al. 2020). By critically appraising previous achievements and calling attention to the incompleteness of the current data basis, we hope to stimulate future investigations in this research area. The validation of current knowledge that is nearly exclusively based on experiments with rodents, for humans is a great challenge for the future. This can be achieved by employing defect mutations in human transporters and/or drugs with molecular characterized targets.

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Organic Cation Transporter Expression and Function in the CNS

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Contents

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Abstract

The blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB) represent major control checkpoints protecting the CNS, by exerting selective control over the movement of organic cations and anions into and out of the CNS compartment. In addition, multiple CNS cell types, e.g., astrocytes, ependymal cells, microglia, contribute to processes that maintain the status quo of the CNS milieu. To fulfill their roles, these barriers and cell types express a multitude of transporter proteins from dozens of different transporter families. Fundamental advances over the past few decades in our knowledge of transporter substrates, expression profiles, and consequences of loss of function are beginning to change basic theories regarding the contribution of various cell types and clearance networks to coordinated neuronal signaling, complex organismal behaviors, and overall CNS homeostasis. In particular, transporters belonging to the Solute Carrier (SLC) superfamily are emerging as major contributors, including the SLC22 organic cation/anion/zwitterion family of transporters (includes OCT1–3 and OCTN1–3), the SLC29 facilitative nucleoside family of transporters (includes PMAT), and the SLC47 multidrug and toxin extrusion family of transporters (includes MATE1–2). These transporters are known to interact with neurotransmitters, antidepressant and anxiolytic agents, and drugs of abuse. Clarifying their contributions to the underlying mechanisms regulating CNS permeation and clearance, as well as the health status of astrocyte, microglial and neuronal cell populations, will drive new levels of understanding as to maintenance of the CNS milieu and approaches to new therapeutics and therapeutic strategies in the treatment of CNS disorders. This chapter highlights organic cation transporters belonging to the SLC superfamily known to be expressed in the CNS, providing an overview of their identification, mechanism of action, CNS expression profile, interaction with neurotransmitters and antidepressant/antipsychotic drugs, and results from behavioral studies conducted in loss of function models (knockout/knockdown).

Keywords

Blood–brain barrier · Blood–cerebrospinal fluid barrier · Brain capillaries · Choroid plexus · Olfactory mucosa · Solute carrier

1 Introduction

The transmembrane flux of charged organic molecules into (uptake) and out of (efflux) cells has been investigated as a physiological phenomenon for centuries. As such, the complex energetics governing renal and hepatic cellular uptake and efflux of small (~300–600 Da) organic anions and cations, i.e., ATP hydrolysis, inorganic ion gradients, and the potential difference across the plasma membrane (negative intracellular environment), were exhaustively characterized. Models

integrating the interplay of these processes were developed and invoked the existence of specific membrane embedded proteins, i.e., transporters, as the gatekeepers of each step (Wright and Dantzler 2004). Moreover, extensive literature based upon a multitude of comparative models confirmed that charged organic solute transport in organs and tissues such as the kidney, bladder, liver, intestine, brain capillaries, and choroid plexus was physiologically identical (across both tissues and species).

The blood–brain barrier (BBB), formed by the brain microvasculature, and the blood–cerebrospinal fluid barrier (BCSFB), comprised of the choroid plexus, arachnoid villi, and circumventricular organs, serve to protect the brain from fluctuations in systemic circulation and to preserve the brain milieu by tightly controlling CNS levels of endogenous substances (e.g., waste-products of cellular metabolism, uremic toxins, and neurotransmitters) and xenobiotics (e.g., drugs, environmental toxins/toxicants). Similar to the barrier function of intestinal, hepatic, and renal membranes, together, the BBB and BCSFB effectively modulate the composition of extracellular fluid of the brain (interstitial fluid and CSF) via anatomical (e.g., tight junctions) and biochemical (e.g., transporter proteins) mechanisms. Thus, identification of the transporters present in these barriers and specialized CNS cellular subtypes (e.g., ependymal cells, astrocytes, and neurons) is fundamental to understanding overall maintenance of CNS homeostasis, including effective neurotransmission, the onset of depression/mood disorders, and other CNS disease states, as well as the efficacy of drugs developed to treat them.

In order for efficient neurotransmission to be maintained, post release, neurotransmitters remaining in the area of the synaptic cleft must be removed as rapidly and completely as possible to terminate the response and prepare for the next neuronal signaling event. It has been known for decades that members of the SLC6 transporter family, the norepinephrine (NET, SLC6A2), dopamine (DAT, SLC6A3) and serotonin (SERT, SLC6A4) transporters, comprise the Na^+-dependent, high affinity/low capacity neuronal uptake (uptake 1) pathways charged with the removal of released monoamine neurotransmitters (Kristensen et al. 2011). For a similar period, it was also known that extraneuronal, Na^+-independent, low affinity/high capacity uptake (uptake 2) of catecholamines and choline existed in brain/glial cells (Yamamura and Snyder 1973; Russ et al. 1996). However, this pathway was not considered to be poised to play a significant role in the cessation of neurotransmission, onset of mood disorders, or the action of therapeutics designed to manipulate catecholamine levels (i.e., antidepressants). Recently, this view has begun to change, with increasing evidence supporting an important contribution of organic cation transporters expressed in CNS barrier membranes and cell types to the overall maintenance of CNS homeostasis via control over CNS permeation of small endogenous organic solutes and drugs, as well as clearance of neurotransmitters and their metabolites from the interstitial fluid/CSF.

The Solute Carrier (SLC) superfamily of transporters, which currently is comprised of 65 distinct transporter families with over 450 individual transport proteins, contains a multitude of known organic cation transporters. Three families in particular within the SLC superfamily, family 22 (SLC22): organic cation/anion/zwitterion transporters, family 29 (SLC29): facilitative nucleoside transporters and

family 47 (SLC47): multidrug and toxin extrusion transporters, contain the organic cation transporters highlighted in this chapter; organic cation transporter 1 (OCT1, SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), organic cation transporter novel 1 (OCTN1, SLC22A4), OCTN2 (SLC22A5), OCTN3 (SLC22A21), plasma membrane monoamine transporter (PMAT, SLC29A4), multidrug and toxin extrusion transporter 1 (MATE1, SLC47A1) and MATE2-K (SLC47A2).

Expression of each of these transporters has been detected in the brains of various species including rat, mouse, rabbit and humans, consistent with their participation in the disposition of their substrates within the CNS (Tables 1 and 2) (Farthing and Sweet 2014; Wang 2016; Koepsell 2020). Further, many endogenous amines (i.e., choline, dopamine, epinephrine, histamine, norepinephrine and serotonin) and therapeutic agents (i.e., amphetamine, desipramine, fluoxetine, morphine, sertraline) that function in or target the CNS are transported by and/or inhibit OCTs, PMAT and MATEs, supporting the contention that they play a prominent role in the CNS, both in terms of maintaining homeostasis and in contributing to the therapeutic response of many CNS acting drugs (Tables 3 and 4) (Farthing and Sweet 2014; Wang 2016; Koepsell 2020). Finally, it has been demonstrated in vivo that genetic or pharmacological blockade of their transport function correlates with altered behaviors and/or increased monoamines/monoamine metabolites in the CNS compartment. For example, introduction of decynium-22 (a pan OCT inhibitor) to the medial hypothalamus of rats induced a dose-dependent increase in extracellular serotonin (Feng et al. 2005). These findings clearly suggest a prominent role for these transporters in the CNS, a complete understanding of which is still lacking.

The focus of this chapter is to provide an overview of the current knowledge regarding organic cation transporter (OCT, PMAT, MATE) expression and function within the CNS. A summary of the biochemical and functional evidence supporting SLC22, SLC29 and SLC47 family members as being relevant to CNS handling of endogenous and xenobiotic organic cations is provided in the following sections. The summaries focus on gene/protein expression solely in CNS tissues and primary cell types relevant to the review topic. To gain greater appreciation for the original cloning, characterization and broad (non-CNS) tissue expression profiles of the nine transporters featured in this work, the reader is referred to the following comprehensive reviews (Farthing and Sweet 2014; Wang 2016; Koepsell 2020).

Table 1 Cation transporter mRNA and protein expression profiles in major CNS tissues/regions

Transporter	Species	Target	Cerebral cortex	Cerebellum	Hippocampus	Hypothalamus	Amygdala	Thalamus	Striatum	Choroid plexus
OCT1 (SLC22A1)	Mouse	mRNA	André et al. (2012)		Baganz et al. (2008) Schmitt et al. (2003)					
		Protein	Wu et al. (2015a)						Wu et al. (2015a)	
	Rat	mRNA	Slitt et al. (2002)	Amphoux et al. (2006) Slitt et al. (2002)						
		Protein								
	Human	mRNA	Geier et al. (2013)							Duan and Wang (2013)
		Protein								
OCT2 (SLC22A2)	Mouse	mRNA	André et al. (2012) Miura et al. (2017)	Miura et al. (2017)	Miura et al. (2017)	Miura et al. (2017)		Miura et al. (2017)	Miura et al. (2017)	Duan and Wang (2013)
		Protein	Bacq et al. (2012) Wu et al. (2015a)		Bacq et al. (2012) Couroussé et al. (2015)	Couroussé et al. (2015)	Bacq et al. (2012) Couroussé et al. (2015)	Couroussé et al. (2015)	Couroussé et al. (2015) Wu et al. (2015a)	
	Rat	mRNA	Slitt et al. (2002)	Amphoux et al. (2006) Slitt et al. (2002)	Amphoux et al. (2006)					Amphoux et al. (2006) Sweet et al. (2001)
		Protein								
	Human	mRNA	Busch et al. (1998) Geier et al. (2013)		Busch et al. (1998)		Busch et al. (1998)	Busch et al. (1998)		Duan and Wang (2013)
		Protein	Busch et al. (1998)		Busch et al. (1998)					
OCT3 (SLC22A3)	Mouse	mRNA	André et al. (2012) Miura et al. (2017) Schmitt et al. (2003)	Miura et al. (2017) Schmitt et al. (2003)	Baganz et al. (2008) Miura et al. (2017) Schmitt et al. (2003)	Miura et al. (2017)		Miura et al. (2017)	Miura et al. (2017)	
		Protein	Vialou et al. (2008)	Cui et al. (2009)	Baganz et al. (2008) Vialou et al. (2008)	Vialou et al. (2008)			Cui et al. (2009) Vialou et al. (2008)	
	Rat	mRNA	Marcinkiewcz and Devine (2015)	Slitt et al. (2002)	Amphoux et al. (2006)	Amphoux et al. (2006)		Amphoux et al. (2006)	Amphoux et al. (2006)	Sweet et al. (2001)

(continued)

Table 1 (continued)

Transporter	Species	Target	Cerebral cortex	Cerebellum	Hippocampus	Hypothalamus	Amygdala	Thalamus	Striatum	Choroid plexus
			Slitt et al. (2002) Wu et al. (1998a)	Wu et al. (1998a)	Marcinkiewcz and Devine (2015) Wu et al. (1998a)	Gasser et al. (2006)			Marcinkiewcz and Devine (2015)	
		Protein	Gasser et al. (2009) Vialou et al. (2004)	Gasser et al. (2009) Vialou et al. (2004)	Gasser et al. (2009) Marcinkiewcz and Devine (2015) Vialou et al. (2004)	Gasser et al. (2006) Vialou et al. (2004)	Gasser et al. (2009) Hill and Gasser (2013) Holleran et al. (2020)	Gasser et al. (2009) Vialou et al. (2004)	Gasser et al. (2009) Holleran et al. (2020)	Gasser et al. (2009) Vialou et al. (2004)
	Human	mRNA	Geier et al. (2013)							Duan and Wang (2013)
		Protein		Cui et al. (2009)						
OCTN1 (SLC22A4)	Mouse	mRNA	Nakamichi et al. (2012)	Nakamichi et al. (2012)	Nakamichi et al. (2012)	Nakamichi et al. (2012)			Nakamichi et al. (2012)	
		Protein	Lamhonwah et al. (2008) Nakamichi et al. (2012)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)				Lamhonwah et al. (2008)
	Rat	mRNA	Wu et al. (2000) Slitt et al. (2002)	Wu et al. (2000) Slitt et al. (2002)	Wu et al. (2000)					
		Protein								
OCTN2 (SLC22A5)	Mouse	mRNA								
		Protein	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)				Lamhonwah et al. (2008)
	Rat	mRNA	Slitt et al. (2002) Wu et al. (1999) Januszewicz et al. (2009)	Slitt et al. (2002) Wu et al. (1999)	Wu et al. (1999)					
		Protein	Januszewicz et al. (2009)							
	Human	mRNA	Geier et al. (2013)							
		Protein								

OCTN3 (SLC22A21)	Mouse	mRNA								
		Protein	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)	Lamhonwah et al. (2008)				Lamhonwah et al. (2008)
	Rat	mRNA	Januszewicz et al. (2009)							
		Protein	Januszewicz et al. (2009)							
PMAT (SLC29A4)	Mouse	mRNA	André et al. (2012) Dahlin et al. (2007) Miura et al. (2017) Wu et al. (2015b)	Dahlin et al. (2007) Miura et al. (2017) Wu et al. (2015b)	Dahlin et al. (2007) Miura et al. (2017) Wu et al. (2015b)	Dahlin et al. (2007) Miura et al. (2017)	Dahlin et al. (2007)	Dahlin et al. (2007) Miura et al. (2017)	Dahlin et al. (2007) Miura et al. (2017)	Dahlin et al. (2007) Duan and Wang (2013)
		Protein	Dahlin et al. (2007) Wu et al. (2015b)	Dahlin et al. (2007)	Dahlin et al. (2007)	Dahlin et al. (2007)	Dahlin et al. (2007)	Dahlin et al. (2007)	Dahlin et al. (2007) Wu et al. (2015b)	Dahlin et al. (2007) Duan and Wang (2013)
	Rat	mRNA	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)	Vialou et al. (2007)
		Protein								
	Human	mRNA	Engel et al. (2004)	Engel et al. (2004)						Duan and Wang (2013)
		Protein								Duan and Wang (2013) Uchida et al. (2015)
MATE1 (SLC47A1)	Mouse	mRNA	André et al. (2012)							Duan and Wang (2013)
		Protein								
	Human	mRNA	Geier et al. (2013)							Duan and Wang (2013)
		Protein								Uchida et al. (2015)
MATE2-K (SLC47A2)	Mouse	mRNA	Geier et al. (2013)	Masuda et al. (2006)						Duan and Wang (2013)
		Protein								
	Human	mRNA								Duan and Wang (2013)
		Protein								

(continued)

Table 2 Cation transporter mRNA and protein expression profiles in major CNS cell types

Transporter	Species[a]	Target	Neurons	Astrocytes/other glial cells[b]	Primary brain vascular endothelial cells	Intact brain microvessels
OCT1 (SLC22A1)	Mouse	mRNA				Wu et al. (2015a)
		Protein			Lin et al. (2010)	Wu et al. (2015a)
	Rat	mRNA		Inazu et al. (2005)		Chaves et al. (2020)
		Protein			Lin et al. (2010)	
	Human	mRNA				Geier et al. (2013)
		Protein			Lin et al. (2010)	
OCT2 (SLC22A2)	Mouse	mRNA				Chaves et al. (2020), Wu et al. (2015a)
		Protein	Bacq et al. (2012) Couroussé et al. (2015)	He et al. (2017) **M**	Lin et al. (2010)	Wu et al. (2015a)
	Rat	mRNA		Inazu et al. (2005)		Chaves et al. (2020)
		Protein			Lin et al. (2010)	
	Human	mRNA	Busch et al. (1998)			Geier et al. (2013)
		Protein	Busch et al. (1998)		Lin et al. (2010)	
OCT3 (SLC22A3)	Mouse	mRNA	Mayer et al. (2018) Schmitt et al. (2003)			André et al. (2012), Wu et al. (2015a), Chaves et al. (2020)
		Protein	André et al. (2012) Cui et al. (2009) Gasser et al. (2017)	Cui et al. (2009) Gasser et al. (2017) Vialou et al. (2008) **E**		

			Vialou et al. (2008)			
	Rat	mRNA		Inazu et al. (2005)		Chaves et al. (2020)
		Protein	Gasser et al. (2017) Hill and Gasser (2013) Vialou et al. (2004)	Gasser et al. (2017) Vialou et al. (2004) **E**		
	Human	mRNA		Inazu et al. (2003) Yoshikawa et al. (2013)	Li et al. (2013)	Geier et al. (2013)
		Protein		Inazu et al. (2003) Yoshikawa et al. (2013)	Li et al. (2013)	Geier et al. (2013)
OCTN1 (SLC22A4)	Mouse	mRNA	Nakamichi et al. (2012)			
		Protein	Lamhonwah et al. (2008) Nakamichi et al. (2012)			
OCTN2 (SLC22A5)	Mouse	mRNA				
		Protein	Lamhonwah et al. (2008)			
	Rat	mRNA	Januszewicz et al. (2010)	Inazu et al. (2006) Januszewicz et al. (2009) Miecz et al. (2008)	Kido et al. (2001)	
		Protein	Januszewicz et al. (2010)	Inazu et al. (2006) Januszewicz et al. (2009) Miecz et al. (2008)		

(continued)

Table 2 (continued)

Transporter	Species[a]	Target	Neurons	Astrocytes/other glial cells[b]	Primary brain vascular endothelial cells	Intact brain microvessels
	Bovine	mRNA			Berezowski et al. (2004)	
		Protein				
	Human	mRNA			Kido et al. (2001)	Geier et al. (2013)
		Protein				
OCTN3 (SLC22A21)	Mouse	mRNA				
		Protein	Lamhonwah et al. (2008)			
	Rat	mRNA	Januszewicz et al. (2010)	Januszewicz et al. (2009)		
		Protein	Januszewicz et al. (2010)	Januszewicz et al. (2009)		
PMAT (SLC29A4)	Mouse	mRNA	Dahlin et al. (2007)		Wu et al. (2015b)	André et al. (2012) Wu et al. (2015b)
		Protein	André et al. (2012) Dahlin et al. (2007)		Wu et al. (2015b)	Wu et al. (2015b)
	Rat	mRNA	Vialou et al. (2007)	Wu et al. (2015b) **P**	Wu et al. (2015b)	
		Protein			Wu et al. (2015b)	
	Human	mRNA		Yoshikawa et al. (2013)	Li et al. (2013) Wu et al. (2015b)	
		Protein		Yoshikawa et al. (2013)	Li et al. (2013) Wu et al. (2015b)	

MATE1 (SLC47A1)	Mouse	mRNA				Chaves et al. (2020)
		Protein				
	Human	mRNA				Geier et al. (2013)
		Protein				Geier et al. (2013)
MATE2-K (SLC47A2)	Human	mRNA				Geier et al. (2013)
		Protein				

[a]Mice may exhibit strain differences in expression
[b]"Other glial cells" denotes non-astrocyte cell types as indicated in the table by: *M* microglia, *E* ependymal cells, *P* pericytes

Table 3 Neurotransmitter interaction with organic cation transporters expressed in the CNS

Neurotransmitter	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
Acetylcholine	hOCT1			580 ± 110	Lips et al. (2005)
	hOCT2	117 ± 13		149 ± 22	Lips et al. (2005)
	hOCTN1	1,000 ± 210			Pochini et al. (2012)
Dopamine	rOct1	1,600 ± 300			Amphoux et al. (2006)
		51			Koepsell (1998)
			1,100		Breidert et al. (1998)
	hOCT1			487 ± 43	Bednarczyk et al. (2003)
	rOct2	2,100 ± 200			Amphoux et al. (2006)
		2,300			Gründemann et al. (1999)
		650			Koepsell (1998)
				2,300 ± 200	Wu et al. (1998a)
	hOCT2	1,400 ± 200			Amphoux et al. (2006)
		330 ± 130			Busch et al. (1998)[b]
		390 ± 160			Busch et al. (1998)
		932 ± 77			Zolk et al. (2009)
	rOct3	1,500 ± 200			Amphoux et al. (2006)
				620 ± 40	Wu et al. (1998a)[c]
				384	Wu et al. (1998a)
	mOct3	785 ± 18			Miura et al. (2017)
	hOCT3	1,033 ± 127			Duan and Wang (2010)
		800			Zhu et al. (2010)

(continued)

Table 3 (continued)

Neurotransmitter	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
	rPmat	271 ± 50			Shirasaka et al. (2017)
	mPmat	160 ± 57			Miura et al. (2017)
		466 ± 106			Shirasaka et al. (2017)
	hPMAT	406 ± 48			Duan and Wang (2010)
		329 ± 8			Engel et al. (2004)
		201 ± 34			Shirasaka et al. (2017)
Epinephrine	rOct1	1,100 ± 300			Amphoux et al. (2006)
	rOct2	1,370 ± 330			Amphoux et al. (2006)
	hOCT2	420 ± 100			Amphoux et al. (2006)
	rOct3	1,500 ± 200			Amphoux et al. (2006)
	hOCT3	240 ± 50			Amphoux et al. (2006)
		458 ± 37			Duan and Wang (2010)
	hPMAT	951 ± 59			Duan and Wang (2010)
		15,323 ± 3,947			Engel et al. (2004)
Histamine	rOct1		1,400		Gründemann et al. (1999)
	hOCT1			3,007 ± 27	Bednarczyk et al. (2003)
	rOct2	890 ± 140			Amphoux et al. (2006)
		540	390		Gründemann et al. (1999)
	mOct2	111 ± 29			Miura et al. (2017)
	rbOct2			427 ± 135	Suhre et al. (2005)
	hOCT2	940 ± 150			Amphoux et al. (2006)

(continued)

Table 3 (continued)

Neurotransmitter	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
		1,300 ± 300			Busch et al. (1998)
		180	140		Gründemann et al. (1999)
				3,251 ± 497	Suhre et al. (2005)
	rOct3	540 ± 90			Amphoux et al. (2006)
	mOct3	1,670 ± 290			Miura et al. (2017)
				1,400	Massmann et al. (2014)
	hOCT3	220 ± 60			Amphoux et al. (2006)
		641 ± 24			Duan and Wang (2010)
				1,100	Massmann et al. (2014)
	mPmat	1,520 ± 270			Miura et al. (2017)
	hPMAT	4,379 ± 679			Duan and Wang (2010)
		10,471 ± 2,550			Engel and Wang (2005)
	hMATE1			761 ± 196	Astorga et al. (2012)
	hMATE2-K			775 ± 148	Astorga et al. (2012)
Norepinephrine	rOct1	800 ± 250			Amphoux et al. (2006)
			2,800		Breidert et al. (1998)
	rOct2	2,100 ± 400			Amphoux et al. (2006)
				11,000 ± 300	Wu et al. (1998a)
	hOCT2	1,500 ± 100			Amphoux et al. (2006)
		1,900 ± 600			Busch et al. (1998)
		510			Gründemann et al. (1998)

(continued)

Table 3 (continued)

Neurotransmitter	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
		5,452 ± 498			Zolk et al. (2009)
	rOct3	1,900 ± 380			Amphoux et al. (2006)
				434	Wu et al. (1998a)
	mOct3	566 ± 106			Miura et al. (2017)
		336 ± 73			Song et al. (2019)
	hOCT3	2,630 ± 80			Amphoux et al. (2006)
		923 ± 172			Duan and Wang (2010)
		182 ± 28			Song et al. (2019)
		1,030			Zhu et al. (2010)
		629 ± 151			Zhu et al. (2012a)
	mPmat	515 ± 128			Miura et al. (2017)
	hPMAT	1,078 ± 107			Duan and Wang (2010)
		2,606 ± 258			Engel et al. (2004)
Serotonin	rOct1	900 ± 500			Amphoux et al. (2006)
			650		Breidert et al. (1998)
	hOCT1	197 ± 42			Boxberger et al. (2014)
	rOct2	760 ± 50			Amphoux et al. (2006)
	mOct2	313 ± 68			Miura et al. (2017)
	rbOct2			664 ± 44	Suhre et al. (2005)
	hOCT2	290 ± 60			Amphoux et al. (2006)
		80 ± 20			Busch et al. (1998)

(continued)

Table 3 (continued)

Neurotransmitter	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
				310 ± 18	Suhre et al. (2005)
	rOct3	500 ± 100			Amphoux et al. (2006)
				970 ± 180	Wu et al. (1998a)
	mOct3	430 ± 188			Miura et al. (2017)
				1,800	Massmann et al. (2014)
	hOCT3	988 ± 264			Duan and Wang (2010)
		900			Zhu et al. (2010)
		510 ± 188			Zhu et al. (2012a)
				4,200	Massmann et al. (2014)
	rPmat	82 ± 21			Shirasaka et al. (2017)
	mPmat	120 ± 50			Miura et al. (2017)
		231 ± 22			Shirasaka et al. (2017)
	hPMAT	283 ± 40			Duan and Wang (2010)
		114 ± 12			Engel et al. (2004)
		116 ± 27			Shirasaka et al. (2017)
	hMATE1			29 ± 5	Astorga et al. (2012)
	hMATE2-K			18 ± 1	Astorga et al. (2012)

[a]Species are designated as follows: *r* rat, *m* mouse, *rb* rabbit, *h* human
[b]Reference reported independent K_m estimates against two different substrates
[c]Reference reported independent IC_{50} estimates against two different substrates

Table 4 Antidepressant/antipsychotic interaction with organic cation transporters expressed in the CNS

Compound	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
Amisulpride	hOCT1	31.3 ± 5.4			Pereira et al. (2014)
	hOCT2	167.9 ± 32.1			Pereira et al. (2014)
	hOCT3	191.9 ± 6.1			Pereira et al. (2014)
	hOCTN1	179.9 ± 20.1			Pereira et al. (2014)
	hOCTN2	185.3 ± 68.0			Pereira et al. (2014)
Berberine	rOct1			18.8	Shi et al. (2019)
	hOCT1	14.8 ± 3.3			Nies et al. (2008)
	rOct2	13.2			Shi et al. (2018)
				1.0	Shi et al. (2019)
	hOCT2	4.4 ± 0.6			Nies et al. (2008)
		1.0 ± 0.2		8.0	Sun et al. (2014)[b]
				0.4	Sun et al. (2014)
				0.4	Sun et al. (2014)
	hOCT3	2.2 ± 0.2		9.9	Sun et al. (2014)[b]
				0.2	Sun et al. (2014)
				0.6	Sun et al. (2014)
	rMate1	4.3 ± 2.2			Xiao et al. (2018)
				10.7	Shi et al. (2019)
Bupropion	hOCT1			161	Haenisch et al. (2012)
	hOCT2			28.6	Haenisch et al. (2012)
				8.6 ± 1.8	Sandoval et al. (2018)[b]
				32.2 ± 5.4	Sandoval et al. (2018)
				13.4 ± 2.8	Sandoval et al. (2018)
				10.8 ± 1.0	Sandoval et al. (2018)
				11.1 ± 1.6	Sandoval et al. (2018)
	hOCT3			738	Haenisch et al. (2012)
	hPMAT			115	Haenisch and Bönisch (2010)
Desipramine	rOct1		2.8 ± 0.6		Grundemann et al. (1994)

(continued)

Table 4 (continued)

Compound	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
	hOCT1		5.4		Zhang et al. (1998)
				1.9	Haenisch et al. (2012)
	hOCT2		16 ± 4		Gorboulev et al. (1997)
				74.5	Haenisch et al. (2012)
	rOct3			68 ± 2	Wu et al. (1998a)
	hOCT3			72.1	Haenisch et al. (2012)
				4.7	Zhu et al. (2012a)
	rOCTN1			180 ± 10	Wu et al. (2000)
	hPMAT		32.6 ± 2.7		Engel et al. (2004)
Fluoxetine	hOCT1			6.2 ± 1.2	Boxberger et al. (2014)
				2.8	Haenisch et al. (2012)
				6.0 ± 0.3	Tzvetkov et al. (2013)
				9.1 ± 3.2	Zhu et al. (2018)
	hOCT2			28.5	Haenisch et al. (2012)
				16.7 ± 0.8	Sandoval et al. (2018)[b]
				18.6 ± 1.5	Sandoval et al. (2018)
				20.1 ± 2.6	Sandoval et al. (2018)
				36.0 ± 5.4	Sandoval et al. (2018)
				56.7 ± 4.5	Sandoval et al. (2018)
				3.3 ± 0.8	Zhu et al. (2018)
	mOct3			50	Massmann et al. (2014)
	hOCT3			37.8	Haenisch et al. (2012)
				27	Massmann et al. (2014)
				21.9	Zhu et al. (2012a)
	hPMAT		22.7 ± 6.1		Engel et al. (2004)
				10.5	Haenisch and Bönisch (2010)
				28.4 ± 7.2	Zhou et al. (2007)

(continued)

Table 4 (continued)

Compound	Transporter[a]	Kinetic estimates (μM)			Reference
		K_m	K_i	IC_{50}	
Imipramine	hOCT1			18.4	Haenisch et al. (2012)
				6.2 ± 1.4	Tzvetkov et al. (2013)
				4.0 ± 1.6	Zhu et al. (2018)
	hOCT2			14.6	Haenisch et al. (2012)
				0.5 ± 0.1	Zhu et al. (2018)
	hOCT3			54.2	Haenisch et al. (2012)
				11.2	Zhu et al. (2012a)
	hPMAT			21.1	Haenisch and Bönisch (2010)
Sertraline	hOCT1			9.3	Haenisch et al. (2012)
	hOCT2			25.8	Haenisch et al. (2012)
	hOCT3			25.7	Haenisch et al. (2012)
	hPMAT			5.1	Haenisch and Bönisch (2010)
				13.5 ± 1.7	Zhou et al. (2007)
				7.4	Zhu et al. (2012a)
Sulpiride	hOCT1	259.7 ± 5.4			Pereira et al. (2014)
		2.6 ± 0.6		182 ± 42	Takano et al. (2017)
	hOCT2	390 ± 59			Li et al. (2017)
		187.2 ± 21.6			Pereira et al. (2014)
		68.6 ± 45.9			Takano et al. (2017)
	hOCT3	160 ± 33			Bai et al. (2017)
	hOCTN1	250 ± 36			Li et al. (2017)
	hOCTN2	235 ± 35			Li et al. (2017)
	hMATE1	14.6 ± 1.6			Li et al. (2017)
		39.9 ± 1.9			Takano et al. (2017)
	hMATE2-K	25.5 ± 4.3			Li et al. (2017)
		60.3 ± 6.1			Takano et al. (2017)

[a]Species are designated as follows: *r* rat, *m* mouse, *rb* rabbit, *h* human
[b]Reference reported independent IC_{50} estimates against multiple substrates

2 Organic Cation Transporters Relevant to the CNS

2.1 OCT1 (*SLC22A1*)

Mechanistically, hOCT1,[1] rOct1 and rbOct1 were demonstrated to function as facilitated diffusion carriers, using the electrochemical gradient as a driving force (Gründemann et al. 1994; Zhang et al. 1997; Terashita et al. 1998). A murine ortholog has also been identified and characterized (Green et al. 1999). Among OCT/Oct1–3, OCT1/Oct1 has the smallest reported expression profile in the CNS, regardless of species (Tables 1 and 2). Most OCT1/Oct1 expression data are message based, with mRNA largely being confined to cortex and brain microvessels (see note below regarding brain capillary preparations), but also observed in human choroid plexus (but not rat) and murine olfactory mucosa (Tables 1 and 2) (Sweet et al. 2001; Slitt et al. 2002; Monte et al. 2004; André et al. 2012; Duan and Wang 2013; Geier et al. 2013; Wu et al. 2015a; Chaves et al. 2020). More recently, protein expression in primary brain vascular endothelial cells and intact brain microvessels has been reported in mice, rats and humans (Lin et al. 2010; Wu et al. 2015a). In general, neurotransmitters are poor substrates or inhibitors of OCT/Oct1, yielding values (K_m, K_i, IC_{50}) in the hundreds of micromolar to millimolar range (Table 3). By far, dopamine (IC_{50} = 487 μM) and serotonin (K_m = 197 μM) exhibit the most potent interaction with hOCT1 (Bednarczyk et al. 2003; Boxberger et al. 2014). Weak inhibition of hOCT1 by acetylcholine was also reported, however, direct confirmation as a substrate was not explored (Lips et al. 2005). In contrast, many antidepressant/antipsychotic therapeutics exhibit stronger hOCT1 interactions (K_m, K_i, IC_{50}), with values in the low to tens of micromolar range (Table 4). To date, most of the data are IC_{50} estimates, however, both amisulpride (K_m = 31 μM) and berberine (K_m = 15 μM) represent relatively potent hOCT1 substrates (Nies et al. 2008; Pereira et al. 2014). Human OCT1 stands out as recognizing amisulpride with ~five- to sixfold stronger affinity than hOCT2, hOCT3, hOCTN1 and hOCTN2. Illustrating that much more work needs to be done in this area, studies examining hOCT1 sulpiride transport have yielded severely contrasting K_m estimates, 2.6 vs. 260 μM (Pereira et al. 2014; Takano et al. 2017).

2.2 OCT2 (*SLC22A2*)

When examined, rOct2-mediated transport was nonresponsive to a trans-applied proton gradient, but virtually abolished by membrane depolarization, again characteristic of a facilitated diffusion transporter (Sweet and Pritchard 1999; Sweet et al.

[1]Throughout this chapter the convention of using all capital letters to designate human transporters and first initial capital followed by lowercase letters to designate non-human transporters is used. Species is indicated by a lowercase letter preceding the transporter name; h = human, r = rat, m = mouse, rb = rabbit.

2001). Subsequently, hOCT2 was also demonstrated to utilize the electrochemical gradient as its driving force (Sweet et al. 2001). Orthologs are known in human, rat, mouse, rabbit and pig (Gorboulev et al. 1997; Gründemann et al. 1997; Mooslehner and Allen 1999; Zhang et al. 2002). In contrast to OCT/Oct1, OCT/Oct2 mRNA expression has been observed in a myriad of CNS regions and cell types (Tables 1 and 2), including cortex and choroid plexus (rat, mouse and human), murine olfactory bulb and olfactory mucosa, human neurons, and rat cerebellum, hippocampus and leptomeninges (Busch et al. 1998; Sweet et al. 2001; Monte et al. 2004; Amphoux et al. 2006; Duan and Wang 2013; Miura et al. 2017). In terms of protein expression, the most comprehensive data are from mice, with mOct2 protein expression detected in neurons (noradrenergic and serotonergic), amygdala, dorsal raphe, frontal cortex, hippocampus, thalamus, median eminence and pituitary (Bacq et al. 2012; Couroussé et al. 2015). Protein was also observed in primary brain vascular endothelial cell preparations from rats, mice and humans and in the cerebral cortex, hippocampus and neurons of mice and humans (Busch et al. 1998; Lin et al. 2010; Bacq et al. 2012; Couroussé et al. 2015; Wu et al. 2015a). Transport of serotonin by hOCT2 may represent one of the strongest OCT/neurotransmitter interactions (Table 3), with one study reporting a K_m of 80 μM, however, other studies have reported K_m/IC_{50} values in the hundreds of micromolar range (290–310 μM), again emphasizing that more work is clearly needed to fully delineate the roles these transporters play in the CNS (Busch et al. 1998; Suhre et al. 2005; Amphoux et al. 2006). Overall, hOCT2 recognizes neurotransmitters with a higher affinity vs. other orthologs (Table 3). Rabbit Oct2 was weakly inhibited by histamine and serotonin, with the rest unexplored (Suhre et al. 2005). Berberine has been identified as a relatively high affinity substrate of rOct2 ($K_m = 13.9$ μM) and hOCT2 ($K_m = 4$ μM). Desipramine, fluoxetine, imipramine and sertraline all produced moderate inhibition of hOCT2 transport activity (Table 4). Amisulpride and sulpiride were demonstrated to be low affinity substrates of hOCT2.

2.3 OCT3 (*SLC22A3*)

Rat Oct3 also supports electrogenic transport of substrate organic cations that is sensitive to membrane potential-difference, thus, Oct3 also functions as a facilitated diffusion carrier (Kekuda et al. 1998). Orthologs are known in human, rat and mouse (Grundemann et al. 1998; Verhaagh et al. 1999). Similar to OCT/Oct2, OCT/Oct3 mRNA expression is widely distributed throughout the CNS (Tables 1 and 2). Oct/Oct3 mRNA was found in cerebral cortex and intact brain microvessels of all three species (Geier et al. 2013; Chaves et al. 2020). A number of studies in rats and mice have reported Oct3 message expression in a variety of brain regions including the area postrema, choroid plexus, dorsal raphe, medial hypothalamus, neurons of the cerebellum, hippocampus, substantia nigra, ventral tegmental area, subfornical organ and thalamus (Sweet et al. 2001; Schmitt et al. 2003; Vialou et al. 2004, 2008; Amphoux et al. 2006; Gasser et al. 2006; Baganz et al. 2008; Miura et al. 2017). Human OCT3 protein expression has been observed in cerebellum, astrocytes,

primary brain vascular endothelial cells and intact brain microvessels (Inazu et al. 2003; Geier et al. 2013; Li et al. 2013; Yoshikawa et al. 2013). Mouse and rat Oct3 protein was detected in neurons, astrocytes and ependymal cells (Vialou et al. 2004, 2008; Cui et al. 2009; Gasser et al. 2017). In addition, rOct3 protein immunoreactivity was observed in a number of circumventricular organs, including the area postrema and subfornical organ (Gasser et al. 2006). All three OCT/Oct3 orthologs are known to transport dopamine, histamine, norepinephrine and serotonin with low affinity (Table 3). For the most part, hOCT3 has stronger affinities for these neurotransmitters among these orthologs, with epinephrine and histamine ranking at the top. A notable exception is norepinephrine, where mOct3 appears to have the highest affinity (K_m = 336 μM) (Song et al. 2019). Berberine and sulpiride are confirmed substrates of hOCT3 (Table 4). With the exception of bupropion (IC_{50} = 738 μM), all of the other drugs listed effectively inhibit hOCT3-mediated transport (5–72 μM) in vitro.

2.4 OCTN1 (*SLC22A4*)

Unlike OCT/Oct1–3, transport mediated by OCTN/Octn1 orthologs was insensitive to manipulation of the membrane potential (hyper- or depolarization), but was stimulated by a trans-applied H^+ gradient, consistent with a H^+/organic cation exchanger (Tamai et al. 1997, 2000, 2004; Yabuuchi et al. 1999; Wu et al. 2000). Orthologs have been identified in human, rat and mouse (Tamai et al. 1997, 2000; Wu et al. 2000). hOCTN1 expression in the CNS has not been reported (Tables 1 and 2). Both rat and murine Octn1 mRNA were detected in cortex, cerebellum and hippocampus, and mOctn1 message was also observed in hypothalamus, striatum, primary cultured cortical neurons and olfactory mucosa (Tamai et al. 2000; Wu et al. 2000; Monte et al. 2004; Nakamichi et al. 2012). Later investigations determined that mOctn1 was not expressed in the BBB or in astrocytes (Inazu et al. 2006; Okura et al. 2008). Currently, information regarding protein expression is restricted to mice, with mOctn1 protein directly observed in the olfactory bulb and nerve, choroid plexus, neurons in adult midbrain and cerebral cortex (as well as in primary cultured cortical neurons), via immunohistochemistry (Lamhonwah et al. 2008; Nakamichi et al. 2012). Characterization of this transporter, and indeed all OCTN/Octns, has primarily focused on carnitine uptake, as this subfamily clearly plays a key endogenous role in supporting metabolism. Thus, at this time interactions with biogenic amines and CNS active therapeutics remain largely unexplored. A notable exception is hOCTN1 transport of acetylcholine (Table 3), albeit with very poor affinity (K_m = 1,000 ± 210 μM) (Pochini et al. 2012). Similarly, information regarding interaction with therapeutics used to treat mood disorders is lacking, however, the antipsychotics amisulpride (K_m = 180 μM) and sulpiride (K_m = 250 μM) are low affinity substrates of hOCTN1 (Pereira et al. 2014; Li et al. 2017), while desipramine is a low affinity inhibitor (IC_{50} = 180 μM) of rOctn1 (Table 4) (Wu et al. 2000).

2.5 OCTN2 (*SLC22A5*)

Initial characterization of hOCTN2-mediated transport found pH-dependent transport of organic cations (Wu et al. 1998b). While this transporter supports the Na^+-dependent transport of carnitine, it also supports Na^+-independent H^+/organic cation exchange (Wu et al. 1998b, 1999). Human, rat, mouse and bovine orthologs are known (Tamai et al. 1998; Wu et al. 1998b, 1999; Berezowski et al. 2004). Transporter message expression (Tables 1 and 2) was confirmed in a variety of tissues including rat cerebellum, cortex, hippocampus, neurons and astrocytes; human cortex and brain microvessels; murine olfactory mucosa; and bovine primary brain vascular endothelial cells (Tamai et al. 1998, 2000; Wu et al. 1998b, 1999; Berezowski et al. 2004; Monte et al. 2004; Alnouti et al. 2006). Octn2 protein expression (Tables 1 and 2) has been observed in rat cerebral cortex, neurons and astrocytes, and murine cerebral cortex, cerebellum, hippocampus, hypothalamus, neurons and choroid plexus (Inazu et al. 2005; Miecz et al. 2008)(Lamhonwah et al. 2008; Januszewicz et al. 2009, 2010; Cano et al. 2010). Despite marked expression in CNS regions and cell types, neurotransmitter interaction on OCTN/Octn2 remains unexplored. Human OCTN2 transports amisulpride (K_m = 185 μM) and sulpiride (K_m = 235 μM) with similar affinities as hOCTN1.

2.6 OCTN3 (*SLC22A21*)

Na^+- and pH-independent carnitine transport was observed for mOctn3, however, direct demonstration of H^+/organic cation exchange for movement of other organic cations remains to be more robustly delineated (Tamai et al. 2000). Rat and human orthologs have been investigated (Lamhonwah et al. 2005; Januszewicz et al. 2010). Expression of mOctn3 (mRNA) was observed in olfactory mucosa, but not in whole brain by PCR or Western blot (Tamai et al. 2000; Monte et al. 2004). A more recent study found mOctn3 protein expression in the olfactory bud and nerve via immunohistochemistry (Lamhonwah et al. 2008). Similar to rOctn2, rOctn3 protein was detected in astrocytes and neurons from adult cerebral cortex (Tables 1 and 2) (Januszewicz et al. 2010). In contrast, hOCTN3 protein expression has been observed in HepG2 cells and peroxisomes, but not in tissues/regions of the CNS (Lamhonwah et al. 2003, 2005). Interaction with neurotransmitters and antidepressant molecules that are known OCT, PMAT and MATE substrates/inhibitors has not been investigated in detail on OCTN3 orthologs. Further investigations of OCTN3/Octn3 protein expression in the CNS are needed to sort out any true species differences, as well as whether or not this transporter might have a role to play in CNS homeostasis or response to CNS therapeutics in humans.

2.7 PMAT (*SLC29A4*)

PMAT/Pmat was originally isolated from human brain and, like OCT/Oct1–3, exhibited Cl^-- and Na^+-independent organic cation transport that was responsive to manipulations of membrane potential, indicating it operates via facilitated diffusion (Engel et al. 2004). Orthologs have been identified in human, rat and mouse (Acimovic and Coe 2002; Engel et al. 2004). Broad distribution of hPMAT mRNA expression in brain was observed by Northern blot (Tables 1 and 2) (Engel et al. 2004). In addition to the regions listed in Tables 1 and 2, hPMAT message expression was noted in medulla, spinal cord, occipital pole, putamen, and the frontal and temporal lobes (Engel et al. 2004). Subsequent studies in rats and mice revealed even more extensive brain distribution of Pmat mRNA including every CNS region or cell type listed in Tables 1 and 2 (Dahlin et al. 2007; Vialou et al. 2007; Wu et al. 2015b; Miura et al. 2017). The patterns of mRNA expression are nearly identical for both species, with two notable exceptions being signal detected in murine olfactory bulb and lack of reported mPmat signal in astrocytes (Miura et al. 2017). Corollary protein expression has been observed in all regions in mice, including the BBB (primary brain vascular endothelial cells and intact brain microvessels), but not in rats (Dahlin et al. 2007; Wu et al. 2015b). However, Pmat expression in the microvasculature of rats and mice is unclear with studies detecting expression (Okura et al. 2011; Wu et al. 2015b) and others not finding significant expression (Dahlin et al. 2007; André et al. 2012). Immunohistochemistry revealed positive protein signal in choroid plexus from both mice and humans and in human primary brain vascular endothelial cells by immunoblotting (Dahlin et al. 2007; Duan and Wang 2013; Li et al. 2013). Dopamine, epinephrine, histamine, norepinephrine and serotonin are all known to be transported by hPMAT, with dopamine and serotonin exhibiting the highest affinities, respectively (Table 3) (Engel et al. 2004). In general, PMAT/Pmat transporters exhibited similar neurotransmitter affinities as the OCT/Octs, regardless of species. However, ranges reported in the literature are often quite broad, e.g., when investigated directly by saturation assay, reported hPMAT K_m values for epinephrine varied widely, 15 mM vs. 950 μM (Engel et al. 2004; Duan and Wang 2010). Desipramine (tricyclic antidepressant) and fluoxetine and sertraline (selective serotonin reuptake inhibitors) blocked hPMAT-mediated substrate transport with values in the low to tens of micromolar range, similar to data reported for hOCT1–3 (Table 4) (Engel et al. 2004).

2.8 MATE1 (*SLC47A1*)

Similar to the OCTNs, hMATE1 was demonstrated to mediate Na^+-independent H^+/organic cation exchange, that was impervious to changes in membrane potential (Otsuka et al. 2005; Tsuda et al. 2007). Orthologs are known in human, rat, mouse and rabbit (Otsuka et al. 2005; Terada et al. 2006; Zhang et al. 2007). Although isolated from human brain RNA by PCR, expression was not observable in human brain sample by Northern blot (Otsuka et al. 2005). Message detection using QPCR

was reported in mouse and human cerebral cortex, intact brain microvessels and choroid plexus (André et al. 2012; Duan and Wang 2013; Geier et al. 2013; Chaves et al. 2020). When examined by immunohistochemistry and quantitative proteomics, expression (Tables 1 and 2) was readily detected in intact brain microvessels and choroid plexus of humans, but not rats (Uchida et al. 2015). Knowledge regarding interaction with neurotransmitters is extremely limited (Table 3), with a single study reporting weak inhibition of hMATE1 by histamine ($IC_{50} = 761 \pm 196$ μM) and relatively potent inhibition by serotonin ($IC_{50} = 28.8 \pm 5.3$ μM) (Astorga et al. 2012). Berberine was found to be a strong substrate of rMate1 ($K_m = 4.28 \pm 2.18$ μM) and sulpiride was determined to be a substrate of hMATE1, with K_m estimates ranging from 15 to 40 μM (Table 4) (Li et al. 2017; Takano et al. 2017; Xiao et al. 2018). Targeted studies examining MATE/Mate1 function in the CNS are needed to conclusively delineate its potential roles.

2.9 MATE2-K (*SLC47A2*)

hMATE2-K represents the first functionally characterized paralog of hMATE1 to be isolated (Masuda et al. 2006). It also operates via a proton/organic cation antiporter mechanism (Masuda et al. 2006). Human, rat, mouse and rabbit orthologs are known (Otsuka et al. 2005; Masuda et al. 2006; Zhang et al. 2007). Although it appears to be highly expressed in human kidney, only weak expression in whole human brain was detected by RT-PCR (Masuda et al. 2006). Subsequent investigations reported mRNA expression (Tables 1 and 2) in cerebral cortex and choroid plexus of mice and in human cerebellum, intact brain microvessels and choroid plexus (Masuda et al. 2006; Duan and Wang 2013; Geier et al. 2013). Actual protein expression in the CNS of any species remains unconfirmed. Knowledge regarding hMATE2-K interaction with neurotransmitters is limited to the same study as for hMATE1 above (Table 3), finding similar weak inhibition of hMATE2-K by histamine ($IC_{50} = 775 \pm 148$ μM) and relatively potent inhibition by serotonin ($IC_{50} = 18.3 \pm 1.4$ μM) (Astorga et al. 2012). Again, information regarding interaction of antidepressant/antipsychotic therapeutics (Table 4) is limited to the same two studies reported above for hMATE1, which found sulpiride also to be transported by hMATE2-K and estimated K_m to be ~26–60 μM (Li et al. 2017; Takano et al. 2017). MATE2-K function in the CNS compartment remains virtually unexplored.

2.10 General Note Concerning Transporter Expression Reported in Brain Capillaries

In their comprehensive study, Andre et al. failed to observe immunostaining for mOct3 and mPmat in the brain vasculature in situ (50–100 μM thick coronal sections) and found no functional in vivo evidence of substrate transport at either the basal or apical side for mOct1–3, mMate1 or mPmat (André et al. 2012). Thus, in

obvious contrast to a number of studies reported in Tables 1 and 2, they concluded that none of these transporters are functionally expressed in the murine BBB and that preparations finding their expression in isolated brain capillaries likely were contaminated with other CNS cell types (e.g., glial cells/astrocytes). Recently, Chaves et al. examined organic cation transporter expression in isolated murine and rat microvessel preparations by QPCR. They reported that mOct1 (regardless of strain examined) was not expressed, and mOct2 and mOct3 levels were extremely low, whereas rat brain capillaries were virtually devoid of rOct1 or rOct2 mRNA, but rOct3 was detectable (Chaves et al. 2020). They found no detectable hOCT1, hOCT2 or hMATE1 protein expression in human brain microvessels by LC-MS/MS (Chaves et al. 2020). Importantly, their isolation technique produced a sevenfold decrease in astrocytic marker contamination and a 31-fold reduction in neuronal contamination (vs. ~twofold reductions in other studies) (Chaves et al. 2020). Therefore, it is possible that the low, inconsistent levels of expression reported in the literature for various transporters in the CNS microvasculature actually may be due to contaminating non-endothelial CNS cell types associated with the isolated brain capillaries, and further refinement and rigor in isolation techniques are needed to unequivocally resolve BBB transporter expression profiles.

3 In Vivo Evidence of Transporter Function in the CNS Derived from Molecular or Pharmacological Knockout Studies

3.1 Oct1 Knockout

This model has been developed and Oct1 knockout animals exhibit altered transport of substrate cations in vivo, however, the studies are largely aimed at investigating function in the liver and kidney. Currently, functional and behavioral studies designed to probe mOct1 contributions in the CNS are lacking, perhaps due to the fact that OCT/Oct1 appears to have the narrowest "CNS expression profile" among OCT/Oct1–3 (Tables 1 and 2).

3.2 Oct2 Knockout

Studies with Oct2 knockout mice have yielded compelling evidence for mOct2 function in the CNS. Cortical and hippocampal cell suspensions prepared from Oct2 knockout mice showed decreased accumulation of norepinephrine and serotonin, while dopamine accumulation was unchanged (Bacq et al. 2012). In fact, dopamine levels were similar in wild type and Oct2 knockout mice, while significant norepinephrine reduction was found within the cortex (~38%) and hypothalamus (~22%) and serotonin levels were reduced in the hippocampus (~19%), hypothalamus (~27%) and striatum (~20%) (Bacq et al. 2012). These changes did not result in any readily apparent effects in basal locomotor activity in Oct2 knockout mice (Bacq

et al. 2012). However, when examined in behavioral models, Oct2 knockout mice exhibited a decreased anxiety-like phenotype, but increased depressive-like phenotype (Bacq et al. 2012). They also exhibited an increased sensitivity to the acute effects of antidepressants, but (in the corticosterone induced depression model) failed to respond to long-term antidepressant treatment (Bacq et al. 2012). Expression of mOct2 in the hypothalamus and pituitary combined with known interactions with the stress hormone corticosterone led to speculation about Oct2/OCT2 playing a role in hormonal response to acute stress. Indeed, when basal corticosterone levels were measured, Oct2 knockout animals showed an 87% increase over wild type and, when subjected to stress, they exhibited a 56% increase in peak stress-induced corticosterone release (Couroussé et al. 2015). Moreover, in a prolonged (8 weeks) model of unpredictable mild stress Oct2 knockout animals showed increased vulnerability and greater impact on depression-related behaviors including self-care, spatial memory and spontaneous social interaction (Couroussé et al. 2015). These data are consistent with Oct2/OCT2 serving an active role in norepinephrine and serotonin clearance from the brain and potentially provide insight to some aspects of interindividual patient variation (through altered transporter levels or function) in response to therapeutics used to treat depression and anxiety disorders. Further, loss of mOct2 function in the pituitary results in an enhanced hormonal response to acute stress and vulnerability to repeated stress, possibly providing novel insight to interindividual patient variation in the etiologies of major depressive disorder or post-traumatic stress disorder.

3.3 Oct1/2 Double Knockout

Given the overlapping tissue expression profiles and substrate specificity of Oct1 and Oct2, double Oct1/Oct2 knockout mice were constructed to further delineate OCT function in vivo. The animals are viable and lack both Oct1 and Oct2 protein – including in their brain microvasculature. Upon systemic (intraperitoneal) administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to wild type and Oct1/2 double knockout animals, CNS levels of both MPTP and its neurotoxic metabolite, 1-methyl-4-phenyl-pyridinium (MPP^+), were markedly lower in the double knockout mice (Wu et al. 2015a). This correlated with a significant reduction in neuronal loss from the substantia nigra in Oct1/2 double knockout mice. However, when MPTP was introduced directly into the brain via chronic low dose intrastriatal infusion (over 7 days), neuronal loss from the substantia nigra in Oct1/2 double knockout mice was significantly increased compared to wild type, i.e., the dopaminergic neurotoxicity was more severe (Wu et al. 2015a). Given the extensive expression of mOct1 and mOct2 in CNS cell types, it is currently unclear if the apparent protective mechanism extends beyond expression in the brain microvasculature, however, it clearly suggests the presence of functional mOct1 and mOct2 in the CNS can modulate MPTP-induced dopaminergic neurotoxicity. Recent studies using Oct1/2 double knockout mice have generated compelling evidence that functional mOct2 is required for oxaliplatin-induced peripheral neurotoxicity, as well,

and that loss of mOct2 function via genetic or pharmacological blockade alleviates the toxicity without compromising the pharmacokinetics or efficacy of oxaliplatin therapy (Sprowl et al. 2013; Huang et al. 2020).

3.4 Oct3 Knockout

Genetic knockout of mOct3 resulted in substantial brain region-specific reductions in dopamine (e.g., ~46% in substantia nigra and ventral tegmentum), serotonin (e.g., ~12% in pons), norepinephrine (e.g., ~25% in substantia nigra and ventral tegmentum) and histamine (e.g., ~20% in hypothalamus and thalamus) (Zwart et al. 2001; Vialou et al. 2008). Primary astrocytes isolated from Oct3 knockout mice exhibited reduced substrate accumulation in comparison with those isolated from wild type mice (Cui et al. 2009). Despite such differences, behavioral assessments failed to identify any changes between wild type and Oct3 knockout mice in basal locomotor activity, balance or coordination, suggesting no manifestations of an overt monoamine imbalance under controlled conditions (Zwart et al. 2001; Vialou et al. 2008). Interestingly, loss of mOct3 was associated with altered neural and behavioral responses to changes in blood osmolarity and regulation of salt ingestion, as well as loss of social interaction preference in males (Vialou et al. 2004; Garbarino et al. 2019). When subjected to high doses of stimulants (amphetamine or cocaine) Oct3 knockout mice exhibited a blunted response, as evidenced by persistence of hyperlocomotion, whereas in wild type mice stereotyped behaviors began to emerge, suppressing locomotor activity (Vialou et al. 2008). Consistent with these findings, amphetamine's actions were found to be Oct3-dependent (Mayer et al. 2018). Moreover, after methamphetamine administration, dopamine levels increased (~two- to threefold) in Oct3 knockout mice, relative to wild type mice, suggesting impaired dopamine clearance (Cui et al. 2009). While aggression and spatial memory testing yielded similar results between wild type and Oct3 knockout animals, impact upon anxiety-like behaviors is less clear (Vialou et al. 2008; Wultsch et al. 2009). Given that histamine is a substrate for Oct3/OCT3 and is thought to ameliorate ischemic brain damage, response to ischemic brain injury was examined in Oct3 knockout mice. Notably, Oct3 knockout mice exhibited an approximately 50% reduction in total infarct volume following middle cerebral artery occlusion (Zhu et al. 2012b). Prior to ischemic damage, brain region histamine levels were similar between wild type and Oct3 knockout mice. However, the dramatic decrease in ischemic damage was associated with a significant elevation in histamine levels in the ischemic area of Oct3 knockout animals (vs. wild type) during and after ischemia, even at 24 h post-ischemia (Zhu et al. 2012b). Together, the findings of these studies are clearly indicative of mOct3 having true functional roles in the CNS, e.g., proper termination of aminergic neurotransmission, development of social behaviors and maintenance of histamine balance/scavenging of ischemia-induced histamine.

In the case of genetic knockout, the animals develop and mature in the complete absence of the gene's function, potentially confounding direct cause and effect

conclusions. To examine loss of Oct3 function in fully mature adult wild type animals, mOct3 antisense oligonucleotides were directly introduced into the third ventricle of the brain (Kitaichi et al. 2005). This resulted in an ~30% reduction in mOct3 protein content in brain (Kitaichi et al. 2005). These animals exhibited an enhanced locomotor response to methamphetamine and decreased depression-like behavior (Kitaichi et al. 2005). When the experiment was performed in rats, administration of rOct3 antisense oligonucleotides decreased rOct3 expression in choroid plexus and ependymal cells (Nakayama et al. 2007). Consistent with mice, decreased CNS expression of rOct3 resulted in significantly elevated extracellular dopamine levels and potentiated hyperlocomotion response to methamphetamine (Nakayama et al. 2007). These results bolster the concept that mOct3/rOct3 participates in the removal of dopamine from the CNS interstitial milieu, and when its function is reduced/absent in the CNS, dopamine clearance is attenuated, enhancing dopaminergic neurotransmission and the associated behavioral response(s).

Finally, pharmacological manipulation of OCT CNS activity led to altered neurotransmitter levels. For example, application of decynium-22 (pan OCT inhibitor) or corticosterone (Oct3 inhibitor) to the medial hypothalamus of rats produced an increase in extracellular serotonin that correlated with altered specific behaviors and enhanced antidepressant efficacy, suggesting that OCTs/rOct3 are functional in the CNS (Feng et al. 2005, 2009). Notably, CNS expression of mOct3 protein is significantly upregulated in SERT knockout mice (Baganz et al. 2008). Local infusion of decynium-22 in brain regions with high Oct3 expression significantly reduced serotonin clearance in SERT knockout animals, but not in wild type mice, suggesting that increased CNS mOct3 protein expression may represent a compensatory mechanism in response to dysfunctional serotonin clearance (Baganz et al. 2008).

3.5 Octn1 Knockout

Initial studies in Octn1 knockout mice found reduced Octn1 substrate permeation into brain (Kato et al. 2010) and follow-on experiments more robustly demonstrated mOctn1 expression and function in CNS cell types. When the Octn1 substrate, ergothioneine, was introduced directly into the ventricles, Octn1 knockout animals exhibited significantly decreased distribution to several brain regions vs. wild type, including cerebellum, medulla, pons, hypothalamus and midbrain (Nakamichi et al. 2012). Subsequently, Octn1 mRNA and protein were detected in mouse brain (e.g., midbrain and cerebral cortex) and primary cultured cortical neurons by immunohistochemistry. Substrate uptake into wild type primary cultured neurons was saturable, whereas uptake was absent in Ocn1 knockout derived primary cultured neurons (Nakamichi et al. 2012). Finally, mOctn1 expression in microglia was directly investigated. Primary cultured microglia isolated from wild type mice supported uptake of ergothioneine, whereas its accumulation was virtually absent in primary cultured microglia isolated from Octn1 knockout mice (Ishimoto et al. 2018). In wild type microglia, mOctn1 mediated uptake of ergothioneine was observed to be

protective against cellular hypertrophy. Thus, mOctn1 expression and function in several CNS cell types has been confirmed and data indicate it may be a key component in protecting general brain health and preventing the onset of certain disease states such as Alzheimer's or Parkinson's disease, the etiology of which may involve chronic overactivation of microglia (Gerhard et al. 2006; Simard et al. 2006).

3.6 Octn2 Knockout

In 1988, Koizumi et al. discovered an inbred homozygous mutant mouse that exhibited a severe metabolic syndrome (systemic carnitine deficiency) and named it the juvenile visceral steatosis (jvs) mouse (Koizumi et al. 1988). Subsequently, a missense mutation was identified in the Octn2 DNA sequence in jvs mice and was found to be the cause for decreased efficiency in the transport of carnitine in these animals (Lu et al. 1998). When examined, carnitine transport by primary brain capillary endothelial cells isolated from Octn2 knockout (jvs) mice was decreased ~46% with respect to wild type cells and the CNS distribution of carnitine was lower in the knockout animals (Kido et al. 2001). While more in-depth studies probing OCTN2/Octn2 function in the brain are needed, these preliminary studies support the contention that mOctn2 is expressed and functional in the CNS and loss of function alters the composition of the CNS milieu. Whether any cognitive or other behavioral anomalies are associated with loss of this transporter remains to be examined.

3.7 Octn3 Knockout

Currently not available.

3.8 Pmat Knockout

Expression of Pmat mRNA in mouse choroid plexus was already established when the Pmat knockout mouse was made, therefore, initial characterization of CNS expression focused on this tissue. When uptake was examined ex vivo in isolated choroid plexus, tissue isolated from Pmat knockout mice showed a significant reduction (~30–40%) in the uptake of mPmat substrates, including serotonin and dopamine (Duan and Wang 2013). This reduced substrate accumulation correlated with loss of mPmat immunoreactive signal from choroid plexus of Pmat knockout mice. No changes in mRNA levels of Oct3, Dat, Net or Sert in Pmat knockout mice were observed, however, protein levels were not examined (Duan and Wang 2013). Recently, a comprehensive behavioral examination of Pmat knockout mice was conducted (Gilman et al. 2018). Given the extensive CNS expression footprint of PMAT/Pmat, and considering the measurable behavioral phenotypes in other organic cation transporter knockout models, e.g., Oct2 and Oct3 knockout mice, it

was predicted that robust behavioral phenotypes would be detected in these animals as well. However, while some effects of mPmat ablation were observed, they were very subtle in nature. No differences in locomotor activity or compulsive/repetitive behaviors were detected between wild type and Pmat knockout mice (Gilman et al. 2018). Moreover, anxiety-related behaviors (evaluated in the elevated plus maze) were only mildly impacted at best. Interestingly, measures of stress coping behaviors indicated that such behaviors were specifically enhanced in female Pmat knockout mice (Gilman et al. 2018). Although the behavioral consequences of Pmat loss of function identified thus far are slight, these in vivo and previous in vitro studies nonetheless support the concept of PMAT/Pmat transport activity contributing to CNS homeostasis and response to stressors.

3.9 Mate1 Knockout

A Mate 1 knockout model has been established and altered transport of substrates in vivo demonstrated, however, functional and behavioral studies designed to probe Mate1 contributions in the CNS have not been conducted as the CNS expression data for MATE/Mate1 are not robust (Tables 1 and 2).

3.10 Mate2-K Knockout

Currently, a mammalian model is not available.

4 Summary

That membrane transport systems exist to aid the movement of charged organic solutes across the lipid bilayers of cells (both uptake and efflux) has been known for centuries. We now know that one vast superfamily of transporters, the Major Facilitator Superfamily, contains dozens of specialized subfamilies of solute carrier transporters (SLCs) that in turn are comprised of hundreds of individual transport proteins (including OCTs, OCTNs, PMAT and MATEs) that serve as some of the gatekeepers for this movement. The past 30 years have seen an exponential growth in our understanding of who the players are, how they sort into functional families, what they look like, what their pharmacological profiles are, where they are located, how their activity is modulated, and their contributions to disease states. It is this latter category where significant inroads have been made in the last decade, particularly in regard to behavioral norms and mood disorders. Indeed, studies in loss of function models have uncovered endogenous roles for these transporters/transporter networks that extend beyond systemic clearance of toxic metabolic intermediates, etc.

While constitutive (vs acute) loss of transporter function may not recapitulate the exact etiology of human mood disorders, the development of SLC transporter

knockout mouse models has yielded invaluable insight as to OCT (SLC22), OCTN (SLC22), PMAT (SLC29) and MATE (SLC47) endogenous function, including in the CNS. Results from investigations of SLC genetic knockout animals in classical behavioral assessment tests (elevated plus maze, forced swim test, open field, light/dark field, mild stressors, marble burying) overwhelmingly support new thinking that low affinity transporters that have high transport capacity can and do represent integral components of pathways meant to preserve "normal" CNS function and that their perturbation does meaningfully impact CNS function. Similarly, pharmacological knockout experiments have provided complementary insight as to the impact of acute perturbations of these transport systems that otherwise developed normally. Again, intriguing data supporting active contribution of these transport pathways to CNS balance and clearance, particularly in catecholaminergic neurotransmission, have been obtained. However, the inhibitor compounds used, e.g., decynium-22 or lopinavir, interact with multiple transporter family members due to their polyspecific nature and, thus, are less "clean" as to absolute cause and effect for a particular transporter. Regardless, together, these approaches are elevating the significance of the purported "uptake 2" pathway in CNS disease etiology and treatment. They are beginning to drive new thinking in the clinical diagnosis of, and therapeutic approach to the treatment of, mood disorders and the varied responses of patients.

Recent investigations have shown that many drugs of abuse (e.g., cocaine, ketamine, amphetamine, methamphetamine, opioids) are substrates of these transporters and much work exploring potential roles for these transporters in stimulus/reward networks and the etiology and treatment of substance abuse is needed. Ability to perform more targeted studies assessing transporter-compound interactions at the molecular level (homology modeling and solved crystal structures) will enhance our understanding of the physicochemical interactions governing CNS pharmacokinetic and pharmacodynamic effects of already existing compounds, as well as inform rational drug design approaches for novel therapeutics targeting these transporters. For example, recent studies have reported development of a hOCT3 homology model and its use, in combination with targeted in vitro transporter studies, in delineating hOCTs as pharmacological CNS targets for the actions of novel phenylguanidine and dihydroquinazoline analogs that possess antidepressant-like actions (Pan et al. 2017; Iyer et al. 2019). Intriguingly, these compounds may exhibit their overall pharmacodynamic effects via a "two strike" approach, targeting both uptake-1 (NET, DAT, SERT) and uptake-2 transporters (OCTs, OCTNs, PMAT and MATEs) simultaneously; reminiscent of enhanced clinical antitumor effects with combination therapies in cancer (Pan et al. 2017; Iyer et al. 2019).

Many important questions regarding the clinical consequences of altered transporter function remain to be addressed. Do patients on chronic therapies, e.g., metformin for diabetes, antivirals for HIV, or antidepressants/antipsychotics for mood disorders, represent pharmacological knockouts? Do other transporter networks upregulate to compensate? Does this lead to patient variability in clinical response? Do transporters expressed in microglia, e.g., Octn1, play a role in microglial activation, which in turn has been tied to the onset and development of

chronic neuroinflammatory diseases such as Alzheimer's and Parkinson's? Are they fundamentally involved in the physiological response to stress? Clearly, the transporters highlighted in this chapter are poised to contribute to multiple CNS processes and pathologies. The next decade will surely see many significant advancements solidifying their prominence in the CNS milieu and advancing available therapies for the treatment of mood disorders, addictive behaviors, neurodegenerative diseases and beyond.

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Genetic and Epigenetic Regulation of Organic Cation Transporters

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Abstract

Organic cation transporters (OCTs) of the solute carrier family (SLC) 22 are the subject of intensive research because they mediate the transport of many clinically-relevant drugs such as the antidiabetic agent metformin, the opioid tramadol, and the antimigraine agent sumatriptan. OCT1 (*SLC22A1*) and OCT2 (*SLC22A2*) are highly expressed in human liver and kidney, respectively, while OCT3 (*SLC22A3*) shows a broader tissue distribution. As suggested from studies using knockout mice, particularly OCT2 and OCT3 appear to be of relevance for brain physiological function and drug response. The knowledge of genetic factors and epigenetic modifications affecting function and expression of OCTs is important for a better understanding of disease mechanisms and for personalized treatment of patients. This review briefly summarizes the impact of genetic variants and epigenetic regulation of OCTs in general. A comprehensive overview is given on the consequences of OCT2 and OCT3 knockout in mice and the implications of genetic OCT2 and OCT3 variants on central nervous system function in humans.

Keywords

Brain · Central nervous system · Drug response · Drug transporters · Epigenetics · Genotype–phenotype correlation · Interindividual variability · Knockout mice · OCT1 · OCT2 · OCT3 · Organic cation transport · Pharmacogenomics · Pharmacokinetics · Single nucleotide polymorphisms

1 Impact of Genetic and Epigenetic Regulation on the Function of Organic Cation Transporters: A Short Overview

The interindividual variability of expression and function of genes involved in drug absorption, distribution, metabolism, and excretion (ADME) accounts for various interindividual differences regarding disease development, drug–drug interaction (DDI), drug response, and treatment outcome. In order to gain a better understanding of these differences and their consequences, it is necessary to unravel the interplay of genetic and non-genetic factors as well as epigenetic modifications affecting the expression and function of these ADME genes (Meyer et al. 2013). This aims at revealing disease mechanisms and to treat patients with a more individualized drug therapy, in which the individual genetic makeup is taken into consideration (Fisel et al. 2017). Drug transporters belong to the group of ADME genes and are

considered important players in normal physiological processes and homeostasis as well as in drug response and safety (César-Razquin et al. 2015; Giacomini et al. 2010).

Among the drug transporters, the organic cation transporters (OCT) 1, OCT2, and OCT3 (encoded by *SLC22A1*, *SLC22A2,* and *SLC22A3*) have been intensively studied because they mediate the transport of organic cations and zwitterions, including many clinically-relevant drugs such as metformin, tramadol, and sumatriptan (Koepsell 2020; Nies et al. 2011; Tzvetkov et al. 2016; Yee et al. 2018). OCT1 is mainly expressed in the liver whereas OCT2 shows a high renal expression (Schaeffeler et al. 2011; Winter et al. 2016). In liver and kidney, OCT1 and OCT2 mediate the uptake of substances from the blood into hepatocytes and proximal tubule epithelial cells, respectively. OCT3 shows a more broad distribution and is expressed in a variety of tissues. There is a growing amount of studies addressing the impact of genetic and epigenetic regulation of OCTs on hepatic and renal physiological processes, disease susceptibility, and response to drug substrates (Yee et al. 2018). Even though OCTs have been shown to be expressed in the blood–brain barrier (BBB) and other brain areas (Lin et al. 2010), information on the effect of genetic and epigenetic regulation of these transporters on brain physiological function and drug response is very limited.

The aim of this chapter is to give a brief overview of the impact of genetic and epigenetic regulation of OCTs in general and to summarize the work about the role of interindividual OCT variation in the central nervous system (CNS).

1.1 Genetic Variants of OCTs

After the initial cloning of *SLC22A1*, *SLC22A2,* and *SLC22A3* (Gorboulev et al. 1997; Gründemann et al. 1998), systematic resequencing of the *SLC22A1* gene identified 14 coding variants that lead to amino acid substitutions and decreased or abolished function of OCT1 in vitro with pharmacodynamic consequences in vivo (Kerb et al. 2002; Shu et al. 2003, 2007; Tzvetkov et al. 2016). Five of these variants are common among different populations and are therefore of particular pharmacological interest: rs12208357 (p.R61C), rs55918055 (p.C88R), rs34130495 (p.G401S), rs72552763 (p.M420del), and rs34059508 (p.G465R). Similarly, variant rs316019 in *SLC22A2* (p.S270A) has been identified as a common variant of OCT2. The impact of these common OCT1 and OCT2 variants has been intensively investigated in vitro, in pharmacokinetic studies and in studies on drug response considering established drug substrates of these transporters, e.g. the antidiabetic drug metformin, some opioids, antiviral medication, and anticancer drugs such as oxaliplatin (Koepsell 2020; Tzvetkov et al. 2016; Yee et al. 2018). The p.S270A variant of OCT2, for example, reduces the risk for cisplatin-induced nephrotoxicity without changing drug disposition (Iwata et al. 2012). *SLC22A2* might also be a susceptibility gene for aspirin intolerance in asthmatics (Park et al. 2011). Loss of function variants of *SLC22A1* seem to play a role in effectiveness of analgesic treatment with opioids. This effect might be of special relevance in children resulting

from their lower OCT1 expression in comparison with adults or in CYP2D6 ultrarapid metabolizers, because this genetic makeup also increases the plasma concentrations of opioids and thereby the toxicity risk (Matic et al. 2017). Based on these and many other studies, the International Transporter Consortium considers genetic variants of OCT1 as highly important for drug disposition, response, and toxicity while the in vitro and in vivo effects of the OCT2 variant are less conclusive (Yee et al. 2018).

Large-scale systematic sequencing projects including thousands of individuals from different geographical regions identified additional variants and confirmed OCT1 as highly polymorphic (Nies et al. 2011; Seitz et al. 2015; Yee et al. 2018). Recent exome/whole genome next generation sequencing efforts of >100,000 individuals have revealed a multitude of novel missense variants, many of which only occur in single individuals (Karczewski et al. 2020). These variants are so rare that they cannot be detected in clinical trials, yet they may contribute to lack of efficacy or adverse drug reactions (Kozyra et al. 2017; Schärfe et al. 2017).

Genetic variants of OCTs leading to an altered protein expression and/or function may not only affect drug response, but may also alter susceptibility for certain diseases (Goswami et al. 2014; Koepsell 2020; Nies et al. 2011). For example, two *SLC22A1* genetic variants correlate with an increased risk for type 2 diabetes mellitus in Chinese patients (Long et al. 2018). Genetic variants of *SLC22A1* were further associated with the development of primary biliary cirrhosis (Ohishi et al. 2014). A genetic variant of *SLC22A3* was shown to lead to reduced OCT3 expression and thereby to a decreased risk of colorectal cancer in a Japanese population (Ren et al. 2019). The *SLC22A3* gene was furthermore identified as risk factor for coronary artery disease as well as prostate cancer (Eeles et al. 2008; Trégouët et al. 2009).

1.2 Epigenetic Regulation of OCTs

Epigenetics is defined as heritable variation of the expression of a gene that is not caused by changes in the DNA sequence (Holliday 2006). This variation can be achieved by noncoding RNAs, histone modification, or DNA methylation. Regulation of ADME genes by genetic variants is studied widely and the resulting pharmacogenomics knowledge is already used in clinical practice (van der Wouden et al. 2019). In contrast, even though epigenetics are getting more and more into focus and are acknowledged to play important roles in not only the normal cell type- and developmental stage-specific gene expression, but also in pathophysiological processes or drug response (pharmacoepigenetics), this field is much less studied and understood in comparison with pharmacogenomics (Fisel et al. 2016). One reason for this might be that it is very difficult to study epigenetic regulation of genes because they are highly dynamic in contrast to actual variations in the DNA sequence. For example, the methylation pattern of renal cell carcinoma (RCC) cell lines differs significantly from RCC tumors and metastases regarding ADME and drug target genes. *SLC22A2,* for example, is hypermethylated in RCC cell lines, but

not in tumors and metastases translating into reduced expression of OCT2 in RCC cell lines in comparison with tumors and metastases. These findings indicate that the use of cell lines to study epigenetic regulation is difficult and might often not lead to reliable results in respect of the real physiological situation (Winter et al. 2016, 2017).

There is some information on epigenetic regulation of OCTs in normal and pathophysiological states. For example, the relatively restricted expression of OCT1 to the liver and OCT2 to the kidney seems to be a result of methylation patterns. Accordingly, *SLC22A1* is strongly methylated in the kidney compared to liver tissue, whereas *SLC22A2* is hypermethylated in the liver and hypomethylated in the kidney (Aoki et al. 2008; Fisel et al. 2016).

The *SLC22A1* gene was shown to be hypermethylated in human hepatocellular carcinoma (HCC) leading to decreased protein expression (Schaeffeler et al. 2011). This knowledge is especially valuable regarding the fact that platinum drugs used as anticancer agents are substrates of OCT1. Reduced OCT1 expression therefore probably leads to reduced treatment success that might be restored by pretreatment of patients with demethylating agents, e.g. decitabine. Furthermore, the methylation state of *SLC22A1* could be used in risk assessment and improvement of early diagnosis of HCC in patients at risk of developing HCC. Similar to the finding of *SLC22A1* hypermethylation in HCC, *SLC22A3* promoter methylation is significantly increased in prostate cancer resulting in reduced expression of OCT3 (Chen et al. 2013).

Epigenetic regulation of OCTs by noncoding microRNAs (miRNAs) has also been described. Li et al. identified genetic variant rs3088442 in the 3′ untranslated region of the *SLC22A3* gene to be associated with coronary heart disease (Li et al. 2015). Mechanistically, the A allele of rs3088442 generates a binding site for miR-147, which inhibits OCT3 expression. The deficiency of OCT3 in turn inhibits lipopolysaccharide induced monocytic inflammatory response explaining the atheroprotective role of this variant. Another example is the regulation of OCT expression by miR-21, which is upregulated in clear cell renal cell carcinoma (Gaudelot et al. 2017) and also inversely correlated with OCT1 expression in human liver (Rieger et al. 2013). Silencing of miR-21 in human kidney cells in vitro resulted in increased expression of OCT1 and OCT2, which could be another strategy to restore treatment success with anticancer agents that are transported by OCTs.

The ontogeny of OCT1 and OCT2 is another process, in which dynamic epigenetic regulation of the OCTs might play a role indicated by a strong increase in OCT1 and OCT2 protein expression from neonates to adults (Cheung et al. 2019; Hahn et al. 2017; Prasad et al. 2016). In contrast to drug-metabolizing enzymes, there is not much known about the ontogeny of drug transporters and its underlying mechanisms. Since it was shown for cytochrome P450 3A4 (CYP3A4) that hypermethylation of transcription factor binding sites leads to decreased protein expression before birth, a similar role of methylation or other epigenetic processes may occur in the ontogeny of drug transporter genes including *SLC22A1* and *SLC22A2* (Brouwer et al. 2015; Mooij et al. 2016).

2 Implications of OCT Regulation in the CNS

In contrast to the variety of studies regarding the genetic and epigenetic regulation of OCTs in liver and kidney, not much has been reported on the role of genetic variants or epigenetic makeup in physiological and pathophysiological processes in the brain. Due to the lack of information on the role of genetic regulation of OCT1 and on epigenetic regulation of OCTs in the CNS in general, this section will focus on genetic regulation of OCT2 and OCT3 and its impact on the CNS.

However, regarding OCT1, it is important that this highly polymorphic transporter transports various opioids, e.g. the active compound of tramadol, O-desmethyltramadol, morphine, and methylnaltrexone, as well as the antimigraine agent sumatriptan (Matthaei et al. 2016; Meyer et al. 2019; Tzvetkov 2017). The common genetic variants of OCT1 leading to decreased or loss of function of OCT1 alter the pharmacokinetics and the effectiveness of opioid treatment probably by decreasing hepatic reuptake of the active substances and thereby increasing their plasma levels and availability (Tzvetkov 2017). A finding underlining this hypothesis is that patients with two nonfunctional alleles showed significantly lower tramadol consumption in order to treat postoperative pain via patient-controlled analgesia compared with carriers of at least one active OCT1 allele (Stamer et al. 2016; Tzvetkov 2017). These findings impressively show that even though the expression of OCT1 in the brain is under debate, interindividual differences in OCT1 function in the liver can still affect the efficacy as well as the toxicity risk of drugs acting in the brain.

Pharmacogenetic studies are often difficult to perform in humans, which also affects the amount of research that has been conducted to date on the effects of OCT gene regulation on the human brain. Animal studies, especially those using knockout (KO) mouse models have proven to be valuable in identifying not only the physiological roles of transport proteins but also the pathophysiological processes caused by altered transport function in vivo (Frick et al. 2013; Nies et al. 2011; Stieger and Gao 2015). There are KO mice existing for all OCT homologs as well as Oct1/Oct2 double KO mice. All of these strains are viable and don't show direct phenotypical aberrations (Nies et al. 2011). KO and also wild type (WT) mice have been used in various studies in order to elucidate the role of Octs on different brain processes and predict possible effects of human OCT variants. Therefore, results gained from such studies are reviewed here, next to findings gained from studies performed with human subjects.

2.1 Genetic Regulation of OCT2 in the CNS

2.1.1 Localization of OCT2 in the CNS

Even though there are studies reporting on the localization of OCT2 in mouse and human brain, the results are not always consistent and do not create a precise expression pattern.

In mice, Oct2 was reported to be expressed in the BBB and also in the blood–cerebrospinal fluid (CSF) barrier (Sweet et al. 2001; Wu et al. 2015), yet BBB expression has been challenged (Chaves et al. 2020). Expression was furthermore shown in limbic and other stress-related regions of the mouse brain as well as in the dorsal root ganglia cells (Bacq et al. 2012; Couroussé et al. 2015; Sprowl et al. 2013). Cholinergic and monoaminergic axon terminals in various mouse forebrain regions likewise showed Oct2 expression, pointing out an implication of Oct2 on presynaptic reuptake and recycling of choline and monoamines (Matsui et al. 2016).

Similar to mice, in human brain, OCT2 seems to be expressed in the BBB, indicated by Lin and colleagues who reported the OCT2 protein in the luminal membrane of brain microvessel endothelial cells (Lin et al. 2010). Yet, a recent study detected only negligible amounts of OCT2 mRNA in human brain microvessels (Chaves et al. 2020). OCT2 expression was also found in human dorsal root ganglia cells (Lin et al. 2010; Sprowl et al. 2013). Expression of the transporter was also detected in pyramidal cells of cerebral cortex and hippocampus as well as in other regions like the corpus striatum, nucleus amygdaloideus, and the thalamus in humans (Busch et al. 1998). Taubert et al. also reported OCT2 expression in human substantia nigra and furthermore in other dopaminergic regions of the brain (Busch et al. 1998; Taubert et al. 2007). The Human Protein Atlas, a freely available resource providing widespread information about the human proteome, reports low OCT2 mRNA expression levels in some brain regions including pons and medulla, midbrain, cerebellum, and olfactory region (Uhlén et al. 2015). There is no data on protein expression provided for these brain regions by this database.

2.1.2 Consequences of Disturbed OCT2 Function in the CNS: What Do We Learn from Animal Studies?

Consistent with the expression of Oct2 in limbic regions of the mouse brain, the transporter seems to be involved in mood-related behavior and stress response. Oct2 KO mice showed less anxiety and increased resignation in behavioral tests as well as higher sensitivity to norepinephrine (NE) and serotonin (5-HT) selective antidepressants in comparison with WT mice with normal Oct2 expression (Bacq et al. 2012). These findings point to the idea that OCT2 controls 5-HT and NE clearance in limbic regions of the brain and should be considered as a genetic factor influencing the response to NE- and 5-HT selective antidepressants. Another study reveals the role of Oct2 in the hormonal response to acute stress (Couroussé et al. 2015). Oct2 KO mice showed increased basal as well as stress-induced plasma corticosterone levels and were more vulnerable to repeated stressful conditions. OCT2 could therefore be a genetic factor influencing the hormonal processes occurring during acute stress implying that genetic variants or epigenetic regulations that alter OCT2 transport function could critically affect vulnerability to stress.

Other animal studies have shown an impact of Oct2 on neurotoxicity of different substances. OCTs transport 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) across the BBB, which is the prodrug of its active metabolite and neurotoxin 1-methyl-4-phenyl-pyridinium (MPP+). MPP+ is also a substrate of OCTs and leads to dopaminergic neurodegeneration and thereby mimics the symptoms of

Parkinson's disease (PD) (Koepsell 2020; Wu et al. 2015). Inhibition of Oct1 and Oct2 with amantadine significantly decreased MPTP transport in mouse and rat brain microvessel endothelial cells (Lin et al. 2010). Furthermore, after intraperitoneal application of MPTP in mice, extracellular MPTP and MPP+ levels in the brain accompanied with dopaminergic toxicity were significantly reduced in Oct1/Oct2 double KO mice (Lin et al. 2010; Wu et al. 2015). Intrastriatal infusion with MPTP, however, increased dopaminergic neurotoxicity in Oct1/Oct2 double KO and also in aged mice that show a lower Oct expression in the BBB in comparison with young mice, probably because of reduced neurotoxin clearance from the brain (Wu et al. 2015). Given these results and the fact that the two endogenous neurotoxins 1-benzyltetrahydroisoquinoline and N-methyl-(R)salsolinol, that are structurally similar to MPTP, are substrates of OCT1 and OCT2 (Wu et al. 2015), OCT2 genetic and epigenetic regulation could crucially influence dopaminergic toxicity caused by these substances and thereby even alter susceptibility for developing PD.

Another finding regarding the role of Oct2 in the effect of neurotoxins is that Oct2 KO mice are protected from neurotoxicity caused by oxaliplatin, a platinum drug that is used in the treatment of colorectal cancer and a substrate of human OCT2 (Huang et al. 2020; Koepsell 2020; Sprowl et al. 2013). Genetic and epigenetic variations of OCT2 could therefore be important factors influencing the risk of treatment-induced neurotoxicity.

Taken together, these results indicate that genetic variants and epigenetic patterns altering OCT2 function might affect mood- and stress-related behavior, the response to NE- and 5-HT selective antidepressants as well as the vulnerability to endogenous and exogenous neurotoxins.

2.1.3 Genetic Variants of OCT2 and Their Implications on CNS Function

Genetic variants of human OCT2 were shown to play a role in smoking cessation therapy (Bergen et al. 2014). The human *SLC22A2* genetic variant rs316019 (p. S270A), that was shown to reduce transport of some OCT2 probe substrates in vitro, and a linked, intronic variant (rs316006) were associated with smoking abstinence in individuals randomized to smoking-cessation treatment, namely to nicotine replacement therapy (NRT) and varenicline. The underlying mechanism is unknown, but the findings suggest that smoking cessation therapy either by NRT or also by nicotinic acetylcholine receptor partial agonist pharmacotherapies like varenicline shows higher effectiveness in subjects with reduced OCT2 function. This could be due to different reasons, like increased plasma concentration or reduced BBB transport of the medications. Another reason might be that reduced OCT2 activity might lead to increased monoamine concentrations in the brain and thereby to potential mood enhancement since OCT2 is thought to be involved in monoamine clearance in limbic brain regions. This hypothesis is supported by the observation that dopamine was reported to be downregulated in the nucleus accumbens, a central component of the limbic system, after smoking cessation (Bacq et al. 2012; Bergen et al. 2014; Zhang et al. 2012).

SLC22A2 might also be a gene regulating the risk of PD, regarding the fact that OCT2 is expressed at relatively high levels in dopaminergic regions in the brain including substantia nigra, the brain area associated with PD (Taubert et al. 2007). Moreover, OCT2 transports two dopaminergic neuromodulators, histidyl-proline diketopiperazine (cyclo(his-pro)) and salsolinol, with high efficiency (Taubert et al. 2007). Balanced levels of the two substances in dopaminergic cells seem to be crucial for cell integrity, with salsolinol showing cytotoxic and cyclo(his-pro) showing neuroprotective effects. In patients with PD, increased salsolinol levels were observed and associated with apoptotic nigral cell death. The *SLC22A2* variant rs8177516 (p.R400C), occurring in African-Americans with an ethnic-specific allelic frequency of 1.5%, was reported to decrease the transport of cyclo(his-pro) whereas salsolinol transport was not affected by this variant. Taken together, these findings lead to the conclusion that the rs8177516 variant might increase susceptibility to dopaminergic cell death and thereby also increase the risk for developing PD (Farthing and Sweet 2014; Grottelli et al. 2016; Taubert et al. 2007).

Another role of OCT2 – and OCT3 – in the brain might be to take part in controlling the development of speech and language competence. This hypothesis arises from the finding that the deletion of a gene cluster including *SLC22A2* and *SLC22A3*, among other genes, was reported to lead to motor speech disorders in two affected children (Peter et al. 2017). Certainly, these findings are not sufficient evidence that OCT2 and/or OCT3 take part in development of speech competence, but it would be interesting to conduct further studies to elucidate the role of OCT2 and/or OCT3 in this process.

Taken together, the findings hint towards an impact of OCT2 on response to smoking cessation therapy and on maintaining dopaminergic cell integrity. OCT2 genetic and epigenetic regulation that alter transport function of certain substrates could therefore affect the effectiveness of smoking cessation therapy as well as the risk for developing PD.

2.2 Genetic Regulation of OCT3 in the CNS

2.2.1 Localization of OCT3 in the CNS

Similar to OCT2, information on the exact localization of OCT3 in the brain is limited. There are only few studies reporting on the region- and cell-specific expression of the transporter in rats and mice and the study situation regarding the human brain is even less satisfactory. Therefore, widespread comprehensive studies on the gene and protein expression of OCT3 in rat, mice, and human brain areas would be necessary to elucidate the expression pattern of the transporter.

In rats, Oct3 was found to be expressed in circumventricular organs and in regions of the brain involved in aminergic neurotransmission. On the cellular level, Oct3 seems to be expressed in neurons and, to a lesser extent, in glial cells in these brain regions (Haag et al. 2004; Vialou et al. 2004). mRNA and protein expression of Oct3 was also detected in rat medial hypothalamus and mRNA expression was moreover reported in choroid plexus epithelium and thereby in the

blood–CSF barrier (Gasser et al. 2006; Sweet et al. 2001). Rat and mouse Oct3 expression was detected in the basolateral complex of the amygdala, which is part of the limbic system. The expression was found in glial cells and pre- and post-synaptic neurons and was localized as expected to the plasma membrane, but also to nuclear membranes and endomembrane systems. This indicates that OCT3, similar to OCT2, plays a role in the clearance of extracellular monoamines and that the transporter might furthermore mediate their intracellular distribution (Gasser et al. 2009, 2017; Hill and Gasser 2013).

In humans, OCT3 was also shown to be expressed in the brain by northern blot analysis (Wu et al. 2000). More specifically, Geier and colleagues detected OCT3 mRNA and protein expression in the BBB (Geier et al. 2013). Another study showed that OCT3 was expressed in normal human astrocytes on mRNA and protein levels (Inazu et al. 2003). The human protein atlas reports low mRNA expression of OCT3 in various brain regions as well as high protein expression in cerebellum. Consistent with the findings from Geier and colleagues, the database also shows OCT3 protein expression in the cerebral cortex and thereby the BBB. Protein expression is furthermore reported in basal ganglia and in the hippocampus, a region assigned to the limbic system. The database provides no data for other brain regions (Uhlén et al. 2015).

2.2.2 Consequences of Disturbed OCT3 Function in the CNS: What Do We Learn from Animal Studies?

Consistent with the localization of Oct3 in circumventricular organs of the rat brain, that are known to regulate fluid exchange, Oct3 seems to be crucial for neural and behavioral response to environmental variation in osmolarity in mice (Vialou et al. 2004). More precisely, Oct3 KO mice showed an increased salt-ingestion behavior under sodium depletion as well as under water deprivation conditions. Even though the underlying mechanism is unclear, this finding was the first physiological evidence discovered for the critical role of OCTs in the brain.

Oct3, similar to Oct2, further seems to be involved in regulating mood-related behavior in the mouse brain, as already indicated by the localization of the transporter in the limbic system. In line with this, Oct3 was shown to be important in aminergic neurotransmission. Increased anxiety levels to high dose psychostimulants were detected in Oct3 KO mice, but the Oct3 KO mice were less sensitive to the locomotor stimulating activity of psychostimulants (Vialou et al. 2008). With increasing dose of amphetamine or cocaine, locomotor activity decreased due to the emergence of stereotyped behavior. This is apparent in the Oct3 WT mice but not as apparent in the Oct3 KO mice (Vialou et al. 2008). On the other hand, Mayer et al. reported no difference in amphetamine stimulated locomotor activity between genotypes across a range of doses, but did show that the OCT blocker, decynium-22, attenuated the locomotor stimulant effects of amphetamine in Oct3 WT but not in Oct3 KO mice, implicating a role for Oct3 in the actions of amphetamines (Mayer et al. 2018). In contrast with these findings, Wultsch and colleagues reported that Oct3 KO mice showed decreased anxiety in comparison with WT mice, but no cognitive impairment or social-dominant behavior in a

comprehensive behavioral test battery (Wultsch et al. 2009). Despite the controversial results, the studies presumed that the altered anxiety behavior of the KO mice might be due to a role of Oct3 in neuronal signaling via modulation of neurotransmitter concentrations in the synaptic cleft. In particular, the authors indicated that Oct3 might serve as an alternative transporter that removes monoamine transmitters that escape neuronal sodium-dependent high-affinity uptake 1 (Vialou et al. 2008; Wultsch et al. 2009). This hypothesis is supported by the finding that Oct3 expression and function is increased in mice deficient for the high-affinity 5-HT uptake transporter 5-HTT (SERT, serotonin transporter encoded by *SLC6A4*). The mechanism underlying the increased transporter expression is not known (Baganz et al. 2008). Inhibition of Oct3 further led to an increased antidepressant-like effect in those mice compared to WT mice indicating that the role of Oct3 in controlling extracellular 5-HT brain levels is notably elevated in absence of the high-affinity uptake transporter. Given the fact that human carriers of a common deletional genetic variant in the promoter region of the 5-HTT gene that receive antidepressant drugs often show increased resistance to conventional treatment with selective 5-HT reuptake inhibitors, these findings offer an attractive option in such cases (Baganz et al. 2008; Daws 2009). Another study addressing the role of Oct3 in mood-related behavior in mice found that Oct3 KO mice showed impaired social behavior, namely reduced interaction preference (Garbarino et al. 2019). Taken together, these results indicate that OCT3 might be an important modulator of mood-related behavior by influencing neurotransmitter concentrations in the synaptic cleft in limbic brain regions. Genetic and epigenetic regulation of OCT3 might thereby alter such behavior as well as the reaction to psychostimulants and antidepressants.

Further animal studies suggest that Oct3, like Oct2, is involved in stress response. Stress causes a rapid increase in 5-HT release in the dorsomedial hypothalamus, where Oct3 has been shown to be expressed in rat brain (Gasser et al. 2006). Feng and colleagues showed that rats treated with an Oct3 inhibitor had increased extracellular 5-HT concentrations in the medial hypothalamus under mild restraint, which represented a stressful condition in this study. The magnitude and duration of the inhibitor-induced increase in 5-HT levels was notably smaller in rats not exposed to mild restraint and animals not treated with the inhibitor did not show any restraint-induced elevation in extracellular 5-HT levels (Feng et al. 2010). These findings support the theory that Oct3 serves as an alternative transporter to the high-affinity monoamine transporters and that this alternative transport is of special importance under conditions when 5-HT levels are high, e.g. in stress situations. Gasser and colleagues showed that accumulation of the OCT3 substrate histamine and efflux of MPP+ from acutely prepared rat medial hypothalamic explants is inhibited by corticosterone, a corticosteroid hormone that displays a rapid increase in its levels after exposure to a stressor. They concluded that regulation of OCT3 through corticosterone might influence the acute regulation of stress response (Gasser et al. 2006). This presumption is supported by the finding that Oct3 expression and function is decreased in mice after activation of the hypothalamic-pituitary-adrenal (HPA) axis via repeated swim conditions. HPA axis activation causes the release of corticosterone and an increase in extracellular 5-HT levels in forebrain regions that is

independent of 5-HTT. These results lead to the assumption that HPA axis activation caused by stressful conditions decreases Oct3 function by increasing levels of corticosterone. Furthermore, chronic exposure to stress seems to lead to reduced Oct3 expression consequently causing elevations in extracellular 5-HT levels (Baganz et al. 2010). In sum, the findings indicate that OCT3 plays a crucial role in regulating extracellular 5-HT brain levels and that corticosterone-induced inhibition of OCT3 leads to increased 5-HT levels under stressful conditions. Therefore, genetic and epigenetic variations affecting OCT3 function could have an impact on 5-HT clearance in the brain in general and could further influence stress response, e.g. if a genetic variant of the transporter leads to an altered inhibitory potential of corticosterone.

Another study analyzing the role of Oct3 in the mouse brain reported an impact of Oct3 together with the dopamine transporter (DAT, encoded by *SLC6A3*) in neurotoxicity mediated by paraquat, an herbicide that has been associated with increasing the risk for developing PD. After treatment with paraquat, Oct3 KO mice showed increased striatal damage in comparison with WT mice (Rappold et al. 2011).

Altogether, these findings regarding the role of Oct3 in mouse and rat brain indicate that Oct3 serves as an alternative transport mechanism to the sodium-dependent high-affinity monoamine uptake 1 and thereby regulates extracellular monoamine levels in the brain. Because of this feature, OCT3, similar to OCT2, seems to be involved in mood-related behavior and stress response. Furthermore, the findings hint to possible roles of OCT3 in response to variations in osmolarity and in paraquat-induced neurotoxicity. Genetic and epigenetic regulations of OCT3 might thereby influence anxiety levels under particular situations, reaction to environmental stress, response to antidepressants and psychostimulants as well as susceptibility to develop neurotoxin-induced PD.

2.2.3 Genetic Variants of OCT3 and Their Implications on CNS Function

Only very few studies have been conducted so far to study the effect of genetic variations of human OCT3 on CNS function.

Although OCT3 has been reported to be localized in the limbic system of the human brain (Uhlén et al. 2015) and was shown to play a role in monoaminergic neurotransmission and thereby in mood-related behavior in mice (Vialou et al. 2008), no correlation was found between the allele or genotype frequencies of seven known OCT3 genetic variants and the occurrence of depression in a Caucasian population (Hengen et al. 2011). These results of course are not sufficient evidence that OCT3 is not involved in the development of depression and that genetic variants other than the seven tested cannot have an impact on disease susceptibility.

However, OCT3 genetic variants have actually been associated with the development of polysubstance use in Japanese individuals with methamphetamine (MAP) dependence (Aoyama et al. 2006). MAP is a substrate of OCT3 and the transporter affects the disposition of MAP as well as behavioral changes caused by the substance. OCT3 knock-down mice, for example, were reported to show increased MAP-induced locomotor activity (Kitaichi et al. 2005). When genotype and allelic

frequency of 5 known OCT3 genetic variants were compared between MAP users and a control group, no significant difference was found. However, when the group of MAP users was divided into single-MAP users and polysubstance users, genotype and allelic frequencies of variant rs509707 and the allelic frequency of variant rs4709426 as well as the haplotypic frequencies of both intronic variants were significantly associated with polysubstance use. This indicates that OCT3 plays a role in the development of polysubstance use in people already dependent on MAP (Aoyama et al. 2006).

Another impact of OCT3 genetic variants seems to be on the development of obsessive-compulsive disorder (OCD). Because of the role of the transporter in the termination of monoamine signaling in the CNS, *SLC22A3* seems to be a candidate gene for various neuropsychiatric disorders. Two novel genetic variants were found to be present in patients with early onset OCD in a Caucasian population. One of them was a promoter variant that significantly increased OCT3 expression in vitro and was found in three unrelated male patients. The other variant, that was found in three related female patients, was localized in the coding region of *SLC22A3* (p. M370I) and led to a significant decrease in norepinephrine transport function in vitro (Lazar et al. 2008).

Taken together, the results of the different studies regarding the role of OCT3 variants in CNS function indicate that such variants as well as epigenetic regulation of OCT3 could alter the susceptibility for drug abuse disorders as well as for OCD.

3 Conclusion

Genetic and epigenetic regulations of OCTs have been shown to be important players in the response to various substrate drugs, in the risk for DDI as well as in disease susceptibility and treatment outcome for certain diseases and medications (Fisel et al. 2016; Koepsell 2020). In contrast to this relatively widespread knowledge of OCT regulation in peripheral organs, not much is known about the effects of this regulation on the CNS.

The first problem regarding this topic is the lack of information on the exact region- and cell-specific localization of OCTs in the brain. Animal studies describe expression of the transporters in various brain regions, but few have comprehensively studied gene as well as protein expression in different areas of the brain and the results gained in these animal studies are sometimes contradictory. Even less is known about the region- and cell-specific localization of OCTs in the human brain. Widespread comprehensive studies on gene and protein expression of OCTs in rat, mice, and human brain areas would therefore be necessary to elucidate their exact expression pattern that in turn could indicate possible OCT functions. Findings gained from such studies could also be used to reveal species-related differences between the expression of human transporters and their homologs in mice and rat in order to align the results gained from animal studies with research performed on human subjects.

The impact of genetic variation of OCTs on CNS function is also only sparsely studied. Findings regarding the general role of the transporters in the CNS gained from animal studies that often use KO mice models can be used to form hypotheses regarding the impact of variation in transporter function in brain. However, the number of these animal studies is limited, and sometimes contradictory, demanding further investigation. Furthermore, there is often a lack of studies that examine the influence of variations in human OCTs on brain processes, in which the animal homologs are supposed to be involved.

Moreover, to our knowledge, there is no information on the role of epigenetic regulation of OCTs on processes determining CNS function. It has been shown that Oct3 expression is downregulated after repeated stressful conditions in mice and elevated in mice deficient for 5-HTT with the underlying mechanisms being unclear (Baganz et al. 2008, 2010). One could speculate that the changes in transporter expression are mediated by epigenetic processes in order to react to environmental circumstances like stress or to the increased extracellular monoamine levels in the brain caused by missing 5-HTT. However, altered expression could also be caused by other mechanisms and therefore it would be interesting to conduct further studies to elucidate the cause for these shifts in expression and function.

In conclusion, OCTs seem to be crucial players in different processes in the brain, with OCT2 and OCT3 being of special importance. These processes include the regulation of extracellular monoamine levels as well as the transport of neurotoxins across the blood–brain barrier and their distribution in the brain. Therefore, the transporters might regulate not only various mood- and stress-related behaviors and response to psychostimulants and antidepressants, but also the risk for acute neurotoxicity as well as the susceptibility for neurodegenerative diseases. Because of this emerging knowledge on the role of OCTs in CNS processes and function, comprehensive studies should be conducted in order to elucidate the effects of genetic and epigenetic regulation of these transporters on these different processes.

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Experimental Methods for Investigating Uptake 2 Processes In Vivo

Anna Marie Buchanan, Brenna Parke, and Parastoo Hashemi

Contents

Abstract

Neuromodulators are critical regulators of the brain's signaling processes, and thus they are popular pharmacological targets for psychoactive therapies. It is

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clear that monoamine uptake mechanisms are complicated and subject to multiple uptake mechanisms. Uptake 1 describes uptake of the monoamine via its designated transporter (SERT for serotonin, NET for norepinephrine, and DAT for dopamine), whereas Uptake 2 details multiple transporter types on neurons and glia taking up different types of modulators, not necessarily specific to the monoamine. While Uptake 1 processes have been well-studied over the past few decades, Uptake 2 mechanisms have remained more difficult to study because of the limitations in methods that have the sensitivity and spatiotemporal resolution to look at the subtleties in uptake profiles. In this chapter we review the different experimental approaches that have yielded important information about Uptake 2 mechanisms in vivo. The techniques (scintillation microspectrophotometry, microdialysis, chronoamperometry, and voltammetry) are described in detail, and pivotal studies associated with each method are highlighted. It is clear from these reviewed works that Uptake 2 processes are critical to consider to advance our understanding of the brain and develop effective neuropsychiatric therapies.

Keyword

Electrochemistry · In vivo · Methods · Neurochemistry · Uptake 2

1 Introduction

Modulators in the brain mediate excitatory and inhibitory chemical processes, thus these messengers are ideal pharmacological targets to restore pathophysiological chemical imbalances. Transporters have been a major therapeutic focus because of their significance in regulating extracellular neurotransmitter levels. As such, agents have traditionally aimed to selectively target one or more transporters (Iversen 2000; Qosa et al. 2016). However, it is becoming clear that uptake mechanisms are much more complicated than one protein one substrate (Daws et al. 2013). There are currently 2 recognized uptake systems; (Daws et al. 2013) Uptake 1 is the designated protein uptake transporter for the specific modulator on neurons (SERT for serotonin, NET for norepinephrine, and DAT for dopamine) and Uptake 2 is a combination of other transporters, not specific to the monoamine, on neurons and glia, including the organic cation transporters (OCTs) and plasma membrane transporters (PMATs) (Grundemann et al. 1999; Masson et al. 1999; Eisenhofer 2001). This more complex uptake profile may account for the clinical variability of reuptake inhibitor therapies. (Locher et al. 2017) Therefore, to improve drug efficacy it is critical to better understand how substrates interact with different uptake transporters. Uptake 1 processes have been studied in great detail over the last few decades, while uptake 2 mechanisms remain less defined.

In general, it is difficult to study uptake mechanisms due to the spatial and temporal limitations of analytical technologies to capture multiple uptake mechanisms in intact tissue. In this chapter, we review the different experimental

approaches that have yielded important information about Uptake 2 mechanisms in vivo. The techniques utilized (scintillation spectrometry, microdialysis, chronoamperometry, and voltammetry) (Shaskan and Snyder 1970, Daws et al. 2005a, b, Feng et al. 2005, Abdalla et al. 2020) are described in detail and major findings applying these methods are highlighted. It is clear from the works reviewed here that Uptake 2 processes are critical to consider for advancing our understanding of the brain and developing more efficacious neuropsychiatric therapies.

2 Methods for Investigating Uptake 2 Processes in Vivo

2.1 Scintillation Microspectrophotometry

It was clear by the mid-1960s that neurotransmitters accumulated in different brain regions (Dengler et al. 1962; Robinson et al. 1965; Ross and Renyl 1967). This phenomenon hinted toward specific mechanisms that enable selective aggregation of molecules. At the time, methods to study such mechanisms were limited. Autoradiography, whereby radiolabeled species were patterned via visualization of the radioactive component, was a popular method to study neurotransmitter localization. In 1967, Aghajanian and Bloom injected ^{3}H-serotonin into the lateral ventricle of the rat and found, via autoradiography, that low concentrations of radiolabeled serotonin localized in nerve terminals in periventricular regions (Aghajanian and Bloom 1967). A series of further studies found serotonin localization in catecholaminergic neurons in the vas deferens (Thoa et al. 1969), pineal gland (Bertler et al. 1964; Neff et al. 1969), and multiple other unspecified brain regions (Fuxe and Ungerstedt 1967; Lichtensteiger et al. 1967). A seminal study, by Blackburn et al. in 1967, showed evidence for active uptake of serotonin into tissue slice preparations using a method called scintillation microspectrophotometry (Blackburn et al. 1967). Here synaptosomes were isolated from brain homogenates and two different groups were incubated at 0° and 37°C with 5 μg mL^{-1} of ^{14}C-serotonin. A toluene scintillator (substance that converts radioactivity (i.e., from ^{14}C-serotonin) to visible light) was added to the incubated preparations to measure the presence of ^{14}C-serotonin as counts per time. When subtracted from the counts per time at 0°C, the preparation at 37°C showed significant uptake of ^{14}C-serotonin. The group found a V_{max} of 770 μg 5 min^{-1} g^{-1} and a K_m of 0.57 × 10^{-6} M. This finding was verified by others using the same method (Ross and Renyl 1967; Chase et al. 1969). Snyder and co-workers explored serotonin uptake in more detail in 1970. They compared serotonin uptake kinetics to norepinephrine uptake kinetics across various brain regions (Shaskan and Snyder 1970). Figure 1 illustrates Snyder's experiment.

Here striatal and hypothalamic regions were extracted from rat brains, homogenized in solution, and incubated at 37°C with different concentrations of ^{3}H-serotonin. Using scintillation spectrophotometry the group found, in both regions, two distinct mechanisms of uptake evidenced by two slopes in a Lineweaver-Burk plot. These two mechanisms consisted of a high affinity (K_{m1}: 1.7 × 10^{-7} M), low efficiency (V_{max1}: 1.12 μmol min^{-1} g^{-1}) mechanism and a low

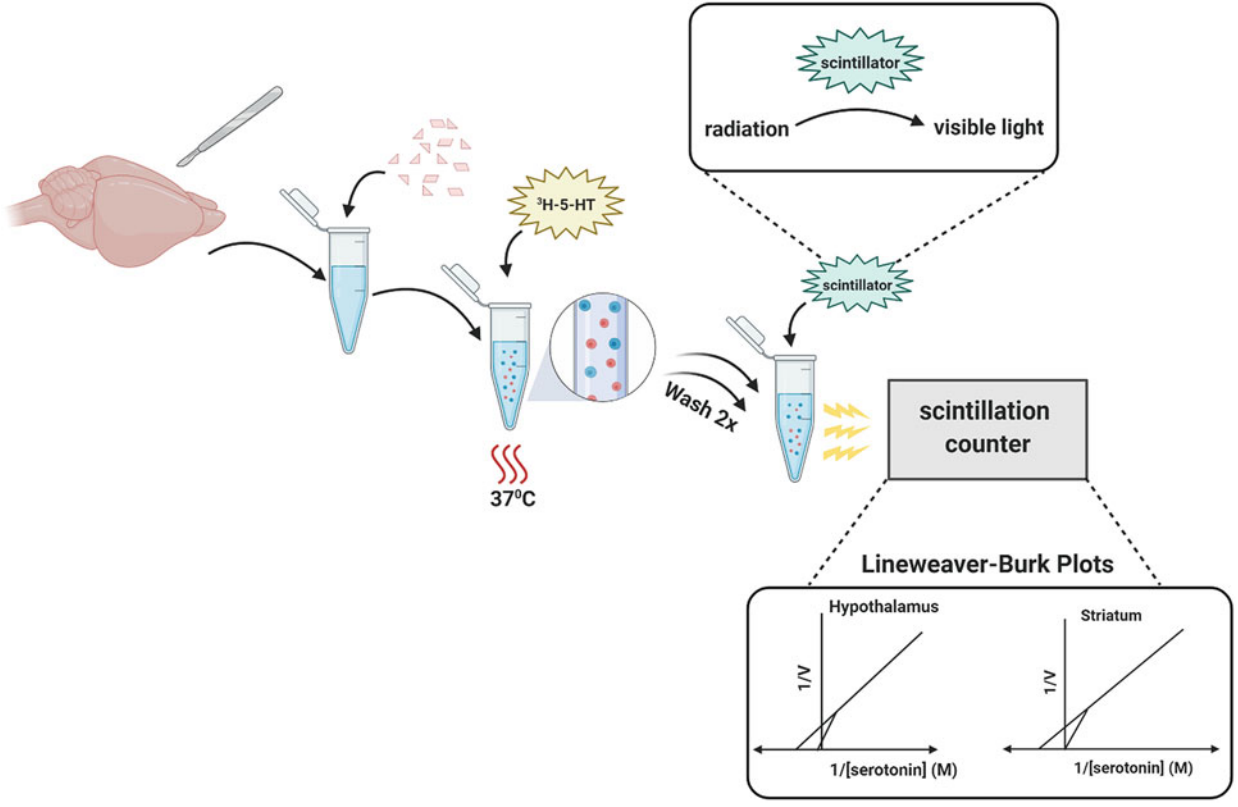

Fig. 1 Illustration of Snyder's 1970 scintillation spectrometry experiment (Shaskan and Snyder 1970). Snyder and colleagues extracted and homogenized hypothalamic and striatal regions of rat brains. The brain homogenates were incubated with radiolabeled serotonin (^{3}H-serotonin) at 37°C at various concentrations and durations. The samples were then washed and a toluene scintillator was added to convert the radioactive photons to visible light, which were measured via spectroscopy. The Lineweaver-Burk plots are reproduced with permission from Aspet Journals. Created using Biorender.com

affinity (K_{m2}: 8.0 × 10^{-6} M), high efficiency mechanism (V_{max2}: 26.6 μmol min^{-1} g^{-1}). Thus was born Uptake 1 and 2. The group went on to show evidence for different serotonin receptor types (Peroutka and Snyder 1979) and how pharmacological manipulation alters their binding efficiencies (Peroutka and Snyder 1980).

2.2 Microdialysis

Scintillation spectrometry, while shedding important light on two uptake mechanisms, is not amenable to in vivo work. Microdialysis, however, is a well-established in vivo method that measures neurochemicals, where a micro-dimensional probe is directly implanted into brain tissue and infused with a perfusion fluid that mimics the osmotic character of interstitial fluid. At the distal end of this probe is a semipermeable, nanoporous membrane. The cut-off of this membrane can be designed to allow exchange of smaller brain chemicals, but not larger bacteria/viruses. As the perfusate travels through the probe, exchange occurs and the dialysate is collected for separation and secondary analysis. This separation is most often carried out via HPLC (Cheng et al. 2009; Nandi and Lunte 2009) with the analysis with US/VIS (Parsons et al. 1998; Tsai et al. 2000; Chang et al. 2005), electrochemical (Tsai et al. 1996; Portas et al. 1998; Chaurasia et al. 1999; Cheng et al. 1999), and mass spectroscopy detection (Zhang and Beyer 2006; Shackman et al. 2007). Figure 2 is an illustration of the method.

In the early 1990s, Wurtman and colleagues used microdialysis in the rat striatum and administered two catechol-O-methyltransferase (COMT) inhibitors *i.p.* (Kaakkola and Wurtman 1992). The group found that these inhibitors increased dialysis levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine. Given that COMT is not localized in presynaptic dopaminergic terminals, this work implied a role for glial cells and non-dopaminergic neurons (i.e., via Uptake 2) in dopamine metabolism. This was foundation work for studying Uptake 2 of dopamine via microdialysis. Käenmäki et al. investigated the effect of DAT, NET, and MAO inhibitors on dopamine concentrations in the prefrontal cortex (pFC) of COMT-k/o mice (Käenmäki et al. 2010). The group found that COMT contributed to over half of the dopamine clearance in the pFC, showing that Uptake 2 played a significant role for dopamine clearance in the pFC. Another role for Uptake 2 was highlighted by Cui et al. *who h*ypothesized that OCT3 (major Uptake 2 transporter type) bidirectionally regulates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine's damaging effects on dopamine neurons (Cui et al. 2009). The group used microdialysis to measure dopamine, homovanillic acid (HVA), DOPAC, and 1-Methyl-4-phenylpyridinium (MPP+) following MPTP administration in OCT3 knock-out mice. Dopamine, HVA, and DOPAC were also measured following administration of methamphetamine. The group found that deletion of OCT3 protects against MPTP induced dopaminergic neurodegeneration by hampering the release of MPP+ from astrocytes. However, OCT3 deletion decreased the removal rate of extracellular dopamine following

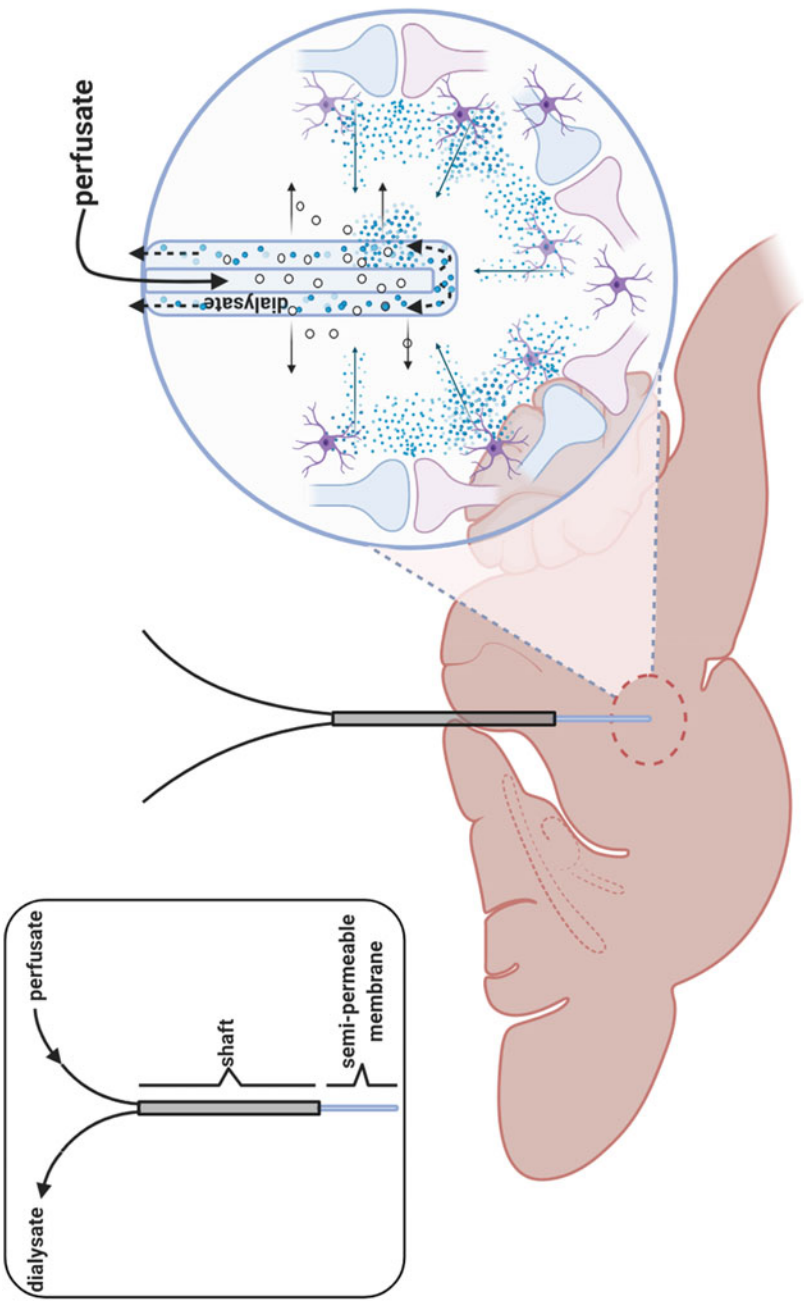

Fig. 2 A general illustration of a microdialysis experiment. A microdialysis probe is implanted into the brain area of interest and perfused with artificial cerebral spinal fluid. Neurochemicals in the extracellular space diffuse into the probe and are carried out via the dialysate. The dialysate is collected, separated, usually via HPLC, and analyzed with a secondary method. Created using Biorender.com

methamphetamine, causing additional damage to dopaminergic terminals. These opposing effects showed the bidirectional control of this damage process by OCT3. A seminal paper by Gasser, Mantsch, and colleagues showed the behavioral significance of Uptake 2 (Wheeler et al. 2017). Corticosteroid (which blocks OCTs and PMATs) was shown to reinstate drug seeking behavior after extinguishment. The group postulated that this could be due to inhibition of dopamine metabolism or due to transporter mediated clearance. Taken together, these dopamine microdialysis studies shed light on the physiological and behavioral importance of Uptake 2 processes.

Microdialysis has also been used to study Uptake 2 in the context of serotonin. Feng et al. measured serotonin in the medial hypothalamus following administration of OCT3 inhibitor decynium-22 (D-22) (Feng et al. 2005). They found that D-22 administration increased local concentrations of serotonin in the medial hypothalamus in a reversible and dose-dependent manner. Adding significance to this work, they found that the highest dose of decynium-22 resulted in an increase in grooming behavior. Further highlighting the role of serotonin in Uptake 2 mediated behavior, Renner and Lowry found that agents that interfere with OCT3 transport (corticosteroid and normetanephrine) increased serotonin concentration in the central amygdala under stress but not under home cage conditions, suggesting a role for OCT3 uptake of serotonin under stress condition (Hassell et al. 2019). Behavioral studies were extended to depression when Rahman et al. measured serotonin and norepinephrine in the rat frontal cortex and found that inhibiting both Uptake 1 and 2 elicited a greater increase in norepinephrine than inhibiting either uptake mechanism alone (Rahman et al. 2008). The group used this finding as a basis to suggest that Uptake 2 mechanisms are involved in antidepressant effects (a notion explored by Lyn Daws and colleagues with chronoamperometry (vide infra)).

In summary, microdialysis has shed important light on mechanistic, behavioral, and pharmacological aspects of Uptake 2.

2.3 Chronoamperometry

High-speed chronoamperometry is an electrochemical technique that applies a square wave potential to a working electrode, often a carbon fiber microelectrode. The potential is stepped from an initial, resting potential to a potential sufficient for oxidation or reduction of analytes. The current resulting from this step is diffusion driven by analyte transfer from bulk solution to the electrode surface (Lefrou et al. 2012). Following this potential step, the current decay is measured as a function of time (Cottrell 1902). The voltage is then stepped to a potential where the inverse REDOX reaction occurs. Chronoamperometry is often used to measure kinetics of chemical reactions (Papanastasiou et al. 1993; Prévoteau et al. 2015), diffusion coefficients (Yap and Doane 1982; Wang et al. 2018), and significant to this chapter, the method has been successfully implemented to study various monoamine systems in the brain (Gerhardt et al. 1987; Daws et al. 1997; Gerhardt and Hoffman 2001).

The vast majority of chronoamperometry studies on Uptake 2 focus on measuring serotonin. In 1998 chronoamperometry was first used to provide evidence of multiple uptake mechanisms (Daws et al. 1998). Here, in rats, administration of a SERT inhibitor slowed serotonin clearance in the dentate gyrus (DG) and the CA3 region of the hippocampus whereas a NET inhibitor slowed clearance only in the DG. This experiment showed differing populations of transporters for serotonin clearance across the two regions. A detailed study by Daws and colleagues in 2005 utilized the method to calculate the kinetics of uptake (Daws et al. 2005a, b). The group inserted multi-barrel carbon fiber microelectrodes into 4 different brain regions (dorsal raphe nucleus, dentate gyrus, CA3, and corpus callosum). They applied increasing concentrations of serotonin (Fig. 3) and the kinetics of serotonin clearance were calculated (inset graph on bottom right of figure). The group clearly showed two mechanisms for serotonin uptake. The first being the high affinity, low capacity (Uptake 1) uptake associated with SERTs and the second being the low affinity, high capacity uptake of NET (Uptake 2). The group then focused on OCTs by using serotonin transporter (5HTT) mutant mice with impaired 5HTT expression (Baganz et al. 2008). Serotonin and histamine clearance were measured in the CA3 region of the hippocampus.

In control mice, no detectable contribution of OCTs was measured for serotonin or histamine uptake. However, in 5HTT deficient mice, OCTs contributed to both serotonin and histamine uptake, showing their ability to uptake these analytes in the absence of SERTs. Further attesting to OCTs ability to uptake serotonin, decynium-22 (D-22) administration has antidepressant-like effects in 5-HTT mutant mice but not control mice as assessed by the tail suspension test. This idea was investigated in more depth in the following study (Horton et al. 2013). The group found that D-22 enhanced the effects of fluvoxamine (SSRI) on serotonin clearance and importantly increased antidepressant effects on behavior. A final study of note was the investigation of the role of HPA axis activation on serotonin clearance (Baganz et al. 2010), whereby serotonin was measured after repeated swim paradigms in mice. Serotonin clearance was likely reduced following these repeated swim paradigms due to release of corticosterone by HPA activation, resulting in inhibited serotonin clearance by OCT3. This method, thus, has been used in versatile and elegantly designed studies to provide a rich body of work on Uptake 2 processes.

2.4 Fast Scan Voltammetry

Concurrent with these chronoamperometry studies, another electrochemical method, namely voltammetry, has been used to uncover important information about parallel uptake mechanisms. Fast-scan cyclic voltammetry (FSCV) applies an electrochemical waveform, at very fast scan rates (typically >400 V s^{-1}), to a carbon fiber microelectrode which rapidly captures redox processes at the electrode surface. The positions of the redox peaks identify analytes of interest and the magnitude of these peaks over time quantifies analytes.

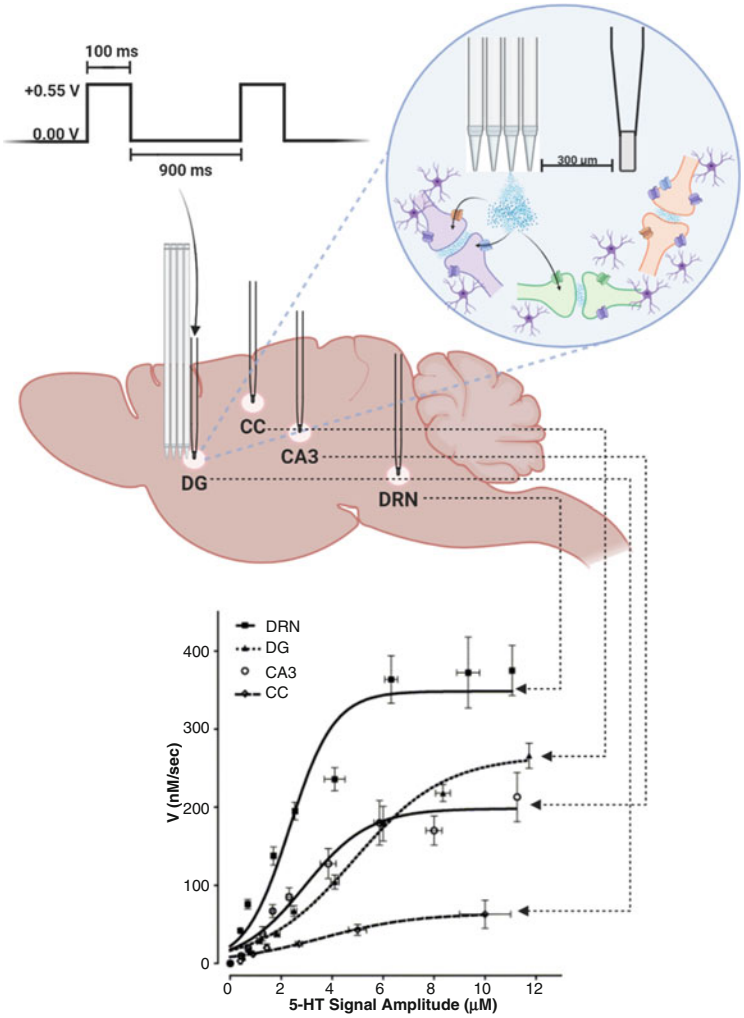

Fig. 3 Illustrative depiction of Daws' chronoamperometry experiment from 2005. Electrochemical recordings were taken, with high-speed chronoamperometry, in the dorsal raphe nucleus (DRN), corpus callosum (CC), CA3 region of the hippocampus (CA3), and dentate gyrus (DG). Exogenous serotonin was pressure-injected into the extracellular space near the electrode (300 µm) and variances in uptake kinetics of serotonin were observed across the four brain regions. Specific transporters responsible for serotonin uptake were discerned by pharmacological manipulation (Daws et al. 2005a, b). Reproduced with permission from Elsevier. Created using Biorender.com

Wightman and colleagues, who pioneered FSCV, first found evidence for different kinetics governing dopamine uptake in the striatum and the nucleus accumbens and attributed these differences to heterogeneity in dopamine overflow (Wightman et al. 1988; May and Wightman 1989; Wightman and Zimmerman 1990; Kennedy et al. 1992). Adrian Michael and his group found domain specific dopamine

signatures in different parts of the striatum (Peters and Michael 2000; Moquin and Michael 2009). Specifically, they found "fast" and "slow" release profiles that, like Wightman's early findings, were correlated with "fast" and "slow" uptake curves (Moquin and Michael 2011). The group modeled these responses (Walters et al. 2015) and suggested that the distinctions in uptake were due to region dependent variations in basal levels of dopamine; functionally dependent on differing levels of autoinhibition (Walters et al. 2016). Similar differences in uptake profiles were found in a collaborative study between Sara Jones and Paul Gasser. In this work, FSCV revealed slower uptake rates for dopamine in the basolateral amygdala (BLA) compared to the NAc (Holleran et al. 2020). These researchers explained their findings via contrasting transporter densities between the regions. Specifically, despite similar levels of OCTs in these brain areas, more DATs were found in the NAc, where faster dopamine uptake profiles were recorded.

FSCV has traditionally been applied to dopamine detection, however our group has pioneered the application of this method to serotonin. Serotonin FSCV is fundamentally more challenging than that of dopamine FSCV. Serotonin is released at much lower levels than dopamine, meaning that a more sensitive probe is needed with a lower limit of detection (Hashemi et al. 2012). Additionally, serotonin metabolites foul the electrode surface, reducing the probe's analytical performance (Hashemi et al. 2009). The modification of the carbon surface with a thin layer of Nafion circumvents these issues by increasing preconcentration of the positively charged serotonin ions and repelling the negatively charged serotonin metabolites. This dual effect, in conjunction with a serotonin-specific waveform (Jackson et al. 1995), adheres enough analytical power to FSCV to measure very low concentrations of evoked serotonin in vivo (~10 s of nM). In 2014 we saw two distinct uptake curves for serotonin in the substantia nigra, pars reticulata (SNr) with the same stimulation of the medial forebrain bundle (Wood et al. 2014). These two curves had two discrete slopes of clearance which were present to varying degrees depending on the microenvironment of the electrode. We termed these responses fast, slow and hybrid to denote each of the two curves (fast and slow) and a combination of the two (hybrid), Fig. 4.

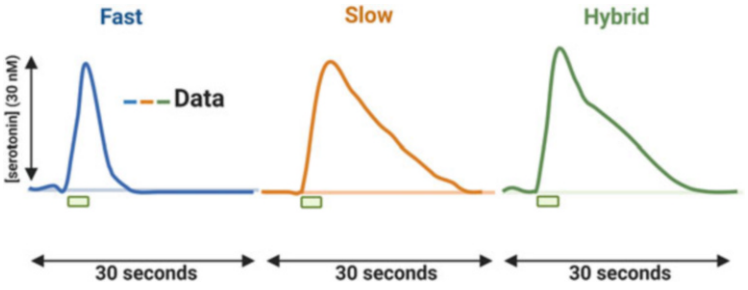

Fig. 4 Representation of the fast, slow, and hybrid signals recorded and kinetically modeled by Wood et al. (2014). These responses were recorded via FSCV in the SNr of a mouse and converted to concentration over time with a predetermined calibration factor. This figure was adapted from Wood et al. (2014) and used with permission from ACS Publications. Created using Biorender.com

Kinetic modeling of the in vivo responses found two distinct sets of Michaelis-Menten parameters were necessary to fit the data (Eq. 1). In this equation, $S(t)$ is the extracellular concentration of serotonin in the SNr, $R(t)$ is the rate of release, and $A(t)$ is the fraction of stimulated autoreceptors. The terms α and β were 0 and 1, respectively, for fast uptake curves and vice versa for slow curves. The Michaelis-Menten parameters for this model were then calculated to be Vmax1 17.5 nMs–1, Km1 5 nM which represents a low efficiency, high capacity system (Uptake 1) and Vmax2 780 nMs, 170 nM Km2, representing a high efficiency, low capacity system (Uptake 2). Administration of escitalopram, a selective serotonin uptake inhibitor (SSRI), largely inhibited Uptake 1 and, to a lesser extent, Uptake 2, which highlights Uptake 1 is controlled by the SERTs.

$$\frac{\mathrm{d}[S(t)]}{\mathrm{d}t} = R(t)\,(1 - A(t)) - \alpha \frac{V_{\max 1}\,[S(t)]}{K_{\mathrm{m1}} + [S(t)]} - \beta \frac{V_{\max 2}\,[S(t)]}{K_{\mathrm{m2}} + [S(t)]} \tag{1}$$

Our serotonin signals in the medial prefrontal cortex (mPFC) mirrored Wightman and Michael's dopamine signals in the striatum. Specifically, we found that serotonin overflow (or evoked release) was strongly connected to uptake kinetics (West et al. 2019). When the CFM was placed in layers 1–3 of the mPFC, a single evoked release peak was observed with a hybrid, Uptake 1 and 2, clearance profile. When the electrode was placed in layers 5–6 of the mPFC, a double evoked event was often observed. The first peak resembled the single peak (observed in layers 1–3) release and uptake kinetics while the uptake of the second profile was attributed to Uptake 1. From these data, V_{max1}, K_{m1}, V_{max2}, and K_{m2} were calculated to be 19.25 nM s^{-1}, 5 nM, 780 nM s^{-1}, and 170 nM, respectively. It is important to note that these values are almost identical to those derived from data taken in the SNr (Wood et al. 2014).

In addition to the SNr and mPFC, we extended our measurements to encompass the hippocampus and compared the responses. We found distinctly different responses in these three areas as seen in Fig. 5 that shows our experiment (Abdalla et al. 2020). By mathematically modeling the responses we were able to attribute the differences in uptake profiles to the relative contribution of Uptake 1 and 2 to the electrochemical signal. This allowed us to hypothesize that the SNr and mPFC have higher densities of SERTs given the larger Uptake 1 (slow uptake) component, and confocal imaging verified this. To strengthen this hypothesis, we used an additional method, fast-scan controlled-adsorption voltammetry (FSCAV).

During FSCV experiments, each time the serotonin-specific waveform is applied to the electrode, a large capacitive current is discharged that masks the faradaic current. Thus, FSCV relies on background subtraction to discern faradaic current signatures associated with specific molecules. This background subtraction inhibits ambient measurements to be made via FSCV. Fast-scan controlled-adsorption voltammetry (FSCAV) was developed to measure ambient concentrations of neurotransmitters in the extracellular space (Atcherley et al. 2013; Abdalla et al. 2017). FSCAV implements the specific waveform for each neurochemical with a

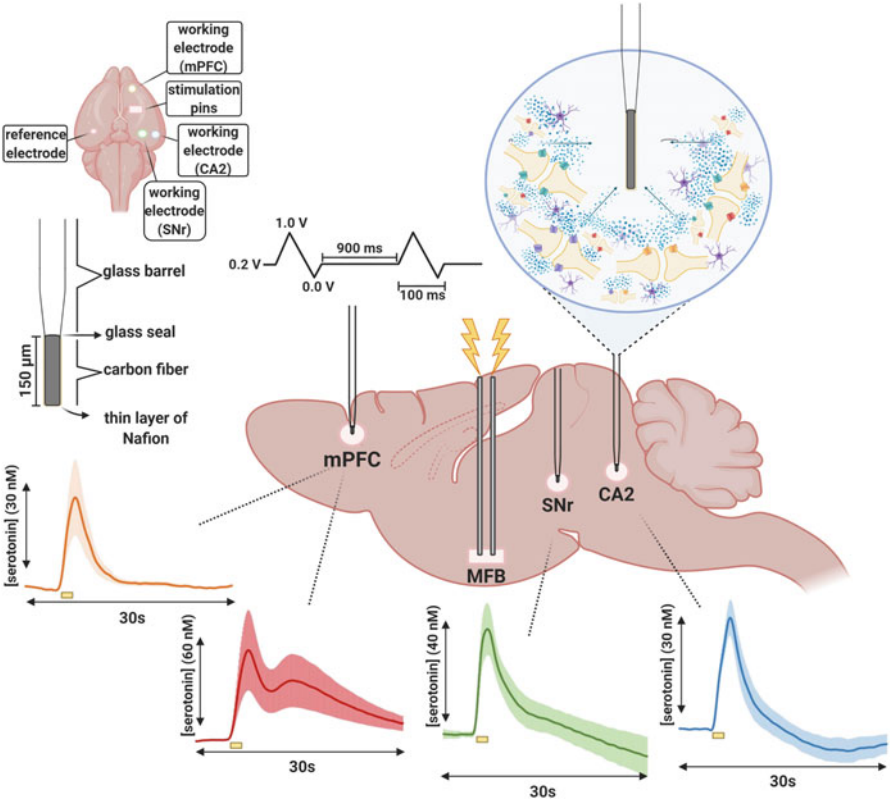

Fig. 5 Illustration of the Abdalla et al. (2020) experiments. The CFMs were electropolymerized with a thin layer of Nafion (Hashemi et al. 2009) prior to implantation into the brain. The serotonin wave form (Jackson et al. 1995) was applied to the CFMs at 10 Hz during experiments. CFMs were placed in either the medial prefrontal cortex (mPFC), substantia nigra pars reticulata (SNr), or CA2 region of the hippocampus (CA2) and stimulated release of serotonin was recorded over time. Two signals from the mPFC showed Uptake 1 (fast decay) and Uptake 2 (slow decay), with the orange trace exhibiting Uptake 1 and the red trace showing both Uptake 1 and 2. The green trace, recorded in the SNr showed a hybrid response, while the blue trace from the CA2 showed properties of Uptake 1 only

modified application scheme and at a higher frequency than FSCV to detect ambient levels of transmitters. Currently this method is applicable to dopamine and serotonin detection. For serotonin, the serotonin-specific waveform is applied at 100 Hz (vs. 10 Hz with FSCV) for 2 s to minimize adsorption to the electrode surface. Then a holding potential (0.2 V for serotonin) is applied for 10 s to allow a period for controlled adsorption of the ambient serotonin in the extracellular space. The waveform is re-applied at 100 Hz for 18 s to reduce and oxidize the adsorbed

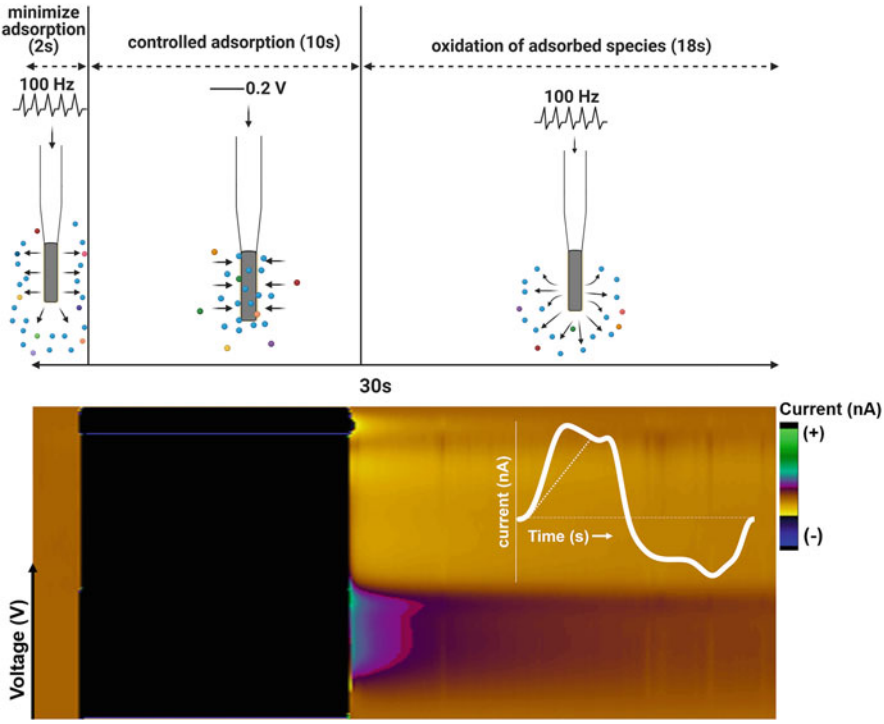

Fig. 6 Representative illustration of the waveform application scheme associated with FSCAV for serotonin detection. A CFM is lowered into the brain region of interest and, for each measurement, the serotonin waveform is applied at a high frequency (100 Hz) for 2 s to minimize any species from adhering to the CFM surface, then a holding potential of 0.2 V is applied for 10 s to allow for species to adsorb to the carbon surface. The waveform is finally re-applied at 100 Hz for 18 s to rapidly reduce and oxidize the adsorbed electroactive species. A representative color plot for a serotonin FSCAV measurement is shown below the cartoon. Created using Biorender.com

serotonin. The peak on the voltammogram is converted to concentration based on the individual calibration of each electrode after implantation into the brain.

A depiction of the FSCAV waveform application scheme along with a representative color plot for serotonin detection is shown in Fig. 6. Using FSCAV, we recorded basal values in these three regions 72.82 $\pm$ 3.21 nM in the CA2 region of the hippocampus, 67.57 $\pm$ 3.42 nM in the mPFC and 39.71 $\pm$ 1.96 nM in the SNr (Abdalla et al. 2020). We attributed the lower serotonin level in the SNr to a much higher ratio of SERT mediated uptake, strengthening our hypothesis of higher SERT density in the SNr. Thus we presented the power of FSCV as a chemical imaging probe that gives information about local physiological architecture.

3 Conclusion

Modulators in the brain regulate the vast majority of neurochemical signaling processes and are thus the targets of many psychoactive therapeutic compounds. The two uptake processes for monoamine, Uptake 1 and 2, show that a more complex uptake profile needs to be considered when studying and targeting monoamines. While much is known about Uptake 1 processes, Uptake 2 mechanisms have been less studied. In general, it is difficult to study these mechanisms because analytical methods are limited in their spatial and temporal resolving power to capture multiple uptake mechanisms in intact tissue. In this chapter, we described different experimental approaches that have yielded important information about Uptake 2 mechanisms and highlighted significant findings using each method. It is clear from these works that Uptake 2 processes are critical to consider when investigating brain monoamines.

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Substrates and Inhibitors of Organic Cation Transporters (OCTs) and Plasma Membrane Monoamine Transporter (PMAT) and Therapeutic Implications

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Contents

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Abstract

The gene products of the *SLC22A* gene family (hOCT1, hOCT2, and hOCT3) and of the *SLC29A4* gene (hPMAT or hENT4) are all polyspecific organic cation transporters. Human OCTs (including hPMAT) are expressed in peripheral tissues such as small intestine, liver, and kidney involved in the pharmacokinetics of drugs. In the human brain, all four transporters are expressed at the blood-brain barrier (BBB), hOCT2 is additionally expressed in neurons, and hOCT3 and hPMAT in glia. More than 40% of the presently used drugs are organic cations. This chapter lists and discusses all known drugs acting as substrates or inhibitors of these four organic cation transporters, independently of whether the transporter is expressed in the central nervous system (CNS) or in peripheral tissues. Of interest is their involvement in drug absorption, distribution, and excretion as well as potential OCT-associated drug–drug interactions (DDIs), with a focus on drugs that act in the CNS.

Keywords

Clinical implications · Drug · Drug interactions · Inhibitors · OCT1 · OCT2 · OCT3 · PMAT · Substrates

1 Introduction

The monoamine serotonin (5-hydroxytryptamine, 5-HT) and the monoamines and catecholamines (CAs) dopamine (DA), epinephrine (E), and norepinephrine (NE) are important neurotransmitters. Monoamines regulate a wide array of physiological, behavioral, cognitive, and endocrine functions in the central and peripheral nervous systems. The actions of released monoamine neurotransmitters are terminated by plasma membrane transporters that remove the transmitters from the extracellular space surrounding a monoaminergic synapse. Historically, two clearly distinct transport systems, designated as "uptake$_1$" and "uptake$_2$", also known as "neuronal" and "extraneuronal" uptake, have been defined to be responsible for the clearance of the monoamine NE (for review, see Iversen 1971; Eisenhofer 2001). The uptake$_1$ transporter is a Na^+- and Cl^--dependent, cocaine-sensitive, high-affinity but low-capacity transporter, which has been cloned by Susan Amara and coworkers (Pacholczyk et al. 1991) and which is known as NET (NE transporter). The NET belongs as SLC6A2 to the solute carrier (SLC) 6 family of Na^+- and Cl^--dependent monoamine neurotransmitter transporters, which also include the 5-HT transporter (SERT; SLC6A4) and the DA transporter (DAT; SLC6A3); meanwhile, the term "uptake$_1$" transporter includes all three of these high-affinity, low-capacity neuronal monoamine transporters.

Uptake$_2$, also known as "extraneuronal uptake," was originally characterized as a Na^+- and Cl^--independent, low-affinity, high-capacity transport system for CAs in

peripheral tissues such as heart and smooth muscle that is sensitive to inhibition by O-methylated catecholamines (e.g., metanephrine or O-methyl-isoprenaline (OMI)) and corticosterone (Iversen 1965, 1973; Trendelenburg 1978; Bönisch 1980; Eisenhofer 2001). The membrane potential was proposed as a driving force for extraneuronal uptake (Bönisch et al. 1985). Expression of a transport system not identical but similar to uptake$_2$ was shown to exist also in the central nervous system (CNS; Hendley et al. 1970; Wilson et al. 1988; Trendelenburg 1989).

In 1990 a clonal cell line from human kidney (Caki-1) was described to express the extraneuronal uptake (uptake$_2$) transporter (Schömig and Schönfeld 1990). Using this cell line Schömig and coworkers identified the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) as an excellent uptake$_2$ substrate (Russ et al. 1992) and cyanine derivatives (such as decynium 22) as highly potent uptake$_2$ inhibitors (Russ et al. 1993).

Hermann Koepsell and coworkers (Gründemann et al. 1994) were the first who published the cloning of an organic cation transporter (termed "OCT1"). Uptake of ^{14}C-TEA^+ (tetraethyl ammonium) in Xenopus oocytes expressing this transporter revealed potential-dependent transport which, however, was only weakly inhibited by corticosterone; in addition, no hybridization was detected in heart and Caki-1 cells. This OCT1 was identified to be the first member of a new prototype of polyspecific transporters of the solute carrier family 22. Also OCT2, the next cloned member of this family of SLC22 transporters did not show the typical properties of an uptake$_2$ transporter since it was only weakly inhibited by OMI (Gorboulev et al. 1997). In 1998 a third member of potential-sensitive organic cation transporters, designated as OCT3, was cloned from rat placenta by Kekuda et al. (1998) and from human Caki-1 cells by Schömig and coworkers (Gründemann et al. 1998) who named this new, OMI- and corticosterone-sensitive transporter "EMT"(extraneuronal monoamine transporter). Engel et al. (2004) published the cloning of a further human organic cation transporter, which was designated as plasma membrane monoamine transporter (PMAT). This hPMAT (SLC29A4) belongs as hENT4 to the family of equilibrative nucleoside transporters (ENTs), which – like the above mentioned OCTs – is a Na^+-independent, membrane-potential-sensitive, polyspecific transporter that is able to mediate bidirectional transport of a broad range of organic cations, including monoamine neurotransmitters. PMAT shows functional similarity to OCTs but also some differences such as more than 10-fold lower sensitivity to inhibition by corticosterone (Engel and Wang 2005). PMAT is expressed in the human brain and mRNA expression of mouse PMAT is much higher than that of mouse OCT2 and OCT3 (Miura et al. 2017). More recent studies suggest that uptake$_2$ consists of multiple organic cation transporters with broad substrate spectrum (Daws 2009; Gasser and Daws 2017), but PMAT and OCT3 are likely to represent the major uptake$_2$ transporters in brain (Duan and Wang 2010).

2 Basic Properties of OCTs and PMAT: Structures and Transport Function

2.1 Membrane Topology and Structure-Function of OCTs

From this topic only some essentials will be given here since excellent and exhaustive reviews on OCTs from Hermann Koepsell, who also contributed to this book, have already been published recently (see, e.g., Koepsell 2020; Koepsell et al. 2007).

The three human organic cation transporters (hOCT1, hOCT2, and hOCT3) belong to the SLC22 family and all three genes (SLC22A1-A3) are located on chromosome 6q25.3. The three hOCTs show between 50–70% amino acid identity and they have a predicted membrane topology of 12 trans-membrane domains (TMDs) with an alpha-helical structure and with a pseudosymmetry between TMDs 1–6 and 7–12. The NH_2- and COOH-terminals are located intracellularly. All three hOCTs contain a large extracellular loop between TMDs 1 and 2 and a large intracellular loop between TMDs 6 and 7. When Koepsell and coworkers (Gründemann et al. 1994) published the cloning of the first organic cation transporter, namely the rat OCT1 (rOCT1, meanwhile mostly termed as rOct1, *Slc22A1*) they showed that uptake of the organic cation ^{14}C-TEA into transfected oocytes was potential-dependent; this was confirmed also for rat OCT2, the next cloned rOCT member (Okuda et al. 1996). Organic cation transport by rOCT2 showed similar properties as that by rOCT1; in addition, transport in both directions could be demonstrated for both rOCTs (Okuda et al. 1996). By measuring not only substrate uptake but also charge transfer in oocytes and eukaryotic cells expressing rOCT1 or rOCT2 the function of both transporters has been investigated in more detail (Koepsell et al. 2003). From these studies as well as from results obtained with mutants of rOCT2 (Gorboulev et al. 1999) and rOCT1 (Volk et al. 2009), and from the additional use of the modeled tertiary structure of rOCT1 in the outward- and inward-facing confirmation, insights into functions and structure-function relationships of rOCT1 have been obtained (Koepsell 2011). Eight amino acids (F160, W218, Y222, T226 (in TMD 2 and 4) and R440, L447, Q448, and D475 (in TMD 10 and 11)) have been identified to be crucial for substrate affinity and/or selectivity and for the interaction of corticosterone, a non-transported inhibitor of all OCTs. Results of site-directed-mutagenesis and homology modeling studies suggest a binding surface with multiple binding sites.

2.2 Membrane Topology and Basic Functions of PMAT

The human PMAT cDNA was cloned from a kidney cDNA library (Engel et al. 2004) and thereafter also the mouse and rat orthologs were cloned (Dahlin et al. 2007; Okura et al. 2011). The human PMAT cDNA encodes a membrane protein that is predicted to possess 11 trans-membrane domains (TMDs) with a long intracellular NH_2-terminus and a short extracellular COOH-terminus and a large intracellular loop between TMD 6–7 (Engel et al. 2004). Mouse and rat PMAT

proteins share 86–87% sequence identity with hPMAT; they are predicted to possess a similar membrane topology (Wang 2016). The human PMAT is not homologous to hOCT1-3; it exhibits low sequence identity (about 20%) to the human members of the SLC29 family of equilibrative nucleoside transporters hENT1, hENT2, and hENT3 but hPMAT is identical to hENT4 (SLC29A4) which is assigned to chromosome 7p22.1. However, there is no significant interaction of hPMAT with nucleosides or nucleobases (Engel et al. 2004), except adenosine which was shown to be transported by hPMAT (Zhou et al. 2010). On the other hand, when expressed in eukaryotic cells or oocytes, hPMAT showed similarities with hOCT3 since hPMAT efficiently transports serotonin, dopamine, and the organic cation MPP^+. Transport of these substrates is membrane-potential-sensitive and does not require Na^+ and Cl^- and is sensitive to inhibition by decynium 22; however, and in contrast to hOCT3, corticosterone exhibits only low inhibitory potency. MPP^+ is transported by hPMAT at an efficiency (V_{max}/K_m) about 17-fold higher than that of adenosine (Zhou et al. 2010). In addition, many organic cations and classic hOCT substrates were shown to be substrates of hPMAT (Engel and Wang 2005). Thus, hPMAT functions as monoamine and polyspecific organic cation transporter.

2.3 Model for Transport by OCTs and PMAT

Koepsell (2011) presented an alternative access model (at low intracellular substrate concentration) predicting electrogenic transport as a result of the following sequence of steps: (1) substrate binding to the outside, (2) translocation of the bound substrate to the intracellular site, (3) dissociation of the substrate, and (4) reorientation of the empty substrate binding site (Koepsell 2011). Reorientation of the unloaded transporter may be the rate-limiting step. Thus, in the absence of a cationic substrate inside of a cell the transporter operates as electrogenic uptake carrier with transport driven by the inside-negative membrane potential. Outward transport, on the other hand, can only occur when extracellular cation substrate is not present or only present in a low concentration and when cells are depolarized or when the cationic substrate inside the cell is high and the outwardly-directed cation gradient overcomes the membrane potential as the driving force. Furthermore, when a different cationic substrate is present on the trans-side and the reorientation of the inward-open to the outward-open conformation of the loaded transporter is more rapid than the reorientation of the unloaded transporter, the phenomenon of "trans-stimulation" is expected. This has indeed been demonstrated for rOCT1 (Keller et al. 2005). This model might hold true also for all other OCTs and also for PMAT. Thus, under physiological conditions with inside-negative membrane potential, all hOCTs and hPMAT are electrogenic uptake transporters (Koepsell 2011). At hOCT2 substrate-dependent ligand inhibition has been observed (Belzer et al. 2013). An additional cation binding site that may induce allosteric inhibition of transport has been observed in OCTs (for review, see Koepsell 2019). In addition, simultaneous interaction of multiple ligands that can interact with OCT2 has been proposed by Sandoval et al. (2018).

3 Human OCTS and hPMAT: Distribution and Endogenous Substrates and Inhibitors

3.1 Tissue Expression of hOCTs and hPMAT

The three human OCTs and hPMAT are expressed in peripheral organs and tissues involved in cationic drug transport such as small intestine, liver, kidney, and blood–brain barrier. Human OCTs are mostly responsible for uptake of cationic drugs and, with the exception of intestinal enterocytes, they are localized to the basolateral membrane of polarized cells including brain microvessel endothelial cells (Fig. 1). Whereas, cationic drug secretion (efflux) at the luminal membrane is mostly mediated by members of the MATE transporters such as MATE1 (*SLC47A1*) and MATE2/K (*SLC47A2*) and other transporters (Koepsell 2015; Ivanyuk et al. 2017). MATE1 and MATE2/K function as proton/organic cation exchangers that couple organic cation efflux with a physiological inwardly directed proton gradient, and together with OCTs they sequentially mediate transepithelial secretion of organic cations (into, e.g., urine or bile).

In **human small intestine**, mRNA expression of hPMAT and the three hOCTs has been demonstrated; however, expression was below detection for hOCT2

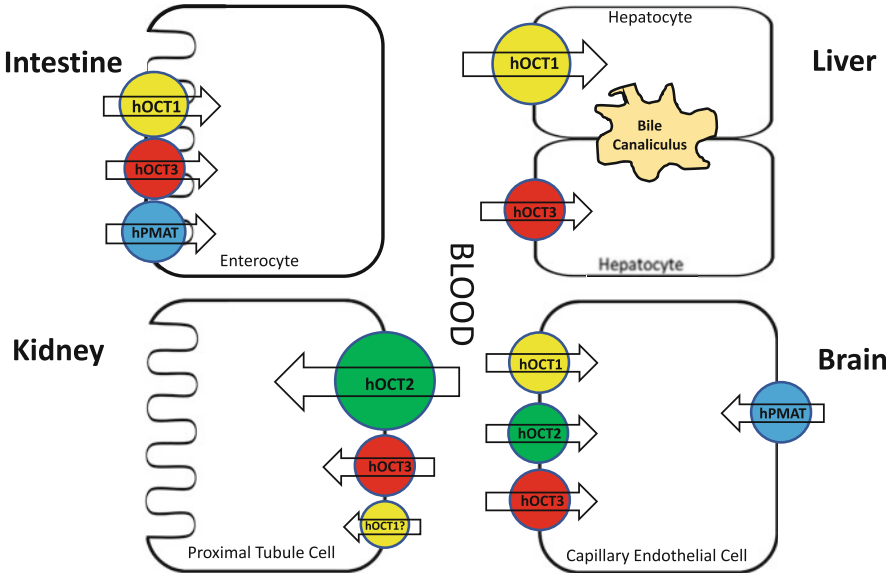

Fig. 1 Expression of human OCTs (hOCT1, hOCT2, and hOCT3) and human PMAT (hPMAT) in the intestine (enterocytes), liver (hepatocytes), kidney (proximal tubule cells), and brain (capillary endothelial cells forming the blood–brain barrier). A question mark (?) behind the name of the transporter indicates that expression remains controversial. On the sinusoidal membrane of hepatocytes, OCT1 (*SLC22A1*) is expressed to a higher extent than OCT3 (*SLC22A3*), whereas on the basolateral membrane of kidney epithelial cells, OCT2 (*SLC22A2*) is the main hOCT; for further details, see text

(Nishimura and Naito 2005). This finding was confirmed for the hOCTs by Müller et al. (2005) at the protein level using hOCT-specific antibodies. In this study strong labelling of hOCT3 in the luminal (apical) brush-border membrane was observed. Interestingly, in the same study, hOCT2 was labelled in the human intestinal cell line Caco-2 (Müller et al. 2005). According to Koepsell et al. (2007), hOCT2 is not detected in the intestine. Thus, it remains to be shown whether hOCT2 is really expressed in the intestinal enterocyte. Human OCT1 expression was believed to localize to the basolateral membranes of enterocytes (Koepsell et al. 2007). Recent studies, however, clearly indicate that hOCT1 is localized on the apical site of human enterocytes (Han et al. 2013; Müller et al. 2017; Xue et al. 2019; Fig. 1). Human PMAT protein expression was described at the apical membrane, on the tips of the mucosal epithelial layer of human small intestine (Zhou et al. 2007; Fig. 1).

In **human liver**, mRNA expression is highest for hOCT1, followed by mRNA expression of hOCT2. Expression of hOCT2 and hPMAT mRNAs is at the limit of quantification (Nishimura and Naito 2005), and protein expression of the corresponding transporters in the liver is questionable and remains to be demonstrated. As shown in Fig. 1, hOCT1 and hOCT3 are proposed to be located in the sinusoidal membrane of hepatocytes (Koepsell et al. 2007; Nies et al. 2009; Pan 2019).

In **human kidney**, the values of mRNA expression for hOCTs was hOCT2 > hOCT3 > hOCT1, and for hPMAT a value at the limit of quantifications was found (Nishimura and Naito 2005). At the protein level hOCT2 is strongly expressed in human tubular kidney cells (Prasad et al. 2016; Yang and Han 2019), whereas hOCT3 is less expressed and plays a minor role (Ivanyuk et al. 2017). In the rat, rOCT1 protein has been shown to be expressed in the kidney (Urakami et al. 1998), however, whether hOCT1 protein is expressed and functional in the human kidney remains to be shown. Xia et al. (2007) provided biochemical evidence that hPMAT protein is likely to be localized on the apical membranes of renal epithelial cells. However, in a subsequent study, using immunofluorescence microscopy, it was shown that hPMAT is specifically expressed in podocytes (Xia et al. 2009) that form a critical part of the glomerular filtration barrier.

In **human brain microvessel endothelial cells** that form the blood–brain barrier little is known about the expression of hOCTs and hPMAT and also little about the clinical relevance. Expression of hOCT1 and/or hOCT2 in human brain endothelial cells has been shown in several studies (e.g., Girardin 2006; Lin et al. 2010; Sekhar et al. 2017; Liu and Liu 2019). Human OCT3 expression in brain microvessels at the mRNA and protein level has been described by Geier et al. (2013), and expression of all three hOCTs has been reported in a recent review (Sweeney et al. 2019; Sweet 2021). Human PMAT was demonstrated to function as an efflux transporter at the blood–brain barrier (Saidijam et al. 2018), and at the blood–cerebrospinal fluid (CSF) barrier of the choroid plexus hPMAT was shown to facilitate solute exchange between the blood and the CSF (Ho et al. 2012).

In **whole human brain**, mRNA expression of hPMAT and of all three hOCTs has been shown, with the highest expression of hPMAT and hOCT3 mRNA (Nishimura and Naito 2005). Within the human brain no specific brain areas, except

microvessels (see above), expressing the hOCT1 are known (Koepsell 2020). In brain, hOCT2 protein was localized in neurons at different brain areas (Busch et al. 1998). Human OCT3 mRNA was observed in diverse brain areas, including nucleus caudatus, substantia nigra, and others (Duan and Wang 2010; Koepsell 2020). Human PMAT expression has been found in many brain regions such as cerebral cortex, hippocampus, substantia nigra, and cerebellum (Wang 2016).

In **further peripheral tissues** such as lung, bladder, placenta, salivary gland, skeletal muscle, and nasal epithelium, expression of hOCTs has been reported (Koepsell 2020; Agu et al. 2011). Human PMAT expression has also been observed in peripheral tissues such as heart, pancreas, and skeletal muscle (Wang 2016).

3.2 Endogenous Substrates and/or Inhibitors of hOCTs and hPMAT

All three human OCTs as well as hPMAT were shown to accept as substrates the endogenous amines and neurotransmitters dopamine, epinephrine, and histamine (Table 1). All hOCTs as well as hPMAT accept the monoamines and neurotransmitters NE and serotonin as substrates (Table 1; Koepsell et al. 2007). All three hOCTs transport the non-cation neurotransmitter acetylcholine, and hOCT1, hOCT2, and hPMAT have been shown to accept as substrate choline, the precursor and degradation product of acetylcholine (Table 1). Transport by all three hOCTs has been demonstrated for the biogenic polyamine spermidine and for agmatine (2-(4-aminobutyl)guanidine), the decarboxylation product of the amino acid arginine (Table 1). Creatinine, a waste product produced by skeletal muscles from the breakdown of endogenously formed creatine, was demonstrated to be a substrate of hOCT2 and hOCT3 (Table 1). The purine nucleoside adenosine, on the other hand, is only accepted as substrate by hPMAT, which is also known as member 4 of the family of equilibrative nucleoside transporters (hENT4) (Zhou et al. 2010, Table 1). All three hOCTs (most pronounced hOCT3) are inhibited by corticosterone and also by estradiol and progesterone (Table 1); these steroid hormones are large, lipophilic, and non-cationic compounds. For hPMAT, inhibition by steroid hormones has hitherto only been shown for corticosterone, which inhibits hPMAT with low potency (Table 1). A potential biomedical role for some of the endogenous substrates and inhibitors and their preferred organic cation transporters will be discussed below (in Sect. 6).

4 Model Substrates or Inhibitors of hOCTs and hPMAT

In early studies at uptake$_2$, the catecholamine isoprenaline was used as substrate and its O-methylated metabolite, OMI, as inhibitor since both substances do not interact as substrate or inhibitor with uptake$_1$ (Bönisch 1978). Both compounds are amines whose amino nitrogen is at physiological pH, at least in part, protonated and thus behave as an organic cation. The proportion of the amine molecule which is charged

Table 1 Endogenous substances as substrates (S) or inhibitors (I) of hOCTs or hPMAT. K_m

Category	K_m or IC_{50} [μM]				References
	hOCT1	hOCT2	hOCT3	hPMAT	
Neurotransmitters					
Acetylcholine (S)	580	149	10,500		Lips et al. (2005), Koepsell et al. (2007)
Dopamine (S)	487	**390**, 1,400, 2,300, 1,400	**1,033,** 620, 384	**406, 329**	Busch et al. (1998), Bednarczyk et al. (2003;), Amphoux et al. (2006), Duan and Wang (2010), Wu et al. (1998), Urakami et al. (2004), Engel and Wang (2005)
Epinephrine (S)	>30,000	**420**	**240, 458**	**951**	Koepsell et al. (2007), Amphoux et al. (2006), Duan and Wang (2010)
Histamine (S)	3,007	940, **1,300, 540,** 3,251	**180**, 220, **641, 640**	**4,379, 4,400**	Bednarczyk et al. (2003), Busch et al. (1998), Gründemann et al. (1999), Amphoux et al. (2006), Duan and Wang (2010), Yoshikawa and Yanai (2017), Suhre et al. (2005)
Norepinephrine (S)		**1,900, 1,500,** 11,000, **3,568**	**2,630, 923,** 434	**1,078, 510, 2,606**	Busch et al. (1998), Amphoux et al. (2006), Duan and Wang (2010), Wu et al. (1998), Wang et al. (2014), Gründemann et al. (1998), Engel and Wang (2005)
Serotonin (S)		**80, 290, 278,** 310	**988,** 970	**283**	Busch et al. (1998), Duan and Wang (2010), Amphoux et al. (2006), Wu et al. (1998), Wang et al. (2014), Suhre et al. (2005)
Hormones					
Corticosterone (I)[a]	7.0, 22	34, 4.2	0.12, 0.29, 4.9, 0.25	450	Gründemann et al. (1998), Zhang et al. (1998), Hayer-Zillgen et al. (2002), Wu et al. (1998), Russ et al. (1996), Engel and Wang (2005)

(continued)

Table 1 (continued)

Category	K_m or IC_{50} [μM]				References
	hOCT1	hOCT2	hOCT3	hPMAT	
Estradiol (I)[a]	5.7	>30, 85	2.9, 1.1		Hayer-Zillgen et al. (2002), Wu et al. (1998)
Progesterone (I)	3.1	27, 1.6	4.3 10		Hayer-Zillgen et al. (2002), Wu et al. (1998)
Various					
Adenosine				**413**	Zhou et al. (2010)
Agmatine (S)	24,000, **18,730**	**1,400, 1,840**	**2,500**		Gründemann et al. (2003), Winter et al. (2011)
Choline (S)	3,540	**210, 465, 300,** 381		>2,000	Bednarczyk et al. (2003), Gorboulev et al. (1997), Severance et al. (2017), Visentin et al. (2018), Suhre et al. (2005), Engel and Wang (2005)
Creatinine (S)		**1,860**, **4,000**, 580	**1,320**		Lepist et al. (2014), Urakami et al. (2004), Ciarimboli et al. (2012)
Spermidine (S)	**996**	**1,036**	**983**		Sala-Rabanal et al. (2013)

[a]See also Tables 2 and/or 3

depends on its p*K*a value and the pH (Mack and Bönisch 1979). In later transport studies at tissues or cells expressing an $uptake_2$ transporter or at transfected cells or oocytes expressing an OCT or PMAT, permanently charged organic cations have been used and are still utilized. These substances (see Table 2) are mostly used either as ^{3}H- or ^{14}C-labelled compounds. A useful cation transporter tool is also the fluorescent organic cation ASP^+ (4(4-Dimethyl-aminostyryl)-N-methylpyridinium) (Table 2), which was introduced by Stachon et al. (1996). It should be noted that transporter-mediated intracellular accumulation of organic compounds can also be measured by means of liquid chromatography and mass spectrometry (LC-MS/MS). Frequently used model substrates are the type I organic cations MPP^+ and TEA^+ which are recognized as substrate by all three OCTs and PMAT (Table 2); however, TEA is only a low-affinity substrate at hOCT3 and hPMAT. The antidiabetic drug metformin is also an often used model compound, since it is a substrate at all four organic cation transporters (Tables 2 and 3). Among the inhibitors listed in Table 2, corticosterone was originally identified as a potent inhibitor of $uptake_2$ with no

Table 2 Model substrates and inhibitors used in studies at hOCTs and hPMAT. K_m

	K_m or IC_{50} [μM]				
	hOCT1	hOCT2	hOCT3	hPMAT	References
Substrates					
ASP^+	**2.3**	**38, 24**	**1.1**		Sandoval et al. (2018), Biermann et al. (2006), Ahlin et al. (2008), Massmann et al. (2014)
Metformin[a]	**1,470**	**285, 518, 215, 1,380**	**2,465**	**1,320**	Sandoval et al. (2018), Severance et al. (2017), Wang et al. (2014), Solbach et al. (2011), Zhou et al. (2007)
MPP^{+a}	**25**	**24,** 110	**114,** 91	**111,** 1,035	Umehara et al. (2007), Duan and Wang (2010), Fraser-Spears et al. (2019)
$NBD\text{-}MTMA^+$		**8.8, 14.5, 9.6**			Sandoval et al. (2018), Severance et al. (2017), Belzer et al. (2013)
TEA^+	**69, 229,** 168	**500, 76, 58, 72**	**921,** 4,000	**8,759**	Umehara et al. (2007), Duan and Wang (2010), Gorboulev et al. (1997), Sandoval et al. (2018), Zhang et al. (1998), Bednarczyk et al. (2003), Severance et al. (2017), Massmann et al. (2014)
Inhibitors					
Corticosterone[a]	22, 7.0	34, 4.2, 80	0.29, 0.12, 4.9, 0.25, 0.62	450, 1,059	Hayer-Zillgen et al. (2002), Zhang et al. (1998), Engel and Wang (2005), Gründemann et al. (1998), Russ et al. (1996), Wu et al. (1998), Fraser-Spears et al. (2019)
Cyanine863		0.21	1.2		Gorboulev et al. (1997), Russ et al. (1996)
Decynium22 (D-22)	0.98, 2.7	0.10, 1.13, 0.004, 10	0.09, 0.07, 0.20	0.10, 1.10	Gorboulev et al. (1997), Hayer-Zillgen et al. (2002), Engel and Wang (2005), Zhang et al. (1998), Russ et al. (1996), Wang et al. (2014), Fraser-Spears et al. (2019)
Disprocynium24	0.31	0.28	0.03, 0,015		Amphoux et al. (2010), Gründemann et al. (1998)
Estradiol[a]	5.7	>30, 85	2.9, 1.1		Hayer-Zillgen et al. (2002), Wu et al. (1998)

(continued)

Table 2 (continued)

	K_m or IC_{50} [μM]				
	hOCT1	hOCT2	hOCT3	hPMAT	References
OMI	>100	570, >100	4.4, 5.3		Gorboulev et al. (1997), Hayer-Zillgen et al. (2002), Russ et al. (1996)
SKF550	No inhibition	0.10	0.05		Hayer-Zillgen et al. (2002)
TPA^+	7.5, 1.8	1.5	28		Gorboulev et al. (1997), Zhang et al. (1998), Bednarczyk et al. (2003), Massmann et al. (2014)

ASP^+ 4(4-Dimethylaminostyryl)-N-methylpyridinium, *MPP^+* 1-Methyl-4-phenylpyridinium, *NBD-MTMA*$^+$ N,N,N-Trimethyl-2-[methyl(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino]ethanaminium, *OMI* 3-O-Methylisoprenaline), *SKF550* (9-Fluorenyl)-N-Methyl-ß-Chloroethylamine, *TEA^+* Tetraethylammonium, *TPA^+* Tetrapentylammonium

[a]See also Tables 1 and/or 3

inhibition of $uptake_1$ (Salt 1972), and it has been proven to potently inhibit all three hOCTs, whereas it is a clearly weaker inhibitor of hPMAT. The most potent inhibitors at all OCTs and PMAT are derivatives of cyanine dyes such as decynium 22, cyanine 863, and disprocynium 24 (Table 2) as well as (not shown in Table 2) some recently synthetized analogs (Fraser-Spears et al. 2019). As can be seen from the IC_{50} values in Table 2, the haloalkylamine SKF550 inhibits very potently hOCT3 (IC_{50} = 50 nM) and hOCT2 (IC_{50} = 100 nM) but not hOCT1. SKF550 was originally described as selective, highly potent, and irreversible inhibitor of $uptake_2$ (Iversen et al. 1972). In the study of Hayer-Zillgen et al. (2002) in which also a series of other $uptake_2$ inhibitors had been examined at the three hOCTs, it was shown that only hOCT3 was irreversibly inhibited by SKF550. In this study a pharmacological discrimination of the three hOCTs was demonstrated by use of the following substances (listed in Tables 2 and/or 3): hOCT1 is not sensitive to inhibition by SKF550 and OMI but selectively inhibited by prazosin and reversibly inhibited by phenoxybenzamine; hOCT2 is not (or only weakly) inhibited by prazosin, OMI, and estradiol but reversibly inhibited by SKF550 and irreversibly by phenoxybenzamine, while hOCT3 is selectively (or preferentially) inhibited by OMI, corticosterone, and decynium22 (Hayer-Zillgen et al. 2002).

From about 50 of the more than 200 substances listed in Tables 1, 2, and 3, K_m values are given, indicating that they are transported substrates. Nearly all substrates listed in Tables 1, 2, and 3 are organic cations. Exception are, e.g., the inorganic substance cisplatin, and the anionic substances acyclovir and ganciclovir.

Table 3 Drugs or toxins interacting as substrate (S) or inhibitor (I) with hOCTs or hPMAT. K_m

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*1*) Abacavir	Antiviral (RTI)	0.07	0.04	0.05		Minuesa et al. (2009)
(*2*) Acebutolol	ß-AR ANT	96				Zhang et al. (1998)
(*3*) Acyclovir (S)	Antiviral (antimetabolite)	**151**				Takeda et al. (2002)
(*4*) Alfuzosin	α_1-AR ANT	15				Chen et al. (2017)
(*5*) Amantadine (S)	Anti-Parkinsonian (NMDA-R ANT)	18, 236	**10, 27,** 28, 20	>1,000	<500	Bednarczyk et al. (2003), Busch et al. (1998), Amphoux et al. (2006), Suhre et al. (2005), Engel and Wang (2005)
(*6*) Amiloride (S)	Diuretic	57	**95**			Biermann et al. (2006), Koepsell (2020)
(*7*) Amisulpride (S)	Antipsychotic	17, **31**	22, **168**	26, **192**		Haenisch et al. (2012), Dos Santos Pereira (2014)
(*8*) Amitriptyline	Antidepressant (TCA)	2.6, 17	9.1, 0.78, 14	30	23	Haenisch and Bönisch (2010), Haenisch et al. (2012), Wang et al. (2014), Ahlin et al. (2008), Zolk et al. (2009)
(*9*) AMPH (S/I)[a]	Psychostimulant	202, 97	11, 20, 0.8, 534	460, 42, 24, 363		Amphoux et al. (2006), Wu et al. (1998), Zhu et al. (2010), Wagner et al. (2017), Koepsell (2020)
(*10*) Amprenavir (I)	Antiviral (HIV protease I)	40	NI	NI	51	Duan et al. (2015)
(*11*) Apomorphine	Anti-Parkinsonian DA-R AG	21				Ahlin et al. (2008)
(*12*) Atazanavir (I)	Antiviral (HIV protease I)	NI	NI	NI	49	Duan et al. (2015)
(*13*) Atenolol (S)	$ß_1$-AR ANT	**128**	**280,** 93			Yin et al. (2015), Ciarimboli et al. (2013)
(*14*) Atropine	M-AChR ANT	1.2, 5.9, 12	29. 4.4	466, 48		Müller et al. (2005), Koepsell (2020), Ahlin et al. (2008)
(*15*) Azidothymidine	Antiviral (NRTI)	0.15	0.27	0.40		Takeda et al. (2002)

(continued)

Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*16*) Beclomethasone	Antiasthmatic corticoid		4.4			Lips et al. (2005)
(*17*) Bendamustine (S)	Cytostatic	138				Arimany-Nardi et al. (2015)
(*18*) Berberine (S)	Antidiarrhoic alkaloid	**15**	**4.4, 1.0**	2.2		Nies et al. (2008), Sun et al. (2014), Koepsell (2020)
(*19*) Budesonide	Antiasthmatic corticoid		7.3			Lips et al. (2005)
(*20*) Bupropion	Antidepressant NET/DAT I)	161	29	738	115	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*21*) Butylscopolamine	Spasmolytic (M-AChR ANT)	23	16	110		Koepsell (2020)
(*22*) Carbachol	M-AChR AG		248			Suhre et al. (2005)
(*23*) Carvedilol	ß-AR/α_1-AR ANTAG	3.4	63			Chen et al. (2017), Zolk et al. (2009)
(*24*) Cathine (S)	Psychostimulant		**46**			Jensen et al. (2021)
(*25*) Cetirizine	H_1-R ANT	256	12	95		Conchon Costa et al. (2020)
(*26*) Chlorpheniramine	H_1-R ANT		6.0			Urakami et al. (2004)
(*27*) Chlorpromazine	Antipsychotic (DA-R ANT)	4.3, 27	14			Bednarczyk et al. (2003), Ahlin et al. (2008), Zolk et al. (2009)
(*28*) Cimetidine (S)	H_2-R ANT	166, 1,010	135, (28, 559, 70), 1,650, **21, 73,** 70, **60**	88, 111, 193	>500	Lepist et al. (2014), Zhang et al. (1998), Thévenod et al. (2013), Umehara et al. (2007), Severance et al. (2017) Tahara et al. (2005), Suhre et al. (2005), Hendrickx et al. (2013), Engel and Wang (2005), Massmann et al. (2014)

(*29*) Cisplatin (S)	Cytostatic	1,000–5,000	**11, 11**	1,000–5,000		Filipski et al. (2008), Sprowl et al. (2013), Burckhardt and Koepsell (2013)
(*30*) Citalopram	Antidepressant (SSRI)	13, 2.8, 19	115, 21	188, 158		Haenisch et al. (2012), Koepsell et al. (2007), Ahlin et al. (2008)
(*31*) Clemastine	H_1-R ANT	4.9				Ahlin et al. (2008)
(*32*) Clomipramine	Antidepressant (TCA)	19				Ahlin et al. (2008)
(*33*) Clonidine	Antihypertensive (α_2-AR AG)	0.55, 0.71,	23, 2.2, 16	33, 110	<500	Zhang et al. (1998), Russ et al. (1996), Bednarczyk et al. (2003), Koepsell et al. (2007), Suhre et al. (2005), Zolk et al. (2009), Engel and Wang (2005)
(*34*) Clotrimazole	Antimycotic	12				Chen et al. (2017)
(*35*) Clozapine	Antipsychotic	6.7	17	14	13	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*36*) Cocaine	Psychostimulant	85	113	>1,000		Amphoux et al. (2006)
(37) Corticosterone[b]	Glucocorticoid	7.0, 22	34, (0.63, 0.07, 7.8)[a], 80	0.12, 0.29, 0.62	450, 1,059	Zhang et al. (1998), Gründemann et al. (1998), Hayer-Zillgen et al. (2002), Engel and Wang (2005), Thévenod et al. (2013), Fraser-Spears et al. (2019)
(*38*) Crizotinib	Cytostatic (TKI)	40	14	2.1		López Quiñones et al. (2020)
(*39*) Cyclophosphamide	Cytostatic		321			Ciarimboli et al. (2011)
(*40*) Dasatinib	Cytostatic (TKI)	1.1	2.1	4.5		Minematsu and Giacomini (2011)
(*41*) Desipramine	Antidepressant (TCA)	5.4, 2.0, 9.2	16, 75, 0.34	72, 68	15, 33	Gorboulev et al. (1997), Zhang et al. (1998), Haenisch and Bönisch (2010), Haenisch et al. (2012), Wu et al. (1998), Wang et al. (2014), Chen et al. (2017), Engel and Wang (2005)

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Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*42*) Dextromethorphan	NMDA-R ANT	10				Chen et al. (2017)
(*43*) Diazepam	Benzodiazepine			2.0		Massmann et al. (2014)
(*44*) Diltiazem	Ca^{2+} channel blocker	12, 16	>1,000	50		Ahlin et al. (2008), Umehara et al. (2008)
(*45*) Diphenhydramine	H_1-R ANT	3.4	15, 21	695		Müller et al. (2005), Zolk et al. (2009)
(*46*) Dipyridamole	Adenosine uptake I	81	2.6		5.9	Kido et al. (2011), Zhou et al. (2010)
(*47*) Disopyramide	Antiarrhythmic	>100, 20	2.9	457		Kido et al. (2011), Zhang et al. (1998), Hasannejad et al. (2009)
(*48*) Dizocilpine	NMDA-R ANT	81	22	224		Amphoux et al. (2006)
(*49*) DMT (S)	Psychostimulant		**13**			Jensen et al. (2021)
(*50*) Dobutamine	$ß_1$-AR AG	4.2				Chen et al. (2017)
(*51*) Dolutegravir	Antiviral (HIV integrase I)		0.07	>100		Lepist et al. (2014)
(*52*) Doxepin	Antidepressant	11, 11	8.4, 13	24		Haenisch et al. (2012), Chen et al. (2017), Zolk et al. (2009)
(*53*) DSP-4 (S)	Noradrenergic neurotoxin	5.0, 0.75	7.1, 1.7	1.6, 0.62		Amphoux et al. (2010), Wenge and Bönisch (2009)
(*54*) DX619 (I)	Fluoroquinolone antibiotic		0.94			Imamura et al. (2011)
(*55*) Emtricitabine	Antiviral (NRTI)	0.02	2.4	0.53		Minuesa et al. (2009)
(*56*) Ephedrine (S)	Psychostimulant		**16,** 29			Jensen et al. (2021), Suhre et al. (2005)
(*57*) Erlotinib	Cytostatic (TKI)	0.4, 16	5.2	4.2		Minematsu and Giacomini (2011), Chen et al. (2017)

(*58*) Estradiol[b]	Steroid hormone	5.7	>30	2.9		Hayer-Zillgen et al. (2002)
(*59*) Ethambutol (S)	Antibiotic (anti-TB)	93, **686**	254, **646**	4,100, **1,356**		Pan et al. (2013), Te Brake et al. (2016)
(*60*) Etilefrine (S)	α/ß-AR AG	447	4,009	**2,800**		Müller et al. (2005)
(*61*) Famotidine (S)	H_2-R ANT	65, 28	1,800, **56,** 111, 70, 114	14, 6.7		Umehara et al. (2007), Motohashi et al. (2004), Tahara et al. (2005), Suhre et al. (2005), Urakami et al. (2004), Bourdet et al. (2005)
(*62*) Fenfluramine	Antiepileptic		10			Zolk et al. (2009)
(*63*) Fenoterol (S)	Antiasthmatic ($ß_2$-AR AG)	**1.8**		**20**		Tzvetkov et al. (2018)
(*64*) Flecainide	Antiarrhythmic	42	>1,000, 191	60		Umehara et al. (2008), Zolk et al. (2009)
(*65*) Fluoxetine	Antidepressant (SSRI)	2.8	29, 4.4	38, 27	11, 23	Haenisch and Bönisch (2010), Haenisch et al. (2012), Wang et al. (2014), Engel and Wang (2005), Massmann et al. (2014)
(*66*) Flurazepam	Benzodiazepine		60			Zolk et al. (2009)
(*67*) Fluvoxamine	Antidepressant (SSRI)	9.9	70	68		Haenisch et al. (2012)
(*68*) Formoterol	Antiasthmatic ($ß_2$-AR AG)	22				Salomon et al. (2015)
(69) Ganciclovir (S)	Antiviral	**516**				Takeda et al. (2002)
(*70*) GBR 12935	DAT I				7.9	Engel and Wang (2005)
(*71*) Gefitinib	Cytostatic (TKI)	1.1	24	5.5		Minematsu and Giacomini (2011)
(*72*) Grepafloxacin	Antibiotic		10.4			Imamura et al. (2011)
(*73*) Guanabenz	Antihypertensive $α_2$-AR AG	4.9				Chen et al. (2017)
(*74*) Guanfacine (S)	Antihypertensive $α_2$-AR AG		**96**			Li et al. (2015)
(*75*) Haloperidol	Antipsychotic (DA-R ANT)	12, 142	14	34	10	Haenisch and Bönisch (2010), Haenisch et al. (2012), Ahlin et al. (2008)

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Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*76*) Harmaline	MAO I	28	0.50	4.8		Wagner et al. (2017)
(*77*) Ifosfamide (S)	Cytostatic		624			Ciarimboli et al. (2011)
(*78*) Imatinib (S)	Cytostatic (TKI)	1.5	5.8	4.4		Minematsu and Giacomini (2011)
(*79*) Imipramine	Antidepressant (TCA)	18, 17, 8.0	15, 29, 0.30, 6.0	54	21	Haenisch and Bönisch (2010), Haenisch et al. (2012), Severance et al. (2017), Wang et al. (2014), Ahlin et al. (2008), Chen et al. (2017), Zolk et al. (2009)
(*80*) Ipratropium (S)	M-AChR ANT	**9.0**	15			Hendrickx et al. (2013), Zolk et al. (2009)
(*81*) Irinotecan	Cytostatic	1.7, 5.1	>400	1.7, 89		Gupta et al. (2012), Shnitsar et al. (2009), López Quiñones et al. (2020)
(*82*) Ketamine	Analgesic NMDA-R ANT	115	23	226, 440		Amphoux et al. (2006), Massmann et al. (2014)
(*83*) Ketoconazole	Antimycotic	2.6				Chen et al. (2017)
(*84*) Lamivudine (S)	Antiviral (HIV RTI)	**249, 1,250**	**248**	**2,100**		Jung et al. (2009), Minuesa et al. (2009)
(*85*) Lamotrigine (S)	Antiepileptic	**62**				Dickens et al. (2012)
(*86*) Lansoprazole	PPI	36	9.5	3.1		Nies et al. (2011)
(*87*) Lapatinib	Cytostatic (TKI)	>30	>30	>30		Minematsu and Giacomini (2011)
(*88*) Levomepromazine	Antipsychotic (DA-R ANT)	1.1	26	24	28	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*89*) Levomethadone	Opioid analgesic		60			Zolk et al. (2009)
(*90*) Lidocaine (S)	Local anaesthetic		294	**139**		Burckhardt and Koepsell (2013), Hasannejad et al. (2004)
(*91*) Loperamide	Opioid Antidiarrhoic	24				Ahlin et al. (2008)

(*92*) Lopinavir (I)	Antiviral (HIV protease I)	174	NI	NI	1.4	Duan et al. (2015)
(*93*) M-AMPH (S)[a]	Psychostimulant	21	**12,** 15	247, 300		Jensen et al. (2021), Wu et al. (1998), Wagner et al. (2017)
(*94*) Maprotiline	Antidepressant	4.7	33	39		Haenisch and Bönisch (2010)
(*95*) MDMA (S)	Psychostimulant	24	1.6	74		Amphoux et al. (2006)
(*96*) Melphalan	Cytostatic			366		Shnitsar et al. (2009)
(*97*) Memantine (S)	Antidementive NMDA-R ANT	3.7, 27	**34,** 7.3	236		Busch et al. (1998), Amphoux et al. (2006), Ahlin et al. (2008)
(*98*) Mepiperphenidol(S)	M-AChR ANT		**4.8**			Gorboulev et al. (1997)
(*99*) Mescaline (S)	Psychostimulant	**24**				Jensen et al. (2021)
(*100*) Metformin (S)[b]	Antidiabetic Biguanide	87, 493	289, **518,** 339, 398	904, **2,500**	**1,320**	Chen et al. (2014), Umehara et al. (2007), Severance et al. (2017), Suhre et al. (2005), Zolk et al. (2009), Zhou et al. (2007)
(*101*) Metoprolol	$ß_1$-AR ANT	268	NI	804		Umehara et al. (2008)
(*102*) Mexiletine	Antiarrhythmic		55			Zolk et al. (2009)
(*103*) Mianserin	Antidepressant α_2-AR ANT	37	183	50		Haenisch et al. (2012)
(*104*) MIBG (S)	Cytostatic	**19**	**17**	**14**		López Quiñones et al. (2020)
(*105*) Midazolam	Benzodiazepine	3.7				Zhang et al. (1998)
(*106*) Mirtazapine	Antidepressant α_2-AR ANT	159	221	61		Haenisch et al. (2012)
(*107*) Mitoxantrone	Cytostatic	16, 85	800	440		Koepsell et al. (2007), Gupta et al. (2012)
(*108*) Moclobemide	Antidepressant (MAO I)		49			Wang et al. (2014)
(*109*) Morphine (S)	Opioid analgesic	46, 28, **3.4**		583		Meyer et al. (2017), Ahlin et al. (2008), Tzvetkov et al. (2013)

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Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*110*) MPP^+ (S)[b]	Dopaminergic neurotoxin	**25,** 12, 16, **15,**	**24**, 2.4, 1.6, 75, 3.1, 1.1, **19,** 110	**114**, 143, **166,** 91, 145	**111,** 1,035	Umehara et al. (2007), Wu et al. (1998), Duan and Wang (2010), Zhang et al. (1998), Gorboulev et al. (1997), Thévenod et al. (2013), Bednarczyk et al. (2003), Urakami et al. (2004), Zhang et al. (1997), Chen et al. (2007), Fraser-Spears et al. (2019), Massmann et al. (2014)
(*111*) Nadolol (S)	ß-AR ANT	>**500**	**122**			Misaka et al. (2016)
(*112*) Nafamostat (S)	Antiviral protease I		**20**			Li et al. (2004)
(*113*) Nefazodone	Antidepressant	13	102	23		Haenisch et al. (2012)
(*114*) Nelfinavir	Antiviral protease I	22, 7.0	13			Jung et al. (2008), Zhang et al. (2000)
(*115*) Nicotine	N-AChR AG	53, 186	42, 22	101	<500	Bednarczyk et al. (2003), Lips et al. (2005), Koepsell et al. (2007), Suhre et al. (2005), Engel and Wang (2005)
(*116*) Nilotinib	Cytostatic TKI	2.9	>30	0.35		Minematsu and Giacomini (2011)
(*117*) Nisoxetine	Antidepressant (SNRI)	2.5	85	71		Haenisch et al. (2012)
(*118*) NMN (S)	Antiinflammatory agent	7,700, 1,035	266, 303, 310, **318**		>2,000	Gorboulev et al. (1997), Zhang et al. (1998), Bednarczyk et al. (2003), Suhre et al. (2005), Urakami et al. (2004), Ito et al. (2012), Engel and Wang (2005)
(*119*) Nomifensine	Antidepressant NET/DAT I	12	394	207		Haenisch et al. (2012)
(*120*) Olanzapine	Antipsychotic	10	82	97	149	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*121*) Omeprazole	PPI	16	6.7	22		Nies et al. (2011)

(*122*) Ondansetron	5-HT_3R ANT	20, >100	0.89			Ahlin et al. (2008), Kido et al. (2011)
(*123*) Orphenadrine	Muscle relaxant	>100	2.5			Kido et al. (2011)
(*124*) Oxaliplatin (S)	Cytostatic	4.0	1.0			Zhang et al. (2006)
(*125*) Oxybutynin (S)	M-AChR ANT	20, **8.8**	128	130		Wenge et al. (2011)
(*126*) Paclitaxel	Cytostatic	50				Gupta et al. (2012)
(*127*) Pantoprazole	PPI	31	2.8	23		Nies et al. (2011)
(*128*) Paraquat (S)	Herbicide		**114**			Chen et al. (2007)
(*129*) Pargyline	MAO I				77	Engel and Wang (2005)
(*130*) Paroxetine	Antidepressant (SSRI)	1.7	121, 2.5	21	14	Haenisch and Bönisch (2010), Haenisch et al. (2012), Wang et al. (2014)
(*131*) Pazopanib (S)	Cytostatic (TKI)	0.25, **3.5**				Ellawatty et al. (2018)
(*132*) Pentamidine (S)	Antiparasitic	**36**	3.8–11	15		Burckhardt and Koepsell (2013)
(*133*) Perazine	Antipsychotic	9.0	162	200		Haenisch et al. (2012)
(*134*) Phencyclidine	Psychostimulant	4.4	25	333		Amphoux et al. (2006)
(*135*) Phenformin (S)	Antidiabetic	13, 15	54, 65	134		Umehara et al. (2007), Suhre et al. (2005), Dresser et al. (2002)
(*136*) Phenoxybenzamine	α-AR ANT irreversible	2.7, 15	4.9	6.1		Hayer-Zillgen et al. (2002), Ahlin et al. (2008)
(*137*) Picoplatin (S)[c]	Cytostatic	0.44	0.28	3.20		More et al. (2010)
(*138*) Pindolol (S)	ß-AR ANT	9.7, 39	51, NI, 145	>1,000		Bednarczyk et al. (2003), Suhre et al. (2005), Umehara et al. (2008), Ciarimboli et al. (2013)
(*139*) PMMA (S)	Psychostimulant		**7,9**			Jensen et al. (2021)
(*140*) Pramipexole (S)	DA-R AG	NI	**15**	**138**		Diao et al. (2010)

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Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*141*) Prazosin	α_1-AR ANT	1.8, 9.9	>100	13		Hayer-Zillgen et al. (2002), Ahlin et al. (2008)
(*142*) Procainamide (S)	Antiarrhythmic	74, 15	50, 5.7	355	>500	Gorboulev et al. (1997), Zhang et al. (1998), Bednarczyk et al. (2003), Hasannejad et al. (2004), Suhre et al. (2005), Engel and Wang (2005)
(*143*) Promazine	Antipsychotic	17				Ahlin et al. (2008)
(*144*) Propafenone	Antiarrhythmic	11, 16	25			Ahlin et al. (2008), Chen et al. (2017), Zolk et al. (2009)
(*145*) Propranolol	ß-AR AG	113	>300, 229	133		Umehara et al. (2008), Zolk et al. (2009)
(*146*) Quetiapine	Antipsychotic	39	148	186		Haenisch et al. (2012)
(*147*) Quinine (S)	Antimalarial	23, 52	3.4, 12, 10		27	Gorboulev et al. (1997), Zhang et al. (1998), Thévenod et al. (2013), Ahlin et al. (2008), Urakami et al. (2004), Engel and Wang (2005)
(*148*) Quinidine (S)	Antiarrhythmic	18, 5.4, 17	93, 446,	32, 14, **216**	25	Zhang et al. (1998), Russ et al. (1996), Bednarczyk et al. (2003), Severance et al. (2017), Umehara et al. (2008), Hasannejad et al. (2009), Engel and Wang (2005)
(*149*) Rabeprazole (I)	PPI	3.0	5.7	3.0		Nies et al. (2011)
(*150*) Ranitidine (S)	H_2-R ANT	22, 42, **63,** 33	**65,** 40, 76	62, 290		Bednarczyk et al. (2003), Umehara et al. (2007), Tahara et al. (2005), Meyer et al. (2017), Suhre et al. (2005), Bourdet et al. (2005)
(*151*) Reboxetine	Antidepressant (SNRI)	7.8	129	64	77	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*152*) Remoxipride	Antipsychotic	54	237	205		Haenisch et al. (2012)
(*153*) Repaglinide	Antidiabetic	9.2				Ahlin et al. (2008)

(*154*) Rilpivirine	Antiviral HIV NNRTI	29	5.1			Moss et al. (2013)
(*155*) Risperidone	Antipsychotic	28	5.6	13	7.0	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*156*) Ritonavir (I)	Antiviral HIV protease I	11, 5.2	20, 79, 25	>20, 60	6.0	Lepist et al. (2014), Duan et al. (2015), Zhang et al. (2000)
(*157*) Salbutamol (S)	$ß_2$-AR AG	277				Salomon et al. (2015)
(*158*) Salmeterol (S)	$ß_2$-AR AG	48				Salomon et al. (2015)
(*159*) Saracatinib (I)	Cytostatic TKI	27	0.46	10		Morrow et al. (2010)
(*160*) Saquinavir (I)	Antiviral protease I	45, 37, 8.3	NI, 205	NI	7.0	Duan et al. (2015), Jung et al. (2008), Zhang et al. (2000)
(*161*) Sertindole	Antipsychotic	5.6	19	17		Haenisch et al. (2012)
(*162*) Sertraline	Antidepressant (SSRI)	9.3	26, 4.9,	26	5.1	Haenisch and Bönisch (2010), Haenisch et al. (2012), Wang et al. (2014)
(*163*) Sibutramine	Anorectic		29			Zolk et al. (2009)
(*164*) Sorafenib (S)	Cytostatic TKI	>30, **3.8**	>30	20		Minematsu and Giacomini (2011), Swift et al. (2013)
(*165*) Spiperone	Antipsychotic	1.9	27	27		Haenisch et al. (2012)
(*166*) Sulpiride (S)	Antipsychotic	**260**	**390, 187**			Li et al. (2017), Dos Santos Pereira et al. (2014)
(*167*) Sunitinib	Cytostatic R-TKI	0.33, 6.1	1.73	5.22		Minematsu and Giacomini (2011), Chen et al. (2017)
(*168*) Tacrine	ACh-esterase I	22, 83	0.68			Chen et al. (2017), Kido et al. (2011)
(*169*) Tamoxifen	Estrogen-R modulator		87			Zolk et al. (2009)
(*170*) Tenatoprazole	PPI	23	20	15		Nies et al. (2011)
(*171*) Tenofovir	Antiviral (NtRTI)	0.85	0.57	0.005		Minuesa et al. (2011)
(*172*) Terazosin	$α_1$-AR ANT	24				Ahlin et al. (2008)

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Table 3 (continued)

Drug or *toxin*	Category	K_m or IC_{50} [μM]				References
		hOCT1	hOCT2	hOCT3	hPMAT	
(*173*) Thioridazine	Antipsychotic	11	39	30		Haenisch et al. (2012)
(*174*) Tianeptine	Antidepressant	278	>1,000	583	191	Haenisch and Bönisch (2010), Haenisch et al. (2012)
(*175*) Tipranavir (I)	Antiviral protease I	45	NI	NI	8.9	Duan et al. (2015)
(*176*) Tramadol	Opioid analgesic	53	140			Severance et al. (2017), Ahlin et al. (2008)
(*177*) Trimethoprim	Antibiotic	51	68, 21, 1,318	12		Lepist et al. (2014), Chen et al. (2017), Urakami et al. (2004), Zolk et al. (2009)
(*178*) Trimipramine	Antidepressant (TCA)	1.6, 28	3.7, 30	8.0, 65	12	Haenisch and Bönisch (2010), Haenisch et al. (2011), Haenisch et al. (2012), Ahlin et al. (2008)
(*179*) Trospium (S)	M-AChR ANT	18, **17**	1.4, **8.0**	710		Wenge et al. (2011)
(*180*) Tyramine (S)	Sympathomimetic	107	106		**283**	Bednarczyk et al. (2003), Suhre et al. (2005), Engel and Wang (2005)
(*181*) Varenicline (S)	N-AChR ANT		**370**			Feng et al. (2008)
(*182*) Vecuronium	Muscle relaxant	127, 232				Zhang et al. (1997), (1998)
(*183*) Veliparib (S)	Cytostatic		**45**			Kikuchi et al. (2013)
(*184*) Venlafaxine	Antidepressant (SSNRI)	44	971, >100	>1,000	216	Haenisch and Bönisch (2010), Haenisch et al. (2012), Wang et al. (2014)
(*185*) Verapamil	Ca^{2+}-channel blocker	2.9, 6.8	206, 85	24	19	Zhang et al. (1998), Koepsell et al. (2007), Ahlin et al. (2008), Zolk et al. (2009), Engel and Wang (2005)
(*186*) Vincristine	Cytostatic			17		Shnitsar et al. (2009)
(*187*) Zalcitabine (S)	Antiviral NRTI	**242**	**232**			Jung et al. (2008)

(*188*) Zotepine	Antipsychotic	8.4	122	94		Haenisch et al. (2012)
(*189*) Zuclopenthixol	Antipsychotic	131	267	244		Haenisch et al. (2012)

5-HT$_3$-R 5-Hydroxytryptamine[Serotonin]3 Receptor, *ACh* Acetylcholine, *AG* Agonist, *AMPH* d-Amphetamine, *ANT* Antagonist, *AR* Adrenergic Receptor = Adrenoceptor, *DA* Dopamine, *DA-R* DA Receptor, *DMT* Dimethyltryptamine, *DSP-4* N-N-Ethyl-2-Brombenzylamin, *H_1* Histamine$_1$ Receptor, *H_2* Histamine$_2$ Receptor, *HIV* Human Immune deficiency Virus, *I* Inhibitor, *M-AChR* Muscarinic ACh-Receptor, *M-AMPH* Methamphetamine, *MAO* Monoamine Oxidase, *MDMA* 3,4 Methylenedioxy-Methamphetamine, *MIBG* meta-Iodobenzylguanidine, radioiodine-labeled, *MPP^+* 1-Methyl-4-Phenylpyridinium, *N-AChR* Nicotinic ACh-Receptor, *I* Inhibitor, *NE* Norepinephrine, *NET* NE transporter, *NI* No Inhibition, *NMDA* N-Methyl-D-Aspartate, *NMN* N-Methyl Nicotinamide, *NNRTI* Non-Nucleoside Reverse Transcriptase Inhibitor, *NRTI* Nucleoside Reverse Transcriptase Inhibitor, *NtRTI* Nucleotide Reverse Transcriptase Inhibitor, *PKI* Protein Kinase Inhibitor, *PMMA* p-Methoxy-Methamphetamine, *PPI* Proton Pump Inhibitor, *R* Receptor, *RTI* Reverse Transcriptase Inhibitor, *SSNRI* Selective Serotonin and Norepinephrine Reuptake Inhibitor, *SNRI* Selective Norepinephrine Reuptake Inhibitor, *SSRI* Selective Serotonin Reuptake Inhibitor, *TCA* Tricyclic Antidepressant, *TKI* Tyrosine Kinase Inhibitor. Note that K_m values are given in bold numbers

[a]Substrate of hOCT2 but presumably not of hOCT1 and hOCT3 (Zhu et al. 2010; Wagner et al. 2017; Koepsell 2020; Jensen et al. 2021)

[b]See also Tables 1 and/or 2

[c]Substrate of hOCT1 and hOCT2; IC_{50} determined with a cytotoxicity assay

5 Drugs as Substrates or Inhibitors of hOCTs and hPMAT

To examine whether a given substance (ligand, e.g., drug or toxin) exhibits affinity to a defined hOCT or to hPMAT, very often inhibition of uptake of a radio labelled model substrate (e.g., ^{3}H-MPP^{+} or ^{14}C-TEA) by the investigated substance is examined in cells overexpressing the organic cation transporter of interest. The resulting IC_{50} values, however, cannot tell us whether the substance is a competing substrate or an inhibitor. Substrates have to be shown to be taken up and to accumulate intracellularly. Furthermore, this accumulation must be sensitive to inhibition by an inhibitor typical for the transporter. Finally, uptake must be saturable with increasing substrate concentrations, resulting in a K_m value, i.e., a concentration at which initial rates of uptake reach half maximum. Such a K_m value indicates the apparent affinity of a substrate to the transporter. However, as discussed by Koepsell (2019), K_m as well as IC_{50} values are also influenced by the employed procedures.

In Table 3, IC_{50} or K_m values as a measure of apparent affinity for a given hOCT or for hPMAT (together with the corresponding references) are listed alphabetically for a large series of drugs (including two toxins). This list is certainly not complete. Further lists of drugs are available in the literature (see, e.g., Nies et al. 2011; Koepsell et al. 2007; Koepsell 2020; Zhou et al. 2021). Of the drugs listed in Table 3 many are substrates as indicated by a K_m value. Non-transported inhibitors of hOCTs, on the other hand, are, e.g., the steroid hormones beclomethasone, budesonide (both are antiasthmatics) as well as corticosterone, progesterone, and estradiol, which are known as inhibitors of uptake$_2$. Subsequently, drugs of biomedical important categories (listed in Table 3 with references) will be discussed with respect to their affinities to hOCTs and hPMAT. Furthermore, clinical implications will be emphasized for a selection of them below (see Sect. 6).

5.1 Antiviral Drugs

Nearly 20 of the drugs discussed in this chapter are antiviral drugs. They are listed in Table 3 (with references) with the following numbers: *1*, 3, *10*, *12*, *15*, *51*, 55, *69*, *84*, *92*, *112*, *114*, *154*, *156*, *160*, *171*, *175*, *187*. Some of the antiviral drugs in Table 3 show high affinity and/or selectivity for a defined hOCT or for hPMAT. **Acyclovir** inhibits hOCT1, **nelfinavir** and **rilpivirine** hOCT1 and hOCT2, whereas **ritonavir** is an inhibitor of all three hOCTs as well as of hPMAT. **Ganciclovir**, **nafamostat, zalcitabine,** and **lamivudine** are substrates of hOCT1, hOCT2, hOCT1 plus hOCT2 and of all three hOCTs, respectively. The subsequent antiviral drugs show either high affinity and/or selectivity for defined hOCTs or hPMAT. **Abacavir** exhibits nanomolar affinity for all three hOCTs, and **azidothymidine** (also known as zidovudine, AZT) inhibits all three hOCTs in submicromolar concentrations. The HIV integrase inhibitor **dolutegravir** inhibits preferentially and with high potency hOCT2 (IC_{50} = 70 nM). **Tenofovir** inhibits hOCT1 and hOCT2 in submicromolar concentrations, and hOCT3 with very high potency (IC_{50} = 5 nM) (Table 3).

Emtricitabine shows very high affinity for hOCT1 (IC_{50} = 20 nM) and it inhibits hOCT2 and hOCT3 with an IC_{50} of 2.4 and 0.53 μM, respectively. **Tipranavir** and **saquinavir** show preferential inhibition of hOCT1 and hPMAT. Whereas the HIV protease inhibitor **lopinavir**, which has been examined at all three hOCTs and hPMAT, exhibits no affinity to hOCT2 and hOCT3, low affinity to hOCT1 (IC_{50} = 174 μM) but relatively high potency as inhibitor of hPMAT (IC_{50} = 1.4 μM). Selective inhibition of hPMAT has also been demonstrated for **atazanavir**, while **amprenavir** was shown to selectively inhibit hOCT1 and hPMAT. The clinical relevance of some antiviral drugs with respect to their affinity for hOCTs and/or hPMAT will be discussed below (see Sect. 6).

5.2 Cytostatic/Antineoplastic Drugs

Various tumors such as breast cancer, hepatocellular carcinoma, or lung small cell carcinoma have been demonstrated to express various hOCTs. However, in most tumor cells, the expression of hOCTs was negatively correlated with tumor malignancies (for review, see, e.g., Koepsell 2020). More than 20 of the drugs listed in Table 3 (with their IC_{50} and references) are cytostatics or other anti-cancer drugs. It is unknown if any of these drugs interacts with hPMAT. The following drugs were shown to inhibit all three hOCTs, and most of them exhibited the highest potency at hOCT1: **dasatinib**, **erlotinib**, **gefitinib**, **lapatinib**, **nilotinib**, **saracatinib**, **sorafenib**, **sunitinib**, and **imatinib**. Interestingly, all of them are tyrosine kinase inhibitors. **Oxaliplatin** was shown to inhibit hOCT1 and hOCT2 and **irinotecan** hOCT1 and hOCT3, whereas from the other cytostatics an inhibition was shown at hOCT1 (**bendamustine** and **paclitaxel**) or hOCT2 (**cisplatin** and **ifosfamide**, **veliparib**, **cyclophosphamide**, **paclitaxel,** and **tamoxifen**) or at hOCT3 (**vincristine**). A selection of these drugs will be discussed in more detail below (see Sect. 6).

5.3 Drugs That Act in the Central Nervous System (CNS)

OCTs and PMAT in the brain will be reviewed in more detail in other chapters of this book. As shown above (Sect. 3.1) and as recently summarized by Koepsell (2015, 2020), OCTs within the brain have been localized to the blood–brain barrier (hOCT1, hOCT2, and hPMAT), to neurons and the choroid plexus (hOCT2), and to neurons and glial cells (hOCT3 and hPMAT).

Inspection of the drugs listed in Table 3 reveals that 69 of them belong to one of the larger groups, namely drugs with targets in the central nervous system (CNS), including the two neurotoxins DSP-4 and MPP^+. Among these CNS active drugs 53 are psychotropic drugs such as **antidepressants** (23), **antipsychotics** (17), **psychostimulants** (10), and **opioid analgesics** (3), as well as **antiepileptics** (3) and **anti-Parkinsonian's** (3). Drugs of these groups will be discussed in more detail below.

The group of CNS active drugs also contains 3 anxiolytic/hypnotic **benzodiazepines,** which exhibit micromolar affinity to hOCT1 (**midazolam**) or hOCT2 (**flurazepam**) or hOCT3 (**diazepam**). The first generation **H1 antihistaminics** (with unwanted anticholinergic properties) are also used as sedatives and/or hypnotics; listed in Table 3 are the following drugs (with their interaction at a cation transporter and with the preferred hOCT underlined): **chlorpheniramine** (hOCT2), **clemastine** (hOCT), and the hypnotic **diphenhydramine** (hOCT1, hOCT2, hOCT3). Further CNS active drugs are the following **NMDA receptor antagonists**: **dextromethorphan** (a cough suppressant with sedative, dissociative, and stimulant properties and affinity to hOCT1), **ketamine** (an analgesic anesthetic with antidepressant properties and with affinity to all three hOCTs but preference to hOCT2), as well as **memantine** (an analog of amantadine with affinity to all three hOCTs but with preference to hOCT1 and hOCT2 at which it was shown to be a substrate); memantine is used for the treatment of Alzheimer's disease. A further **anti-dementia drug** is the acetylcholinesterase inhibitor **tacrine,** which interacts with hOCT1 and even more potently with hOCT2 (for references, see Table 3). As already noted above, **DSP-4** and MPP^+ are **neurotoxins**. We have shown that the noradrenergic neurotoxin DSP-4 is an irreversible inhibitor of hNET but an almost reversible inhibitor of hDAT and hSERT as well as of the three hOCTs, which are inhibited with high potency by DSP-4 (Wenge and Bönisch 2009). The dopaminergic neurotoxin MPP^+ is a substrate of all three hOCTs and of hPMAT (for reference, see Table 3). MPP^+ is formed within the brain by monoamine oxidase B as a metabolite of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which penetrates through the blood–brain barrier by means of OCT1 and OCT2 (Lin et al. 2010). **Sibutramine**, a NET, DAT, and SERT inhibiting anorectic drug, was shown to inhibit hOCT2 (Table 3, Zolk et al. 2009).

5.3.1 Antidepressants (ADs)

More than 20 of the substances listed in Table 3 are antidepressants such as the tricyclic antidepressants (TCAs) amitriptyline, clomipramine, desipramine, imipramine, and trimipramine, or the selective serotonin reuptake inhibitors (SSRIs) citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline, the DAT and NET inhibitor bupropion, and the selective NET inhibitor reboxetine as well as nefazodone, mianserin, mirtazapine and further antidepressants. Many of these ADs have been examined for their interaction with hOCTs by Haenisch and Bönisch (2010) and for their inhibitory affinity to hPMAT by Haenisch et al. (2012). The following ADs inhibited all three hOCTs as well as hPMAT with the range of IC_{50} values [in μM] given in parentheses: **amitriptyline** (0.78–30), **bupropion** (29–738), **desipramine** (0.34–75), **fluoxetine** (2.8–38), **imipramine** (0.30–54), **paroxetine** (1.7–121), **reboxetine** (7.8–129), **sertraline** (4.9–26), **tianeptine** (191–>1,000), and **venlafaxine** (44–>1,000). The following ADs have not been examined at hPMAT and were shown to inhibit at least all three hOCTs (again with the range of IC_{50} values given in parentheses): **citalopram** (2.8–188), **doxepin** (8.4–24), **fluvoxamine** (9.9–70), **maprotiline** (4.7–39), **mianserin** (37–50), **mirtazapine** (61–221), **nefazodone** (13–102), **nisoxetine** (2.5–85), **nomifensine** (12–394),

sertindole (5.6–19), and **trimipramine** (1.6–65). Only at hOCT2 and only for **amitriptyline**, **desipramine**, and **imipramine** IC_{50} values in submicromolar concentrations were observed (see Table 3 with references).

Ketamine, an NMDA receptor antagonist and analgesic/anesthetic drug, inhibits all three hOCTs with IC_{50}s between 23 and 440 μM (Table 3). Ketamine is also a rapid acting antidepressant with a largely unknown mode of action. Ketamine has previously been shown by us to inhibit the NET (Hara et al. 1998) and it has recently been demonstrated that SERT and PMAT are necessary for the antidepressant-like effects of ketamine in mice (Bowman et al. 2020); this study provided evidence for an important role of SERT and PMAT in the serotonin clearance inhibiting and antidepressant actions of ketamine.

5.3.2 Antipsychotics

Nearly 20 of the drugs listed in Table 3 are antipsychotics. Subsequently they are given with their range of IC_{50} for inhibition of hOCTs and/or hPMAT (in μM, in parentheses). **Clozapine** (6.7–17), **haloperidol** (10–142), **levomepromazine** (1.1–28), **olanzapine** (10–149), and **risperidone** (5.8–28) inhibit all four transporters. An inhibition of the three hOCTs (but not yet of hPMAT) has been shown for **amisulpride** (17–192), **perazine** (9.0–200), **quetiapine** (39–186), **remoxipride** (54–237, **sertindole** (5.6–19), **spiperone** (1.9–27), **thioridazine** (11–39), **zotepine** (8.4–122), and **zuclopenthixol** (131–267). Thus, none of the antipsychotics listed in Table 3 show affinities in the submicromolar range at these organic cation transporters. Interestingly, however, the antipsychotics sulpiride and its analog amisulpride (listed in Table 3 with references), which both are relatively hydrophilic, as well as perphenazine (Hendrickx et al. 2013; not listed in Table 3 since no IC_{50} is known), have been shown to be substrates of hOCTs. Some clinical aspects with respect to inhibition of hOCTs or hPMAT of some of the above-mentioned antidepressants and antipsychotics will be discussed in Sect. 6.

5.3.3 Psychostimulants

Ten compounds (shown in Table 3 with references) are **psychostimulant drugs** and/or illicit drugs. They are listed subsequently with either their range of IC_{50} or K_m values [in μM] for interaction with all three hOCTs: AMPH (**amphetamine**; 0.8–534), **cocaine** (85–>1,000), M-AMPH (**methamphetamine**; 12–300), MDMA (**ecstasy**; 1.6–74), and **phencyclidine** (4.4–333). An interaction as substrate of hOCT2 has been reported for **cathine** (46), **DMT** (dimethyltryptamine; 13), **ephedrine** (16–29), and **PMMA** (p-methoxy-methamphetamine; 7.9), whereas **mescaline** has been reported to be a substrate at hOCT1 ($K_m = 24$ μM).

5.3.4 Opioid Analgesics

Further CNS active drugs listed in Table 3 are three **opioid analgesics**. The corresponding drugs with their IC_{50} value [in μM] or range of IC_{50} values (in parentheses) are **levomethadone** which interacts with hOCT2 (IC_{50} 60), **morphine** which interacts with hOCT1 (IC_{50} 3.4–46) and weakly with hOCT3 (IC_{50} 583) and **tramadol** with an interaction at hOCT1 and hOCT2 (53, 140). From these

three drugs only morphine had been identified to be a substrate of hOCT1; however, O-desmethyltramadol, the metabolite of tramadol, has also been demonstrated to be a substrate of hOCT1 (Tzvetkov et al. 2011, 2013). Very recently, further opioids have been identified as substrates of hOCT1 (not listed in Table 3) such as hydromorphone, oxymorphone, meptazinol, and methylnaltrexone as one of the strongest hOCT1 substrate hitherto known (Meyer et al. 2019).

5.3.5 Antiepileptics

Further CNS active drugs are the anti-epileptics **dizocilpine**, **fenfluramine,** and **lamotrigine** (Table 3). **Dizocilpine**, an anticonvulsant that inhibits NMDA receptors, has been shown to inhibit all three hOCTs with IC_{50} values of 81, 22, and 223 at hOCT1, hOCT2, and hOCT3, respectively (Amphoux et al. 2006). **Fenfluramine** is a substituted amphetamine, which acts primarily as serotonin releasing agent; it is preferentially used orally for the treatment of seizures associated with Dravet syndrome, a severe myoclonic epilepsy of infancy. Fenfluramine has only been examined at hOCT2 where it inhibits this transporter with an IC_{50} of 10 μM; no drug–drug interactions at hOCT2 have been shown yet. **Lamotrigine** is a sodium channel blocking antiepileptic acting presynaptically to reduce glutamate release and it is a substrate of hOCT1 (with a K_m of 62 μM; see Table 3). Lamotrigine is considered a first-line drug for primary generalized tonic-clonic seizures and it is also used in patients with bipolar disorder. Lamotrigine is a substrate of hOCT1 (K_m 62 μM), and its passage through endothelial cells of microvessels forming the blood–brain barrier (BBB) is mediated by hOCT1 (Dickens et al. 2012). These authors also showed a putative pharmacokinetic drug–drug interaction (DDI) between the antipsychotic drug quetiapine (an inhibitor of hOCT1; see Table 3) and lamotrigine.

5.3.6 Anti-Parkinsonian Drugs

For the treatment of Parkinson's disease, which is characterized by the loss of nigrostriatal dopaminergic neurons, drugs must be able to enter the brain to replace or to mimic the missing dopamine (DA). Gold standard for therapy is the amino acid l-3,4-dihydroxyphenylalanine (known as levodopa or L-DOPA). Levodopa traverses the BBB and subsequently the plasma membrane of dopaminergic neurons via the L-type amino acid transporter 1 and 2. Inside dopaminergic neurons levodopa is converted into dopamine (DA) to be taken up in storage vesicle (via a VMAT transporter) to restore in part the missing DA. A fraction of DA is metabolized by monoamine oxidases (MAO-A and B). Since Parkinson's disease is also characterized by impaired movement associated with an excess of glutamate, some drugs are used as NMDA receptor antagonists. Only three from the drugs listed in Table 3 (with references) are anti-Parkinsonian drugs, namely the NMDA receptor antagonist **amantadine** and the two DA receptor agonists **apomorphine** and **pramipexole**. **Amantadine**, a substrate of NET (Bönisch and Rodrigues-Pereira 1983), has been shown to interact with all four organic cation transporters, but with higher potencies only at hOCT1 and hOCT2 (Table 3). At hOCT2 amantadine was shown to be a transported substrate (Bednarczyk et al. 2003; Busch et al. 1998).

Apomorphine has hitherto only been shown to inhibit hOCT1 (Ahlin et al. 2008). The DA receptor agonist **pramipexole** (listed in Table 3) has been shown to be a substrate for rat OCT1 (Ishiguro et al. 2005); however, according to Diao et al. (2010) pramipexole is only a substrate of hOCT2 and hOCT3. Interestingly, in a population-based cohort study (Becker et al. 2011), a hOCT1 variant (rs622342 minor C variant allele in the SLC22A1 gene) was associated with higher prescribed doses of the anti-Parkinsonian drugs amantadine, pramipexole, and levodopa and shorter survival time after start of levodopa therapy. The authors proposed that these three drugs are substrates of hOCT1 and that the hOCT1 variant is associated with a decreased transport efficacy (Becker et al. 2011). Further studies are necessary to confirm this finding and to examine whether coadministration of hOCT1 inhibiting drugs also may cause shorter survival time.

5.4 Other Drugs Acting at hOCTs and/or hPMAT

Beside drugs reviewed in the sections above, further drugs are listed in Table 3. These are presented below only with their number (given in Table 3) and they are listed in an alphabetical order according to their therapeutic use or function. The numbers of those drugs that are a substrate of at least one transporter are underlined.

Antiarrhythmics (*47*, *64, 102, 142, 144, 148*); **antiasthmatics:** corticosteroids (*16*, *19*), ß2-adrenoceptor agonists (*63*, *68, 157*, *158*); **antibiotics** (*54*, *59*, *72*, *177*); **anti-cardiogenic shock** $ß_1$ adrenoceptor agonist (*50*); **anticholinergic M-AChR antagonists**: spasmolytic (*21*), antiasthmatic (*80*), parasympatholytic (*98*, *125, 179*), antibradycardic (14); **antidepressant monoamine oxidase inhibitors** (*76, 108, 129*); **antidiabetics** (*100*, *135*, *153*); **antidiarrhoics**: alkaloid (*18*), opioid (*91*); **antigastric ulcer** proton pump inhibitors (*86*, *121*, *127*, *149*, *170*); **antiemetic** 5-HT_3R antagonists (122); **antiglaucoma** M-AChR agonist (*22*); **antihypertensive**: α-adrenoceptor antagonists (*136*, *141, 172*), $α_2$-adrenoceptor agonists (*33, 73, 74*), ß-adrenoceptor antagonists (*2*, *13*, *23*, *101*, *111*, *138, 145*), diuretic (*6*), Ca^{2+} channel blockers (*44, 185*); **antiinflammatory** food supplement (*118*); **antiischemic** adenosine uptake inhibitor (*46*); **antimalarial** (*147*); **antimycotics** (*34, 83*); **antiparasitic** (*132*); **antiprostatic hyperplasia** α-adrenoceptor antagonist (*4*); **H_1-antihistaminics** (*25, 26, 31, 45*); **H_2-antihistimanics** (*150,* see also below); **herbicide** (not a drug) (*128*); **local anesthetic** (*90*); **muscle relaxants**: mixed antagonist at mAChR, NMDAR and H_1R (*123*), N-AChR antagonists (*182*); **nicotine replacement** N-AChR agonists (*115, 181*); **NMDA receptor antagonist** (*48*); **sympathomimetics**: α/ß-adrenoceptor agonist (*60*, *135*) and indirect (*180*); for abbreviations, see head of Table 3.

The H_2 antihistaminic and antigastric ulcer drugs **cimetidine** (*28*) and **famotidine** (*61*) and the antidiabetic **metformin** (*100*) that inhibit all three hOCTs (with famotidine and metformin additionally inhibiting hPMAT) are all at least substrates of hOCT2 and in their radiolabeled form are frequently used in studies at OCTs.

From the above given drugs, the following showed IC_{50} values <10 μM at hOCT1: carvedilol (*23*), clemastine (*31*), dobutamine (50), guanabenz (73),

ipratropium (80), ketoconazole (83), oxybutynin (125), prazosin (141), repaglinide (153); at hOCT2: beclomethasone (16), budesonide (20), dipyridamole (46), disopyramide (47), mepiperphenidol (98), omeprazole (121), ondansetron (122), orphenadrine (123), pantoprazole (127); at hOCT2/3: atropine (14), clonidine (33), lansoprazole (*86*); at hOCT2/3: berberine (18), harmaline (76), and at all 3 hOCTs: phenoxybenzamine (136) and rabeprazole (149).

6 Clinical Relevance of hOCTs and hPMAT as Targets for Drug–Drug Interactions (DDIs) and as Drug Transporters

6.1 The Antidiabetic Metformin as an Example for DDIs at hOCTs

Metformin is the "gold standard" in oral therapy of diabetes mellitus type 2. By inhibition of hepatic gluconeogenesis (and blockade of other hepatic functions involved in glucose control) metformin lowers blood glucose levels, increases the efficacy of insulin, and causes (in therapeutic doses) a clinically less important increase in blood lactate. Metformin in the liver is not metabolized and only marginally secreted into the bile but almost exclusively renally excreted. As a biguanide, which at physiological pH is positively charged, metformin is a highly hydrophilic molecule and it needs cation transporters to be taken up in cells. Organic cation transporters contributing to drug distribution and pharmacokinetics are shown in Fig. 1. Metformin is a substrate of all three hOCTs and of hPMAT (see Table 3). As discussed by Koepsell (2015), intestinal absorption of metformin through uptake into enterocytes is mediated by hOCT1 and hOCT3 but mainly by the far more abundant PMAT. Since metformin is not significantly secreted into the bile, it must leave the enterocytes again through an organic cation transporter (which one is not yet known). Uptake of metformin into hepatocytes (which contain metformin's target sites) through the sinusoidal membrane is mainly mediated by hOCT1 and less through hOCT3, which is less densely expressed there. Metformin may leave hepatocytes to the blood site again by the same transporter to reach the kidney. Metformin appears in the urine not only by glomerular filtration but also by secretion in proximal tubules. This secretion is mediated by hOCT2 in the basolateral membrane and in concern with the transporters MATE1 and MATE2-K expressed in the brush-border membrane of proximal tubules.

Thus, several organic cation transporters are involved in the pharmacokinetics and the liver uptake of metformin. These may offer a diversity of drug–drug interactions (DDIs), particularly with drugs that inhibit at therapeutic concentrations the corresponding OCTs. In this context it should be noted that drug concentrations in the portal vein and not those in the systemic blood are more relevant for interactions of orally applied drugs and their hepatic transport mediated by hOCT1 and hOCT2. It has been shown that drug concentration can there be nearly 100-fold higher (Tzvetkov et al. 2013). Thus, for metformin DDIs at hepatic OCTs may be much more important than DDIs at intestinal or renal transporters. However, hitherto

only few DDIs of potential clinical relevance have been reported. Some drugs used in combination with metformin can affect the disposal process of metformin via hOCT1 (Dawed et al. 2019). Increased intestinal adverse effects of metformin associated with early metformin discontinuation have been reported for a hOCT1 variant and for a combination with opioids (Dujic et al. 2016; Barengolts et al. 2018) and decreased renal excretion of metformin for a combination with proton pump inhibitors (Kim et al. 2014). The hOCT1 inhibiting antihypertensive and antiarrhythmic drug verapamil has been shown to decrease the glucose-lowering effect of metformin (Cho et al. 2014). However, the clinical relevance of these DDIs has still to be demonstrated in clinical trials.

For further reviews on DDIs at organic cation transporters the reader is referred to, e.g., Koepsell (2015), Yin and Wang (2016), Ivanyuk et al. (2017), Gessner et al. (2019), Koepsell (2020), and Zhou et al. (2021).

6.2 Human OCTs and hPMAT as Transporters for Endogenous Substances

As summarized in Table 1 (with references), all three hOCTs and hPMAT transport the endogenous amines dopamine, epinephrine, and histamine, hOCT2, hOCT3, and hPMAT additionally norepinephrine (NE) and serotonin (5-HT), whereas the non-cationic neurotransmitter acetylcholine (ACh) is a substrate of hOCT1 and hOCT2 but presumably not of hOCT3 and hPMAT. Their role as neurotransmitter transporters (preferentially in the brain) and their inhibition by the endogenous steroid hormones corticosterone, estradiol and progesterone (Table 1) may be discussed in more detail by other authors of this book.

Corticosterone, which preferentially inhibits hOCT3 and only marginally hPMAT, has been proposed as a drug to increase the specificity of radioiodinated MIBG as diagnostic tool and anti-cancer drug for the identification and treatment of neuroendocrine tumors by decreasing unwanted hOCT-mediated uptake in non-target cells. Endogenous corticosteroids may exert favorable effects in asthma and COPD (chronic obstructive pulmonary disease), since at least the inhaled corticosteroids beclomethasone and budesonide have been shown to potently inhibit hOCT1- and/or hOCT2- mediated luminal "release" (outward transport) of bronchoconstrictor acetylcholine in the respiratory epithelium (Lips et al. 2005; Kummer et al. 2006).

6.3 Human OCTs and hPMAT Involved in Therapeutic or Toxic Effects of Drugs

6.3.1 Cytostatics/Antineoplastic Drugs

The most widely used chemotherapeutic regimen for the treatment of non-small cell lung cancer (NSCLC) consists of a combination of cisplatin or carboplatin with a

nonplatinum cytotoxic agent. Cisplatin and oxaliplatin have been shown to be substrates of hOCT2 (for references, see Table 3 and Yokoo et al. 2008).

It is well known that such platin-based regimens are associated with hematologic toxicity, nephrotoxicity, ototoxicity, nausea, and vomiting, and these toxicities are dose limiting. Picoplatin, a third generation platinum compound, which is currently in phase III clinical trials, has shown an improved safety profile with no indication of significant nephrotoxicity, ototoxicity, or neurotoxicity and to be very effective in the treatment of drug resistant or refractory lung cancer (More et al. 2010). The polar platinum compounds need transporters to penetrate into cells. Cisplatin and oxaliplatin have been shown to be substrates of hOCT2 and of all three hOCTs, respectively (for references, see Table 3 and Yokoo et al. 2008). As shown by More et al. (2010), expression of hOCT1 and hOCT2, but not hOCT3, significantly enhanced picoplatin cytotoxicity, which was reduced in the presence of an hOCT inhibitor. In addition, the volume of hOCT1-expressing xenografts in mice was significantly reduced by picoplatin treatment, suggesting that OCT1 may enhance the antitumor efficacy of picoplatin (More et al. 2010). Thus, OCT transporters play an important role in platinum-based chemotherapy and the related toxicity (Zhou et al. 2021).

Pharmacokinetic drug–drug interactions (DDIs) could occur in anti-cancer therapy using the tyrosine kinase inhibitors pazopanib, erlotinib, and nilotinib. Pazopanib is a substrate and inhibitor of hOCT1 and its unbound plasma concentration is near its IC_{50} for inhibition of hOCT1 suggesting clinically relevant DDIs are mediated by hOCT1 (Ellawatty et al. 2018). The same might hold true for erlotinib and nilotinib, which potently inhibit hOCT1 at clinically relevant concentrations and which could cause significant inhibition of hOCT1-mediated uptake of, e.g., oxaliplatin (Minematsu and Giacomini 2011; Zhou et al. 2021).

Another example for the importance of organic cation transporters in anti-cancer drug actions is the reduction of the toxicity of an anti-cancer drug, which is taken up in non-target cells expressing OCTs. Radioiodine-labeled *meta*-iodobenzylguanidine ([^{128}I or ^{131}I] MIBG) is a targeted compound used both in the diagnosis (through scintigraphy imaging) and treatment (through radiation) of neuroblastoma, pheochromocytoma, and paraganglioma, since MIBG is taken up into these tumor cells by the norepinephrine transporter (NET) which is expressed in these tumors (Streby et al. 2015). As demonstrated by Bayer et al. (2009) and López Quiñones et al. (2020), MIBG is also taken up by all three OCTs in normal, non-target cells or tissues such as the OCT3 expressing salivary glands and heart muscle cells, or, even more pronounced, in OCT1 expressing hepatocytes, and especially high in OCT2 expressing proximal tubules of the kidney, where more than 90% of the administered dose of MIBG is excreted unchanged in the urine (Lashford et al. 1988). This non-specific uptake increases toxicity and it causes a lot of background noise in scintigraphy imaging in the search and treatment of neuroendocrine tumors. To reach this goal, corticosterone, which is a known inhibitor of hOCTs (see Table 2) and which does not inhibit the NET, has been proposed by Bayer et al. (2009). By screening drugs used in the treatment of neuroblastoma for their inhibitory potency at hOCTs, López Quiñones et al. (2020) identified irinotecan

(see Table 3) to selectively and potently inhibit hOCT1-mediated, and the TKI crizotinib (see Table 3) to inhibit hOCT2-mediated MIBG uptake. These two drugs may reduce toxicities and may be helpful in the future clinical application of radioiodine-labeled MIBG.

Furthermore, the TKI sorafenib, indicated for the treatment of advanced hepatocellular carcinoma, is a substrate of hOCT1 (Swift et al. 2013; Table 3) and has to be taken up in the liver by means of hOCT1 (Lozano et al. 2013). The effect of sorafenib is limited because of impaired activity of hOCT1 in liver cancer patients, and hOCT1 expression in liver tumors has been shown to be a significant positive prognostic factor (Grimm et al. 2016). We have shown that the *Src* family TKI saracatinib, which inhibits all three hOCTs but preferentially hOCT2 (see Table 3), potently impairs oxaliplatin uptake in colorectal cancer cells and that it reduces oxaliplatin efficacy maximally in cells overexpressing hOCT2 (Morrow et al. 2010). Interestingly, Yokoo et al. (2008) showed that the level of hOCT3 mRNA was about 10-fold higher in cancerous colon cells than in normal colon cells of patients, and that cells with high expression of hOCT3 showed increased accumulation of and cytotoxicity induced by oxaliplatin treatment. In this context it should be noted that hOCTs differentially accept cisplatin and oxaliplatin as substrate. Whereas both compounds are good substrates of hOCT2, cisplatin is additionally a weak substrate of hOCT1 and oxaliplatin of hOCT3 (Yonezawa et al. 2006). The preferential uptake of cisplatin mediated by hOCT2 in renal proximal tubules explains its organ-specific toxicity (Ciarimboli et al. 2005).

In addition, pharmacokinetic drug–drug interactions (DDIs) could occur in anticancer therapy using the TKIs pazopanib, erlotinib, and nilotinib. Pazopanib is a substrate and inhibitor of hOCT1 and its unbound plasma concentration is near its IC_{50} for inhibition of hOCT1 suggesting clinically relevant DDIs are mediated by hOCT1 (Ellawatty et al. 2018). This holds true also for nilotinib and erlotinib, which potently inhibit hOCT3 and hOCT1 at clinically relevant maximal free plasma concentrations up to about 10 times their IC_{50} (Minematsu and Giacomini 2011; Zhou et al. 2021). Thus, DDIs as well as the degree of expression of liver and/or intestinal hOCTs may affect the disposition, efficacy, and toxicity of drugs that are substrates of hOCTs.

6.3.2 Antiviral Drugs

To act against an infection by a retrovirus such as HIV, nucleoside reverse transcriptase inhibitors (NRTIs) must, e.g., penetrate into $CD4^+$ T cells, the main target of HIV-1. According to Minuesa et al. (2009), hOCT1-3 are expressed and active in $CD4^+$ T cells, and lamivudine was shown to be a substrate of all three hOCTs. Other antiviral drugs with high affinity to hOCT1 and/or hOCT2 such as the NRTIs azidothymidine and abacavir, the protease inhibitors nelfinavir, ritonavir and saquinavir, and the antibiotics trimethoprim and pentamidine are potentially co-administrated drugs within the regimen of highly active antiretroviral therapy (HAART). Thus, DDIs may be of important clinical relevance. It has been shown that the addition of nelfinavir and ritonavir could reduce the accumulation of lamivudine in CD4 cells of HIV-infected patients (Jung et al. 2013; Zhou et al.

2021). Lamivudine is also taken up via hOCT1 by hepatocytes, where its active metabolites prevent hepatitis B replication. Thus, possible DDIs should be considered in concomitant administration of drugs with high affinity to hOCT1 and/or hOCT2 in the treatment of patients infected with HI or hepatitis B virus.

6.3.3 CNS Active Drugs

Antidepressants

Only at hOCT2 and only for amitriptyline, desipramine and imipramine IC_{50} values in submicromolar concentrations have been published (Table 3). The IC_{50} values of most of the other antidepressants and antipsychotics at hOCTs or hPMAT (listed in Table 3) are in the middle to high micromolar range and thus by far above their mean free steady state plasma concentration at therapeutic dosages. As discussed by Haenisch et al. (2012), from the antidepressants and antipsychotics examined only bupropion, nefazodone, and clozapine may cause at their upper plasma concentrations between 10 and 22% inhibition of hOCT2 and/or hOCT3, but none of these psychoactive drugs may cause at therapeutic plasma concentrations more than marginal inhibition of hPMAT (Haenisch and Bönisch 2010).

At clinically relevant concentrations, antidepressants may inhibit nearly exclusively $uptake_1$ transporters in the CNS and presumably only marginally $uptake_2$ transporters. Human OCTs and hPMAT could play a compensatory role in the inactivation of monoamines, if the high-affinity $uptake_1$ transporters are inhibited by antidepressants. Under this condition, monoamine neurotransmitters will diffuse more broadly in the brain and could be taken up by, e.g., hOCT3 and/or hPMAT expressed e.g., in glial cells where monoamines like 5-HT are metabolized by MAO and NE as well as DA additionally by catechol-O-methyltransferase (COMT). Inhibition of OCTs could, under these conditions, contribute to antidepressant action (Gasser and Daws 2017; Bowman et al. 2020). For the hitherto clinically used antidepressants it remains to be shown whether at least some of them reach high enough concentrations in the brain for significant inhibition of hOCTs or hPMAT.

Antipsychotics

None of the antipsychotics listed in Table 3 shows affinities in the submicromolar range at the organic cation transporters. Haloperidol (Sekhar et al. 2019) as well as sulpiride and its analog, amisulpride, which both are relative hydrophilic compounds, have been shown to be substrates of hOCTs enabling OCT-mediated passage through the BBB into the CNS (Hendrickx et al. 2013). Furthermore, Sekhar et al. (2019) demonstrated that changes of region-specific BBB transporters (OCT1 and PMAT) lead to increased sensitivity to amisulpride in Alzheimer's disease (AD). This in vitro BBB and in silico transporter study showed that (1) amisulpride and haloperidol were transported at BBB by the influx transporter OCT1 and efflux transporters PMAT and MATE1, (2) amisulpride brain uptake was increased in AD mice (compared to wildtype mice) and (3) PMAT and MATE1 expression was reduced in capillaries obtained from specific human brain regions (putamen and caudate) from AD cases compared to age matched controls. These results may

explain the increased sensitivity of individuals with Alzheimer's to amisulpride. Thus, dose adjustments may be required for drugs that are substrates of these transporters when prescribing for individuals with AD. Furthermore, in an epilepsy patient a DDI has recently reported between the antipsychotic quetiapine, an inhibitor of OCT1, and lamotrigine, an anticonvulsant that enters the brain via hOCT1 (of endothelial cells of the BBB) (Dickens et al. 2012). However, the clinical relevance of this finding needs further investigation.

Psychostimulants

Psychostimulants are known to increase brain concentration of the monoamines dopamine (DA), norepinephrine (NE), and/or serotonin by either inhibiting one or more of the corresponding uptake$_1$ transporter (such as cocaine which inhibits DAT, NET, and SERT) or by inducing transporter-mediated outward transport (such as amphetamine at DAT, NET, and SERT) or psychostimulants may act as agonists at brain monoamine receptors. It is largely unknown whether their interaction with hOCTs may significantly contribute to their psychostimulant actions. However, convincing evidence has been presented for the participation of OCT3 in amphetamine-stimulated DA efflux (and behavior). OCT3 transports DA, NE, and serotonin and it is nearly insensitive to cocaine ($IC_{50} > 1{,}000$ μM) but potently inhibited by decynium 22 (see Tables 1, 2, and 3). Mayer et al. (2018) have recently shown that decynium22 inhibited amphetamine-induced DA release in OCT3$^{+/+}$ but not in OCT3$^{-/-}$ mice; evidence was presented that the outward transport of DA was via reverse transport through OCT3. Thus, hOCTs (at least OCT3) appear to be involved in the action of psychostimulants and it remains to be shown whether interactions at hOCTs may be considered when, e.g., amphetamine or methamphetamine is used for the treatment of ADHD (attention deficit hyperactivity disorder).

Beside interaction at liver and kidney, DDIs at OCTs expressed in cells of the blood–brain barrier (BBB) may be of clinical relevance for the application of CNS active drugs, which are substrates of organic cation transporters expressed there. This might be of particular relevance to the elderly who are prescribed multiple drugs; especially for prescription of drugs for older multimorbid patients with impaired liver or kidney function and who are suffering from Alzheimer's disease. These elderly often obtain (mostly in older people's residences or nursing homes) daily up to 10 drugs, especially often more than one antipsychotic together with an antidepressant, sedative or opioid analgesic beside drugs for the treatment of diabetes mellitus (e.g., metformin), hypertension or heart failure. In addition, genetic variations of *SLC22A1* (hOCT1) have been shown to affect the pharmacokinetics of CNS active hOCT1 substrates such as the opioid analgesic morphine and others (Meyer et al. 2019). The role of hOCT or hPMAT variants will be discussed in more detail in another chapter in this book.

7 Summary and Conclusion

This chapter first discusses essential basic properties such as the structure and functions of the human transporters for organic cations, hOCT1, hOCT2, hOCT3, and hPMAT, and their essential expression sites in the human organism. In addition to important endogenous ligands, model ligands are presented as substrates or inhibitors of these transporters. However, the main focus is on drugs that are substrates or inhibitors of these transporters. These drugs are listed and their interaction with organic cation transporters, which are particularly important for the pharmacokinetics and pharmacodynamics of drugs, is discussed. OCT-mediated drug–drug interactions (DDIs) of various, but in particular CNS-effective drugs such as antidepressants, antipsychotics, psychostimulants, antiepileptics, and anti-Parkinson's are presented and their clinical significance is discussed using examples from the literature. However, more clinical studies are needed to demonstrate more impressively the clinical significance of hOCTs and of hPMAT and relevant DDIs.

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Organic Cation Transporters in Brain Histamine Clearance: Physiological and Psychiatric Implications

Fumito Naganuma and Takeo Yoshikawa

Contents

Abstract

Histamine acts as a neurotransmitter in the central nervous system and is involved in numerous physiological functions. Recent studies have identified the causative role of decreased histaminergic systems in various neurological disorders. Thus, the brain histamine system has attracted attention as a therapeutic target to improve brain function. Neurotransmitter clearance is one of the most important processes for the regulation of neuronal activity and is an essential target for diverse drugs. Our previous study has shown the importance of histamine *N*-methyltransferase for the inactivation of brain histamine and the intracellular localization of this enzyme; the study indicated that the transport system for the

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movement of positively charged histamine from the extracellular to intracellular space is a prerequisite for histamine inactivation. Several studies on in vitro astrocytic histamine transport have indicated the contribution of organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) in histamine uptake, although the importance of these transporters in in vivo histamine clearance remains unknown. Immunohistochemical analyses have revealed the expression of OCT3 and PMAT on neurons, emphasizing the importance of investigating neuronal histamine uptake. Further studies using knockout mice or fast-scan cyclic voltammetry will accelerate the research on histamine transporters. In this review article, we summarize histamine transport assays and describe the candidate transporters responsible for histamine transport in the brain.

Keywords

Histamine · Histamine N-methyltransferase · Organic cation transporter · Plasma membrane monoamine transporter

1 Histamine in the Central Nervous System

Histamine (2-[4-imidazolyl]ethylamine) was first synthesized from an essential amino acid L-histidine in 1907 (Windaus and Vogt 1907). In 1910, Sir Henry Dale and his colleagues reported the biological actions of histamine, including hypotensive effects and uterine contraction (Barger and Dale 1910; Dale and Laidlaw 1910). Histamine controls a wide variety of physiological events through interaction with four different histamine receptors (H_1R, H_2R, H_3R, and H_4R), a class of G-protein coupled receptors (Haas et al. 2008). The strong impact of H_1R on immunological reactions has led to the development of H_1R antagonists for allergic rhinitis by Daniel Bovet (Bovet and Staub 1937), for which he was awarded the Nobel Prize in 1957. Pharmacological experiments focusing on the involvement of H_2R in gastric acid secretion resulted in the discovery of H_2R antagonists for peptic ulcers by James Black (Black et al. 1972), who was also awarded the Nobel Prize in 1988. In the central nervous system (CNS), histamine acts as a neurotransmitter (Yoshikawa et al. 2021). Histaminergic neurons are exclusively expressed in the tuberomammillary nucleus in the posterior hypothalamus and project their synaptic fibers to all brain regions (Haas and Panula 2003; Watanabe et al. 1983). Brain H_1R, a $G_{q/11}$-coupled receptor expressed on postsynaptic membranes, regulates the sleep-wake cycle, cognitive function, and locomotor activity. Recent studies have shown the importance of astrocytic H_1R in gliotransmitter release and several mouse behaviors, such as aggression and circadian rhythm (Karpati et al. 2018, 2019). H_2R, a G_s-coupled receptor on postsynaptic membranes, is involved in memory retrieval and nociception (Nomura et al. 2019; Obara et al. 2020). H_3R, a $G_{i/o}$-coupled receptor mainly localized to the presynaptic terminal of histamine neurons as an autoreceptor, negatively controls histamine synthesis and release. H_3R is also expressed in microglia and controls their activity (Iida et al. 2015). Since H_3R is

widely distributed in the CNS and has strong constitutive activity as an inhibitory receptor, H_3R inverse agonists induce the release of histamine and enhance histaminergic activity. Thus, H_3R inverse agonists have attracted attention as potential drugs to improve brain function (Sadek et al. 2016). Due to the importance of histamine in brain functions, chronic histamine dysfunction in mice induces various brain dysfunctions (Schneider et al. 2014; Yamada et al. 2020). Indeed, numerous human studies have shown the involvement of histaminergic dysfunction in neuropsychiatric disorders. Lower brain histamine concentration was observed in patients with Alzheimer's disease than in age-matched controls (Panula et al. 1998). In patients with narcolepsy, histamine concentration in the cerebrospinal fluid was significantly decreased (Kanbayashi et al. 2009; Nishino et al. 2009). Human positron emission tomography studies indicated that the binding potential of radiolabeled ligands to H_1R was decreased in patients with depression, schizophrenia, and Alzheimer's disease (Yanai and Tashiro 2007). This evidence strongly indicates the critical importance of histamine concentration for brain conditions and the therapeutic potential of histaminergic improvement for neurological disorders.

To maintain normal intra- and extra-cellular histamine concentrations, histamine synthesis and clearance have essential roles. Histamine is synthesized from an essential amino acid, histidine, by the catalyzation of histidine decarboxylase (HDC) (EC 4.1.1.22) (Komori et al. 2012). Pharmacological inhibition of histamine synthesis by alpha-fluoromethylhistidine, a suicide inhibitor of HDC, dramatically reduces brain histamine content and alters various brain functions, such as circadian rhythms and neuroendocrine responses (Watanabe et al. 1990). Insufficient intake of L-histidine reduced brain histamine content and led to anxiety-like behaviors in mice (Yoshikawa et al. 2014). Nonsense mutations in human HDC have a causative role in Tourette's syndrome (Baldan et al. 2014). On the other hand, direct histamine injection into the hippocampus ameliorated brain inflammation (Saraiva et al. 2019) or activated the hypothalamic-pituitary-gonadal axis (Niaz et al. 2018). Moreover, a placebo-controlled, double-blind crossover trial showed that daily histidine intake for humans alleviated mental fatigue and improved clear thinking and attentiveness (Sasahara et al. 2015). This evidence demonstrates the importance of histamine synthesis in maintaining brain histamine concentration and normal brain function. Additionally, histamine clearance also contributes to the regulation of histamine concentration. Histamine is metabolized by two different enzymes, diamine oxidase (DAO) (EC 1.4.3.4) and histamine *N*-methyltransferase (HNMT) (EC 2.1.1.8). DAO is dominantly expressed in the intestinal tracts and contributes to the reduction in histamine toxicity derived from rotten foods (Maintz and Novak 2007). DAO is not highly expressed in the CNS. On the other hand, HNMT is ubiquitously expressed in various organs, including the CNS. Previous studies using metoprine, an HNMT inhibitor penetrating the blood–brain barrier (Kitanaka et al. 2016), indicated the involvement of HNMT in brain functions, although the low specificity of metoprine could not rule out the involvement of off-target effects of the drug. We first created and analyzed *Hnmt*-deficient mice. Hnmt disruption induced an eightfold increase in brain histamine concentration, and this substantial histamine elevation by *Hnmt*

deficiency altered the sleep-wake cycle and aggressive behaviors in mice (Naganuma et al. 2017), demonstrating the dominant contribution of HNMT to brain histamine concentration and brain function. We also examined the subcellular localization of HNMT by fractionation and immunocytochemistry and determined its cytosolic localization (Yoshikawa et al. 2013). Immunohistochemical analysis by Nishibori et al. also supported the intracellular localization of HNMT (Nishibori et al. 2000). Since histamine is a positively charged molecule, histamine transport across the lipid bilayer from the extracellular space to the cytosol is required for inactivation by HNMT, indicating the substantial importance of histamine transporters for the regulation of brain histamine concentration and histaminergic system. Although the detailed histamine transport system in the brain remains to be elucidated, here we summarize previous studies examining transporters involved in histamine transport.

2 Monoamine Neurotransmitter Transporters in the Central Nervous System

There are two distinct transport systems responsible for the uptake of extracellular monoamine neurotransmitters in the CNS. The uptake-1 system transports neurotransmitters through high-affinity and low-capacity transporters (low Michaelis constant (K_m) value and low maximum transport velocity (V_{max}) value). The serotonin transporter (SERT), norepinephrine transporter (NET), and dopamine transporter (DAT) belong to the uptake-1 system (Kristensen et al. 2011). These transporters expressed on presynaptic terminals play a crucial role in the regulation of monoaminergic signaling by controlling extracellular monoamine concentrations. To this end, various antidepressants and psychostimulants, including serotonin-selective reuptake inhibitors (Jakobsen et al. 2017) and methamphetamine (Ashok et al. 2017) target these transporters and modulate neuronal activities. In contrast, the uptake-2 system facilitates intracellular transport through low-affinity and high-capacity transporters (high K_m and high V_{max}) (Koepsell 2020). Organic cation transporters (OCTs) and plasma membrane monoamine transporter (PMAT) belong to the uptake-2 transporter system. The uptake-2 system has been considered to function mainly in peripheral organs. However, recent studies have shown the involvement of the uptake-2 system in the monoaminergic nervous system in the CNS. The disruption of mouse *Oct2* altered norepinephrine and serotonin concentrations in various brain areas and changed anxiety-like behaviors and depression-like behaviors (Bacq et al. 2012). Courousse et al. demonstrated the involvement of Oct2 in stress vulnerability in mice (Courousse et al. 2015). Extensive investigations using Oct3 knockout mice have shown that this transporter controls dopaminergic and serotonergic tones and regulates a wide variety of physiological brain functions, including anxiety, depression, and addiction (Baganz et al. 2008, 2010; Daws 2009; Horton et al. 2013; Mayer et al. 2018). To date, a histamine transport system belonging to the uptake-1 system has not been identified, and several transporters of the uptake-2 system have been reported to convey

histamine from the extracellular space. Organic cation transporter 2 (Oct2), organic cation transporter 3 (Oct3), and plasma membrane monoamine transporter (Pmat) are capable of histamine transport. The involvement of these transporters in central and peripheral histamine clearance is summarized in Tables 1 and 2.

2.1 Organic Cation Transporter 2

Oct2, encoded by the *Slc22a2* gene, was first cloned from a rat kidney in 1996 (Okuda et al. 1996). Human OCT2 transports various cations, such as tetraethylammonium and 1-methyl-4-phenylpyridinium (MPP), and diverse drugs, including cimetidine, a histamine H_2 receptor antagonist. Several neurotransmitters, such as acetylcholine, dopamine, norepinephrine, serotonin, and histamine, are also substrates for human OCT2 (Koepsell 2013). Previous reports have shown the expression of OCT2 in the brain, including the cortex, hippocampus, thalamus, hypothalamus, dorsal raphe nucleus, and locus coeruleus (Bacq et al. 2012; Busch et al. 1998; Courousse et al. 2015; Nakata et al. 2013). Nakata et al. also showed that Oct2 was localized to presynaptic nerve end terminals. The K_m values of OCT2 to histamine were 111 μM (mouse OCT2), 0.89 mM (rat OCT2), and 0.94 mM or 1.3 mM (human OCT2). Basal histamine concentration in the CNS was not determined, although Samaranayake et al. estimated that electrical stimulation of histaminergic neurons resulted in an increase of 5–15 μM extracellular histamine concentration (Samaranayake et al. 2016). Because the pK_i values of human H_1 receptor and human H_2 receptor were reported to be 4.2 and 4.3 μM, respectively (Lim et al. 2005), extracellular histamine concentration in the brain might be at least 10 μM after excitatory stimulation. The K_m values of uptake-1 transporters are usually less than 5 μM, suggesting that uptake-2 transporters are better suited for histamine removal in the brain.

2.2 Organic Cation Transporter 3

The rat and human OCT3 genes encoded by *Slc22a3* were cloned in 1998 (Gründemann et al. 1998; Kekuda et al. 1998). OCT3 is expressed in the placenta, intestine, heart, and brain. OCT3 is widely expressed in different brain regions, such as the hippocampus, cerebellum, and cerebral cortex (Wu et al. 1998). Gasser et al. reported that Oct3 expression was observed in both neurons and astrocytes (Gasser et al. 2017). Their immuno-electron microscopy observations also revealed that neurons express OCT3 in axons, dendrites, neuronal bodies, and astrocytes in their processes. Recent studies have revealed that brain OCT3 plays an important role in the clearance of neurotransmitters, including serotonin (Baganz et al. 2008) and dopamine (Cui et al. 2009). The K_m values of OCT3 to histamine were 1.6 mM (mouse OCT3), 0.54 mM (rat OCT3), and 0.22 or 0.64 mM (human OCT3)

Table 1 Histamine transport by astrocytes or synaptosomes

Cell	Results
Adult rat astrocytes and synaptosomes (Rafałowska et al. 1987)	Histamine was more actively taken up into astrocytes. The K_m and V_{max} values of histamine calculated by Lineweaver-Burk plot were 0.5 μM and 1.6 pmol/mg protein/min
Embryonic chicken astrocytes (Huszti et al. 1990b)	The K_m and V_{max} values of embryonic chicken astrocytes to histamine calculated by Eadie-Hofstee plot indicated the two transport systems; high-affinity system ($K_m = 0.24$ μM and $V_{max} = 0.31$ pmol/mg protein/min) and low-affinity system (K_m and V_{max} were not calculated)
Neonatal rat astrocytes and dissociated hypothalamic cells (Huszti et al. 1994)	Histamine transport by dissociated hypothalamic cells ($K_m = 0.33$ μM and $V_{max} = 2.65$ pmol/mg protein/min) was significantly decreased by an astrocytic toxin alpha-aminoadipic acid. The K_m and V_{max} values to histamine calculated by linear regression methods were 0.19 μM and 3.12 pmol/mg protein/min
Rat anterior hypothalamic area (Huszti et al. 1998)	Fluoroacetate, an astrocytic toxin, increased extracellular histamine concentration around the rat anterior hypothalamic area
Rat synaptosomes (Sakurai et al. 2006)	The K_m and V_{max} values of rat synaptosomes to histamine calculated by nonlinear regression indicated the two transport systems; one had higher affinity $K_m = 0.16$ μM and $V_{max} = 2.4$ fmol/mg protein/min, and the other had $K_m = 1.2$ μM and $V_{max} = 3.4$ fmol/mg protein/min. V_{max} values of this study were quite low compared to other studies
Rat neonatal type 1 astrocytes (Osredkar et al. 2009)	Kinetic analysis by nonlinear regression showed a single transport system with K_m of 3.5 μM and V_{max} of 7.9 pmol/mg protein/min. Several drugs, such as amitriptyline and desipramine, partially inhibited histamine transport
Rat adult astrocytes (Perdan-Pirkmajer et al. 2012)	The K_m and V_{max} values of rat adult astrocytes to histamine calculated by linear regression methods were 141 μM and 22.5 pmol/mg protein/min. Higher *Oct2* and lower *Oct3* expression were determined by quantitative RT-PCR
Rat neonatal astrocytes from cortex, striatum, and cerebellum (Perdan-Pirkmajer et al. 2013)	The regional differences of kinetic parameters to histamine transport by astrocytes were reported. Although uptake-2 transporter inhibitors, such as corticosterone and decynium-22, did not inhibit histamine transport by cerebellar and striatal astrocytes, *Oct2* expression in astrocytes was confirmed

(continued)

Table 1 (continued)

Cell	Results
Primary human astrocytes (Yoshikawa et al. 2013)	Kinetic analysis using nonlinear regression showed that two low-affinity transport system (K_m = 0.56 mM and 4.0 mM). Knockdown of *OCT3* and *PMAT* significantly reduced histamine transport
1,321 N1 cells, a human astrocyte-derived cell line (Naganuma et al. 2014)	The K_m and V_{max} values to histamine calculated by nonlinear regression methods were 3,870 μM and 363 pmol/mg protein/min. *PMAT* knockdown and decynium-22 treatment significantly decreased histamine transport

Table 2 Histamine transport by peripheral tissues

Cell	Results
Human submandibular salivary gland epithelial cells (Stegaev et al. 2013)	Histamine transport of human submandibular salivary gland epithelial cells was inhibited by MPP. A strong expression of OCT3 implied the dominant contribution of this transporter to histamine transport
Human endometrial cells (Noskova et al. 2006)	Histamine transport in human endometrial cells is partially inhibited by corticosterone. The involvement of OCT3 is deduced. Later, the authors confirmed PMAT expression though the functional involvement of PMAT was not examined
Bronchial epithelial cells (Yamauchi and Ogasawara 2019)	*OCT3* polymorphism was associated with asthma. Decreased histamine transport by OCT3 SNPs might increase extracellular histamine levels and exacerbate asthma symptoms
Murine basophils (Schneider et al. 2005)	Oct3 is responsible for histamine uptake in murine basophils. Oct3-mediated control of intracellular histamine levels has a role in basophil functions, such as cytokine synthesis
Rat mast cell lines (Slamet Soetanto et al. 2019)	The uptake-2 transporters play a role in histamine uptake in mast cells. The possible contribution of Oct1 and Pmat to histamine transport is mentioned
Mouse mast cells (Nakamura et al. 2017)	Oct3 expression level in mast cells is controlled by the circadian gene *Clock*. The circadian variation of Oct3 expression might be involved in the daily fluctuation of plasma histamine level and allergic symptoms
Human keratinocytes in oral lichen planus (Salem et al. 2017)	Histamine release through OCT3 in keratinocytes of oral lichen planus might contribute to the disease pathogenesis

(Amphoux et al. 2006; Duan and Wang 2010; Miura et al. 2017). Sakata et al. reported that five SNPs (T44M, A116S, T400I, A439V, and G475S) resulted in the reduction of histamine transport by OCT3 (Sakata et al. 2010), although the involvement of these SNPs in neuropsychiatric disorders is still unknown.

2.3 Plasma Membrane Monoamine Transporter

PMAT encoded by the *SLC29A4* gene was newly identified in 2004 (Engel et al. 2004) and was strongly expressed in skeletal muscle and brain. PMAT transports MPP^+ and various neurotransmitters, including dopamine and serotonin. Vialou et al. examined the expression of PMAT in the rat brain and showed a wide distribution. Interestingly, rat PMAT was expressed in Hdc-positive histaminergic neurons in the tuberomammillary nucleus (Vialou et al. 2007). Pmat is abundantly expressed in epithelial cells of the mouse choroid plexus (Dahlin et al. 2007) and contributes to the transport of serotonin and dopamine (Duan and Wang 2013). Several studies have indicated the involvement of Pmat in serotonin clearance (Daws et al. 2013; Matthaeus et al. 2015) and the association of the *SLC29A4* mutation with autism spectrum disorder (Adamsen et al. 2014). However, the impact of mouse *Pmat* deficiency on mouse behavior is limited (Duan and Wang 2013; Gilman et al. 2018). The K_m values of PMAT to histamine were 1.52 mM (mouse PMAT) and 4.38 mM (human PMAT).

2.4 Other SLC22 Family Transporters

Several studies have examined the monoamine transport activity of OCT1 encoded by *SLC22A1*. Gründemann et al. reported that rat Oct1 did not transport histamine, although MPP^+ uptake through rat Oct1 was inhibited by high concentrations of histamine ($K_i = 4.2$ mM) (Gründemann et al. 1999). Amphoux et al. reported that rat Oct1 transported dopamine, serotonin, and norepinephrine but not histamine (Amphoux et al. 2006). They also showed no histamine transport activity in human OCT1. Additionally, OCT1 expression is predominantly localized to brain microvessels but not neurons (Koepsell 2004; Lin et al. 2010). Therefore, OCT1 appears to be unimportant for histamine clearance in the CNS. Although novel OCTs (OCTN), OCTN1 and OCTN2, which are expressed in the CNS, could transport carcinine (β-alanylhistamine) (Tamai 2013), no histamine transport through these transporters has been reported to date.

3 Histamine Uptake in the Central Nervous System

Histamine transport in peripheral tissues was first determined in mast cells and platelets (Cabut and Haegerma 1966; Tuomisto 1968). In the CNS, Tuomisto et al. showed histamine transport activity in rabbit hypothalamic slices (Tuomisto et al. 1975). Although several studies have confirmed histamine transport activity in rat and guinea pig brain slices (Biggs and Johnson 1980; Mulder et al. 1983; Subramanian and Mulder 1977), cells responsible for histamine transport were not determined. In 1987, Rafalowska et al. isolated rat synaptosomes and astrocytes and compared the activity of histamine transport and Hnmt (Rafałowska et al. 1987). They reported higher astrocytic activity for histamine uptake and histamine

metabolism. In later studies, astroglial histamine transport activity was confirmed (Huszti et al. 1990a, 1994). Hustzi et al. also examined the importance of astrocytes for histamine transport by using the astrocytic toxin alpha-aminoadipic acid or fluoroacetate. These toxins significantly decreased histamine transport in hypothalamic slices and primary astrocytes and increased extracellular histamine concentration (Huszti 1998; Huszti et al. 1998), supporting the contribution of astrocytes to histamine transport. Although their kinetic studies indicated the existence of high-affinity transporters and low-affinity transporters, responsible transporters were not determined. After the cloning of OCTs in the late 1990s, several laboratories reported the distribution of OCTs in the CNS and their neuronal and astrocytic expression (Cui et al. 2009; Gasser et al. 2006). Although Perdan-Pirkmajer et al. isolated neonatal and adult rat astrocytes and investigated the impact of various inhibitors against OCTs on histamine uptake (Perdan-Pirkmajer et al. 2012, 2013), the results are still inconclusive. We examined the histamine transporters responsible for histamine uptake in primary human astrocytes. Primary human astrocytes can transport [^{3}H]-histamine in an extracellular sodium-independent manner (Yoshikawa et al. 2013). In the kinetic analysis, the K_m values of histamine uptake by primary human astrocytes were estimated as 0.56 and 4.0 mM, indicating that the two uptake-2 transporters were involved in histamine transport. Gene silencing technology, as well as pharmacological assays, clearly demonstrated the major contribution of PMAT and the minor contribution of OCT3 to histamine transport by primary human astrocytes. We also confirmed the importance of OCT3 and PMAT for histamine transport in 1321 N1 cells, a cell line derived from the human astrocytoma cell line (Naganuma et al. 2014), supporting the importance of cooperative transport through OCT3 and PMAT for histamine uptake in astrocytes.

Although our in vitro experiments indicated the involvement of uptake-2 transporters for histamine clearance, the importance of these transporters in the CNS is still unknown. Gasser et al. showed that histamine uptake in rat hypothalamic mince was significantly inhibited by decynium-22 and corticosterone, which are typical inhibitors of uptake-2 transporters (Gasser et al. 2006), indicating the involvement of uptake-2 transporters in histamine clearance in the CNS. However, these inhibitors could not abolish histamine transport activity in hypothalamic mince, implying that additional unknown transporters play a role in histamine clearance in the CNS. In *Oct3*-deficient mice, Vialou et al. reported that histamine concentration in homogenates of cortex and diencephalon was significantly decreased (Vialou et al. 2008). In contrast, Zhu et al. showed that *Oct3* disruption did not change the basal histamine concentration in cortex, although brain ischemia induced a substantial increase in cortical histamine by *Oct3* deficiency (Zhu et al. 2012). Thus, these studies using *Oct3* knockout mice did not show consistent results. However, high-speed chronoamperometric analysis done by Baganz et al. (2008) showed that the increase of Oct3 expression in Sert$^{-/-}$ mice resulted in faster histamine clearance, suggesting involvement of Oct3 in brain histamine clearance in vivo. Additionally, previous studies using *Pmat* deficient mice did not measure histamine concentration in the CNS (Duan and Wang 2013). Therefore, further studies using knockout mice, focusing on the histaminergic nervous system, are

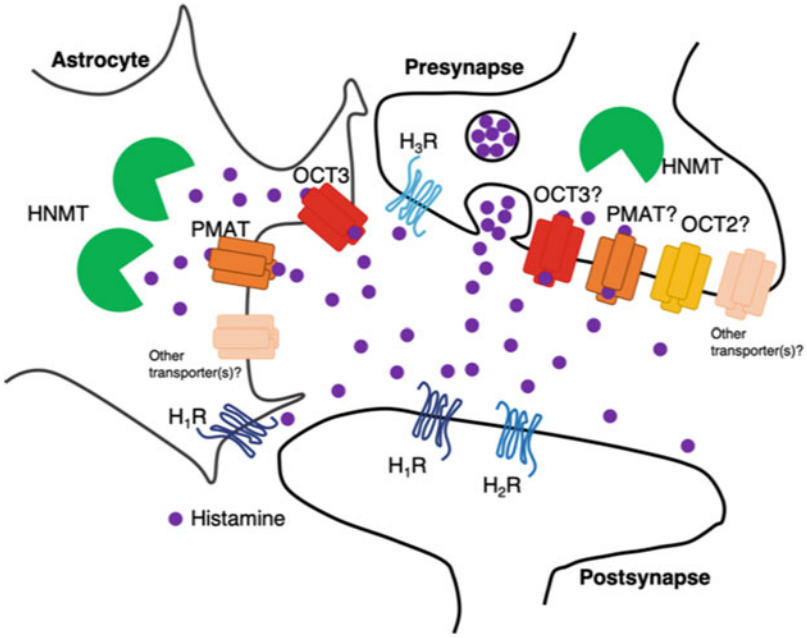

Fig. 1 Histamine is released from the presynaptic terminal into the synaptic cleft. Released histamine binds to postsynaptic and/or astrocytic histamine H_1 receptor (H_1R), postsynaptic H_2R and presynaptic H_3R. Excessive extracellular histamine might be transported through organic cation transport 2 (OCT2), organic cation transporter 3 (OCT3), plasma membrane monoamine transporter (PMAT), and other transporter (s) on the astrocytes and/or presynapses. Then, transported histamine is inactivated by histamine *N*-methyltransferase (HNMT) in the intracellular space

necessary to demonstrate the importance of Oct2, Oct3, and Pmat for histamine clearance in vivo.

Several studies have suggested the contribution of neurons for histamine clearance. Shan et al. showed *HNMT* mRNA expression in neurons but not glial fibrillary acidic protein (GFAP)-positive astroglia in the prefrontal cortex of patients with Alzheimer's disease (Shan et al. 2012). Immunohistochemical analysis of the bovine brain also supported the neuronal distribution of Hnmt (Nishibori et al. 2000). OCT3 and PMAT are also expressed not only in astrocytes but also in neurons, and the possible contribution of neurons in histamine clearance cannot be ignored. Although previous studies indicated lower synaptosomal histamine transport than astrocytic transport, Sakurai et al. suggested the existence of high-affinity histamine transporters in rat synaptosomes (Sakurai et al. 2006). Additionally, the subcellular localization of Oct3 to neuronal somata has been demonstrated (Gasser et al. 2017), emphasizing that histamine uptake studies using primary neurons as well as synaptosomes are essential to determine the involvement of neurons for histamine clearance (Fig. 1).

Previous studies did not indicate the involvement of low-affinity transporters for histamine transport (Table 1). Before the existence of low-affinity transporters with higher K_m values was widely recognized, dose-response transport assays necessary for kinetic analysis used a narrow concentration range, such as from 10 nM to 200 nM (Huszti et al. 1994). Thus, it is quite difficult to predict the involvement of Oct2, Oct3, and Pmat in histamine transport of astrocytes, which have K_m values of at least 100 μM for histamine. Additionally, they calculated K_m and V_{max} values from linear regression analyses, such as the Lineweaver-Burk plot and Eadie-Hofstee plot, but not nonlinear regression methods, which are clearly more accurate than the former. The unique characteristics of typical uptake-2 inhibitors also interfered with the molecular determination of histamine transporters. Several studies showed that the effects of typical uptake-2 inhibitors, such as corticosterone, disprocynium24, and decynium-22, were dependent on the phosphorylation state of analyzed cells (Amphoux et al. 2010; Miura et al. 2017). Specifically, corticosterone enhanced mouse Oct3 transport activity, although the facilitative effect of corticosterone on mouse Oct3 was abolished by KN-93, an inhibitor of CaMKII. Thus, methodological limitations might contribute to the lower K_m values of astrocytic histamine transport in previous studies, although it is not possible to rule out that high-affinity histamine transporters are involved in histamine clearance. Recently, Moriyama et al. identified a vesicular polyamine transporter for spermine and spermidine by using proteoliposomes and indicated its possible involvement in histamine storage in the vesicles of neurons and astrocytes (Hiasa et al. 2014; Moriyama et al. 2020). Thus, further studies to identify high-affinity histamine transporters using new technologies are quite important.

4 Future Perspectives

Recently, the European Medicines Agency and the U.S. Food and Drug Administration approved pitolisant, an H_3R inverse agonist to increase brain histamine release, for the treatment of excessive daytime sleepiness in adults with narcolepsy (Syed 2016). H_3R inverse agonists, including pitolisant, are recognized to have potential therapeutic benefits for various neuropsychiatric disorders, such as epilepsy, obstructive sleep apnea, and Parkinson's disease (Harwell and Fasinu 2020), emphasizing the importance of brain histamine concentration as a drug target. Indeed, a wide variety of CNS drugs, such as serotonin-selective reuptake inhibitors and monoamine oxidase inhibitors, increase neurotransmitter concentration via the suppression of the monoamine clearance system and exert their therapeutic effects. Therefore, it is important to elucidate the involvement of transporters in the regulation of histamine concentration. To evaluate the importance of neurotransmitter transporters for brain functions, the measurement of extracellular monoamine concentration is essential. Microdialysis, chronoamperometry, and fast-scan cyclic voltammetry have been applied to reveal the involvement of the uptake-2 system for monoamine clearance (Baganz et al. 2008; Hassell Jr et al. 2019; Holleran et al. 2020; Horton et al. 2013). Brain microdialysis has been widely used for the

histaminergic nervous system (Mochizuki et al. 1991). Recently, Samaranayake et al. showed that fast-scan cyclic voltammetry could be applied to evaluate brain histamine concentration (Samaranayake et al. 2015, 2016). These applications will accelerate research on histamine clearance in the future. In addition, a specific inhibitor for OCT2, OCT3, or PMAT is still undeveloped. Therefore, drug discovery research also needs to promote the research of each transporter. Some clinical studies have suggested that a lower HNMT activity caused by *HNMT* polymorphism might be associated with various disorders, such as multiple sclerosis (García-Martín et al. 2010), Parkinson's disease (Palada et al. 2012), schizophrenia, and attention-deficient hyperactivity disorder (Stevenson et al. 2010). Thus, gene mutations of OCT2, OCT3, or PMAT might also be observed in CNS disorders related to abnormal histaminergic systems. We hope that future studies of histamine transporters would promote a better understanding of the histaminergic nervous system and might lead to the development of new therapeutic targets for various brain diseases caused by histaminergic dysfunction.

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Organic Cation Transporters in Brain Catecholamine Homeostasis

Paul J. Gasser

Contents

Abstract

Catecholamines, including dopamine, norepinephrine, and epinephrine, are modulatory transmitters released from specialized neurons throughout the brain. Collectively, catecholamines exert powerful regulation of mood, motivation, arousal, and plasticity. Transporter-mediated uptake determines the peak concentration, duration, and physical spread of released catecholamines, thus playing key roles in determining the magnitude and duration of their modulatory effects. Most studies of catecholamine clearance have focused on the presynaptic high-affinity, low-capacity dopamine (DAT), and norepinephrine (NET) transporters, which are members of the uptake$_1$ family of monoamine transporters. However, recent studies have demonstrated that members of the uptake$_2$ family of monoamine transporters, including organic cation transporter 2 (OCT2), OCT3, and the

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plasma membrane monoamine transporter (PMAT) are expressed widely throughout the brain. In contrast to DAT and NET, these transporters have higher capacity and lower affinity for catecholamines and are multi-specific, each with the capacity to transport all catecholamines. The expression of these transporters in the brain suggests that they play significant roles in regulating catecholamine homeostasis. This review summarizes studies describing the anatomical distribution of OCT2, OCT3, and PMAT, their cellular and subcellular localization, and their contribution to the regulation of the clearance of catecholamines in the brain.

Keywords

Catecholamine · Clearance · Dopamine · Epinephrine · Norepinephrine · OCT1 · OCT2 · OCT3 · Organic cation transporter · PMAT · Uptake

1 Introduction

Early studies of catecholamine clearance mechanisms revealed the presence of two kinetically and pharmacologically distinct uptake processes in cardiovascular tissue: $Uptake_1$, a low capacity (V_{max} = 1.22 nmol/min/mg tissue), high-affinity (K_d = 0.27 μM) transport process inhibited by cocaine and desipramine; and $Uptake_2$, a high capacity (V_{max} = 100 nmol/min/mg tissue), low-affinity (K_d = 252 μM) process insensitive to cocaine and desipramine, but inhibited by corticosterone and normetanephrine (Iversen 1965; Iversen and Salt 1970). Subsequent studies identified the proteins responsible for these two processes in cardiac tissue as the norepinephrine transporter (NET) for $uptake_1$ (Pacholczyk et al. 1991) and, for $uptake_2$, a protein initially named the extraneuronal monoamine transporter (EMT) (Gründemann et al. 1998b), and subsequently identified as the third member of the organic cation transporter (OCT) family, OCT3 (Wu et al. 1998). It is now understood that $uptake_1$ and $uptake_2$ are two families of monoamine transporters: the $uptake_1$ family containing the sodium-dependent norepinephrine (NET), dopamine (DAT), and serotonin (SERT) transporters; and the $uptake_2$ family containing three organic cation transporters (OCT1, 2, and 3) and the plasma membrane monoamine transporter (PMAT). Each of the uptake2 transporters has been identified in brain tissue (Amphoux et al. 2006; Gasser et al. 2006, 2009; Vialou et al. 2007), suggesting that, in areas where they are expressed, these transporters may play important roles in the disposition of monoamines in the brain, and thus may contribute to the regulation of monoaminergic neurotransmission. This review describes studies examining the roles of $uptake_2$ transporters in the regulation of catecholamine homeostasis and signaling in the brain.

2 Transport of Catecholamines by Uptake$_2$ Transporters: Cell Culture Studies

Studies of monoamine transport by uptake$_2$ transporters exogenously expressed in cultured cells have demonstrated that all of the uptake$_2$ transporters can transport catecholamines and that each transporter displays a distinct substrate specificity profile (Amphoux et al. 2006; Schömig et al. 2006; Duan and Wang 2010). OCT3 preferentially transports norepinephrine, epinephrine, and dopamine, all with similar efficiencies (V_{max}/K_m) (Duan and Wang 2010). OCT2 preferentially transports epinephrine, with much lower transport of dopamine and norepinephrine (Gründemann et al. 1998a; Amphoux et al. 2006). PMAT strongly prefers dopamine and serotonin, which it transported with five- to sevenfold greater efficiency than any other monoamine (Duan and Wang 2010). Of the catecholamines, OCT1 preferentially transports epinephrine, with lower transport of dopamine and only weak transport of norepinephrine (Breidert et al. 1998). It is important to note that there is considerable variation in reported transport efficiencies between studies and that direct comparisons of transport efficiencies in cell culture studies are difficult due to variation in transporter expression, etc. Nonetheless, these studies indicate that OCT2, OCT3, and PMAT have the potential to contribute to the regulation of catecholamine homeostasis in the brain.

Studies of catecholamine transport by neuronal and glial cells in primary culture confirm roles for uptake$_2$ transporters while at the same time revealing the complexity of catecholamine uptake in more naturalistic systems. Uptake of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) by cultured cerebellar granule neurons (CGNs) is inhibited by decynium-22 (D-22) as well as by corticosterone, aldosterone, estradiol, and other steroids, but not by DAT inhibitors (Shang et al. 2003; Hill et al. 2011). The expression of mRNA for OCT1, OCT3, and PMAT, but not for NET or DAT, in CGNs confirms that catecholamine clearance in these cells is the function of multiple transport processes. Similarly, cultured astrocytes express mRNA for both OCT3 and NET (Inazu et al. 2002, 2003; Takeda et al. 2002), indicating that both uptake$_1$ and uptake$_2$ can contribute to catecholamine clearance in a single cell type. Understanding the specific roles of a given transporter in regulating catecholamine signaling will require information about its subcellular localization, including its spatial relationship to catecholamine receptors.

3 Expression and Localization of Uptake$_2$ Transporters in Brain

With the exception of OCT1, all of the uptake$_2$ transporters are widely expressed in the brain. OCT1 mRNA is barely detectable in most brain regions except for cerebellum and some white matter tracts (Amphoux et al. 2006; Hill et al. 2011). Here, we review the literature describing OCT3, OCT2, and PMAT localization in the central nervous system.

3.1 OCT3

In situ hybridization and immunohistochemical studies have shown that OCT3 is expressed widely throughout the rat brain. Particularly high levels of mRNA and protein expression have been observed in layer II of all cortical areas, especially retrosplenial, insular, and piriform cortex; in striatum, hippocampus, and cerebellum; and in periventricular organs including the subfornical organ, choroid plexus, fornix, and OVLT (Vialou et al. 2004; Amphoux et al. 2006; Gasser et al. 2009). OCT3 expression has been observed in neurons (Hill and Gasser 2013; Vialou et al. 2004), including cerebellar, hippocampal, and olfactory granule neurons (Cui et al. 2009; Gasser et al. 2009; Hill et al. 2011), striatal medium spiny neurons (Graf et al. 2013), and VTA dopaminergic neurons (Mayer et al. 2018). The transporter is also expressed in non-neuronal cells, including ependymal cells (Gasser et al. 2006, 2009); vascular endothelial cells in the brain (Li et al. 2015); and glial cells, including microglia (Gasser et al. 2016; He et al. 2017), oligodendrocytes (Gasser et al. 2009); and astrocytes (Cui et al. 2009; Gasser et al. 2016). The detection of OCT3 in brain astrocytes in situ is consistent with studies demonstrating OCT3 expression and function in human (Inazu et al. 2002) and rat (Perdan-Pirkmajer et al. 2012) astrocytes in primary culture.

3.2 OCT2

Immunohistochemical studies have demonstrated that OCT2 is widely distributed throughout the mouse brain, but is particularly enriched in prelimbic, infralimbic, cingulate and motor cortices, as well as in amygdala, periventricular thalamus (PVT), and hippocampus, including CA1, CA2, and CA3 regions (Bacq et al. 2012; Couroussé et al. 2015). OCT2 was expressed in most noradrenergic neurons in the locus coeruleus but was not detected in the dopaminergic VTA or substantia nigra (Bacq et al. 2012). In these studies, OCT2 expression was exclusively neuronal, with no evidence for astrocytic expression. This is in contrast with cell culture studies, which identified strong OCT2 mRNA expression in cultured rat astrocytes (Perdan-Pirkmajer et al. 2012).

3.3 PMAT

Immunohistochemical and in situ hybridization studies have identified PMAT mRNA and protein expression distributed throughout the brain, with particularly high expression in cerebellum, olfactory bulb, and medial septum. Interestingly, PMAT was expressed only in some, but not all, catecholamine cell groups. Most locus coeruleus noradrenergic neurons expressed PMAT, but the transporter was poorly expressed in dopaminergic cell groups. These studies also examined the expression of PMAT in specific neuronal subtypes. They identified high levels of PMAT expression in glutamatergic neurons including mitral cells in the olfactory

bulb, cholinergic neurons in striatum, hindbrain, and medial septum; and hippocampal and cerebellar granule cells (Dahlin et al. 2007; Vialou et al. 2007; Hill et al. 2011; Wang 2016).

4 Roles for Uptake$_2$-Mediated Transport in Brain Catecholamine Homeostasis: In Vivo and Ex Vivo Studies

Evidence that uptake$_2$ transporters play significant roles in shaping catecholamine homeostasis in the brain comes from studies using ex vivo uptake assays, microdialysis, voltammetry, or chronoamperometry to measure the effects of pharmacological or genetic manipulations on extracellular dopamine or norepinephrine dynamics. The most common compounds used to inhibit uptake$_2$ transporters and thereby test their involvement in monoamine clearance are the adrenal steroid hormone corticosterone and the quinoline derivative 1, 1′-diethyl-2,2′-cyanine iodide (decynium-22; D-22). All uptake$_2$ transporters are acutely inhibited by corticosterone and other steroids, though they differ in their sensitivities (for review, see Gasser and Lowry 2018; Benton et al. (this volume)). OCT3 and OCT2 are much more sensitive to corticosterone (IC_{50} = 0.1 μM (OCT3), 1 μM (OCT2)) than is PMAT (IC_{50} = 500 μM). D-22 inhibits OCTs and PMAT with IC_{50} of approximately 0.1 μM (Engel and Wang 2005). However, studies have demonstrated that D-22 has significant actions at other targets. These effects include antagonism at α1 adrenoceptors (Russ et al. 1996) and more recently, D2 dopamine receptors (Lloyd et al. 2019). It is important to note that D-22 has been shown in chronoamperometry studies to inhibit monoamine (serotonin) clearance at very low concentrations of D-22 (~0.05 μM) (Baganz et al. 2008; Horton et al. 2013), while the D-22-induced antagonism of dopamine D2 receptors was observed at very high concentrations (25 μM) (Lloyd et al. 2019). Thus, D-22 is an effective inhibitor of monoamine uptake, but off target actions of D-22 should be considered in studies of extracellular catecholamine concentrations. Experiments using these compounds must be carefully designed and must consider dose/concentration, as well as information about the expression of uptake$_1$ and uptake$_2$ transporters in the brain region where measurements are being taken.

Strong evidence that uptake$_2$ transporters contribute to catecholamine homeostasis comes from studies of mice genetically engineered to lack individual transporters. OCT2-deficient mice display decreased tissular norepinephrine levels in hippocampus, cortex, striatum, and brainstem and, in ex vivo uptake assays, hippocampal and cortical tissue from these mice display decreased D-22-sensitive norepinephrine uptake (Bacq et al. 2012). OCT3-deficient mice exhibit significantly lower tissular concentrations of dopamine in olfactory bulb, cortex, striatum, ventral tegmental area (VTA)/substantia nigra (SN), thalamus/hypothalamus and brainstem; and of norepinephrine in VTA/SN (Vialou et al. 2008). Methamphetamine and the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+), two dopamine-releasing agents, induce larger, longer lasting increases in striatal dopamine concentrations in OCT3-deficient mice than in wild-type mice (Cui et al. 2009), suggesting a role for

OCT3 in dopamine clearance in this area. In the only study to examine monoamine transport in PMAT-deficient mice, choroid plexus tissue from PMAT-deficient mice accumulated significantly less dopamine than tissue isolated from wild-type mice (Duan and Wang 2013). Together, these studies indicate that the clearance of extracellular catecholamines in the central nervous system is mediated by both $uptake_1$ and $uptake_2$ transporters.

A large number of studies have examined the effects of $uptake_1$ and $uptake_2$ inhibitors, alone and in combination, on extracellular catecholamine concentrations using microdialysis or electrochemical techniques. Several of these studies have examined potential joint contributions of OCT3 and DAT to dopamine clearance in the striatum. Graf and colleagues showed that systemic injection of a low dose of cocaine (2.5 mg/kg), administered alone, had no effect on extracellular dopamine concentrations. However, when preceded by an injection of corticosterone, this same dose of cocaine caused significant increases in extracellular dopamine (Graf et al. 2013). The dose of corticosterone administered in these studies (2.5 mg/kg) results in plasma corticosterone concentrations that mimic those induced by exposure to stress (Graf et al. 2013). Microdialysis studies have reported that the concentration of corticosterone in brain tissue during stress reaches approximately 100 nM (Droste et al. 2008, 2009; Qian et al. 2012), a concentration previously shown to inhibit OCT3-mediated transport (Gasser et al. 2006). Thus, the studies of Graf and colleagues implicate OCT3 in the regulation of dopaminergic transmission in this region. However, it is likely that OCT2 and PMAT, both of which are expressed in the striatum, also contribute to dopamine clearance in this area. In other microdialysis studies, direct administration of D-22 into the striatum of nigrostriatal lesioned rats markedly enhanced L-DOPA-induced increases in extracellular dopamine concentrations (Sader-Mazbar et al. 2013). Although the results of this study are consistent with a role for $uptake_2$ in striatal dopamine clearance, the high concentration of D-22 administered (50 μM) means that other, non-transport mechanisms, including DA D2 receptor antagonism (Lloyd et al. 2019), may contribute to the observed increases in dopamine.

Fast-scan cyclic voltammetry (FSCV) and high-speed chronoamperometry are electrochemical techniques that can monitor catecholamine concentrations on a sub-second timescale, thus allowing quantification of clearance rates. Studies using FSCV in the rat striatum have provided strong evidence that OCT3, as well as DAT, mediates dopamine clearance in this region. These studies examined the effects of the DAT inhibitor GBR12909, in the presence or absence of corticosterone, on the clearance of electrically induced increases in dopamine in the nucleus accumbens of anesthetized rats (Graf et al. 2013). Systemic injection of GBR12909 alone significantly decreased NAc dopamine clearance (increases in full width at half height (FWHH), apparent K_m, and tau, all indicating decrease clearance). Once the effects of DAT blockade had stabilized, injection of corticosterone, but not vehicle, further decreased dopamine clearance (further increases in FWHH, apparent K_m, and tau) (Graf et al. 2013). These studies revealed for the first time in an in vivo setting, the presence of corticosterone-sensitive, DAT-independent clearance of dopamine. Subsequent studies used FSCV to examine the effects of corticosterone and cocaine

on NAc dopamine clearance in awake and behaving animals. In these studies, injection of corticosterone alone significantly increased the duration and magnitude of spontaneous dopamine transients, indicating that, even in the NAc, where DAT is very densely expressed, corticosterone-induced inhibition of DA clearance can be observed in the absence of DAT blockade. Further, in these studies, administration of low-dose cocaine alone had no effect on the amplitude, and only slightly increased the duration, of dopamine transients. However, when administered after corticosterone, this dose of cocaine robustly increased both amplitude and duration of dopamine transients (Wheeler et al. 2017). These studies clearly demonstrate that both uptake$_1$ (DAT-mediated) and uptake$_2$ (likely OCT3-mediated) processes work to shape catecholamine signals in vivo.

The relative contributions of uptake$_1$ and uptake$_2$ to catecholamine clearance likely vary regionally, resulting from region-specific patterns of transporter expression and catecholamine release. Holleran et al. (2020) used FSCV in slices of basolateral amygdala and nucleus accumbens to describe region-specific expression of DAT and OCT3, and regional differences in the contributions of uptake$_1$ and uptake$_2$ transporters to catecholamine clearance, in the nucleus accumbens (NAc) and basolateral amygdala (BLA) (Holleran et al. 2020). While OCT3 was expressed at similar levels in both regions, DAT expression was much greater in the NAc than in the BLA. Consistent with this expression pattern, the uptake$_2$ inhibitor corticosterone inhibited a greater proportion of catecholamine clearance in the BLA, while the DAT/uptake$_1$ inhibitor cocaine inhibited clearance in the NAc. In the hippocampus, where both uptake1 and uptake2 transporters are expressed, locally administered D-22 (at very low doses) enhanced desipramine-induced increases in norepinephrine concentrations measured by high-speed chronoamperometry, suggesting that regulation of catecholamine homeostasis in this region is also mediated by both classes of transporter (Bowman et al. 2020).

5 Subcellular Localization of Uptake$_2$ Transporters

The subcellular localization of a transporter, particularly its spatial relationship to receptors and transmitter release sites, is a critical determinant of the degree to which it regulates neurotransmission. While no studies have directly examined the co-localization of any uptake$_2$ transporter with catecholamine receptors or its proximity to catecholamine release sites, immuno-electron microscopy studies have demonstrated that OCT3 is positioned to exert significant influence over the temporal and physical spread of released monoamines (Gasser et al. 2016). In these studies, OCT3 was localized to plasma membranes of dendritic spines in amygdala, in some cases adjacent to axonal processes consistent with the morphology of catecholamine release sites. OCT3 was also observed in plasma membranes of neuronal somata, axonal profiles, and astrocyte processes surrounding axodendritic and axospinous profiles. Positioned at these sites, OCT3-mediated transport may be an important determinant of the degree to which released catecholamines activate receptors on pre- and post-synaptic cells.

Interestingly, OCT3 was also observed in a variety of endomembranes in both neurons and glia, including outer nuclear membranes, Golgi, mitochondrial, and vesicular membranes (Gasser et al. 2016). The localization of OCT3 to outer nuclear membranes is consistent with studies demonstrating nuclear localization of adrenergic receptors in cardiac myocytes (Boivin et al. 2006; Wu et al. 2014). Vesicular and Golgi localization are consistent with recent studies in non-neuronal cells demonstrating OCT3-mediated transport is required for activation of adrenergic receptors localized to these endomembranes (Irannejad et al. 2017). In the only other electron microscopic study of an uptake2 transporter, OCT2 was also observed densely localized to synaptic vesicles in spinal cord cholinergic neurons (Nakata et al. 2013). Mitochondrial localization raises the possibility that OCT3 may mediate access of catecholamines to mitochondrial monoamine oxidase for metabolism, though this hypothesis has not been tested.

6 Summary

By regulating the duration, physical spread, peak concentrations, and intracellular disposition of released catecholamines, transport processes determine the extent to which catecholamine receptors are activated and, thus, the extent to which catecholamines exert neuromodulatory influences over surrounding synapses. While clearance of released catecholamines was once believed to be solely mediated by the high-affinity, low-capacity dopamine and norepinephrine transporters (DAT and NET), the studies reviewed here have demonstrated that OCT2, OCT3, and PMAT are expressed throughout the brain in a variety of cell types and have begun to reveal how these transporters act, alone and in concert with NET and DAT, to influence catecholaminergic neurotransmission.

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The Interaction of Organic Cation Transporters 1-3 and PMAT with Psychoactive Substances

Julian Maier, Marco Niello, Deborah Rudin, Lynette C. Daws, and Harald H. Sitte

Contents

Abstract

Organic cation transporters 1-3 (OCT1-3, SLC22A1-3) and the plasma membrane monoamine transporter (PMAT, SLC29A4) play a major role in maintaining monoaminergic equilibrium in the central nervous system. With many psychoactive substances interacting with OCT1-3 and PMAT, a growing literature focuses on characterizing their properties via in vitro and in vivo studies. In vitro studies mainly aim at characterizing compounds as inhibitors or substrates of murine, rat, and human isoforms. The preponderance of studies

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has put emphasis on phenylalkylamine derivatives, but ketamine and opioids have also been investigated. Studies employing in vivo (knockout) models mostly concentrate on the interaction of psychoactive substances and OCT3, with an emphasis on stress and addiction, pharmacokinetics, and sensitization to psychoactive drugs. The results highlight the importance of OCT3 in the mechanism of action of psychoactive compounds. Concerning in vivo studies, a veritable research gap concerning OCT1, 2, and PMAT exists. This review provides an overview and summary of research conducted in this field of research.

Keywords

Amphetamine · Ketamine · New psychoactive substances · Opioids · Stress-addiction axis

1 Introduction

Organic cation transporters 1-3 (OCT1-3, SLC22A1-3, OCTs) and the plasma membrane monoamine transporter (PMAT, SLC29A4), historically also referred to as "uptake-2" transporters, are expressed in both peripheral organs and the central nervous system (CNS) (Koepsell 2020). OCT 2 and 3, as well as PMAT have been observed in pre- and post-synaptic neurons and OCT3 was furthermore localized in glial cells (Courousse and Gautron 2015; Mayer et al. 2018; Amphoux et al. 2006; Vialou et al. 2007; Wang 2016). Studies concerning the expression of OCTs and PMAT in the blood–brain barrier have presented heterogeneous results, with many suggesting the presence of either all or some of the aforementioned transporters (Hosoya and Tachikawa 2011; Lin et al. 2010; Sekhar et al. 2017, 2019; Chaves et al. 2020). Thus, in addition to e.g., multi-antimicrobial extrusion protein (MATE), organic anion and ABC transporters, they likely influence the passage of exogenous and endogenous compounds through the blood–brain barrier (ibid.). In addition, OCTs, widely expressed in intestines, liver, and kidney, play a significant role in the absorption, clearance, and excretion of xenobiotics (Koepsell 2013; Jonker et al. 2003).

OCT1-3 and PMAT are low-affinity and high-capacity transporters of monoamines. In conjunction with the high affinity, low capacity SLC6 neurotransmitter-sodium symporters (norepinephrine transporter – NET, SLC6A2; dopamine transporter – DAT, SLC6A3; serotonin transporter – SERT, SLC6A4), they play a major role in maintaining monoaminergic equilibrium in the CNS (Gasser 2019; Koepsell 2020). It is therefore easily discernible why investigations concerning the role of OCTs and PMAT in the CNS have also explored their interactions with compounds acting on the central nervous system, including psychoactive substances (Sitte and Freissmuth 2015). This review provides an overview and summary of research conducted to further this, still relatively young, field of research.

2 In Vitro Studies

Many studies investigating the interaction between OCT1-3, PMAT, and psychoactive substances make use of radiotracer-based in vitro assays of cells overexpressing the transporter of interest. While offering valuable pharmacodynamic information, interpretations arising from comparisons of in vitro results stemming from different research groups have to be made with caution due to the fact that differences in cell line, transfection method, and assay operating procedure can potentially lead to strikingly divergent results (Ilic et al. 2020). In addition, different isoforms (human, mouse, rat) of OCT1-3 have been investigated in vitro. Many studies reported marked differences in compound affinities among species which might, in part, be explained by differences in amino acid sequence homology (see Fig. 1). For PMAT, rat and mouse isoforms share 87.31% sequence homology, human and mouse 86.14%, and human and rat 80.23% (see also (Shirasaka et al. 2017)).

The lion's share of in vitro studies characterizing the interaction between psychoactive substances and OCTs and PMAT focused on phenylalkylamine derivatives (such as amphetamines) and cocaine.

Amphoux and colleagues investigated the interaction of the psychostimulants *d*-amphetamine, MDMA, and cocaine with rat and human OCT1-3 (Amphoux et al. 2006) (see Table 1). Radiotracer-based uptake inhibition assays revealed the compounds (except for cocaine at rOCT3) to inhibit rOCT1-3 in the low micromolar range. Furthermore, MDMA potently inhibits hOCT1 and 2 whereas *d*-amphetamine only interacts with hOCT2 (Amphoux et al. 2006). The human isoform of OCT3 is not inhibited by d-amphetamine or cocaine at lower concentrations (Mayer et al. 2018). Wu et al. have also found *d*-amphetamine to be a relatively potent inhibitor of rOCT3 but not hOCT3 (Wu et al. 1998). Wagner et al. (2017) showed that methamphetamine and its metabolites amphetamine and *para*-hydroxymethamphetamine inhibit hOCT1 and 2. Only *para*-hydroxymethamphetamine was shown to inhibit hOCT3. Additionally, amphetamine was unveiled to be a substrate of hOCT2, as was shown to be the case for methamphetamine at hOCT1 and 2 (Wagner et al. 2017).

Mayer and colleagues demonstrated that pretreatment with decynium-22 (D22, a potent inhibitor of OCT1-3 and PMAT with IC_{50} values ranging from 0.09 to 10 μM

Sequence homology (%)	hOCT1	hOCT2	hOCT3	mOCT1	mOCT2	mOCT3	rOCT1	rOCT2	rOCT3
hOCT1		69.55	47.88	77.34	69.55	46.33	77.52	67.39	46.24
hOCT2			48.32	68.4	82.73	47.23	67.68	81.84	47.59
hOCT3				46.99	48.93	85.64	46.99	48.15	85.46
mOCT1					69.6	45.7	95.35	67.81	46.06
mOCT2						47.66	68.17	92.43	47.84
mOCT3							45.34	47.13	98.73
rOCT1								67.99	45.7
rOCT2									47.13
rOCT3									

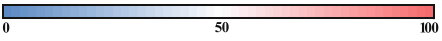

Fig. 1 Heat map portraying the degrees of amino acid sequence homology (given as %, extracted from UniProt alignment) between the human, mouse, and rat isoforms of OCT1-3

Table 1 Comparison of the pharmacodynamic properties of psychoactive substances characterized by their interaction with OCTs and PMAT

Compound	Main pharmacodynamic mechanism	hOCT1 (IC_{50} in μM)	hOCT2 (IC_{50} in μM)	hOCT3 (IC_{50} in μM)	hPMAT (IC_{50} in μM)
d-amphetamine (Amphoux et al. 2006; Zhu et al. 2010; Wagner et al. 2017; Wu et al. 1998)	Releasing agent at hDAT and hNET	202 ± 68 (rOCT1: 3.9 ± 0.1) 96.7 ± 37	10.5 ± 2.6 (rOCT2: 4.7 ± 2.6) 20:3 ± 16:9 (substrate: EC_{50}: 0.72 ± 0.29)	460 ± 140 (rOCT3: 4.5 ± 1.0) 363 ± 56:4 41.5 ± 7.5 (dopamine-HPLC) 24.1 ± 7.0 (serotonin-HPLC) (rOCT3: 42 ± 7)	–
Methamphetamine (Wagner et al. 2017; Wu et al. 1998)	Releasing agent at hDAT and hNET	21.1 ± 8.8 (substrate: EC_{50}: 5.29 ± 0.66)	15.0 ± 6.8 (substrate: EC_{50}: 1.21 ± 0.19)	300 ± 139 (rOCT3: 247 ± 26)	–
Para-hydroxymethamphetamine (Wagner et al. 2017)	Metabolite of methamphetamine	12.0 ± 3.4	83.8 ± 22.3	44.4 ± 25.5	–
MDMA (Amphoux et al. 2006)	Releasing agent at hDAT, hNET, and hSERT	24.2 ± 9.2 (rOCT1: 0.82 ± 0.1)	1.6 ± 0.6 (rOCT2: 2.8 ± 1.1)	73.6 ± 50.3 (rOCT3: 1.6 ± 0.3)	–
Cocaine (Amphoux et al. 2006)	Inhibitor of hDAT, hNET, and hSERT	85 ± 22 (rOCT1: 13.3 ± 1.4)	113 ± 21 (rOCT2: 23.8 ± 6.5) 277 (Koepsell et al. 2003)	>1,000 (rOCT3: >1,000)	–
Phencyclidine (PCP) (Amphoux et al. 2006)	NMDA receptor antagonist	4.4 ± 1.4 (rOCT1: 0.16 ± 0.03)	24.9 ± 5.6 (rOCT2: 16.1 ± 2.4)	333 ± 60 (rOCT3: 3 ± 0.2)	–
Dizocilpine (MK-801) (Amphoux et al. 2006)	NMDA receptor antagonist	80.5 ± 48.7 (rOCT1: 5.6 ± 1.7)	21.5 ± 8.5 (rOCT2: >1,000)	224 ± 74 (rOCT3: >1,000)	–

Ketamine (Amphoux et al. 2006; Massmann et al. 2014; Keiser et al. 2018)	NMDA receptor antagonist	114.5 ± 43.7 (rOCT1: 4.7 ± 0.1) K_m: 73.9 ± 15.2 μmol/l (pH = 6.5, intestinal lumen)	22.7 ± 6.3 (rOCT2: 207 ± 39.5) K_m: 33.5 ± 20.3 μmol/l (pH = 6.5)	225.7 ± 65 400 (rOCT3: 65.7 ± 38.7) (mOCT3: 200) K_m: 52.9 ± 15.0 μmol/l (pH = 6.5) K_m: 365 ± 125 μmol/l (pH = 7.4)	–
Morphine (Tzvetkov et al. 2013)	Opioid receptor agonist	4.2 ± 0.6 K_m: 3.4 ± 0.3 μM	–	538 ± 76	–
Codeine (Tzvetkov et al. 2013)	Opioid receptor agonist	10.5 ± 1.3			
Pentedrone(Castells et al. 2020)	Inhibitor of hDAT and hNET	–	26.95 (24.0-29.9)	>1,000	–
N-ethyl-pentedrone (Castells et al. 2020)	Inhibitor of hDAT and hNET	–	57.58 (46.97-68.19)	>1,000	–
N,N-diethyl-pentedrone (Castells et al. 2020)	Inhibitor of hDAT and hNET	–	47.81 (46.75-48.87)	>1,000	–
α-PVP (Castells et al. 2020)	Inhibitor of hDAT and hNET	1.31 (1.06-1.64)	17.49 (13.50-21.48) 5.62 (3.89-7.92)	>1,000 1,631 (1,209–2,286)	13.42 (9.76-18.70)
α-PpVP (Castells et al. 2020)	Inhibitor of hDAT and hNET	–	12.88 (12.15-13.61)	>1,000	–
α-PPP (Maier et al. 2021)	Inhibitor of hDAT and partial releaser at hNET	1.30 (1.10-1.55)	7.11 (5.47-9.23)	1,050 (854-1,311)	75.48 (61.43-93.10)

(continued)

Table 1 (continued)

Compound	Main pharmacodynamic mechanism	hOCT1 (IC_{50} in μM)	hOCT2 (IC_{50} in μM)	hOCT3 (IC_{50} in μM)	hPMAT (IC_{50} in μM)
MDPPP (Maier et al. 2021)	Inhibitor of hDAT and partial releaser at hNET	0.96 (0.77-1.13)	5.57 (4.48-6.92)	326 (261-410)	21.86 (17.21-28.18)
3-Br PPP (Maier et al. 2021)	Inhibitor of hDAT and partial releaser at hNET	0.63 (0.49-0.81)	6.51 (5.54-7.64)	332 (265-419)	60.62 (45.47-81.51)
4-Br PPP (Maier et al. 2021)	Inhibitor of hDAT and hNET	0.57 (0.46-0.71)	7.41 (5.54-9.83)	149 (120-183)	17.53 (12.39-28.53)
4-Me PPP (Maier et al. 2021)	Inhibitor of hDAT and hNET	0.91 (0.66-1.27)	8.12 (6.19-10.60)	433 (278-496)	36.89 (27.17-51.00)
Diazepam (Massmann et al. 2014)	Allosteric $GABA_A$ receptor modulator	–	–	2 (mOCT3:40)	–

IC_{50} Half maximal inhibitory concentration, *EC_{50}* Half maximal effective concentration, K_m Michaelis-Menten constant. All values are given in μM

(Koepsell et al. 2007; Fraser-Spears et al. 2019; Shirasaka et al. 2017)) dose-dependently potentiated the ability of cocaine to decrease amphetamine-mediated ^{3}H-MPP^{+} release in rat superior cervical ganglia cells (Mayer et al. 2018). In HEK293 cells expressing hOCT3 and hVMAT2 it was shown in a microperfusion assay that amphetamine-induced MPP^{+} release from hVMAT2 might lead to efflux via hOCT3. This raises the question, how was amphetamine translocated intracellularly? Possibilities include diffusion through the lipid bilayer or transport by an undetected transporter endogenously expressed in HEK293 cells.

Duart-Castells et al. demonstrated that five cathinones (pentedrone and α-PVP, i.e. α-pyrrolidinovalerophenone, derivatives) relatively potently inhibit hOCT2 while not interacting with hOCT3 at pharmacologically relevant concentrations (Castells et al. 2020). Recently, Maier et al. (2021) investigated the effects of α-PVP, α-PPP (α-pyrrolidinopropiophenone) and four derivatives on hOCT1-3 and PMAT. All compounds potently inhibited hOCT1 and 2, as well as relatively potently interacted with hPMAT. hOCT3 was not inhibited at pharmacologically relevant concentrations (with possibly the exception of 4-Br PPP). In HEK293 cells overexpressing mOCT3, Mayer et al. showed that 3,4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (mephedrone, 4-MMC) did not inhibit the transporter at pharmacological concentrations (Mayer et al. 2019). Mephedrone caused ^{3}H-MPP^{+} release in rat superior cervical ganglia cells that could be attenuated by administration of MDPV and D22, in a dose-dependent manner, suggesting MPP^{+} efflux is mediated by both SLC6 neurotransmitter-sodium-symporters and OCT3.

An analysis of frequently abused NMDA receptor antagonists (ketamine, phencyclidine, and dizocilpine) revealed the drugs to be potent inhibitors of rOCT1 (Amphoux et al. 2006). Concerning the human OCTs, hOCT3 is only weakly inhibited by any of the compounds, which interact more with hOCT2 than hOCT1. Similarly, Massmann and colleagues compared mouse and human OCT3 and found that while ketamine more potently inhibits the murine isoform, it is the reverse with diazepam (Massmann et al. 2014). Recently, ketamine was shown to be a substrate of hOCT1-3 at intestinal pH levels (pH = 6.5) and of hOCT3 at a pH of 7.4 (Keiser et al. 2018). In another study, morphine was shown to be a substrate of hOCT1 but not hOCT3 (Tzvetkov et al. 2013).

Ho and colleagues investigated phenylalkylamine derivatives and concluded that increasing distance between the aromatic ring and the nitrogen atom led to an increase in potency to inhibit hPMAT (Ho et al. 2011). This was corroborated by Maier et al. (2021) for α-PPP and α-PVP, where an increase in carbon side chain length resulted in a higher inhibitory potency. While some studies have, on a small scale, investigated the structure–activity relationships (SAR) of psychoactive substances with the OCTs and PMAT, large-scale SAR analyses with psychoactive substances are missing thus far (Ho et al. 2011). Some groups have screened libraries of cationic medical drugs at OCT1-3 and PMAT, in part employing computational methods (e.g., machine learning, structural modelling), which might also serve as potential inspiration for the field of research on psychoactive substances (Kido et al. 2011; Liu et al. 2016; Sala-Rabanal et al. 2013).

Converging lines of evidence are currently highlighting the heterogeneity in pharmacology for neurotransmitter transporters, with several inhibitors targeting allosteric sites or showing atypical mechanisms (Niello et al. 2020; Reith et al. 2015). In the case of OCT2 and PMAT, only a few studies have tried to understand the pharmacological type of inhibition exerted by different drugs. A recent study suggested that rOCT1 contains three MPP+ binding sites, where two of them act as low-affinity binding sites involved in transport and one high-affinity binding site that does not participate in transport but acts allosterically on the other two sites (Keller et al. 2019). Another study attempted to characterize the pharmacological properties of different hOCT2 inhibitors. Results suggest that corticosterone inhibits ^{14}C-TEA uptake with non-competitive kinetics, while diazepam, lidocaine, malathion, and triazolam instead showed mixed kinetics of inhibition (Chiba et al. 2013). Further studies will be required to confirm and expand these findings.

It becomes apparent that the degree of amino acid sequence homology between transporters plays a major role concerning the similarity of their interaction with compounds. The more homologous OCT1 and 2 have a higher degree of pharmacodynamic overlap than the less homologous OCT3. Most investigated psychoactive compounds more potently interact with hOCT1 and 2 than hOCT3. Concerning interspecies translatability, it becomes apparent that comparisons between mouse and rat isoforms (due to the large degree of sequence homology, i.e. >92.00%, see Fig. 1) can be more readily drawn than comparisons between rat or murine and human OCTs (homology between 77-85%). As an example, rat and murine isoforms of OCT3 are more potently inhibited by phenylalkylamine derivatives than human OCT3 (see Table 1).

3 In Vivo Studies

3.1 Pharmacokinetics of Drug Disposition

In vivo studies in mice and rats have so far mostly focused on the role of OCT3 (or lack thereof in knockout (−/−) animal models) in the CNS and its interaction with methamphetamine, amphetamine, and cocaine. These compounds have been shown to disrupt function of the blood–brain barrier, leading to increased permeability and, possibly, their subsequent accumulation in the CNS (Kousik et al. 2012). A pharmacokinetic study in mice investigated tissue distribution of *d*-amphetamine, methamphetamine and its metabolite *para*-hydroxymethamphetamine (Wagner et al. 2018). Concentrations of compounds found in brain tended to be greater in the wild-type OCT3+/+ mice than in their OCT3−/− counterparts. Zhu et al., on the other hand, found no differences in tissue distribution of *d*-amphetamine between OCT3+/+ and OCT3−/− mice (Zhu et al. 2010). However, in comparison to OCT3+/+, OCT3−/− mice had lower concentrations of *para*-hydroxymethamphetamine in muscle and salivary glands, confirming the OCT3 substrate status of the methamphetamine metabolite (Wagner et al. 2017, 2018).

3.2 Neurotoxicity

The importance of OCTs for high-capacity monoamine uptake and stabilization of monoaminergic equilibrium becomes apparent in a study conducted by Cui and colleagues (Cui et al. 2009). OCT3+/+ and OCT3−/− mice were treated with increasing doses of methamphetamine and microdialysis was conducted in striatum to quantify resulting increases in extracellular dopamine. DAT-mediated release of striatal dopamine elicited by methamphetamine was increased in OCT3−/− mice, relative to OCT3+/+ mice. Consequently, loss of striatal dopaminergic terminals, due to extracellular dopamine toxicity, was markedly increased in OCT3−/− mice. This study was the first to show a crucial role of OCT3 as a modulator of neurotoxicity in the nigrostriatal dopamine pathway.

3.3 Stress-Addiction Axis

Several groups have explored the role OCT3 plays in the stress-hormone (i.e., corticosterone)-addiction axis. Corticosterone is, on the one hand, a potent inhibitor of OCTs and, on the other, the major stress hormone in rodents (comparable to cortisol in humans). In stressed animals increased levels of corticosterone subsequently inhibit the OCTs (and most potently OCT3) which, in turn, leads to an extracellular accumulation of monoamines in the CNS. Repeated stress has been reported to decrease OCT3 expression in mice, consistent with prolonged blockade of OCT3 by corticosterone (Baganz et al. 2010). Interestingly, methamphetamine injections also increase corticosterone levels in rats, inhibiting OCTs further (Fujimoto et al. 2007). Kitaichi et al. (2003) and Fujimoto et al. (2007) found that repeated methamphetamine exposure (5 mg/kg i.p. on five consecutive days) led to decreased expression of OCT3 in the brain, lung, and kidney of different rat strains. Both phenomena cause increased extracellular monoamine concentrations, resulting in increased rewarding sensations. The described circuit is reminiscent of a vicious circle, a self-amplifying (positive feedback) loop, typical of addiction. The role for OCT3 in the rewarding effects of amphetamine (2 mg/kg i.p.) was investigated in a modified conditioned place preference (CPP) paradigm by Vialou et al. (2008). They found no difference in CPP for amphetamine between OCT3+/+ and OCT3−/− mice. In a recent study by Clauss and colleagues (2021), the sex dependency of CPP for amphetamine (1 mg/kg i.p.) was investigated. No difference in CPP between female OCT3+/+ and OCT3−/− mice was apparent, whereas in males, OCT3+/+ mice developed CPP for amphetamine, while OCT3−/− mice did not. Consistent with this premise, pretreatment with the OCT blocker, D22 (0.1 mg/kg, i.p.), abolished CPP for amphetamine in OCT3+/+ male mice.

In line with this concept, investigations by Graf and colleagues found corticosterone (and normetanephrine, a metabolite of norepinephrine and a potent OCT3 inhibitor) to potentiate cocaine-induced increases in extracellular dopamine in the nucleus accumbens of rats (Graf et al. 2013). Similarly, Wheeler et al. demonstrated that systemic corticosterone injections (2.0 mg/kg i.p.), coupled with cocaine at a

low dosage (2.5 mg/kg i.p.), led to a decrease in dopamine clearance in the nucleus accumbens (Wheeler et al. 2017). Interestingly, corticosterone – alone and in conjunction with amphetamine – induced an increase in extracellular serotonin in the ventral hippocampus of rats that could be antagonized by glucocorticoid receptor antagonist mifepristone (Barr and Forster 2011). In another study, it was shown that corticosterone treatment inhibits clearance of catecholamines more pronouncedly in areas with low DAT expression relative to OCT3 (e.g., basolateral amygdala) (Holleran et al. 2020). Furthermore, in OCT3+/+ mice, but not in OCT3−/− mice, corticosterone treatment (systemically and locally in the nucleus accumbens) led to reinstatement of extinguished cocaine CPP after reintroduction of a low dose of cocaine (2.5 mg/kg i.p.) (Graf et al. 2013). These results help to solidify the role OCT3 plays in monoaminergic equilibrium in the CNS of rodents (McReynolds et al. 2017; Gasser 2019).

While consumption of psychoactive substances, as mentioned above, causes inhibition of OCT3 via an increase in corticosterone levels and leads to decreased expression of OCT3, the opposite phenomena were reported for drug withdrawal. Withdrawal from chronic amphetamine treatment in rats was accompanied with increased expression of OCT3 in ventral hippocampus, resulting in increased clearance of extracellular serotonin. This was correlated with increased anxiety states in the first 24 h post-withdrawal (Barr et al. 2013). In a follow-up study, Solanki and colleagues found that during the first 24 h after withdrawal, rOCT3 expression is upregulated not only in the ventral hippocampus (together with an increase in rSERT expression) but also in the central nucleus of the amygdala and in the dorsomedial hypothalamus (Solanki et al. 2016). Two weeks post-withdrawal, expression levels of rOCT3 and rSERT remained elevated in the central nucleus.

Consistent with findings that increased OCT3 expression is associated with decreased extracellular monoamine concentrations and increased anxiety, Wultsch et al. found mice constitutively lacking OCT3 to have decreased levels of anxiety (Wultsch et al. 2009). Vialou and colleagues found OCT3−/− mice to show subtle increases in anxiety (Vialou et al. 2008). While the reason for this discrepancy remains unclear, use of different tests for anxiety and/or assay conditions likely play a role.

3.4 Sensitivity to Psychoactive Substances

In addition to investigations of the role of OCT3 in the stress-addiction axis, a growing number of studies have probed the dependency of the action of psychoactive substances on OCTs. Kitaichi, Nakayama, and colleagues knocked down (~25%) OCT3 in rats using antisense oligonucleotides and found that methamphetamine exposure (1 mg/kg s.c.) led to significant increase in extracellular dopamine levels in the nucleus accumbens, as well as increased locomotor activity, i.e. hyperlocomotion, compared to controls (Kitaichi et al. 2005; Nakayama et al. 2007). In contrast, Vialou and colleagues only noticed increased hyperlocomotor response in OCT3−/− mice, relative to OCT3+/+ mice, following high doses of

amphetamine (10 mg/kg) and cocaine (40 mg/kg) (Vialou et al. 2008). Mayer and colleagues found no difference between OCT+/+ and OCT3−/− mice in locomotor response caused by amphetamine administration (concentrations up to and including 10 mg/kg i.p.) (Mayer et al. 2018). Pretreatment with D22 (0.1 mg/kg i.p.) robustly attenuated the locomotor response to amphetamine in OCT3+/+ mice but was without effect in OCT3−/− mice (Mayer et al. 2018). Additionally, Mayer and colleagues observed that D22 potentiated the ability of the DAT inhibitor cocaine to inhibit amphetamine-induced dopamine efflux in striatum of OCT3+/+ mice but not in OCT3−/− mice. Each of these studies provides support for a role of OCT3 in the acute actions of amphetamine and methamphetamine to increase locomotor activity and extracellular dopamine, with contrasting findings likely accounted for by the different means used to decrease OCT3 expression and species used.

Concerning sensitization to locomotor stimulant effects of amphetamine (2 mg/kg), Vialou et al. (2008) found no difference between OCT3+/+ and OCT3−/− mice. Clauss et al. (2021) could confirm no differences for male mice, whereas female mice, consistent with literature showing females to be more sensitive to the effects of psychostimulants, did develop sensitization to amphetamine (1 mg/kg). Strikingly, the magnitude of sensitization was dramatically less in female OCT3−/− mice than in OCT3+/+ mice, suggesting that OCT3 is important for sensitization to the locomotor stimulant effects of amphetamine.

Thus, there is evidence for a role of OCT3 in the actions of amphetamine. However, additional studies are needed to fully understand the underlying mechanisms and nuanced aspects. To date there have been no in vivo studies examining potential roles of OCT1, OCT2, or PMAT in the actions of psychostimulants.

3.5 Polymorphisms

Many studies have investigated polymorphisms and mutations of OCTs with respect to various diseases and their effect on the transport of pharmaceutical drugs (such as metformin) (Mofo Mato et al. 2018; Chen et al. 2010; Zhang et al. 2006). However, very little is known about the effects of OCT polymorphisms on addiction (Koepsell 2020). A genetic analysis of a small Japanese cohort of patients addicted to methamphetamine revealed no association between drug dependence and hOCT3 polymorphisms, although significant differences were found between methamphetamine and polysubstance users (Aoyama et al. 2006).

4 Conclusions

Most in vitro studies on substances' interaction with OCTs and PMAT focus either on pharmaceutical drugs or "classical" psychoactive substances. More recent studies have begun investigating new psychoactive substances, i.e. uncontrolled drugs of abuse, which have become increasingly prevalent in the last decades (Maier et al.

2018). A growing literature reports phenylalkylamine derivatives to be potent inhibitors of rodent OCTs and human OCT1 and 2 but not hOCT3. Hence, a different chemical scaffold might be better suited for explorations of compounds potently interacting with OCT3. Large-scale structure–activity relationship studies are needed to further our understanding of transporter-psychoactive compound interactions. Furthermore, the type of inhibition exerted by compounds on OCTs and PMAT will require a more thorough exploration: non-competitive pharmacology would offer several advantages over classical inhibitors such as increased specificity and reduced off-target effects (Niello et al. 2020).

In vivo studies have provided evidence for a role of OCT3 in addiction and stress and shown its importance in maintaining monoaminergic equilibrium in the CNS. Due to interspecies differences in (1) the degree of amino acid sequence homology of the OCTs and (2) the hormonal environment (e.g., the main stress hormone of rodents is corticosterone, which potently inhibits OCTs, whereas the human equivalent is cortisol), the translational value of the stress-related results is difficult to appraise. Still, it is evident that the conducted studies offer one piece of the puzzle in explaining psychostimulant addiction, withdrawal difficulties, and drug side effects. Future studies might possibly explore the effects of polymorphisms and mutations of OCTs and PMAT found in human cohorts on monoaminergic equilibrium and the transporters' interaction with psychostimulants. With respect to psychoactive substances and the current focus on OCT3, the organic cation transporters 1 and 2, as well as PMAT, constitute especially veritable desiderata in research.

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Organic Cation Transporters in Psychiatric Disorders

Lynette C. Daws

Contents

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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Organic Cation Transporters and Nongenomic Glucocorticoid Action

Kelsey C. Benton, Christopher A. Lowry, and Paul J. Gasser

Contents

Abstract

Corticosteroid hormones exert powerful influences on neuronal physiology and behavior by activating intracellular glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which act as ligand-gated transcription factors, altering gene expression. In addition to these genomic effects on physiology and behavior, which are usually delayed by minutes to hours, corticosteroid hormones also initiate rapid effects through diverse nongenomic mechanisms. One such mechanism involves the direct inhibition by corticosteroid hormones of monoamine transport mediated by the "uptake$_2$" transporter, organic cation transporter 3 (OCT3), a high-capacity, low-affinity transporter for norepinephrine, epinephrine, dopamine, serotonin, and histamine. In this review we describe studies that

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demonstrate OCT3 expression and corticosterone-sensitive monoamine transport in the brain and present evidence supporting the hypothesis that corticosterone exerts rapid, nongenomic actions on glia and neurons, ultimately modulating physiology and behavior, by inhibiting OCT3-mediated monoamine clearance. We also describe the corticosteroid sensitivity of the other members of the uptake$_2$ family and examine their potential contributions to nongenomic effects of corticosteroids in the brain.

Keywords

Corticosterone · Dopamine · Histamine · Norepinephrine · OCT2 · OCT3 · Organic cation transporter 2 · Organic cation transporter 3 · Serotonin · slc22a3 · Solute carrier family 22 (organic cation transporter)

Abbreviations

CeA	Central nucleus of the amygdala
CNS	Central nervous system
CPP	Conditioned place preference
DAT	Dopamine transporter
DHEA	Dehydroepiandrosterone
DMH	Dorsomedial hypothalamus
FSCV	Fast-scan cyclic voltammetry
GR	Glucocorticoid receptor
MPP^+	1-Methyl-4-phenylpyridinium
MR	Mineralocorticoid receptor
NAc	Nucleus accumbens
NET	Norepinephrine transporter
OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
OCT3	Organic cation transporter 3, also known as slc22a3, solute carrier family 22 (organic cation transporter), serotonin
PMAT	Plasma membrane monoamine transporter
SERT	Serotonin transporter
Slc22a3	Solute carrier family 22 (organic cation transporter), serotonin, also known as organic cation transporter 3
VTA	Ventral tegmental area

1 Uptake$_2$: Corticosteroid-Sensitive Monoamine Transport

Early studies of norepinephrine uptake in cardiovascular tissue revealed two kinetically and pharmacologically distinct transport processes: uptake$_1$, a high-affinity, low-capacity uptake process observed under conditions of low substrate concentration and inhibited by cocaine and desipramine; and uptake$_2$, a high-capacity,

low-affinity process observed at high substrate concentrations, insensitive to cocaine but inhibited by corticosterone and other steroids (Iversen 1963, 1965a, b). Subsequent studies demonstrated that uptake$_1$ and uptake$_2$ processes could be detected at all substrate concentrations (Lightman and Iversen 1969) and that these two uptake processes are mediated by two families of transporters. Uptake$_1$ includes the sodium-dependent dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters; and uptake$_2$ includes the organic cation transporters (OCT1, 2, and 3) and the plasma membrane monoamine transporter (PMAT). Uptake$_2$ transporters are bidirectional, sodium independent, and vary in substrate selectivity for norepinephrine, epinephrine, dopamine, histamine, and serotonin (Gründemann et al. 1998a; Schömig et al. 2006; Duan and Wang 2010). All of the uptake$_2$ transporters have been identified in brain, and roles for these transporters in the uptake and disposition of extracellular monoamines in brain have been demonstrated. The corticosteroid sensitivity of these transporters suggests a mechanism by which circadian-, ultradian-, or stress-induced increases in corticosteroid concentrations may modulate monoaminergic neurotransmission. This review focuses on the sensitivity of uptake$_2$ transporters to inhibition by corticosteroid hormones and the evidence that this inhibition contributes to the physiological and behavioral actions of corticosterone.

2 Corticosteroid Sensitivity of Uptake$_2$ Transporters

Corticosteroid sensitivity is a defining feature of uptake$_2$ transporters. Early studies of norepinephrine uptake in cardiovascular tissue revealed that, in addition to corticosterone, uptake$_2$ was inhibited by aldosterone, cortisol, deoxycorticosterone, androsterone, and dehydroepiandrosterone (DHEA) (Fowler and Chou 1961; Besse and Bass 1966). Each of the uptake$_2$ family members is inhibited by corticosterone, though their sensitivities vary widely. In both human and rodent, OCT3 appears to be the most corticosteroid sensitive and is inhibited by concentrations of corticosterone within the physiological range (K_i = 0.1–0.2 μM) (Gründemann et al. 1998b; Wu et al. 1998; Arndt et al. 2001; Hayer-Zillgen et al. 2002). Corticosterone inhibition of OCT1 (IC_{50} = 150 μM) and PMAT (IC_{50} = 450 μM) requires concentrations well outside the physiological range (Engel et al. 2004), while the corticosterone sensitivity of OCT2 is intermediate (reported K_i = 0.5–4 μM, Gründemann et al. 1998a; Schömig et al. 2006; Duan and Wang 2010). Synthetic corticosteroids, widely used as treatments for inflammatory conditions, also inhibit uptake$_2$ transporters. In studies of cultured cerebellar granule neurons, which express OCT1, OCT3, and PMAT, both natural and synthetic corticosteroids, including dexamethasone, betamethasone, and prednisolone, inhibited the uptake of the OCT substrate 1-methyl-4-phenylpyridinium (MPP^+) (Hill et al. 2011). Given that OCT3, but not OCT1 or PMAT, is sensitive to corticosterone in a physiologically relevant range, these findings raise the intriguing hypothesis that blockade of OCT3-mediated monoamine clearance may contribute to the widely reported neuropsychiatric

symptoms of high-dose treatment with synthetic corticosteroids (Wolkowitz et al. 1997; Dubovsky et al. 2012).

Corticosterone is not a competitive inhibitor of OCT-mediated transport, nor is it a substrate of the transporters (Barendt and Wright 2002; Schmitt and Koepsell 2005; Schmitt et al. 2009). Instead, it acts as a "mixed type" inhibitor, substantially decreasing the maximal rate of transport (J_{max}) and modestly increasing the Michaelis constant (K_t) (Barendt and Wright 2002). Corticosterone rapidly inhibits OCT-mediated transport by a mechanism that appears to involve direct binding to the transporters at specific sites. Volk and colleagues identified three amino acid residues in the 10th transmembrane domains of OCT1 and 2 (Ala443, Leu447, and Gln448 in rOCT1 and Ile443, Tyr447, and Glu448 in rOCT2) that were critical for corticosteroid sensitivity and that determined the IC50 of corticosterone (Gorboulev et al. 2005). Replacing the three residues in OCT1 with the corresponding OCT2 residues increased the sensitivity of OCT1 to corticosterone. These residues, which are deep within a substrate-binding cavity in the transporter, are identical in OCT2 and 3 and appear to be involved in directly interacting with corticosteroids (Volk et al. 2009). The evidence that corticosterone interacts with residues in a transmembrane domain deep in the substrate-binding cleft is consistent with the amphipathic nature of the steroid and with observations from patch clamp studies indicating that corticosterone inhibits OCT2 when applied either extracellularly or intracellularly (Volk et al. 2003, 2009). This suggests that corticosterone could also inhibit OCTs localized to intracellular membranes, including nuclear, Golgi, and vesicular membranes, where OCT3 has been identified (Gasser et al. 2017).

3 Sensitivity of Uptake$_2$ Transporters to Other Steroids

While corticosteroids are most frequently used to inhibit uptake$_2$-mediated transport, the transport system is also sensitive to inhibition by sex steroids. In early studies, Iversen and Salt demonstrated that uptake$_2$ was inhibited by testosterone and 17-β-estradiol (Iversen and Salt 1970; Salt 1972). Studies in cultured cells exogenously expressing individual OCTs demonstrated that the three isoforms display distinct sensitivities to steroids and that there appear to be species differences in sensitivity to some steroids. 17-β-Estradiol and progesterone are potent inhibitors of human isoforms of OCT1 and OCT3 but not OCT2 (Hayer-Zillgen et al. 2002). In the rat, estradiol selectively inhibits OCT1 and OCT3, while progesterone selectively inhibits OCT1 and OCT2 (Wu et al. 1998). Studies in central nervous system tissues and cells have also demonstrated that uptake$_2$-mediated transport is inhibited by a wide array of steroids. In dorsomedial hypothalamic tissue punches, which express OCT3 but not OCT1 or OCT2 (PMAT was not measured), uptake of histamine was inhibited by estradiol ($IC_{50} = 1.26$ μM) as well as by corticosterone (Gasser et al. 2006). Taken together, these findings suggest that a number of circulating steroid hormones in addition to corticosteroids may regulate extracellular monoamine concentrations by inhibiting uptake$_2$-mediated transport in the central nervous system, thereby influencing monoaminergic neurotransmission, physiology,

and behavior. Because there are no inhibitors that exclusively target individual transporters, determining the contributions of individual uptake$_2$ transporters to monoamine clearance in a specific brain region is difficult and requires information about transporter expression as well as sensitivity to various inhibitors. The differential steroid sensitivity of OCTs may be used to determine the relative contributions of individual transporters to monoamine clearance in in vivo studies.

4 Expression of OCT2 and OCT3 in the Central Nervous System

It is increasingly evident that in addition to their roles in peripheral tissues, uptake$_2$ transporters contribute to the regulation of monoaminergic neurotransmission in the central nervous system. Brain expression of OCT2 (Gründemann et al. 1997; Amphoux et al. 2006; Bacq et al. 2012) and OCT3 (Wu et al. 1998; Gasser et al. 2006, 2009; Wheeler et al. 2017) has been well documented. OCT3 is expressed in both neuronal and glial cells (Russ et al. 1996; Vialou et al. 2004; Cui et al. 2009; Yoshikawa et al. 2013; Gasser et al. 2017), while OCT2 is expressed in neurons, but not astrocytes (Busch et al. 1998; Bacq et al. 2012; Yoshikawa et al. 2013). These transporters are expressed in a variety of brain regions and mounting evidence suggests they may significantly contribute to regulation of extracellular monoamines. For example, evidence indicates that monoamine uptake in cerebellar granule cells is mediated primarily by OCT3 (and PMAT), not by uptake$_1$ transporters (Shang et al. 2003; Hill et al. 2011).

5 Corticosteroid Regulation of Monoaminergic Neurotransmission and Behavior

While corticosteroid-sensitive, OCT-mediated, uptake of all of the major monoamines has been demonstrated in peripheral tissues, only a limited number of studies have examined corticosteroid-induced inhibition of monoamine uptake in the brain or the contribution of OCT inhibition to nongenomic behavioral actions of corticosteroids. Here, we review studies focused on OCT3 as these studies have focused primarily on OCT3.

5.1 Corticosteroid Regulation of Dopamine Clearance and Cocaine-Seeking Behavior

It is increasingly evident that the regulation of extracellular dopamine concentrations, long attributed primarily to DAT and, in some brain regions, NET, also involves uptake$_2$ transport mechanisms. Studies of dopamine clearance have focused primarily on the nucleus accumbens (NAc), which receives a dense dopaminergic projection from the ventral tegmental area (VTA). OCT3 is expressed in

neuronal and glial cells of the NAc (Gasser et al. 2009; Graf et al. 2013). Graf and colleagues examined the contributions of DAT and OCT3 to dopamine clearance by monitoring extracellular dopamine concentrations in the NAc of freely moving rats in response to treatment with cocaine, which blocks DAT-mediated dopamine clearance, and corticosterone. Using microdialysis, they demonstrated that a low dose of cocaine, insufficient by itself to increase extracellular dopamine concentrations, caused significant increases in NAc dopamine when preceded either by acute stress or by peripheral injection of stress levels of corticosterone (Graf et al. 2013). These studies also used fast-scan cyclic voltammetry (FSCV) to demonstrate that corticosterone potentiates the actions of cocaine by acutely inhibiting the clearance of extracellular dopamine in the NAc, leading to increases in the magnitude and duration of both naturally occurring dopamine transients and dopamine released in response to electrical stimulation (Graf et al. 2013; Wheeler et al. 2017). Importantly, the study by Wheeler and colleagues demonstrated that peripheral injection of corticosterone alone, in the absence of DAT blockade, results in significant decreases in dopamine clearance (Wheeler et al. 2017). A more recent study using the FSCV technique in ex vivo slices demonstrated roles for both uptake$_1$ and uptake$_2$ transporters in the clearance of catecholamines in NAc and basolateral amygdala (BLA). These studies showed that, while it was measurable in the NAc, OCT3-mediated catecholamine clearance was more prominent in the BLA, indicating the presence of regional differences in the relative contributions of the two transport systems (Holleran et al. 2020).

The demonstration that corticosterone acutely inhibits dopamine clearance in the NAc suggests the hypothesis that OCT3 inhibition may contribute to the effects of stress on cocaine-seeking behavior. Graf and colleagues tested this hypothesis by examining the effects of stress and corticosterone on the reinstatement of cocaine-seeking behavior in rats. In rats trained to self-administer cocaine, followed by extinction and reinstatement testing, neither acute stress alone nor injection with a low dose of cocaine (2.5 mg/kg) leads to significant reinstatement of drug-seeking behavior. However, low-dose cocaine does induce reinstatement when it is preceded by acute stress (Graf et al. 2013). Similarly, acute injection of stress levels of corticosterone, either intraperitoneally or directly into the NAc, does not by itself induce reinstatement but acutely potentiates low-dose cocaine-induced reinstatement. Importantly, the behavioral effects of peripheral corticosterone injection are not blocked by pretreatment with RU-38486, a glucocorticoid receptor antagonist, but are blocked by intra-NAc injection of the DA receptor antagonist fluphenazine (Graf et al. 2013). These studies provide strong evidence that inhibition of uptake$_2$-mediated dopamine clearance contributes to acute stress- and glucocorticoid-induced regulation of behavior. As these studies used doses of corticosterone that reproduce stress-induced concentrations of the steroid, and as OCT3 is expressed on medium spiny neurons and glial cells in the nucleus accumbens, it is likely that the behavioral actions of stress and glucocorticoids are mediated by inhibition of OCT3-mediated dopamine transport.

To confirm the role of OCT3 in corticosterone-induced potentiation of cocaine-primed reinstatement, McReynolds et al. (2017) examined the reinstatement of

cocaine conditioned place preference (CPP) in wild type mice and transgenic OCT3-deficient mice. Systemic administration of either corticosterone or normetanephrine, a competitive inhibitor of OCT3-mediated transport, potentiated reinstatement of CPP in response to a previously subthreshold dose of cocaine in wild type mice but had no effect in OCT3-deficient mice (McReynolds et al. 2017).

5.2 Corticosterone-Sensitive Serotonin Clearance

Roles for uptake$_2$ transporters in regulating extracellular serotonin levels have been revealed in several brain regions of male rodents. Daws and colleagues demonstrated the presence of a low-affinity, high-capacity serotonin transport system independent of SERT in rat hippocampus (Daws et al. 2006). Further investigation of this uptake system revealed that OCT3 expression is elevated in SERT-deficient mice and that intra-hippocampal infusion of corticosterone acutely decreased local serotonin clearance in these mice (Baganz et al. 2008). This group went on to demonstrate that repeated swim stress decreases hippocampal serotonin clearance in both wild type and SERT-deficient mice in a corticosterone-dependent manner (Baganz et al. 2010). These studies showed that stress-induced increases in corticosterone decreased both the expression and function of OCT3 in hippocampus, strongly suggesting a role for OCT3 in stress-induced regulation of serotonergic neurotransmission in hippocampus. Couroussé and colleagues examined potential contributions of OCT2 to clearance of serotonin and norepinephrine in hippocampus (Couroussé et al. 2015). They provided evidence that subchronic (4 days) administration of corticosterone in drinking water leads to decreases in serotonin and norepinephrine clearance when high-affinity uptake$_1$ transporters are blocked, and that this effect of corticosterone was not evident in OCT2-deficient mice. However, because corticosterone treatment was prolonged and not acute, and because corticosterone did inhibit uptake in OCT2-deficient mice in the absence of uptake$_1$ inhibition, it is not possible to determine whether these effects are due to acute uptake inhibition or to changes in gene expression.

In the dorsomedial hypothalamus (DMH), acute stress and corticosterone treatment lead to increases in serotonin and other monoamines (Lowry et al. 2001, 2003). OCT3 is expressed in ependymal cells lining the third ventricle in this region of the hypothalamus, and OCT3-mediated uptake of both histamine and serotonin has been demonstrated in acute DMH explants (Gasser et al. 2006). In in vivo microdialysis studies, local application of corticosterone into the DMH potentiated the effects of the serotonin-releasing agent D-fenfluramine (administered either systemically or directly into the DMH) on extracellular serotonin concentrations, consistent with the hypothesis that OCT3 inhibition by corticosterone contributes to stress-induced increases in serotonin in the DMH (Feng et al. 2009). A similar phenomenon has been observed in the central nucleus of the amygdala (CeA). Infusion of either corticosterone or normetanephrine into the CeA potentiates stress-induced increases in extracellular serotonin concentrations in male rats (Hassell et al. 2019).

6 Summary

Since uptake$_2$-mediated transport of monoamines was first described, it was defined by its sensitivity to inhibition by corticosterone and other steroids. The corticosteroid sensitivity of the uptake$_2$ transporters represents not only a distinguishing pharmacological feature, but also a mechanism by which stress, through increases in corticosterone or cortisol, may acutely regulate monoaminergic neurotransmission, physiology, and behavior. The studies reviewed here describe the relative sensitivity of each uptake$_2$ transporter to corticosterone and other steroids and provide evidence that corticosteroids regulate extracellular monoamine concentrations in specific brain regions by inhibition of one or more uptake$_2$ transporters.

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Brain Plasma Membrane Monoamine Transporter in Health and Disease

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Abstract

Precise control of monoamine neurotransmitter levels in the central nervous system (CNS) is crucial for proper brain function. Dysfunctional monoamine signaling is associated with several neuropsychiatric and neurodegenerative disorders. The plasma membrane monoamine transporter (PMAT) is a new polyspecific organic cation transporter encoded by the *SLC29A4* gene. Capable of transporting monoamine neurotransmitters with low affinity and high capacity, PMAT represents a major uptake$_2$ transporter in the brain. Broadly expressed in multiple brain regions, PMAT can complement the high-affinity, low-capacity monoamine uptake mediated by uptake$_1$ transporters, the serotonin, dopamine, and norepinephrine transporters (SERT, DAT, and NET, respectively). This chapter provides an overview of the molecular and functional characteristics of PMAT together with its regional and cell-type specific expression in the mammalian brain. The physiological functions of PMAT in brain monoamine homeostasis are evaluated in light of its unique transport kinetics and brain location, and in comparison with uptake$_1$ and other uptake$_2$ transporters (e.g., OCT3) along with corroborating experimental evidences. Lastly, the possibility of PMAT's involvement in brain pathophysiological processes, such as autism, depression, and Parkinson's disease, is discussed in the context of disease pathology and potential link to aberrant monoamine pathways.

Keywords

5-HT · Autism · Brain · Catecholamine · Choroid plexus · Depression · Monoamine neurotransmitter · MPP^+ · OCT3 · Organic cation · PMAT · *Slc29a4* knockout · Uptake$_1$ · Uptake$_2$

1 Introduction

The plasma membrane monoamine transporter (PMAT) is the newest addition to the broadly selective organic cation and monoamine transporters. Unlike the organic cation transporters (OCTs) in the SLC22 family, PMAT belongs to the mammalian solute carrier 29 (SLC29) gene family, which primarily encodes the equilibrative nucleoside transporters (ENTs) (Baldwin et al. 2004; Kong et al. 2004; Ho and Wang 2014). Initially named ENT4, we first identified the existence of this transporter from the draft human genome database around 2000–2001 and had initially hypothesized it to function as a nucleoside or nucleobase transporter (Kong et al. 2004). We subsequently cloned the full-length human ENT4 cDNA and expressed the transporter in several expression systems including MDCK and HEK cell lines and *Xenopus laevis* oocytes. However, despite the transporter's sequence similarity to the ENTs, extensive screening work did not detect significant transport activities for nucleosides, nucleobases, or other structurally related analogs. The turning point came in 2003 when a postdoctoral fellow, Dr. Karen Engel, in our laboratory discovered by serendipity that 1-methyl-4-phenylpyridinium (MPP^+), a

dopaminergic neurotoxin and an OCT substrate, is robustly transported by ENT4. Using MPP^+ as a lead, we quickly discovered that the structurally related catecholamines (dopamine, norepinephrine, epinephrine) and indolamines (e.g., 5-HT) are all substrates for ENT4. We thus renamed ENT4 to "plasma membrane monoamine transporter (PMAT)" to reflect its true substrate profile.

In 2004, we reported the cloning and functional characterization of PMAT as a novel monoamine neurotransmitter transporter in the human brain (Engel et al. 2004). Shortly after, we found that besides monoamines, PMAT also transports a variety of structurally diverse organic cations (OC) and shares a remarkable functional resemblance to the OCTs (Engel and Wang 2005). The discovery of PMAT as a previously unknown monoamine and OC transporter has thus introduced a new member to organic cation and monoamine transporters. Since the initial cloning and characterization of PMAT, enormous advances have been made to our understanding of the molecular mechanisms, functional characteristics, cell and tissue-specific expression of PMAT. Critical reagents including antibodies, cell lines, and fluorescent probes have been developed. In 2013, we also generated the first mouse knockout model for Pmat (Duan and Wang 2013). With the availability of the molecular tools and animal models, the in vivo function of PMAT in the central nervous system and its significance in health and disease are just beginning to be understood. In this chapter, we summarize our current understanding of PMAT with an emphasis on its functional characteristics and potential roles in the central nervous system (CNS) in health and disease.

2 Molecular and Functional Characteristics of PMAT

2.1 Molecular Features

The human PMAT protein is encoded by the *SLC29A4* gene located at chromosome 7p22.1. Human PMAT is a membrane protein of 530 amino acid residues and has a predicted molecular mass of 58 kDa. Based on hydropathy analysis, an 11-transmembrane domain (TM) model with a long intracellular N-terminus and a short extracellular C-terminus has been proposed (Fig. 1). There are six consensus sites for protein kinase C phosphorylation (S6, S262, S268, S306, S476, and T338) and one cAMP-dependent kinase phosphorylation site (T199), in addition to one potential N-linked glycosylation site (N523) (Engel et al. 2004). Homologs of PMAT are also found in mouse and rat (Dahlin et al. 2007; Okura et al. 2011). Those homologs share 86–87% amino acid sequence identity with human PMAT and are predicted to have a similar membrane topology as the human protein. At the protein level, human and rodent PMATs exhibit a low but significant sequence identity (~20%) to the ENTs. In the TM regions, sequence identity between PMAT and the ENTs increases up to 35–40%. In contrast, the sequence identity of PMAT to the OCTs is only 11–14% (Zhou et al. 2007b).

The main substrate-binding domain in PMAT was identified by constructing and analyzing domain-swapping chimeric transporters between human PMAT and

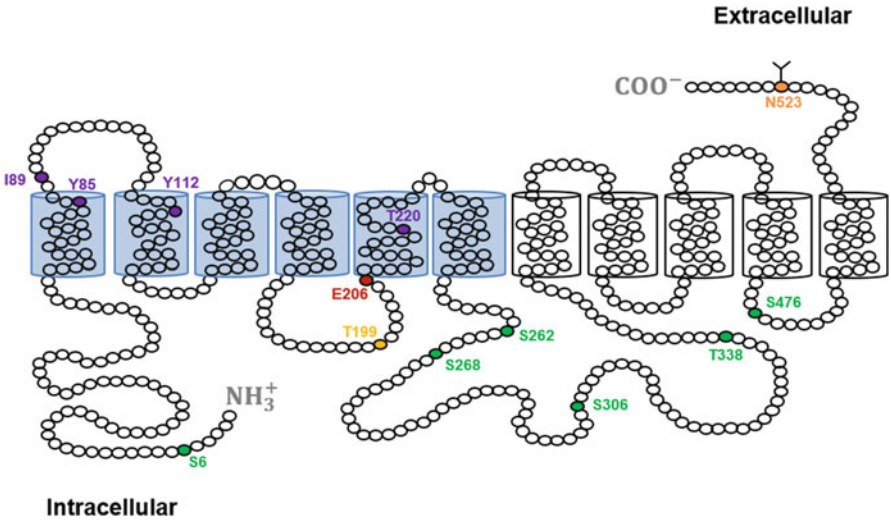


Fig. 1 Proposed topology of human PMAT with 11-TM domains. Circles represent individual amino acid residues. TM1-6 important for substrate recognition are shaded in blue. Purple: residues involved in substrate recognition (Y85, I89, Y112, T220). Red: residue involved in charge recognition (E206). Green: potential sites for protein kinase C phosphorylation (S6, S262, S268, S306, T338, S476). Yellow: potential site for cAMP-dependent protein kinase phosphorylation (T199). Orange: potential N-glycosylation site (N523)

ENT1 (Zhou et al. 2007b). A chimera consisting of TM1-6 of PMAT and TM7-11 of hENT1 behaved like PMAT, transporting MPP^+ but not uridine (a nucleoside). These data suggest that although the C-terminal half of PMAT likely contributes to the formation of the substrate permeation pathway, the major substrate recognition site in PMAT is located within its N-terminal half (Fig. 1). Site-directed mutagenesis analyses further identified that a negatively charged glutamate residue (E206) on TM5 is critical for the cation selectivity of PMAT (Zhou et al. 2007b). T220, another residue in TM5, also directly impacts PMAT's interaction with its substrates. Helical wheel analysis further revealed an amphipathic pattern of residue distribution on TM5, with E206 and T220 clustered closely in the center of the hydrophilic face, suggesting that TM5 is a critical component of the substrate permeation pathway in PMAT (Zhou et al. 2007b). In addition, Y85, Y112, and I89 on TM1-2 were also identified to play important roles for PMAT to interact with its cationic substrates and inhibitors (Ho and Wang 2010; Ho et al. 2012b).

2.2 Substrate Specificity

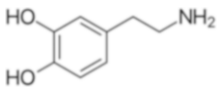
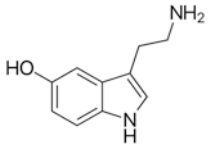
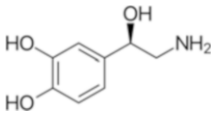
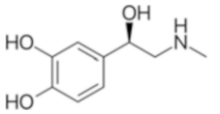
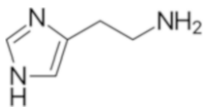
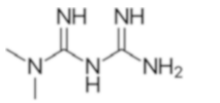
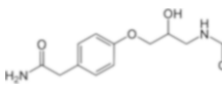
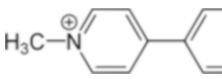
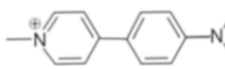
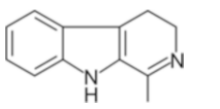
Nearly all PMAT substrates identified to date are small and hydrophilic organic cations (OCs). Biogenic amines, cationic drugs, and neurotoxins with diverse chemical structures are some of the OCs transported by PMAT (Engel and Wang

2005; Zhou et al. 2007c; Ho et al. 2011). Table 1 illustrates the structures of selected PMAT substrates along with their apparent binding affinities (K_m). As seen by the structures shown in Table 1, many of the PMAT substrates are OCs that contain one or more aromatic rings. However, the presence of the aromatic groups is not a required feature for interacting with this transporter, as some aliphatic OCs such as metformin and tetraethylammonium (TEA) are also transported by PMAT (Engel and Wang 2005; Zhou et al. 2007c). As a general observation, the presence of a positively charged nitrogen at physiological pH and a balance between hydrophilicity and hydrophobicity seem to be important determinants for interaction with PMAT. The diverse structures of compounds able to interact with PMAT indicate that this transporter functions as a polyspecific organic cation transporter. The OCTs from the SLC22 family are also considered polyspecific transporters, and a significant overlap is seen in the molecules transported by PMAT and OCT1-3. For instance, both metformin and the neurotoxin MPP^+, which are considered prototype substrates of OCTs, are efficiently transported by PMAT (Engel et al. 2004; Zhou et al. 2007c). In addition, the monoamine neurotransmitters, some of the most studied PMAT substrates, are known to be transported by OCT1-3 (Gründemann et al. 1999).

Despite the substantial overlap in substrates, there are also significant and important differences in the specificity and kinetics of PMAT and OCTs. For example, ASP^+, a fluorescent analog of MPP^+, is an excellent substrate for OCT1, 2, and 3, and is used as a fluorescence probe for these transporters (Ciarimboli et al. 2005; Kido et al. 2011; Duan et al. 2015). However, ASP^+ is not a PMAT substrate. On the other hand, another MPP^+ analog, APP^+ (IDT307), is robustly transported by PMAT (Duan et al. 2015). TEA is considered a good substrate for OCT1 and 2, but is a moderate to poor substrate for PMAT and OCT3. For the shared substrates, transport kinetics may also differ significantly among these transporters. For example, while the monoamine neurotransmitters are all transportable substrates for human PMAT and OCT3, the two transporters show marked differences in their apparent affinity and transport efficiency towards these substrates (Duan and Wang 2010). hPMAT shows a kinetic preference for serotonin and dopamine whereas hOCT3 appears to be more efficient towards histamine, norepinephrine, and epinephrine (see Sect. 5.3).

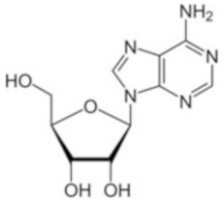
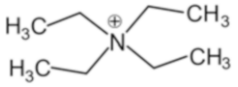
As stated earlier, PMAT (SLC29A4) was identified as the fourth member in the mammalian ENT family, which also contains ENT1, ENT2, and ENT3. ENT1 and 2 are classic nucleoside transporters and contribute to the cellular uptake of purine and pyrimidine nucleosides and their structural analogs (Baldwin et al. 2004; Kong et al. 2004; Ho and Wang 2014). ENT3 is an intracellular transporter essential in the transport of nucleosides into mitochondria and lysosomes (Govindarajan et al. 2009; Hsu et al. 2012). Although initially named ENT4, our functional studies have clearly shown that PMAT does not transport classical ENT substrates such as nucleosides, nucleobases, and nucleotides (Engel et al. 2004; Engel and Wang 2005). Among the naturally occurring nucleosides and nucleobases only adenosine is recognized as a substrate of PMAT; however, it is transported at an efficiency much lower than the OC substrates such as MPP^+ and serotonin (Zhou et al. 2010).

Table 1 Structure and apparent affinity (K_m) of selected PMAT substrates

Class	Substrate	K_m (μM)	Structure
Biogenic amines	Dopamine	406 ± 48[a] 329 ± 8[b]	
	Serotonin (5-HT)	283 ± 40[a] 114 ± 12[b]	
	Norepinephrine	1,078 ± 107[a] 2,606 ± 258[b]	
	Epinephrine	951 ± 59[a] 15,323 ± 3,947[b]	
	Histamine	4,379 ± 679[a] 10,471 ± 2,550[c]	
Organic cation drugs	Metformin	1,320[d]	
	Atenolol	907 ± 93[e]	
Neurotoxins and molecular analogs	MPP^+	111 ± 3[a] 33 ± 7[b]	
	APP^+ (IDT307)	*n.a.*	
	Harmalan	*n.a.*	

(continued)

Table 1 (continued)

Class	Substrate	K_m (μM)	Structure
Other substrates	Adenosine	413 ± 107[f]	
	TEA	8,759 ± 3,175[a] 6,593 ± 1,702[c]	

n.a. indicates value was not available in literature
Values from [a]Duan and Wang (2010)
[b]Engel et al. (2004)
[c]Engel and Wang (2005)
[d]Zhou et al. (2007b)
[e]Mimura et al. (2017)
[f]Zhou et al. (2010)

Table 2 K_i or IC_{50} values (μM) of selected inhibitors of human PMAT

Inhibitor	K_i or IC_{50} (μM)
Decynium-22 (D22)	0.10 ± 0.03[a]
GBR12935	7.9 ± 1.0[a]
Fluoxetine	22.7 ± 6.1[a]
Desipramine	32.6 ± 2.7[a]
Corticosterone	450.5 ± 76.5[a]
Rhodamine123	1.02 ± 0.12[b]
Quinine	26.9 ± 4.6[b]
Quinidine	25.3 ± 4.8[b]
Verapamil	18.6 ± 3.1[b]
Lopinavir	1.4 ± 0.2[c]
Ritonavir	6.0 ± 0.5[c]
Saquinavir	7.0 ± 1.0[c]

Values from [a]Engel et al. (2004)
[b]Engel and Wang (2005)
[c]Duan et al. (2015)

2.3 PMAT Inhibitors

Most of the known PMAT inhibitors are also positively charged organic molecules. However, while PMAT substrates are mostly smaller and more polar OCs (type I cations), high affinity PMAT inhibitors are generally bulkier and more hydrophobic (type II cations) (Table 2). Similar to what was observed for PMAT substrate profile, there is also a large overlap between OCT and PMAT inhibitors. A wide range of known OCT inhibitors were tested and most of them cross-inhibited PMAT (Engel

and Wang 2005). Several compounds such as decynium-22 (D22), quinidine, and rhodamine123 potently inhibited PMAT, but none of them show selectivity towards PMAT. For instance, D22, a cationic derivate of quinoline and a commonly used inhibitor of PMAT is able to inhibit OCT3 with the same potency ($K_i \sim 100$ nM) and is also able to inhibit OCT1 and 2 with K_i equal to 0.98 and 1.13 μM, respectively (Hayer-Zillgen et al. 2002; Engel and Wang 2005).

One strategy used in many studies for differentiating PMAT from OCT activity is the use of corticosterone. Corticosterone is an uncharged steroid hormone which shows much greater potency towards OCTs over PMAT (Ki = 450 μM for PMAT versus $IC_{50} < 35$ μM for OCT1-3), being especially selective towards OCT3 ($IC_{50} = 0.29$ μM) (Hayer-Zillgen et al. 2002; Engel and Wang 2005). Due to the selectivity of corticosterone towards OCTs, sensitivity towards D22 but not towards corticosterone is often used in literature for discerning PMAT and OCTs activity (Baganz et al. 2008; Duan and Wang 2010, 2013; Naganuma et al. 2014; Matthaeus et al. 2015). This approach however is not ideal for in vivo studies since corticosterone elicits a variety of genomic and nongenomic effects, which could affect many physiological processes and complicate the interpretation of results. Selective inhibitors of uptake$_1$ monoamine transporters (i.e., SLC6A family – serotonin, norepinephrine, and dopamine transporters, SERT, NET, and DAT) such as fluoxetine, desipramine, and GBR12935 were also found to inhibit PMAT (Engel et al. 2004). However, the K_i values obtained for PMAT were much higher (2–3 orders of magnitude) than observed towards SERT, NET, and DAT, indicating a strong preference of those inhibitors towards transporters from the SLC6A family.

In order to streamline the identification and characterization of potential PMAT inhibitors, a fluorescence assay was recently developed using IDT307, an MPP^+ fluorescent analog transported by PMAT (Duan et al. 2015). Using this assay, the HIV protease inhibitors were identified as inhibitors of PMAT, showing higher sensitivity towards PMAT in comparison with OCTs. In particular, lopinavir is a potent PMAT inhibitor and exhibited more than 120-fold selectivity toward PMAT over OCT1 ($IC_{50} = 1.4$ μM and 174 μM respectively), with no significant inhibitory effect for OCT2 or OCT3 at the highest tested concentration. While lopinavir is selective towards PMAT and can be a useful tool to differentiate PMAT activity from those of OCTs in in vitro and ex vivo studies, its use as a PMAT-selective inhibitor in vivo can be limited by several factors. Lopinavir is highly bound to plasma proteins and is a substrate of the efflux transporter P-gp (Van Waterschoot et al. 2010). The presence of P-gp at the blood–brain barrier may limit the entry of lopinavir into the brain and thus its inhibition efficiency towards PMAT in vivo in the brain. Therefore, the search for highly specific PMAT inhibitors suitable for in vivo studies continues.

The structure-activity relationship of OCs interacting with PMAT as substrates and inhibitors was previously investigated (Engel and Wang 2005; Ho et al. 2011). A positively charged nitrogen atom and a hydrophobic mass are the two most prominent features of PMAT substrates and inhibitors. A planar aromatic mass is often associated with high affinity interaction with PMAT (Engel and Wang 2005). Using a variety of phenylalkylamine analogs, the optimal distance between the

positively charged nitrogen and the aromatic ring was determined to be between 5.2 and 7.7 Å, which is consistent with a spacer chain length of 2–3 carbons (Ho et al. 2011). Several 3D pharmacophore models were also generated through molecular modeling based on the analysis of molecules known to interact with PMAT (Ho et al. 2011). These models are described by the presence of a hydrogen bond donor (representative of the positively charged nitrogen) and 2–3 hydrophobic features. In agreement to what was observed using the phenylalkylamine analogs, the distance between the hydrogen bond donor and the hydrophobic mass in these models ranges from 5.20 to 7.02 Å. As expected from the large substrate and inhibitor overlaps with the OCTs, the pharmacophore models of PMAT showed remarkable similarities to those developed for the OCTs (Bednarczyk et al. 2003; Zolk et al. 2009).

2.4 Mechanism of Transport

Solute carrier transporters (SLC) can mediate transmembrane flux of their substrates through both passive and active mechanisms. Ion coupled transporters, such as the high-affinity monoamine transporters (i.e., SERT, DAT, NET), mediate uphill transport of their substrates by coupling to the downhill flow of inorganic ions (e.g., Na^+, Cl^-). The inside-negative membrane potential universally present in animal cells can also influence membrane transport process. For instance, it is used by the OCTs as a driving force to power cellular uptake of positively charged molecules (Wagner et al. 2016). Radiotracer uptake studies in PMAT-expressing MDCK cells and *Xenopus laevis* oocytes showed that different from the high affinity biogenic amine transporters, Na^+ and Cl^- do not affect PMAT-mediated transport (Engel et al. 2004). In contrast, PMAT activity is highly sensitive to membrane potential changes (Engel et al. 2004; Zhou et al. 2007c). In agreement with these observations, two-microelectrode voltage-clamp studies in *Xenopus laevis* oocytes explicitly demonstrated that PMAT-mediated histamine uptake is associated with substrate-evoked, inwardly directed currents under voltage-clamp conditions (Itagaki et al. 2012). Kinetic analysis further showed that the inside-negative membrane potential enhances PMAT-mediated histamine transport by increasing maximal transport velocity with little effect on apparent binding affinity. These studies unequivocally demonstrated PMAT as an electrogenic transporter that uses the physiological inside-negative membrane potential to drive its uptake of OCs.

Protons also exert a strong stimulatory effect on PMAT activity. Extracellular pH has been shown to impact PMAT-mediated uptake of many substrates (Barnes et al. 2006; Xia et al. 2007; Zhou et al. 2007c, 2010; Itagaki et al. 2012). In general, an acidic extracellular pH is associated with an increase in PMAT-mediated transport whereas an alkaline pH is associated with reduced PMAT activity (Wang 2016). This stimulatory effect is not substrate-specific, but observed for nearly all tested substrates, including MPP^+, 5-HT, metformin, adenosine, and histamine (Xia et al. 2007; Zhou et al. 2007c, 2010; Itagaki et al. 2012). The proton ionophore FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone) greatly reduces the uptake of MPP^+ by PMAT (Xia et al. 2007; Itagaki et al. 2012). Two-microelectrode

voltage-clamp analysis in oocytes further demonstrated that the pH effect is present under voltage-clamp conditions, providing strong evidence that the effect of pH on PMAT uptake is unlikely to be due to an indirect effect of proton on membrane potential (Itagaki et al. 2012). Currently this effect is hypothesized to occur either via a direct coupling mechanism of OCs with protons or through a pH-induced change in protein ionization and folding state, which could provoke a change in the intrinsic catalytic activity of PMAT (Wang 2016). More studies are needed to understand the precise mechanism underlying the effect of protons on PMAT-mediated transport.

3 Expression and Distribution in the CNS

3.1 Regional Expression in Rodent and Human Brains

While PMAT mRNA/protein has been reported to be expressed in multiple tissues including brain, heart, small intestine, kidney, and liver (Engel et al. 2004; Barnes et al. 2006; Dahlin et al. 2007; Zhou et al. 2007c; Xia et al. 2009; Duan and Wang 2010), the brain has consistently been shown to be the major site of PMAT expression. Multiple studies, including northern blot, RT-PCR, in situ hybridization (ISH), and immunostaining analysis, have consistently demonstrated high expression of PMAT mRNA and protein in human and rodent brains (Engel et al. 2004; Dahlin et al. 2007, 2009; Vialou et al. 2007; Duan and Wang 2010, 2013). Using non-radioactive ISH, Dahlin et al. showed that mPmat mRNA is widely expressed in the mouse brain. The highest expression levels are observed in forebrain cortex, olfactory tubercle, dentate gyrus of hippocampus, cerebellum, and choroid plexus (Dahlin et al. 2007, 2009). This mRNA distribution pattern is in good agreement with the ISH data for *Slc29a4* from the Allen Mouse Brain Atlas (http://brainatlas.com/aba/) (Dahlin et al. 2009). A similar mRNA expression pattern is also observed for rat Pmat by ISH studies in rat brain sections (Vialou et al. 2007). Using an anti-PMAT polyclonal antibody, immunohistochemical staining further confirmed the widespread expression of mPmat protein across the mouse brain, consistently showing high protein expression in mouse forebrain cortex, hippocampus, olfactory areas, cerebellum, and choroid plexus (Dahlin et al. 2007).

The expression of PMAT mRNA in the human brain was initially analyzed by northern blot. Widespread expression was found in all tested regions including cerebellum, cerebral cortex, medulla, occipital pole, frontal and temporal lobes, putamen, and spinal cord (Engel et al. 2004). Using quantitative real-time PCR, Duan et al. quantified the copy numbers of PMAT in several human brain regions and choroid plexus (Duan and Wang 2010, 2013). While the PMAT transcript was found in all areas, it is particularly abundant in the cerebral cortex, hippocampus, substantia nigra, medulla oblongata, cerebellum, and choroid plexus (Engel et al. 2004; Duan and Wang 2010, 2013). The expression of PMAT protein in human brain was confirmed by immunoblotting and immunostaining studies in human cerebellum and choroid plexus tissue samples (Dahlin et al. 2007; Duan and Wang 2013).

3.2 Cell Type-Specific Expression in the Brain

In the mouse brain, ISH and immunohistochemistry detected mPmat expression in diverse groups of neurons including pyramidal neurons, interneurons, granular neurons, and Purkinje cells (Dahlin et al. 2007). Co-localization studies with the neuronal marker MAP2 further demonstrated that mPmat co-localizes extensively with MAP2. Consistently, immunostaining studies in human cerebellum tissue sections revealed intense PMAT immunoreactivity in axons and dendrites of neuronal cells (Dahlin et al. 2007). The expression and distribution of rPmat mRNA in the rat brain was also determined by Vialou et al. using in situ hybridization/immunohistochemistry co-labeling (Vialou et al. 2007). The rPmat mRNA was found in various neuron subtypes throughout the brain. Interestingly, rPmat was expressed in some, but not all, aminergic pathways. On the other hand, high expression was observed in cholinergic, glutamatergic, and GABAergic neurons in several discrete brain areas (Vialou et al. 2007).

Beside neurons, very high PMAT expression is found in the epithelial cells of the choroid plexus, which form the blood–cerebral spinal fluid (CSF) barrier. ISH and immunohistochemical studies in mouse and rat brains revealed choroid plexus as one of the brain regions with highest Pmat mRNA and protein expression (Dahlin et al. 2007; Vialou et al. 2007). Western blot analysis of human choroid plexus homogenate also detected a strong band with expected molecular mass of human PMAT (Duan and Wang 2013). Immunofluorescence staining of human choroid plexus sections showed that PMAT is predominantly expressed on the surface of choroid plexus epithelial cells and co-localizes strongly with the apical membrane marker Na^+/K^+-ATPase (Duan and Wang 2013).

With regard to astrocytes, initial co-localization studies in mouse brain revealed very little overlap of mPmat immunoreactivity with that of the astrocyte marker glial fibrillary acidic protein (GFAP) (Dahlin et al. 2007). Nevertheless, the high stringency used in these immunostaining studies may not be able to detect lower level of mPmat protein expression. In addition, there is also evidence that GFAP may not be present in all astrocytes (Walz and Lang 1998). More sensitive RT-PCR analyses have shown the expression of human PMAT mRNA in primary human astrocytes and human astrocytoma-derived cell lines (e.g., 1321N1, A172) (Engel et al. 2004; Yoshikawa et al. 2013; Naganuma et al. 2014).

4 Mouse Model for PMAT

Functional analyses of mouse and rat Pmat transporters in expression systems showed that the rodent Pmat transporters share substrate specificity and transport kinetics similar to those of human PMAT (Shirasaka et al. 2017). To elucidate the in vivo and physiological function of PMAT, our laboratory developed a knockout mouse model for Pmat (Duan and Wang 2013). The targeting vector was designed to replace exons 3–7 of the murine *Slc29a4* gene upon homologous recombination, producing an altered allele in which exon 2 is joined with exon 8. This would result

in a defective mRNA transcript with multiple premature stop codons after the first 72 amino acids, generating a nonfunctional short peptide with only one partial transmembrane domain. The targeting vector was transfected into G4 embryonic stem cells and a homologous recombinant clone was identified and used for blastocyst injection into C57BL/6J embryos to generate chimeric mice. The chimeric males were crossed with C57BL/6J females, and germ line transmission was identified by PCR genotyping. The F0 heterozygotes were then interbred to generate wild-type ($Pmat^{+/+}$) and knockout ($Pmat^{-/-}$) mice. The knockout mice have been continuously backcrossed with wild-type C57BL/6J mice to achieve a genetic background of more than 99% C57BL/6J. The disruption of the mPmat function in the gene product was also confirmed by isolating the defective mRNA species from $Pmat^{-/-}$ mice followed by expression and functional analysis in Flp-in HEK293 cells (Duan and Wang 2013).

$Pmat^{-/-}$ mice are viable, fertile with no overt physiological abnormalities (Duan and Wang 2013). Initial blood chemistry showed no significant differences in baseline values of various serum biomarkers from wild-type mice. Quantitative real-time PCR revealed that whole brain expression of functionally related transporters such as the high affinity monoamine transporters (*Sert, Dat, Net*) and *Oct3* are not significantly changed in knockout mice. The generation of the *Slc29a4* global knockout model provided an invaluable model to study the role of PMAT in vivo. This model has been used to analyze the function of Pmat in several studies (Duan and Wang 2013; Gilman et al. 2018; Bowman et al. 2020; Wei et al. 2020).

5 Physiological Roles in Monoamine Neurotransmission

5.1 $Uptake_1$ and $Uptake_2$

In the CNS, the monoamine neurotransmitters such as serotonin (5-HT), norepinephrine, and dopamine, are important chemical messengers that participate in a myriad of physiological and cognitive processes, including behavior, emotion, learning, and memory (Carlsson 1987; Greengard 2001). Like other chemical transmitters, monoamine neurotransmitters are synthesized in specific monoaminergic neurons and are stored in secretory vesicles at the axon terminal of the presynaptic neuron. During neurotransmission, the neurotransmitters are released into the synaptic cleft. They diffuse across the cleft and bind to specific receptors on postsynaptic neurons to elicit a variety of responses (Fig. 2). The signaling process is terminated by the removal of the released monoamines from the synaptic space. This process is carried out by membrane proteins capable of transporting monoamines into presynaptic neurons or adjacent cells, where the transmitter can either be recycled by repackaging into secretory vesicles or be degraded by intracellular enzymes such as the monoamine oxidases (MAOs) or the catechol-O-methyltransferase (COMT) (Torres et al. 2003).

The uptake of released monoamines into presynaptic neurons is mainly carried out by a family of Na^+- and Cl^--dependent, high affinity, low capacity transporters

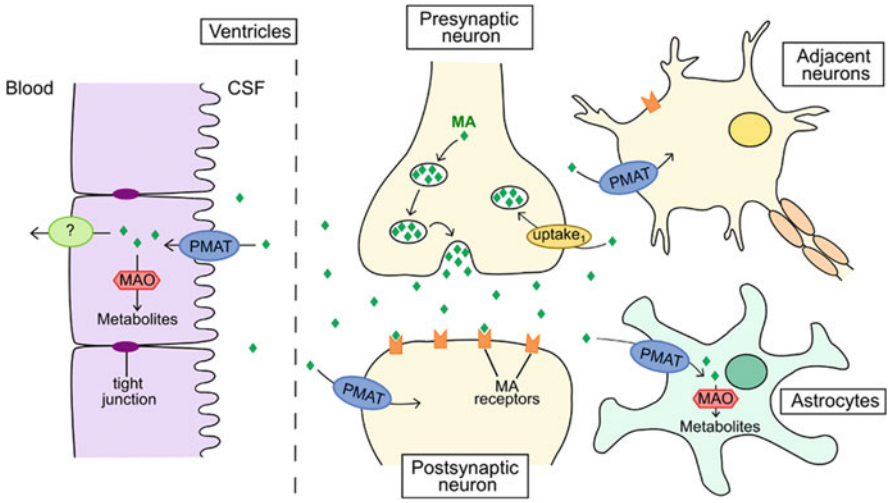


Fig. 2 Proposed roles of PMAT in monoamine (MA) clearance in the brain. PMAT is present in neuronal (yellow) and non-neuronal (purple and green) cells. It acts as an uptake$_2$ transporter and mediates the uptake of MA that has diffused out of the synaptic cleft into the interstitial fluid or CSF. MA can be metabolized by intracellular enzymes (e.g., MAO) or in the case of choroid plexus epithelial cells (purple) may be further exported into the blood

collectively known as "uptake$_1$". Uptake$_1$ include DAT, SERT, and NET (Blakely et al. 1994; Torres et al. 2003; Kristensen et al. 2011). These transporters share high sequence similarity and belong to the solute carrier 6 (SLC6) family. The reported apparent affinities (K_m) of uptake$_1$ transporters are typically in nanomolar to low micromolar range. In the CNS, DAT, SERT, and NET are expressed almost exclusively in the nerve terminals of dopaminergic, serotonergic, or noradrenergic neurons. Due to their major role in the reuptake of monoamines from the synaptic cleft, uptake$_1$ transporters are the targets of many psychostimulants (e.g., cocaine and amphetamines), neurotoxins (e.g., MPP^+) and antidepressants (e.g., selective serotonin reuptake inhibitors, tricyclic antidepressants) (Blakely et al. 1994; Torres et al. 2003; Kristensen et al. 2011).

Besides uptake$_1$, a different monoamine uptake system, termed uptake$_2$, was first described by Iversen (1965) in peripheral tissues with sympathetic innervation. Similar uptake$_2$ activities were also detected in brain areas such as striatum and cortex (Hendley et al. 1970; Wilson et al. 1988). Different from the uptake$_1$ system, uptake$_2$ transport is broadly selective, Na^+ and Cl^- independent and of low-affinity and high-capacity (Eisenhofer 2001; Daws 2009). The role of uptake$_2$ was initially considered to be related to facilitating the uptake of monoamines for their metabolism by intracellular enzymes such as MAOs and COMT (Lightman and Iversen 1969; Trendelenburg et al. 1987). However, the presence of uptake$_2$ activity in the brain suggested the possibility of uptake$_2$ to function as a secondary monoamine uptake system for the clearance of monoamine neurotransmitters in the CNS

(Gründemann et al. 1998; Eisenhofer 2001; Engel et al. 2004; Daws 2009; Matthaeus et al. 2015).

5.2 PMAT as a Brain Uptake$_2$ Transporter

In 1998, two research groups reported the cloning and characterization of OCT3 as the extraneuronal monoamine transporter (Gründemann et al. 1998; Wu et al. 1998). OCT3 showed classic features of the uptake$_2$ activity characterized in extraneuronal tissues, broadly transporting catecholamines with low affinity and high capacity. OCT3 is Na^+ and Cl^- independent but highly sensitive to corticosterone, and was thus considered as the uptake$_2$ transporter in sympathetically innervated tissues and in brain cells (Gründemann et al. 1998; Wu et al. 1998). Besides OCT3, the closely related OCT1 and OCT2 are also capable of mediating uptake$_2$-like transport of monoamine neurotransmitters (Gründemann et al. 1999; Koepsell et al. 2007).

In 2004, we reported the identification and characterization of PMAT as a novel monoamine transporter in the human brain. PMAT-mediated monoamine transport also shows classic uptake$_2$ characteristics, such as Na^+ and Cl^- independency and low substrate affinity but high transport capacity (Engel et al. 2004). With the identification and functional characterization of PMAT and OCTs, it is now clear that uptake$_2$ consists of a group of organic cation transporters with broad monoamine selectivity. Although all these transporters are capable of mediating Na^+- and Cl^--independent, low-affinity and high-capacity monoamine uptake, PMAT and OCT3 are likely to represent the major uptake$_2$ transporters in the brain and in sympathetically innervated tissues due to their marked expression in these tissues (Duan and Wang 2010).

The functional properties and brain distribution of PMAT appear to be complementary to uptake$_1$ monoamine transporters. SERT, DAT, and NET function as Na^+ and Cl^- dependent, high affinity, low capacity transporters. In contrast, PMAT-mediated transport is Na^+ and Cl^- independent and is of low affinity and high capacity in nature. For instance, the apparent affinities (K_m) of PMAT for 5-HT and dopamine are 2–3 orders of magnitude lower than those of SERT and DAT. Meanwhile, the V_{max} values of PMAT-mediated 5-HT and dopamine transport are also 2–3 orders higher than the V_{max} values of SERT or DAT (Engel et al. 2004). Furthermore, while uptake$_1$ transporters are exclusively expressed in monoaminergic neurons, PMAT is broadly expressed in many brain areas that may or may not express the uptake$_1$ (Engel et al. 2004; Dahlin et al. 2007). Lastly, PMAT is generally resistant to inhibitors of DAT, SERT, and NET. The functional characteristics of PMAT and its strategically broad localization in the brain suggest that this uptake$_2$ transporter may supplement the roles of the high affinity transporters under certain conditions. Based on these observations, we first hypothesized that PMAT is a brain uptake$_2$ transporter responsible for clearing released neurotransmitters that have escaped reuptake by the presynaptic uptake$_1$ transporters (Engel et al. 2004; Zhou et al. 2007a) (Fig. 2). PMAT may also play an

active role in monoamine uptake in brain areas that do not express uptake$_1$ (Dahlin et al. 2007).

The in vivo concentrations of monoamine neurotransmitters vary significantly in different brain areas. The extracellular concentrations of the monoamine are highest at its site of release and decline as the transmitter diffuses away (Bunin and Wightman 1998, 1999). It has been shown that synaptic concentrations of neurotransmitters can transiently reach high concentrations in the millimolar range (Bunin and Wightman 1998), where the high affinity transporters would be saturated. Although the precise localization of PMAT in neurons is still unknown, it is possible that the transporter is present in postsynaptic membranes and participates in the clearance of monoamines at these high concentration areas (Fig. 2). Furthermore, monoamine neurotransmitters are known to diffuse away from the synaptic cleft and exert neuromodulatory effects on other cells at distances well beyond their sites of release (i.e., volume transmission) (Agnati et al. 2010). The widespread expression of PMAT in the CNS makes it a perfect candidate to modulate monoamine signaling in volume transmission by taking up diffused neurotransmitters in non-monoaminergic neurons and in astroglial cells (Fig. 2).

5.3 Comparison Between PMAT and OCT3

The pronounced expression of PMAT and OCT3 in the CNS and sympathetically innervated tissues indicates that they are the two most prominent uptake$_2$ transporters for endogenous monoamines. PMAT is widely expressed in the human and rodent brains, including areas that do not express uptake$_1$ transporters (Dahlin et al. 2007; Duan and Wang 2010). Although PMAT is mostly found in neurons (Dahlin et al. 2007; Vialou et al. 2007), some studies have also reported expression in astrocytes (Yoshikawa et al. 2013). OCT3 was also found to be expressed in multiple brain regions in both astroglial and neural cells (Cui et al. 2009; Gasser et al. 2009).

The large overlaps in substrate specificity and broad brain distribution of PMAT and OCT3 raised important questions regarding their specific contribution to uptake$_2$ monoamine clearance in the brain. To explore the kinetic difference of PMAT and OCT3 in transporting endogenous monoamines, our laboratory developed stably transfected HEK293 cell lines that express hPMAT and hOCT3 at comparable levels (Duan and Wang 2010). Parallel and detailed kinetics analysis in these cell lines revealed distinct and seemingly complementary patterns for the two transporters in transporting monoamine neurotransmitters. hPMAT is more selective toward serotonin (5-HT) and dopamine, with the rank order of transport efficiency (V_{max}/K_m) being dopamine, 5-HT $\gg$ histamine, norepinephrine, epinephrine (Table 3). The substrate preference of hPMAT toward these amines is mainly driven by large differences in K_m (Duan and Wang 2010). In contrast, hOCT3 is less selective toward the monoamines with a rank order of V_{max}/K_m being histamine $>$ norepinephrine, epinephrine $>$ dopamine $>$5-HT (Table 3). Interestingly, hOCT3 showed

Table 3 Relative transport efficiency of hPMAT and hOCT3 towards monoamine neurotransmitters

Relative transport efficiency to MPP^+		
Substrate	hPMAT (%)	hOCT3 (%)
MPP^+	100	100
Dopamine	93	23
Serotonin (5-HT)	85	12
Histamine	16	57
Norepinephrine	14	35
Epinephrine	13	30

Values obtained from Duan and Wang (2010). Percentage values indicate transport efficiency (V_{max}/K_m) relative to the classical substrate MPP^+

comparable K_m, but significantly different V_{max} values, for the endogenous monoamines.

Using real-time PCR assay, the copy numbers of hPMAT and hOCT3 transcripts were quantified in several human tissue (Duan and Wang 2010). In spinal cord and eight brain regions, hPMAT expression is much higher than hOCT3, while hOCT3 is more abundant than hPMAT in adrenal glands and skeletal muscle. Other groups have also reported high OCT3 expression in circumventricular organs and broad expression throughout the rodent brain (Vialou et al. 2004; Amphoux et al. 2006; Gasser et al. 2006). Taken together, these data suggest that PMAT likely represents the major uptake$_2$ transporter for 5-HT and dopamine (two major centrally active neurotransmitters) in the CNS. OCT3, on the other hand, may be the major uptake$_2$ transporter for histamine, norepinephrine, and epinephrine, especially in peripheral tissues with sympathetic innervation.

5.4 PMAT in Brain 5-HT Clearance

Among the monoamine neurotransmitters, 5-HT is robustly transported by PMAT with highest affinity (Engel et al. 2004; Duan and Wang 2010) (Table 1). 5-HT is a major brain monoamine neurotransmitter involved in complex pathways associated with affection, emotion, learning, and mood modulation (Frazer and Hensler 1999; Meneses and Liy-Salmeron 2012). The major pathway for presynaptic clearance of 5-HT is mediated by SERT expressed in serotoninergic neurons (Blakely et al. 1994; Torres et al. 2003). Several studies have provided evidence of significant involvement of PMAT in 5-HT clearance in the brain (Zhou et al. 2007a; Duan and Wang 2010; Horton et al. 2013; Hosford et al. 2015).

Our laboratory first used an antisense hybrid depletion approach to assess PMAT's contribution to total brain 5-HT uptake in vitro (Zhou et al. 2007a). *Xenopus laevis* oocytes were injected with total human brain poly(A)$^+$ mRNA to allow the expression of the full spectrum of genes in the brain. Radiotracer uptake studies showed a ~2.5-fold increase in 5-HT uptake compared to control oocytes injected with water. Pre-hybridization of poly(A)$^+$ mRNA with PMAT or SERT antisense oligonucleotides, respectively, reduced mRNA-induced 5-HT uptake by

40–60% and 60–70% (Zhou et al. 2007a). These data suggest that functional transcripts of PMAT are present in the human brain and that PMAT may be significantly involved in brain uptake of 5-HT.

In vitro analysis showed that PMAT is resistant to selective serotonin reuptake inhibitors (SSRIs) that are highly potent and selective towards SERT (Zhou et al. 2007a; Haenisch and Bönisch 2010). We have taken the advantage that PMAT is highly sensitive to D22, but resistant to SSRIs and the OCT inhibitor corticosterone to assess potential contribution of Pmat in synaptosomes prepared from whole mouse brain (Duan and Wang 2010). Using this approach, we provided the evidence that PMAT may contribute to approximately 20% of total 5-HT uptake in mouse whole brain synaptosomes (Duan and Wang 2010). Corroborating with our findings, an in vivo study by Horton et al. (2013) was able to show that fluvoxamine (an SSRI) inhibition of 5-HT clearance in mouse hippocampus was potentiated when in combination with D22 – a potent inhibitor of PMAT and OCT3 – in both wild-type and Oct3 knockout mice, suggesting an involvement of mouse Pmat in 5-HT clearance. In addition, fast-cyclic voltammetry studies suggest that 5-HT clearance in the nucleus tractus solitarii of anesthetized rats is possibly under the regulation of PMAT, but not OCT3 or SERT (Hosford et al. 2015).

5.5 PMAT as a Clearance Mechanism at the Blood–CSF Barrier

Beside neurons, very high PMAT expression is found in the epithelial cells of the choroid plexus (Dahlin et al. 2007; Duan and Wang 2013). Located in the lateral, third, and fourth brain ventricles, the primary function of the choroid plexus is to produce and secrete CSF that baths and protects the brain (Ho et al. 2012a). The choroid plexus epithelial cells are joined by tight junctions and form the blood–CSF barrier. These epithelial cells are polarized with their apical membranes facing the CSF and the basolateral membrane facing the blood circulation. The blood–CSF barrier expresses numerous membrane transporters to facilitate solute exchange between the CSF and the systemic circulation (Keep and Smith 2011; Ho et al. 2012a).

Expression profiling analysis of ISH data from the Allen Brain Atlas showed that Pmat is one of the highest expressed *Slc* genes in the mouse choroid plexus (Dahlin et al. 2009; Ho et al. 2012a). Real-time PCR, western blot, and proteomic analyses also confirmed the abundant expression of PMAT mRNA and protein in human choroid plexus (Duan and Wang 2013). Importantly, other organic cations transporters (OCT1-3 and MATE1/2K) and uptake$_1$ monoamine transporters (SERT, DAT, NET) are minimally expressed in choroid plexus. The PMAT protein is localized to the apical (CSF-facing) membrane of the choroid plexus epithelial cells, consistent with a role in mediating active uptake of monoamines from the CSF into choroid plexus cells. Once inside those cells, monoamine neurotransmitters can be metabolized by the intracellular MAOs expressed within choroid plexus cells (Kaplan et al. 1981; Vitalis et al. 2002) or may be further exported across the basolateral membrane into the blood (Fig. 2).

The generation of the Pmat knockout mouse model provided a new tool for assessing the contribution of PMAT in the uptake of monoamines and other organic cations from the CSF (Duan and Wang 2013). Ex vivo uptake studies with live choroid plexus tissues revealed that the uptake of MPP^+, 5-HT, and dopamine is impaired in the choroid plexus of Pmat knockout mice. Furthermore, D22 reduced the uptake of 5-HT and dopamine into the choroid plexus of wild type but not Pmat knockout mice. In contrast, neither corticosterone (an OCT1-3 inhibitor) nor RTI-55 (a potent inhibitor of $uptake_1$ transporters) showed an effect in the uptake of 5-HT or dopamine in choroid plexus (Duan and Wang 2013). Together, these data demonstrated that PMAT is the major monoamine uptake transporter at the blood–CSF barrier. Consistent with these findings, CSF clearance studies in anesthetized rats showed that intracerebroventricular administered MPP^+ is quickly eliminated from the CSF, and the addition of dopamine and 5-HT inhibited this process, indicating a significant role of PMAT in regulating CSF organic cation concentrations in vivo (Okura et al. 2011). A similar study by Usui et al. (2016) using lopinavir as an inhibitor also pointed towards a significant role of PMAT in the clearance of histamine from the CSF.

6 Potential Involvement in Brain Pathophysiological Processes

In the CNS, aberrant levels of monoamine neurotransmitters are associated with a number of brain disorders such as depression, autism, schizophrenia, Parkinson's disease, and drug addiction. Due to their major roles in regulating extracellular monoamine levels, altered function of $uptake_1$ transporters has been implicated in several neuropsychiatric conditions (Kristensen et al. 2011; Pramod et al. 2013). For instance, reduced transporter expression and function due to the SERT promoter region polymorphism (e.g., 5-HTTLPR) has been associated with many neuropsychiatric conditions such as bipolar disorder, depression, obsessive–compulsive disorder, suicide, eating disorders, substance-abuse disorders, autism, and attention-deficit/hyperactivity disorder (Murphy and Moya 2011). In addition, therapeutic responses and side effects following treatment with SSRIs have been found to be associated with SLC6A4 variants.

To date, no study has systematically analyzed the allele frequency and functional consequence of single nucleotide polymorphisms (SNPs) of *SLC29A4* in the human populations. Our laboratory first suggested that PMAT may play a role in intestinal absorption of the antidiabetic drug metformin based on its expression in the gastrointestinal tract and its robust in vitro transport activity towards metformin (Zhou et al. 2007c). Several pharmacogenomics studies have explored the relationship between *SLC29A4* intronic variations and metformin pharmacokinetics and pharmacodynamics (Christensen et al. 2011; Duong et al. 2013; Dawed et al. 2019). A recent study in 286 severe metformin-intolerant and 1,128 metformin-tolerant individuals showed a significant association of the G allele at rs3889348 with gastrointestinal intolerance of metformin (Dawed et al. 2019).

Little is currently known regarding the involvement of PMAT in monoamine-related brain disorders. The impact of genetic polymorphisms or mutations in the human *SLC29A4* gene on neuropsychiatric and neurodegenerative disorders has not been explored. Nevertheless, a number of in vitro and in vivo studies suggest that PMAT should be considered as a candidate gene potentially involved in several monoamine-related brain disorders. Below we discuss these possibilities in the context of monoamine pathophysiology and speculate the potential involvement of PMAT under these scenarios.

6.1 Autism

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that impacts social interaction, verbal and nonverbal communication, and repetitive behavior. ASD manifestation and severity varies broadly among individuals and includes an umbrella of conditions that used to be diagnosed separately, such as autism disorder, Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified. Despite strong evidence pointing towards genetic heritability of autism, only few genes associated with ADS have been identified and they do not explain the majority of cases (Muhle et al. 2004; Sandin et al. 2017; Waye and Cheng 2018).

Serotonin (5-HT) is known to be an important neurotransmitter involved in the development and regulation of the CNS, playing a role in many behavior and psychological processes (Whitaker-Azmitia 2001). The study of this neurotransmitter is especially interesting in the context of ASD, considering that many serotonin-related abnormalities have been identified in ASD patients. For instance, close to one-third of autistic patients and their first-degree relatives present hyperserotonemia (increase in whole-blood and platelet serotonin), which is believed to be associated with atypical maturation of the serotoninergic system (Cook and Leventhal 1996; Leboyer et al. 1999; Whitaker-Azmitia 2001; Cross et al. 2008). In addition, low CSF levels of 5-hydroxyindolacetic acid (5-HIAA – a 5-HT metabolite) were found in up to 20% of patients with neurological disorders, including ASD patients (De Grandis et al. 2010). This evidence indicates that abnormal serotonin neurotransmission may be important in autism manifestation, and therefore genes of proteins involved in serotonin homeostasis (such as SERT and PMAT) may be of special interest.

Using a genetic candidate gene approach, Adamsen et al. (2014) identified a high prevalence of three rare non-synonymous mutations within the PMAT gene in ASD patients versus unaffected individuals. Genome analysis confirmed that these mutations were inherited and not de novo mutations. When transfected into MDCK cells, all mutants were localized to the plasma membrane at comparable levels to wild-type PMAT; however, two of them (A138T and D326E) showed reduced uptake of 5-HT. At least two ASD subjects with either the A138T or the D326E mutation had low 5-HIAA levels in the CSF and elevated serotonin levels in blood and platelets. As stated earlier, PMAT is widely expressed in the brain

including regions that lack significant SERT expression (Dahlin et al. 2007). It was speculated that PMAT dysfunction may play a significant role in the neurodevelopment of ASD patients (Adamsen et al. 2014). It was hypothesized that the reduced functionality of PMAT leads to a compromised serotonin clearance – especially in areas with low SERT expression – and may promote high prenatal exposure to serotonin. Consequently, high serotonin level leads to negative feedback in auto-receptors, perturbing the development of serotonin network and its local synthesis and culminating in low levels of serotonin and metabolites in the brain. In the blood, 5-HT is mainly stored in platelets through the uptake by SERT that is abundantly expressed in these cells (Jedlitschky et al. 2012). It is presently unknown if PMAT is expressed in platelets. However, the elevated platelet level of 5-HT in autism patients with the A138T or D326E mutation, corresponding to a reduced function of PMAT, indicates that PMAT may not play a significant role in 5-HT uptake and storage in platelets. Nevertheless, given the relatively small size of the ASD patient samples carrying the *SLC29A4* mutations, more studies are necessary to confirm these findings and elucidate the role of PMAT in serotonergic abnormalities and ASD development.

6.2 Depression

Depressive disorders are complex and have diverse manifestation in patients, with severity of symptoms varying from mild to severe. Tricyclic antidepressants (TCA), MAO inhibitors, serotonin and norepinephrine reuptake inhibitor (SNRIs), and selective serotonin reuptake inhibitors (SSRIs) are some of the major classes of antidepressants currently used for depression treatment. Most of them exert their effect by affecting the uptake and/or metabolism of serotonin in the brain, which is known to be involved in depression pathophysiology (Cowen and Browning 2015). The SSRIs, such as fluoxetine, citalopram, and sertraline, are some of the most widely prescribed medications in the treatment of depression, increasing extracellular levels of serotonin in the brain through inhibition of its reuptake by SERT. While the uptake$_1$ transporter SERT plays an essential role in serotonin homeostasis in the CNS, a significant portion of individuals undergoing SSRI treatment have poor or delayed response, with 30–40% of patients not responding satisfactorily to treatment (Steffens et al. 1997; Al-Harbi 2012).

Due to their complementary role in brain 5-HT uptake, uptake$_2$ transporters, such as PMAT and OCT3, have been hypothesized as potential targets to improve the response and reduce latency to antidepressant treatment (Schildkraut and Mooney 2004; Zhou et al. 2007a; Daws 2009). PMAT is highly expressed in human brain and transports 5-HT efficiently (Engel et al. 2004; Dahlin et al. 2007). We and others previously showed that many antidepressants including SSRIs and TCAs do not affect PMAT transport activity at clinically relevant concentrations (Zhou et al. 2007a; Haenisch and Bönisch 2010), indicating that PMAT may play a compensatory role in serotonin uptake, buffering the effect of these antidepressants and

therefore contributing to the clinical presentation of treatment resistance (Zhou et al. 2007a; Daws 2009).

Current evidence supporting this hypothesis comes mostly from pharmacological inhibition studies associating the use of uptake$_1$ inhibitors (i.e., SSRIs) and PMAT inhibitors (i.e., D22), while using knockout or selective inhibition (i.e., corticosterone) to rule out the impact of OCT3 in serotonin uptake. Both in vivo experiments with rodents and in vitro studies using mouse brain synaptosome have demonstrated that D22 potentiates the effect of SSRIs, decreasing uptake and clearance of serotonin and improving antidepressant-like effect (Duan and Wang 2010; Horton et al. 2013). The recent development of Pmat knockout mouse model provides a new tool for investigating the psychological and neurological function of Pmat in the CNS. While Pmat deficient mice do not have an overt phenotype (Duan and Wang 2013), they display subtle differences in anxiety and coping behaviors (Gilman et al. 2018). In addition, a recent study measuring real-time clearance of 5-HT in mouse hippocampus showed that ketamine was able to significantly inhibit serotonin clearance in wild-type but not in Sert$^{-/-}$ or Pmat$^{-/-}$ mice; pointing towards PMAT as a potential contributing factor in ketamine's antidepressant-like effects (Bowman et al. 2020). Further neurochemistry and behavioral studies of these animals, as well as comparative studies between SSRI treatment in Pmat$^{-/-}$ and wild-type mice may provide a direct link between PMAT and the treatment of depressive disorders. If proven, PMAT might represent a promising target in the development of new and improved antidepressant drugs.

6.3 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of nigrostriatal dopaminergic neurons. PD is a multifactorial disease with both genetic and environmental factors playing roles. CNS exposure to toxic organic cations can damage nigrostriatal dopaminergic pathways and produce parkinsonian syndromes, as seen with the classic PD protoxin MPTP (Sian et al. 1999). MPTP is a lipophilic compound that can readily pass through the blood–brain barrier. Once inside in the brain, MPTP is rapidly converted to the toxic metabolite MPP$^+$ in astrocytes by MAO-B. After releasing from the astrocytes through OCT3 (Cui et al. 2009), MPP$^+$ is selectively transported into the dopaminergic neurons through DAT, where it impairs mitochondrial respiration chain and precipitates cell death (Sian et al. 1999).

CNS exposure to environmental or endogenously produced MPP$^+$-like toxins, such as paraquat, certain β-carbolines, and tetrahydroisoquinoline derivatives, has long been implicated in the etiology of PD (Nagatsu 1997; Collins and Neafsey 2000). β-carbolines are naturally found structural analogs of MPP$^+$, and 1-benzyl-1,2,3,4-tetrahydroisoquinoline is an example of an endogenously produced neurotoxin found to be elevated in the CSF of PD patients (Kotake et al. 1995). Interestingly, many of those cationic neurotoxins were found to be substrates of PMAT. MPP$^+$ is efficiently transported by cell lines expressing PMAT (Engel et al. 2004). Cytotoxicity assays demonstrated that harmalan and norharmanium (two

β-carbolines) are also transportable substrates of PMAT (Ho et al. 2011). Similarly, 1-benzyl-1,2,3,4-tetrahydroisoquinoline was also reported to be a PMAT substrate (Wu et al. 2015). Cells expressing PMAT are ~15-fold more sensitive to the toxic effect of those compounds (Ho et al. 2011).

The choroid plexus in the brain plays a major role in maintaining CSF homeostasis and clearing potentially harmful compounds from the CNS. PMAT is highly expressed in human and mouse choroid plexuses and is localized to the apical membrane (CSF-facing) of the choroid plexus epithelial cells (Duan and Wang 2013). By clearing cationic neurotoxins from the CSF and preventing its accumulation in the brain, PMAT may play a protective role in the CNS, potentially lowering the risk of PD. Further investigation of this hypothesis is needed to elucidate the functional and toxicological significance of PMAT in PD etiology.

7 Summary

Since the discovery of PMAT in 2004, great progress has been made in our understanding of this transporter at the molecular and functional levels. Work conducted in the past 15 years has clearly established that PMAT functions as a polyspecific organic cation transporter that transports biogenic amines and xenobiotic organic cations. PMAT-mediated substrate-uptake is Na^+-independent and driven by inside-negative membrane potential. Broadly expressed in the brain in both neural and non-neural cells, PMAT represents a major brain $uptake_2$ transporter for centrally acting monoamine neurotransmitters such as 5-HT and dopamine. By serving as a complementary clearance mechanism for released monoamine neurotransmitters, PMAT may play a regulatory role in monoamine neurotransmission and neuromodulation. Nevertheless, we are still at the beginning of understanding the in vivo significance of this transporter in brain monoamine physiology and pathophysiology. Elucidating the roles of PMAT in monoamine neurotransmission and its involvement in neuropsychiatric and neurodegenerative diseases would be important and exciting directions for the time ahead.

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Regulation of Neurogenesis by Organic Cation Transporters: Potential Therapeutic Implications

Takahiro Ishimoto and Yukio Kato

Contents

Abstract

Neurogenesis is the process by which new neurons are generated from neural stem cells (NSCs), which are cells that have the ability to proliferate and differentiate into neurons, astrocytes, and oligodendrocytes. The process is essential for homeostatic tissue regeneration and the coordination of neural plasticity throughout life, as neurons cannot regenerate once injured. Therefore, defects in neurogenesis are related to the onset and exacerbation of several neuropsychiatric disorders, and therefore, the regulation of neurogenesis is considered to be a novel

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strategy for treatment. Neurogenesis is regulated not only by NSCs themselves, but also by the functional microenvironment surrounding the NSCs, known as the "neurogenic niche." The neurogenic niche consists of several types of neural cells, including neurons, glial cells, and vascular cells. To allow communication with these cells, transporters may be involved in the secretion and uptake of substrates that are essential for signal transduction. This chapter will focus on the involvement of polyspecific solute carriers transporting organic cations in the possible regulation of neurogenesis by controlling the concentration of several organic cation substrates in NSCs and the neurogenic niche. The potential therapeutic implications of neurogenesis regulation by these transporters will also be discussed.

Keywords

Neural stem cells · Neurogenesis · Neurogenic niche · Neuropsychiatric disorders · Organic cation transporters

1 Introduction: Neurogenesis, Neural Stem Cells, and the Neurogenic Niche

Neurons play pivotal roles in the fundamental functions of the brain, but do not have the ability to self-renew; consequently, neurons cannot regenerate themselves if injured. In contrast, neural stem cells (NSCs) generate new neurons and can thereby replenish damaged neurons (Johansson et al. 1999; Zhang et al. 2008; Knoth et al. 2010). NSCs exist in the subgranular zone (SGZ) of the hippocampal dentate gyrus and subventricular zone (SVZ) of the adult mammalian brain. NSCs have the capacity to proliferate and differentiate into neurons, astrocytes, and oligodendrocytes. The process of generating new neurons from NSCs is called neurogenesis. Neurogenesis contributes to homeostatic tissue regeneration and the coordination of neural plasticity throughout life (Zhang et al. 2008; Ming and Song 2011) and is affected by various factors in daily life, including exercise, sleep, food, and stress (LaDage 2015; Stankiewicz et al. 2017; Pons-Espinal et al. 2019).

In contrast, defects in neurogenesis are related to the onset and exacerbation of several neuropsychiatric disorders, such as major depressive disorder and Parkinson's disease (PD) (Eisch and Petrik 2012; Le Grand et al. 2015; LaDage 2015). Therefore, the regulation of neurogenesis is considered a novel strategy for the treatment of neuropsychiatric disorders (Snyder et al. 2011; Kohl et al. 2012; Tuszynski et al. 2015; Alam et al. 2018). Indeed, there are a few neurogenic drugs under development. For example, the benzylpiperazine-aminopyridine neurogenic compound NSI-189 had positive effects on major depressive disorder in a Phase 1b clinical study (Fava et al. 2016), and a novel neurotrophic agent, T-817MA, preserved memory function and accelerated motor function recovery in brain-damaged animals (Fukushima et al. 2011; Abe et al. 2018). Moreover, clinically used drugs such as selective serotonin reuptake inhibitors (SSRIs) also promote neurogenesis

(Banasr et al. 2004; Gur et al. 2007), which was recently proposed to be one of the mechanisms of their antidepressant activity.

There are considerably fewer NSCs (<1,200) than neurons (~70,000,000) and glial cells (approximately 10 times more than neurons) in the murine brain (Brazel and Rao 2004; Herculano-Houzel et al. 2006). This may lead to speculation that NSCs are regulated by crosstalk with cells other than NSCs. Histological analysis of the SGZ and SVZ revealed that many types of cells surround the NSCs (Bátiz et al. 2016; Bonzano et al. 2018; Lin et al. 2019). These include astrocytes, microglia, brain microvascular endothelial cells (BMECs), pericytes, and choroid plexus epithelial cells (CPECs). Neurogenesis is thought to be regulated by a "neurogenic niche," which represents the functional microenvironment surrounding the NSCs. Thus, the cells surrounding the neurogenic niche may play regulatory roles in several functions of NSCs, including differentiation and proliferation. Neurogenesis is thought to be regulated by various neurotransmitters, growth factors, neurotrophins, cytokines, and exosomes secreted from astrocytes, microglia, BMECs, pericytes, neurons, and CPECs (Song et al. 2012; Bátiz et al. 2016; Wicki-Stordeur et al. 2016; Cope and Gould 2019; Lin et al. 2019; Lehtinen et al. 2011; Yanpallewar et al. 2010). For communication with other cells, membrane receptors are essential to receive and transduce signals, whereas transporters are involved in the secretion and uptake of these molecules for signal transduction (Song et al. 2012; Trujillo-Gonzalez et al. 2019; Pajarillo et al. 2019). Therefore, it is important to comprehend the expression and exact roles of transporters in NSCs and the neurogenic niche to understand the homeostasis of NSCs. In this chapter, we will focus on the regulation of neurogenesis by solute carriers (SLCs) transporting organic cations with broad specificity, which are defined as "OCTs," in NSCs and other niche cells.

2 Organic Cations Transporting Solute Carriers Expressed in NSCs

2.1 OCTN1/SLC22A4 and Its Substrate Ergothioneine (ERGO)

Among OCTs, the expression of the carnitine/organic cation transporter OCTN1, OCTN2/SLC22A5, and OCTN3/SLC22A21 genes was detected in murine primary cultured NSCs, whereas the expression of OCT1/SLC22A1, OCT2/SLC22A2, OCT3/SLC22A3, multidrug and toxin extrusion protein 1 (MATE1), concentrative nucleoside transporter 2 (CNT2/SLC28A2), equilibrative nucleoside transporter 1 (ENT1/SLC29A1), and ENT4 was below the limit of detection (Ishimoto et al. 2014). The mRNA expression of OCTN1 was much higher than that of other genes (Ishimoto et al. 2014). No information is currently available on the expression of these genes in NSCs in higher animals, including humans. In cultured murine NSCs, immunocytochemical analysis showed that OCTN1 was expressed on the cell surface membranes of the NSC marker nestin-positive cells. In addition, the NSCs showed uptake of a typical substrate ERGO, which is a food-derived antioxidant, whereas NSCs derived from *octn1* gene knockout mice (*octn1*$^{-/-}$) minimally

incorporated ERGO (Ishimoto et al. 2014), demonstrating that OCTN1 was functionally expressed in murine NSCs.

In the rodent brain, OCTN1 is expressed in neurons and their precursor cells NSCs, but not in glial cells such as astrocytes (Inazu et al. 2006; Nakamichi et al. 2012; Ishimoto et al. 2014, 2018). This may imply the involvement of OCTN1 in the differentiation of NSCs to neurons. Indeed, the transfection of OCTN1 siRNA into P19 embryonic carcinomas differentiated into neural stem-like cells (a model NSC cell line) suppressed their ability to differentiate into βIII-tubulin-positive cells (a neuronal marker) with a concomitant decrease in the functional expression of OCTN1 (Ishimoto et al. 2014), suggesting that this organic cation transporter may be involved in neuronal differentiation.

A typical OCTN1 substrate, ERGO, is present in serum in vivo and in the culture medium. Therefore, ERGO was considered to be involved, at least partially, in OCTN1-mediated neurogenesis. The exposure of NSCs to ERGO significantly increased the proportion of βIII-tubulin-positive cells and decreased that of GFAP-positive cells (an astrocyte marker) after the induction of differentiation, whereas ERGO did not affect the neuronal differentiation of NSCs derived from *octn1*$^{-/-}$, suggesting that OCTN1-mediated ERGO uptake promoted neuronal differentiation (Ishimoto et al. 2019). Moreover, the uptake of ERGO by OCTN1 activated an amino acid sensor, mammalian target of rapamycin complex 1 (mTORC1), and neurotrophin 5/TrkB signaling prior to the promotion of neuronal differentiation, suggested the possible involvement of intracellular signaling in ERGO-mediated differentiation (Ishimoto et al. 2019).

Notably, despite the hydrophilic properties of ERGO, it has a tissue-to-plasma concentration ratio of more than two in the mouse brain, indicating active transport of this compound into the brain, whereas ERGO was not detected in the brain of *octn1*$^{-/-}$ mice (Kato et al. 2010; Nakamichi et al. 2016). This result implied that OCTN1-mediated ERGO uptake in the brain may have a significant role in brain function. Indeed, intake of ERGO-containing diet for 2 weeks in mice showed an increase in the newborn neuron marker doublecortin (Dcx)-positive cells in the SGZ of the hippocampus with the concomitant antidepressant-like effect of ERGO intake, as demonstrated in the forced swimming test (FST) and tail suspension test (TST) (Nakamichi et al. 2016). In addition, oral administration of ERGO enhanced cognitive function, as shown by a longer exploration time for novel objects in a novel object recognition test, a behavioral experiment for assessing memory function (Nakamichi et al. 2020). Oral administration of ERGO also protected against memory deficits induced by β-amyloid and D-galactose in mice (Yang et al. 2012; Song et al. 2014). This enhancement of memory function may be related to ERGO-induced neurogenesis as it is well known that neurogenesis positively regulates memory function (Kumar et al. 2019), although there are still other possible mechanisms, including the maturation of neurons and/or the antioxidant activity of ERGO (Yang et al. 2012; Song et al. 2014; Nakamichi et al. 2020).

In humans, there is no information regarding the involvement of OCTN1 in brain function; however, oral administration of ERGO-containing mushroom extract tablets for 8 and 12 weeks was reported to show improvement in cognitive function

of healthy volunteers and patients with mild cognitive impairment (MCI) compared with that in the placebo group (Watanabe et al. 2020). The serum ERGO concentration in patients with PD and the blood ERGO concentration in patients with MCI were also reported to be significantly lower than those in healthy volunteers (Hatano et al. 2016; Cheah et al. 2016). Further epidemiological analysis is needed to clarify whether the lower ERGO concentration in brain may be a risk factor for PD and MCI.

2.2 OCTN2/SLC22A5 and Its Substrates Carnitine and Acetyl-L-Carnitine

OCTN2 efficiently transports carnitine and acetyl-L-carnitine as endogenous substrates (Januszewicz et al. 2010; Kido et al. 2001; Ohashi et al. 1999; Tamai et al. 1998). Carnitine plays an essential role in the transport of fatty acids into the mitochondria for their subsequent β-oxidation, a process for generating energy (Frigeni et al. 2017). In humans, systemic carnitine deficiency, mainly caused by the dysfunction of OCTN2, leads to hypoketotic hypoglycemia, cardiomyopathy, and encephalopathy (Kimura and Amemiya 1990; Frigeni et al. 2017). In contrast, in the brain, the substrates carnitine and acetyl-L-carnitine are proposed to be involved in neurogenesis (Athanassakis et al. 2002; Cuccurazzu et al. 2013; Fathi et al. 2017; Singh et al. 2017).

The exposure of primary cultured NSCs to carnitine (40–200 μM) for 1 month resulted in the formation of a neural network that was positively stained with cresyl violet, whereas exposure to vehicle resulted in only neurospheres (Athanassakis et al. 2002). Moreover, the exposure of mesenchymal stem cells to carnitine (200 μM) induced gene expression of neurogenic markers, such as nerve growth factor (ngf), brain-derived neurotrophic factor (bdnf), and nestin, with a concomitant increase in the expression of proteins related to the protein kinase A (PKA) and Wnt/β-catenin pathways, such as β-catenin, low-density lipoprotein receptor-related protein (LRP) 5c, Wnt1, and Wnt3a. In contrast, the PKA inhibitor H89 suppressed neurogenic markers (Fathi et al. 2017). These results suggested that carnitine promotes neurogenesis, at least partially, through the Wnt/β-catenin and PKA pathways (Athanassakis et al. 2002; Fathi et al. 2017). At 40–200 μM of carnitine, the promotion of neurogenesis was observed, which is close to the endogenous carnitine concentration in the human brain (~0.05 μmol/g; Nakano et al. 1989), which corresponds to ~50 μM if the gravity of the brain is assumed to be unity, suggesting that carnitine may induce the promotion of neurogenesis in vivo.

As carnitine is known to restore mitochondrial membrane potential, Kim et al. tested whether supplementation with carnitine in vitro could reverse the defects in neuronal differentiation induced by mitochondrial dysfunction. Immunocytochemical analysis showed that exposure of adult SVZ-derived NSCs to an inhibitor of mitochondrial fission-promoting protein DRP1 decreased the number of βIII-tubulin-positive cells, whereas supplementation of carnitine at 50 nM fully reversed the

decrease (Kim et al. 2015). Thus, even low concentrations of carnitine could recover the defects in neuronal differentiation induced by mitochondrial dysfunction.

Daily intraperitoneal administration of acetyl-L-carnitine (100 mg/kg) for a month restored the decrease in the number of Dcx-positive cells (a newborn neuron marker) in the SGZ of the hippocampus of rats with model PD induced by intrastriatal injection of 6-hydroxydopamine (Singh et al. 2017). Daily subcutaneous administration of acetyl-L-carnitine (100 mg/kg) for 21 days increased the number of cells with double-positive immunostaining for the neuronal marker NeuN and the cell proliferation marker BrdU in the SGZ of the hippocampus. In addition, behavioral assessment in the FST and TST showed antidepressant-like activity of acetyl-L-carnitine with an increase in protein expression of metabotropic glutamate receptor 2 receptor in the hippocampus (Cuccurazzu et al. 2013). Thus, not only carnitine, but also acetyl-L-carnitine, may be involved in the regulation of neurogenesis. The concentration of these compounds in the brain is at least partially regulated by OCTN2 expressed in BMECs, which act as the blood-brain barrier (Kido et al. 2001). Therefore, further studies are required to clarify whether OCTN2 expressed in BMECs may be associated with the effect of carnitine and/or acetyl-L-carnitine on neurogenesis. In contrast, OCTN2 was reported to be expressed in primary cultured NSCs and hippocampal neurons (Ishimoto et al. 2014; Lamhonwah et al. 2008). Therefore, in these neural cells, OCTN2 may also regulate the concentration of carnitine and acetyl-L-carnitine around the NSCs in the hippocampus.

2.3 SERT/SLC6A4 and Serotonin

SLCs transporting organic cations include various neurotransmitter transporters, notably the serotonin transporter SERT, the glutamate transporters (vGlut1-3 and EAAT1-3), and the GABA transporter GAT1, and were reported to be expressed in NSCs (Ren-Patterson et al. 2005; Sanchez et al. 2006; Benninghoff et al. 2012; Sánchez-Mendoza et al. 2017). Among these transporters, the involvement of SERT in neurogenesis has been relatively well characterized.

SERT is expressed in neurons, astrocytes, and NSCs (Ren-Patterson et al. 2005; Benninghoff et al. 2012; Malynn et al. 2013) and is believed to be a primary regulator of serotonin levels in the brain. Serotonin is a neurotransmitter related to the pathogenesis of several neuropsychiatric conditions, such as major depressive disorder, schizophrenia, and various anxiety disorders (Benninghoff et al. 2012). The brain serotonin level is thought to be relevant to neurogenesis owing to the following evidence. First, p-chlorophenylalanine, a serotonin synthesis inhibitor, significantly suppressed hippocampal neurogenesis by decreasing serotonin levels in the hippocampus (Jha et al. 2006). Second, inhibitors of SERT, some of which are SSRIs used clinically as antidepressants, increased the level of extracellular serotonin and caused hippocampal neurogenesis (Snyder et al. 2011; Li et al. 2008).

The expression of SERT in NSCs increases as NSCs become more differentiated into neuronal progenitor cells (Ren-Patterson et al. 2005), supporting the possible involvement of this transporter in neurogenesis. However, in *sert* gene knockout

mice (*sert*$^{-/-}$), the proliferative capacity of NSCs was higher than that in older wild-type mice (~14.5 months), whereas no significant difference in proliferation was observed in younger (~7 weeks) and adult (~3 months) mice between the two strains (Schmitt et al. 2007). Unexpectedly, there was minimal difference in the proliferative capacity of NSCs between *sert*$^{-/-}$ and wild-type mice, despite the large difference in serotonin concentration between the two strains. The serotonin concentration in the hippocampus of *sert*$^{-/-}$ was approximately three times lower than that of wild-type mice (Kim et al. 2005). Interestingly, the rate of serotonin synthesis in the brain of *sert*$^{-/-}$ was 1.5 times higher than that in wild-type mice (Kim et al. 2005), whereas the expression of OCT3, another serotonin transporter, in the hippocampus of *sert*$^{-/-}$ was ~1.4 times higher than that in wild-type mice (Schmitt et al. 2003; Baganz et al. 2008). Thus, alterations in the biosynthesis of serotonin and expression of OCT3 in *sert*$^{-/-}$ may be one of the possible compensation mechanisms.

The antidepressant effect induced by SSRIs generally requires repeated administration to patients for weeks to months. This requirement cannot be simply explained by direct inhibition of SERT by SSRIs; notably, more time-consuming processes, including neurogenesis, followed by the replenishment of damaged neurons are speculated to be involved in the pharmacology of these drugs (Warner-Schmidt and Duman 2006; Perera et al. 2008; Li et al. 2008; Gur et al. 2007; Han et al. 2011). Indeed, the antidepressant-like activity of an SSRI, fluoxetine, assessed using the TST, was diminished in the mice, and hippocampal neurogenesis was suppressed by conditional gene deletion of TrkB in NSCs (Li et al. 2008). Thus, the serotonin-induced promotion of neurogenesis may be a prerequisite, at least partly, for the antidepressant activity of SSRIs.

3 OCTs Expressed in the Neurogenic Niche

3.1 OCT2/SLC22A2 in the Hippocampus

The expression of OCTs in various neural cells is summarized in Table 1. In neurons, the expression of various OCTs, such as OCT2, 3, OCTN1-3, CNT2, and ENT1 and 4 were reported (Table 1). In particular, OCT2, 3, and OCTN1-3 are expressed in the hippocampus of rodents (Matsui et al. 2016; Vialou et al. 2008; Nakamichi et al. 2012; Lamhonwah et al. 2008). Immunohistochemical analysis revealed that OCT2 was detected by punctate, bouton-like staining in cholinergic, dopaminergic, and serotonergic axon terminals that were co-labeled with presynaptic neurochemical markers in mice (Matsui et al. 2016). OCT2 transports several neurotransmitters, such as acetylcholine and monoamines (Table 1, Matsui et al. 2016), and genetic deletion of *oct2* in mice showed a significant reduction in concentrations of norepinephrine and serotonin in the hippocampus (Bacq et al. 2012), implying the possible regulation of neurotransmitters by OCT2 around the neuronal axon terminals.

Interaction between the axon terminals of neurons and NSCs has been proposed in SGZ and SVZ (Song et al. 2012; Pardal and López Barneo 2016). For example,

Table 1 Expression of OCTs in NSCs and surrounding cells

OCTs	NSCs		Neurons		Astrocytes		Microglia	
	References/ species	Expression	References/ species	Expression	References/ species	Expression	References/ species	Expression
OCT1 (SLC22A1)	Ishimoto et al. (2014) Mouse	N.D. [mRNA] primary cultured cells	–		–		–	
OCT2 (SLC22A2)	Ishimoto et al. (2014) Mouse	N.D. [mRNA] primary cultured cells	Matsui et al. (2016), Nakata et al. (2013) Mouse, rat	[Protein] cerebral cortex, hippocampus, striatum, amygdala, synaptic terminals	Naganuma et al. (2014) Human	N.D. [mRNA] cell line (1321N1)	–	
OCT3 (SLC22A3)	Ishimoto et al. (2014) Mouse	N.D. [mRNA] primary cultured cells	Gasser et al. (2017), Matthaeus et al. (2015), Vialou et al. (2008) Mouse	[Protein] amygdala, hippocampus, cerebral cortex, primary cultured serotonergic neurons	Gasser et al. (2017), Naganuma et al. (2014), Vialou et al. (2008) Human, mouse	[Protein] amygdala, [mRNA] cell line (1321N1)	Gasser et al. (2017) Mouse	[Protein] amygdala
OCTN1 (SLC22A4)	Ishimoto et al. (2014) Mouse	[mRNA, protein] primary cultured cells	Nakamichi et al. (2012), Lamhonwah et al. (2008) Mouse	[mRNA] cerebellum, medulla and pons, hypothalamus, striatum, hippocampus, cerebral cortex	–		Ishimoto et al. (2018) Mouse	[mRNA] primary cultured cells, cell line (BV2)

OCTN2 (SLC22A5)	Ishimoto et al. (2014)	[mRNA] primary cultured cells	Januszewicz et al. (2010), Lamhonwah et al. (2008)	[Protein] olfactory bulb, hippocampus, hypothalamus, cerebellum, cerebral cortex	Inazu et al. (2006)	[mRNA, protein] primary cultured cells	–
	Mouse		Mouse, rat		Rat		
OCTN3 (SLC22A21)	Ishimoto et al. (2014)	[mRNA] primary cultured cells	Januszewicz et al. (2010), Lamhonwah et al. (2008)	[Protein] olfactory bulb, hippocampus, hypothalamus, cerebellum, cerebral cortex	Januszewicz et al. (2010)	[mRNA, protein] primary cultured cells	–
	Mouse		Mouse, rat		Rat		
MATE1 (SLC47A1)	Ishimoto et al. (2014)	N.D. [mRNA] primary cultured cells	–		–		–
	Mouse						
CNT2 (SLC28A2)	–		Li et al. (2013)	[mRNA] cerebral cortex	Li et al. (2013)	[mRNA] cerebral cortex	–
			Mouse		Mouse		
ENT1 (SLC29A1)	–		Li et al. (2013), O'Donovan et al. (2018)	[mRNA] cerebral cortex	Li et al. (2013), O'Donovan et al. (2018)	[mRNA] cerebral cortex	–
			Human, mouse		Human, mouse		
ENT4 (SLC29A4)	Ishimoto et al. (2014)	N.D. [mRNA] primary cultured cells	Engel et al. (2004), Matthaeus et al. (2015)	[mRNA] cerebral cortex, cerebellum, primary cultured serotonergic neurons	Naganuma et al. (2014)	[mRNA] cell line (1321N1)	–
	Mouse		Human, mouse		Human		

(continued)

Table 1 (continued)

Brain microvascular endothelial cells		Choroid plexus epithelial cells		Substrates	
References/species	Expression	References/species	Expression	Endogenous and food components	Drugs
Morris et al. (2017)	Luminal	–		Histidyl-proline diketopiperazine, salsolinol, agmatine	Metformin, oxaliplatin, aciclovir, ganciclovir
Human, rat					
Morris et al. (2017)	Luminal	Morris et al. (2017)	Abluminal	Histidyl-proline diketopiperazine, salsolinol, choline, agmatine acetylcholine, monoamines, creatinine	Metformin, ranitidine, amantadine, oxaliplatin, memantine, cimetidine, linagliptin, Debrisoquine
Human, rat		Rat			
Morris et al. (2017)	–	Morris et al. (2017)	–	Histidyl-proline diketopiperazine, salsolinol, agmatine, monoamines, creatinine	Metformin, cimetidine, ketamine, memantine, cisplatin, lidocaine, desipramine, pramipexole
Human		Rat			
–		–		Ergothioneine, carnitine, stachydrine, homostachydrine	Pregabalin, tiotropium ipratropium, pyrilamine, quinidine, verapamil, doxorubicin, mitoxantrone, gabapentin, oxaliplatin, donepezil, metformin
Morris et al. (2017)	–	–		Carnitine, acetyl-l-carnitine, choline	Etoposide, cephaloridine, tiotropium, emetine, verapamil, spironolactone, mildronate, pyrilamine
Human, mouse, rat					
–		–		Carnitine	–
–		Morris et al. (2017)	–	Nucleosides, creatinine, guanidine, thiamine, estrone-1-sulfate	Cimetidine, metformin, fexofenadine, cephalexin, acyclovir, ganciclovir, procainamide, topotecan
		Human			

Morris et al. (2017)	–	Morris et al. (2017)	–	Adenosine, uridine, inosine, guanosine	Ribavirin, clofarabine, didanosine, 5-floxuridine
Human, rat		Human, mouse, rat			
–		Morris et al. (2017)	–	Purine nucleosides, pyrimidine nucleosides, nucleobases	Cladribine, clofarabine, cytarabine, fludarabine, gemcitabine, ribavirin
		Rat			
–		Morris et al. (2017)	Abluminal	Monoamines, adenosine	Metformin
		Human, mouse			

OCTs, solute carriers transporting organic cations with broad specificity; *NSCs* neural stem cells; –, Unknown, *N.D.* Not detected

the axon terminals of parvalbumin-positive GABAergic interneurons and NSCs are in proximity in the hippocampus, and the neurons regulate the fate of adult quiescent NSCs (Song et al. 2012). The synaptic regulator α-synuclein in dopaminergic nerve terminals is also essential for the maintenance of NSCs in the SVZ (Perez-Villalba et al. 2018). As in these examples, OCTs expressed on the axon terminals could potentially interact with NSCs in the neurogenic niche. OCTs show relatively lower affinity to neurotransmitters compared with the other neurotransmitter-specific transporters, and may play a role in the regulation of their extracellular concentration as neurotransmitters are present at high levels in the vicinity of neurons. For example, OCT2 expressed in the neuronal axon terminals proximate to NSCs in hippocampus might be involved in uptake of such neurotransmitters to maintain the appropriate concentration around the neurogenic niche.

3.2 OCT3/SLC22A3 in the Hippocampus

Among OCTs, OCT3/SLC22A3 is ubiquitously expressed in neurons, astrocytes, microglia, BMECs, and CPECs in the brain, whereas other OCTs, such as OCT1, OCT2, OCTN1-3, and ENT4, are expressed in a few types of cells (Table 1). Immunohistochemical analysis has shown that OCT3 is expressed in neurons and astrocytes of the hippocampus in mice (Vialou et al. 2008). In the hippocampus, neurons and astrocytes are in proximity to NSCs (Bonzano et al. 2018; Cope and Gould 2019). This may imply the possible regulation of NSCs by OCT3 expressed in neurons and astrocytes with NSCs, as OCT3 transports several neurotransmitters, including serotonin, dopamine, and histamine, which are known to activate neurogenesis (Backhouse et al. 1982; Banasr et al. 2004; Höglinger et al. 2004; Molina-Hernndez and Velasco 2008; Klempin et al. 2010; Veena et al. 2011; Masuda et al. 2012; Matthaeus et al. 2015; Saraiva et al. 2019). In neurons, OCT3 is localized on post-synaptic sites and may be involved in the reuptake of serotonin in the synaptic cleft. It is generally considered that OCT3 inhibition in neurons results in antidepressant activity via an increase in serotonin concentration in the synaptic cleft when the high-affinity transporters, including SERT, are saturated or inhibited in neurons (Daws 2009; Couroussé and Gautron 2015). An inhibitor of OCT3, decynium-22, enhanced the antidepressant activity of SSRI fluvoxamine in mice, possibly via inhibition of serotonin clearance (Horton et al. 2013, and see chapter “OCTs in Psychiatric Disorders”).

3.3 OCTN1/SLC22A4 in the Hippocampus

OCTN1 is expressed in the hippocampus in vivo, as shown by PCR and immunohistochemical analysis (Nakamichi et al. 2012; Lamhonwah et al. 2008) and is commonly detected in primary cultures of NSCs, neurons, and microglia in mice (Ishimoto et al. 2014, 2018; Nakamichi et al. 2012) (Table 1). In particular, microglia are very densely populated and proliferative in the neurogenic niche of

SGZ and SVZ, and appear to be closely associated with NSCs (Mosher et al. 2012). It is well known that microglia communicate with NSCs via the secretion of growth factors and chemokines, which regulates neurogenesis (Matsui and Mori 2018; Osman et al. 2019).

Communication between NSCs and microglia via common substrates of common transporters can be speculated to involve ERGO and OCTN1. Both NSCs and microglia incorporated the antioxidant ERGO via OCTN1, to scavenge intracellular reactive oxygen species, followed by the regulation of their proliferation and cell hypertrophy, respectively (Ishimoto et al. 2014, 2018). In cultured microglia, OCTN1 negatively regulated the expression of the inflammatory cytokine IL-1β (Ishimoto et al. 2018), which promotes neuronal differentiation of NSCs (Park et al. 2018). The crosstalk between NSCs and microglia via OCTN1 needs to be further evaluated.

4 Possible Regulation of Neurogenesis by Clinically Used OCT Substrate Drugs

4.1 Metformin

OCTs transport several clinically used organic cation drugs. Brain function is affected by such drugs, especially those that are permeable through the blood-brain barrier, indicating high brain distribution.

The antidiabetic drug metformin is a substrate of OCT1-3, OCTN1, MATE1, and ENT4 (Table 1). Metformin has recently garnered attention because some clinical studies have suggested that metformin usage is associated with a reduced risk of dementia in humans (Campbell et al. 2018; Guo et al. 2014; Ng et al. 2014). In the mouse model of Alzheimer's disease, 3xTg, metformin restored deficits in neurogenesis and spatial memory via activation of both atypical protein kinase C (aPKC) and CREB-binding protein (CBP) followed by a decrease in the expression of monoacylglycerol lipase (Wang et al. 2012; Syal et al. 2020). Treatment with metformin also restored the impairment of neurogenesis and spatial memory in an $AlCl_3$-induced mouse model of neurodegeneration (Ahmed et al. 2017).

4.2 Ketamine

The anesthetic drug ketamine is an antagonist of the N-methyl-D-aspartate (NMDA) receptor and exhibits rapid antidepressant efficacy in patients with treatment-resistant depression (Kraus et al. 2017). This mode of action can be quite different from clinically used SSRIs, which take weeks to months to exert antidepressant activity. A single intraperitoneal ketamine administration (10 mg/kg) elevated the densities of neuronal progenitors and newborn granule cells in the ventral hippocampus related to emotion in adult mice, as shown by immunohistochemical analysis, and the FST showed antidepressant activity at 13 days post-injection (Yamada

and Jinno 2019). Clarke et al. also showed that three ketamine injections (10 mg/kg for 2 weeks) reduced immobility time in FST both 2 and 8 days after the final injection and increased the number of Dcx-positive cells in the hippocampal dentate gyrus (Clarke et al. 2017). These results suggested that ketamine-induced neurogenesis was partially involved in its antidepressant activity. OCT3 is considered an important transporter involved in the disposition of ketamine (Keiser et al. 2018) and is expressed commonly in various neural cells (Table 1). It is possible that OCT3 may regulate ketamine concentration in the neurogenic niche, and consequently, ketamine-induced neurogenesis and antidepressant activity.

4.3 Memantine

Like ketamine, the anti-Alzheimer's disease drug memantine is also an NMDA receptor blocker and has neurogenic activity. A single intraperitoneal injection of memantine (50 mg/kg) increased the number of BrdU-positive cells (a proliferating cell marker) in the dentate gyrus of the hippocampus of both 3- and 12-month-old mice (Maekawa et al. 2009). The mechanism underlying the neurogenic actions of memantine and ketamine has not yet been fully clarified, but the neurogenic activities may be caused by inhibition of NMDA receptors, as activation of NMDA receptors rapidly decreased the number of cells synthesizing DNA in the adult hippocampus, whereas inhibition of NMDA receptors rapidly increased the number of cells in the S phase, as identified with [^{3}H]-thymidine (Cameron et al. 1995). Memantine was transported by OCT2 in in vitro transport studies (Busch et al. 1998; You and Morris 2014); however, the involvement of this transporter in the regulation of memantine concentration in the brain has not yet been demonstrated.

4.4 Gabapentin

The anti-epileptic drug gabapentin is a ligand of the α2δ-subunit of N-type calcium channels and increased the number of newborn mature neurons generated from adult hippocampal NSCs in vitro according to immunocytochemical analysis (Valente et al. 2012). Chronic intraperitoneal treatment with another α2δ ligand, pregabalin, at 10 mg/kg for 21 days significantly increased the number of adult-generated neurons positive for BrdU and NeuN double staining in the hippocampal region in mice, and the TST and FST showed the antidepressant-like activity of pregabalin in mice subjected to chronic restraint stress. Gabapentin is a substrate of OCTN1, and a polymorphism in the *octn1* affects renal clearance of this drug (Urban et al. 2008). Pregabalin is also transported by OCTN1 (You and Morris 2014), although the association of this transporter with the induction of neurogenesis by these drugs is still unknown.

5 Future Perspectives

In this chapter, the possible involvement of OCTs and their substrates in neurogenesis has been reviewed. As neurogenesis plays pivotal roles in brain homeostasis, these transporters are useful in therapies targeting neurogenesis, which may be applicable to various neuropsychiatric disorders, and their neurogenic substrates are potential therapeutic and preventive agents for these disorders. Moreover, inhibition of these OCTs by clinically used drugs and other compounds expressed in NSCs and the neurogenic niche may affect neurogenesis by inhibiting the influx and efflux of neurogenic substrates. However, there is still limited evidence for the clinical relevance of transporter-mediated neurogenesis because of the difficulty of direct analysis of phenotypes in the human brain. To demonstrate OCT-mediated neurogenesis in humans, the use of postmortem brains, human iPS-NSCs, and biomarkers for neurogenesis would be helpful. Further analyses using these promising tools may enable the development of novel drugs targeted to OCTs for the treatment of neuropsychiatric disorders.

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Organic Cation Transporter (OCT/OCTN) Expression at Brain Barrier Sites: Focus on CNS Drug Delivery

Robert D. Betterton, Thomas P. Davis, and Patrick T. Ronaldson

Contents

Abstract

Therapeutic delivery to the central nervous system (CNS) continues to be a considerable challenge in the pharmacological treatment and management of neurological disorders. This is primarily due to the physiological and biochemical characteristics of brain barrier sites (i.e., blood–brain barrier (BBB), blood–cerebrospinal fluid barrier (BCSFB)). Drug uptake into brain tissue is highly restricted by expression of tight junction protein complexes and adherens junctions between brain microvascular endothelial cells and choroid plexus

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epithelial cells. Additionally, efflux transport proteins expressed at the plasma membrane of these same endothelial and epithelial cells act to limit CNS concentrations of centrally acting drugs. In contrast, facilitated diffusion via transporter proteins allows for substrate-specific flux of molecules across the plasma membrane, directing drug uptake into the CNS. Organic Cation Transporters (OCTs) and Novel Organic Cation Transporters (OCTNs) are two subfamilies of the solute carrier 22 (SLC22) family of proteins that have significant potential to mediate delivery of positively charged, zwitterionic, and uncharged therapeutics. While expression of these transporters has been well characterized in peripheral tissues, the functional expression of OCT and OCTN transporters at CNS barrier sites and their role in delivery of therapeutic drugs to molecular targets in the brain require more detailed analysis. In this chapter, we will review current knowledge on localization, function, and regulation of OCT and OCTN isoforms at the BBB and BCSFB with a particular emphasis on how these transporters can be utilized for CNS delivery of therapeutic agents.

Keywords

Blood–Brain Barrier (BBB) · Blood–Cerebrospinal Fluid Barrier (BCSFB) · Brain parenchymal transporters · CNS drug delivery · Organic cation transport

1 Introduction

Targeted drug delivery to the CNS requires overcoming anatomical barriers that restrict blood-to-brain transport of therapeutic molecules. The two principal barrier tissues that separate the peripheral circulation from brain parenchyma include the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCFSB) (Fig. 1). The BBB is composed of endothelial cells where the paracellular cleft between adjacent cells is "sealed" by tight junction protein complexes. These brain microvascular endothelial cells acquire a barrier phenotype through communication with glial cells (i.e., astrocytes, microglia), pericytes, and neurons as well as via protein and enzymatic components of the extracellular matrix, a concept referred to as the neurovascular unit (NVU). For example, expression of tight junction proteins and adherens junction constituents is regulated through trophic factors that are released from pericytes and astrocytes. Such mechanisms aid in maintenance of BBB functional integrity. Interactions between brain microvascular endothelial cells and other constituents of the NVU enable a rapid response to environmental changes by allowing cerebral blood flow to be matched with brain metabolic demands (Iadecola 2017). Indeed, current knowledge on brain barriers emphasizes the fact that NVU components work in concert to enable dynamic responses to pathological and pharmacological stressors including selective solute uptake from blood into brain tissue. Similarly, the BCSFB is comprised of a monolayer of choroid plexus epithelial cells that also possess tight junctions. Tight junctions at the BCSFB

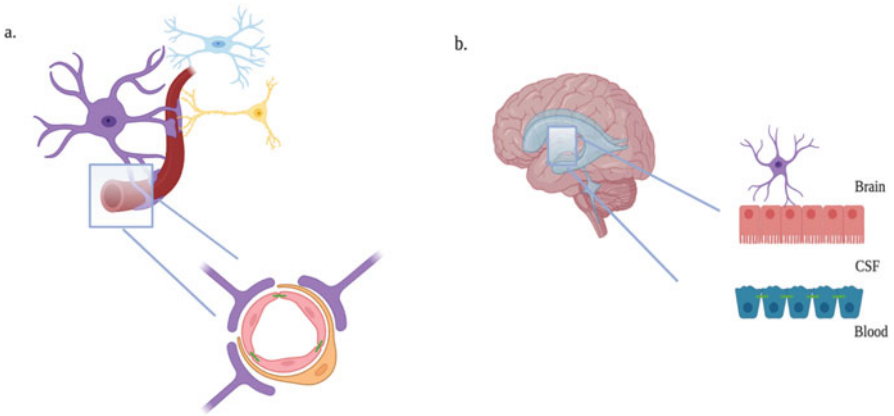

Fig. 1 Structure of CNS barriers. (**a**) Blood–brain barrier composed of endothelial cells lining the systemic circulation expressing numerous tight junction protein complexes and adherens junctions (green) regulated by other cells within the neurovascular unit including the astrocytes (purple), neurons (yellow), microglia (blue), and pericytes (orange). (**b**) Blood–cerebrospinal fluid barrier composed of choroid plexus epithelial cells (blue) expressing tight junction protein complexes to regulated molecular passage from the fenestrated capillaries into the cerebrospinal fluid (Created with biorender.com)

function in a manner similar to that of the BBB by limiting free passage of circulating substances from fenestrated capillaries into the cerebrospinal fluid (CSF) that is separated from brain tissue by a layer of ependymal cells (Hosoya and Tachikawa 2011). Transendothelial/transepithelial electrical resistance (TEER) values for the BCSFB are less than that of the BBB (i.e., approximately 150 Ω cm^2 for the BCSFB versus 1,500–2,000 Ω cm^2 for the BBB), which suggests that the BCSFB is somewhat leaky relative to the BBB (Redzic 2011; Lochhead et al. 2017). Nonetheless, paracellular transport across the BBB and BCSFB is limited to small molecular weight substances that can pass transcellularly through brain microvascular endothelial cells or choroid plexus epithelial cells (Liddelow 2015; Brzica et al. 2017). Passive diffusion across brain barrier cellular layers is a potential mechanism for uptake; however, drug physicochemical properties (i.e., molecular weight, hydrophilicity, pK_a, number of hydrogen bond acceptors and donors) can limit the effectiveness of this therapeutic delivery route (Mikitsh and Chacko 2014). Additionally, drugs that are capable of partitioning into the plasma membrane are often substrates for ATP-dependent efflux transporters such as P-glycoprotein (P-gp), Multidrug Resistance Proteins (MRPs in humans; Mrps in rodents), and Breast Cancer Resistance Protein (BCRP in humans, Bcrp in rodents) (Chaves et al. 2014; Abdullahi et al. 2017; Yang et al. 2018). These transporters are involved in cellular extrusion of drugs and constitute a considerable biochemical barrier to effective brain delivery of therapeutic agents. In general, P-gp transports cationic or basic and neutral compounds while MRPs/Mrps are involved in cellular efflux of anionic drugs and their glucuronidated, sulfated, and glutathione-conjugated metabolites (Polli et al. 2009; Ronaldson and Davis 2015). BCRP/Bcrp has

considerable substrate overlap with P-gp and is thought to function in synergy with P-gp to limit drug uptake into brain tissue (Polli et al. 2009; Ronaldson and Davis 2015; Williams et al. 2020). Clearly, the physical and biochemical characteristics of the BBB and BCSFB emphasize a need to consider novel approaches that can enable drugs to attain effective concentrations in the brain. As a result, there is considerable interest in facilitated transport mechanisms that can be exploited for selective delivery of therapeutics to the CNS for treatment of neurological diseases (Razzak and Florence 2019; Williams et al. 2020).

The solute carrier (SLC) superfamily of transporters is responsible for blood-to-brain transport of circulating solutes into brain tissue (Abdullahi et al. 2017; Williams et al. 2020). The subfamily SLC22A is primarily involved in uptake transport of therapeutics from blood to brain and includes numerous antiporters, cotransporters and facilitated diffusion systems such as organic anion transporters (OATs in humans; Oats in rodents), organic cation transporters (OCTs in humans; Octs in rodents), and novel organic cation transporters (OCTNs in humans; Octns in rodents) (VanWert et al. 2010; Zhu et al. 2015). OCTs and OCTNs themselves are comprised of three main subtypes: facilitated diffusion transporters OCT1 (*SLC22A1*), OCT2 (*SLC22A2*), and OCT3 (*SLC22A3*); a cation and carnitine transporter OCTN1 (*SLC22A4*), a sodium carnitine or carnitine derivative cotransporters OCTN2 (*SLC22A5*) and OCTN3 (*SLC22A21*) (Gründemann et al. 1994; Okuda et al. 1996;Gründemann et al. 1998; Tamai et al. 1997; Wu et al. 1998). The membrane potential and concentration gradients of cationic substrates contribute to the driving force for OCT/Oct and OCTN1/Octn1 substrate flux across biological membranes with ion-independent electrogenic transport and predominantly coupled transport with either sodium or protons reported for OCTN2/Octn2- and OCTN3/Octn3-mediated transport (Tamai et al. 2001, 2004). While expression of OCTs and OCTNs has been well characterized in peripheral tissues, their localization and function within CNS barriers require more extensive research. To date, most information involving OCT- and OCTN-mediated transport at the CNS has been derived from non-human cell culture systems, OCT or OCTN-overexpressing cells or *Xenopus* oocytes, and proteoliposome experimentation (Friedrich et al. 2003). It has been shown, at the mRNA level, that all of the known OCT isoforms as well as OCTN2 are expressed in brain microvascular endothelial cells (Geier et al. 2013). Further studies have shown that OCT1–3 and OCTN2 proteins can be found within CNS barrier tissues, with greater expression of transporters observed at the BBB as compared to the BCSFB (Morris et al. 2017). Despite these expression data, the exact localization of OCTs and OCTNs in brain microvascular endothelial cells or choroid plexus epithelial cells has yet to be determined. Such information on localization and substrate specificity of OCT and OCTN transporters can aid in determining their role in facilitating drug delivery to the brain for treatment of neurological diseases. Furthermore, a detailed understanding of OCT and OCTN transport dynamics at brain barrier sites can inform discovery and development of novel centrally-acting therapeutics that display more efficient brain penetration due to selective CNS uptake mediated by cation transport mechanisms.

2 BBB Localization and Expression of OCTs and OCTNs

Central to targeting OCTs for CNS drug delivery is the understanding of their localization at brain barrier tissues. Several studies have shown that cultured brain endothelial cells or brain microvessels are highly enriched with OCT mRNA (Friedrich et al. 2003; Sung et al. 2005; Miecz et al. 2008; Wu et al. 2015). More recently, protein expression of OCT1/Oct1, OCT2/Oct2, and OCT3/Oct3 were reported in the murine brain endothelial cell line (bEND3) and in the human brain microvessel endothelial cell line (hCMEC/d3) (Sekhar et al. 2017). Interestingly, this same study localized Oct1 to both the luminal and abluminal plasma membrane in bEND3 cells; however, Oct1 expression was reported to be significantly higher at the luminal plasma membrane (Sekhar et al. 2017). This observation is consistent with a previous study by Lin and colleagues that also showed elevated luminal expression of Oct1 and Oct2 in cultured rat brain endothelial cells (Lin et al. 2010). Our laboratory has reported protein expression of Oct1 in intact microvessels isolated from rat brain (Brzica et al. 2017; Fig. 2). More recently, global proteomic analysis of human brain microvessels from healthy individuals revealed detectable quantities of OCT1 and OCT3 protein (Al-Majdoub et al. 2019). In contrast, quantitative targeted proteomics has failed to detect either OCT1 or OCT2 at the BBB in hippocampal Brodmann Areas 17 and 39 (Billington et al. 2019). When compared to the work of Al-Majdoub and colleagues, these data imply regional differences in BBB expression of OCTs; however, such results must be confirmed by detailed molecular studies and functional analyses. Although BBB expression of OCT and OCTN isoforms have been detected at the mRNA and/or protein level, there is insufficient evidence to determine the exact localization of these transporters at the apical or basolateral plasma membrane of brain microvessels in vivo. Such information is critical to advancing this family of transporters as a platform for drug delivery.

Typically, translocation of cationic substrates across polarized epithelia involves a two-step process in which OCTs are involved in initial cellular uptake. A good example is organic cation secretion in the human kidney that involves both OCT2 and the multidrug and toxin extrusion transporter (MATE) 1 (Sandoval et al. 2018). In this context, MATE1 functions to ensure extrusion of cationic substrates into the urinary filtrate so that they can be efficiently excreted. At the BBB, evaluation of OCT/Oct localization has indicated that these transporters are preferentially expressed at the luminal plasma membrane of microvascular endothelial cells (Fig. 2). Indeed, functional expression of Octs has been demonstrated at the BBB as evidenced by OCT1/Oct1 and OCT2/Oct2-mediated uptake of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a substance that is able to access brain tissue and does not remain "trapped" within the endothelial cell (Lin et al. 2010). Taken together, these studies suggest that additional transporters such as MATEs may be required to ensure effective delivery of cationic solutes to the brain. Recently, protein expression of MATE1 and MATE2 was detected in hCMEC/d3 cells (Sekhar et al. 2019). Additionally, Mate1 and Mate2 protein expression was observed in microvessels isolated from C57BL6/129 mice (Sekhar et al. 2019) and Mate1

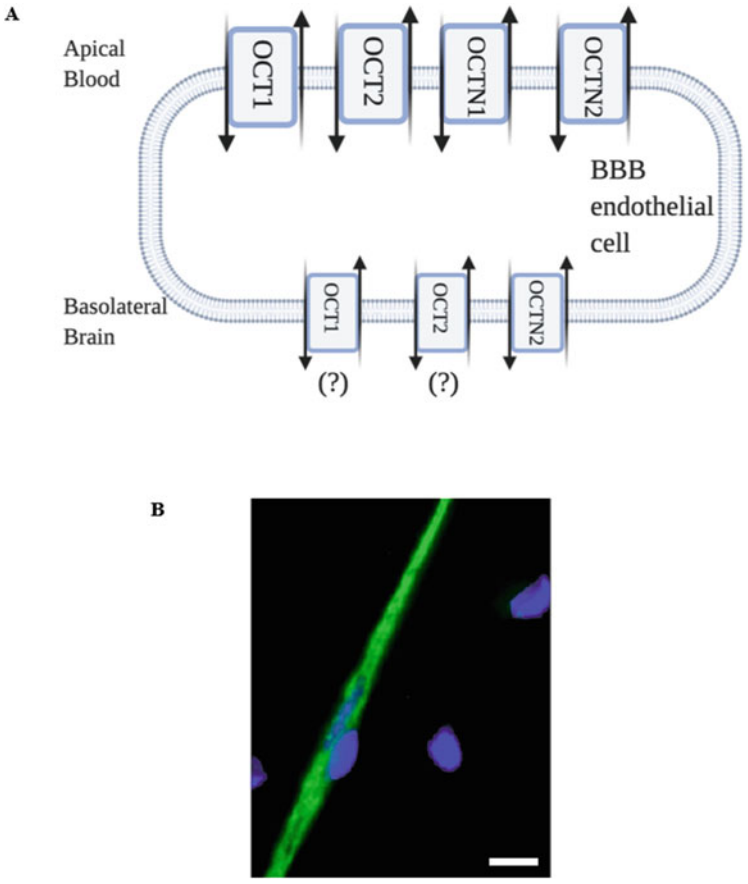

Fig. 2 Proposed localization of OCT and OCTN transporters at the BBB. (**a**) Based on previous studies, various OCT and OCTN isoforms have been detected in brain microvessel endothelial cells (Created with biorender.com). (**b**) Fluorescence confocal microscopy data from our laboratory has shown that Oct1 is expressed in isolated microvessels from the brain of Sprague-Dawley rats. Green = Oct1; Blue = DAPI. Scale bar = 4 μm (Adapted from Brzica et al. J Cent Nerv Syst Dis. 9:1179573517693802, 2017)

mRNA and protein expression was reported in brain capillaries from male ddY mice (Hiasa et al. 2006). More recently, Mate1 mRNA was shown to be expressed in brain microvessels from Swiss, FVB, and C57BL/6JRj mice (Chaves et al. 2020). Of particular significance, MATE1 and MATE2 protein was detected by western blot analysis in human frontal cortex, caudate nucleus, and putamen brain regions (Sekhar et al. 2019). These results are consistent with immunofluorescence staining of human brain microvessels, which demonstrated expression of MATE1 at the BBB (Geier et al. 2013). In contrast, Chaves and colleagues failed to detect MATE isoforms in brain microvessels isolated from human temporal lobe glioma specimens (Chaves et al. 2020). Since these data were derived from human tumor tissue, it is

possible that transporters for organic cations such as MATEs were downregulated in response to cancer pathogenesis or pharmacotherapy. The variability in MATE/Mate expression data represented by these studies indicates the need to further clarify involvement of MATE isoforms as critical transporters that function in concert with OCTs/Octs to deliver cationic substances including drugs across polarized epithelial/endothelial cellular layers in the brain. This information is particularly critical to developing cation transporters as targets for CNS drug delivery given current data implying preferential expression of OCTs/Octs at the luminal plasma membrane of brain microvascular endothelial cells.

While it is generally accepted that neurons require efficient delivery of carnitine to allow for beta-oxidation of fatty acids, expression of OCTN isoforms at the BBB has not been well elucidated (Tracey et al. 2018). Some clarification of this critical issue has been provided in the scientific literature where mRNA and protein expression of OCTN2 has been confirmed within the cerebral microvasculature (Tsuji 2005; Okura et al. 2014). This is consistent with the known physiology for OCTN2, which has increased substrate specificity for carnitine derivatives and can facilitate delivery of these substances to brain parenchyma (Kido et al. 2001). More recently, Kurosawa and colleagues reported measurable expression of OCTN1 and OCTN2 mRNA in human brain microvessel endothelial cells derived from induced pluripotent stem cells as well as in the hCMEC/d3 cell line (Kurosawa et al. 2018). Functional studies that have evaluated substrate permeation across the BBB have allowed for more detailed identification of specific OCTN isoforms. For example, brain accumulation of ergothioneine, a specific OCTN1 substrate, provides functional evidence for OCTN1 expression at the level of the BBB (Tang et al. 2018). In studies using in vitro human brain endothelial cell culture systems, OCTN2 expression was shown to control transport of carnitine derivatives across the cell monolayer, suggesting that expression of this novel organic cation transporter is required for transcellular passage of large quantities of carnitine to promote neuronal homeostasis (Okura et al. 2014). Further evidence for functional OCTN2 expression in human brain endothelial cells was provided by Kurosawa and colleagues who showed that cellular uptake of L-carnitine (K_M = 4.08 ± 2.61 μM; V_{max} = 0.000797 ± 0.000256 nmol/mg protein/min) could be blocked by addition of an excess concentration of unlabeled carnitine (Kurosawa et al. 2018). In an in vitro mouse model, Octn2 has been detected at the protein level in cultured brain endothelial cells and was predominantly localized to the abluminal plasma membrane (Miecz et al. 2008). Overall, these studies provide evidence for localization and functional expression of OCTN isoforms at the BBB (Fig. 2) and suggest that these transporters can be targeted to facilitate drug delivery to the CNS.

3 BCSFB Localization and Expression of OCTs and OCTNs

In addition to the BBB, transporters that are localized to the BCSFB are known to highly regulate permeation of circulating molecules between the systemic circulation, the CSF, and brain interstitial space. The majority of transporters within the

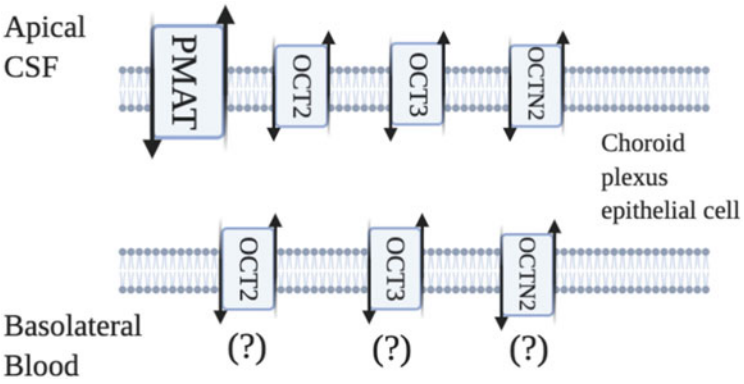

Fig. 3 Proposed localization of OCT and OCTN transporters at the BCSFB (Created with biorender.com)

BCSFB are localized to choroid plexus epithelial cells that form a barrier between the blood and the CSF. Similar to the BBB, BCFSB epithelial cells express tight junction protein complexes, which limit paracellular diffusion of molecules. Although the BCSFB barrier is leakier in comparison with the BBB endothelium, it remains highly efficient in controlling molecular composition of the CSF through selective transcellular transport mechanisms. This includes transporters for cationic substances such as OCT/Oct and OCTN/Octn isoforms (Fig. 3). At the mRNA level, Oct1, Oct3, Octn1, and Octn2 have been detected in choroid plexus epithelium from Sprague-Dawley rats (Choudhuri et al. 2003). In contrast, Sweet and colleagues detected mRNA expression of Oct2 and Oct3, but not Oct1, at the adult rat choroid plexus (Sweet et al. 2001). In this particular study, transfection of GFP-tagged Oct2 was primarily localized to the apical membrane of choroid plexus epithelium (Sweet et al. 2001). Protein expression of Oct2 was exclusively detected in choroid plexus epithelial cells isolated from the third ventricle of pig brain (Uchida et al. 2020). Consistent with data of Sweet and colleagues, Oct2 was proposed to be localized to the apical membrane of choroid plexus epithelial cells and to function in concert with Mate1 to facilitate removal of cationic substances from the CSF (Uchida et al. 2020). Interestingly, this same study demonstrated Octn2 protein expression in choroid plexus isolated from all cerebral ventricles (Uchida et al. 2020). Additionally, the plasma membrane monoamine transporter (PMAT; Slc29a4), a novel identified multispecific organic cation transporter, has been identified at the apical membrane of choroid plexus epithelial cells (Engel et al. 2004; Duan and Wang 2013). The functional role of PMAT at the choroid plexus has been demonstrated in a Pmat knockout mouse model. Specifically, choroid plexus epithelial uptake of cationic substrates (i.e., dopamine, 5-HT, 1-methyl-4-phenylpyridinium (MPP^+)) was reduced in Pmat(−/−) mice (Duan and Wang 2013). Furthermore, substrate uptake in these mice was insensitive to pharmacological Oct or neurotransmitter transporter inhibitors (Duan and Wang 2013), data that further confirms the functional importance of PMAT in the uptake of cationic substances into choroid plexus epithelial

cells. High levels of Oct3 protein have been reported in choroid plexus epithelium isolated from male Wistar rats (Nakayama et al. 2007) and in mouse choroid plexus epithelium and ependymal cells (Vialou et al. 2004). Of particular note, antisense oligonucleotides targeting Oct3 and injected directly into the CSF increased brain concentrations of the Oct3 transport substrate methamphetamine, suggesting that this organic cation transporter is localized to the apical membrane of choroid plexus epithelial cells and can participate in extrusion of potentially toxic organic cations from the brain (Nakayama et al. 2007). In the context of drug delivery, further work needs to be done to confirm localization of OCT/Oct and/or OCTN/Octn isoforms to the basolateral plasma membrane of choroid plexus epithelial cells. Such information is critical in determining the therapeutic potential of targeting the BCSFB to enable therapeutic delivery directly into the CSF.

4 Localization of OCTs and OCTNs in Brain Parenchyma

Once cationic substrates have bypassed brain barrier tissues, they can also display cell-type specific uptake due to functional expression of OCTs/Octs and OCTNs/Octns within brain parenchyma cell types (Fig. 4). For example, neurons have been shown to express numerous OCT and OCTN isoforms that facilitate uptake of specific substrates (i.e., choline, carnitine, thiamine, ergothioneine) and neurotransmitters within the brain to mediate chemical signaling within neural networks (Busch et al. 1998; Duan and Wang 2010; Januszewicz et al. 2010). In neurons, expression of OCTN2 is required for uptake of carnitine to allow for beta-oxidation of fatty acids and subsequent increases in energy production and control of neurological functions (Pochini et al. 2019). Several OCT isoforms have substrate selectivity for precursor molecules required for neuronal synthesis of acetylcholine

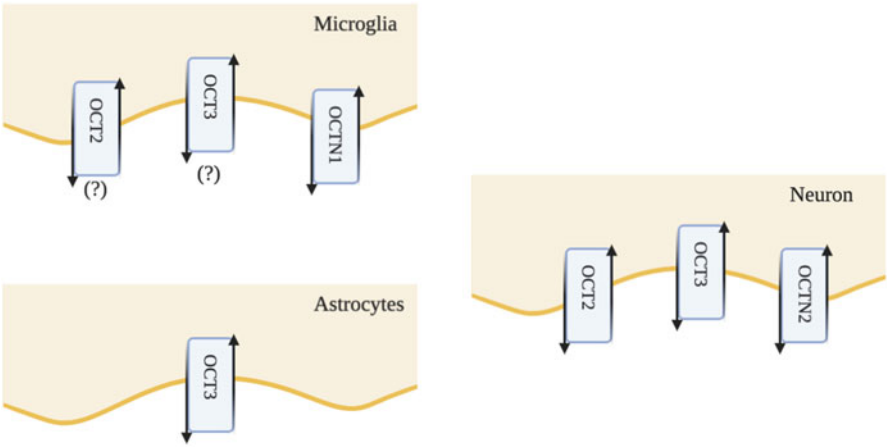

Fig. 4 Proposed localization of OCT and OCTN transporters in neurons and in glial cells within brain parenchyma (Created with biorender.com)

and dopamine. Indeed, expression of OCT2 and OCT3 within neuronal cells and OCT3 in glial cells illustrates the necessity of these transporters for adequate delivery of precursory subunits used in neurotransmitter synthesis (Blakely and Edwards 2012). Reuptake of neurotransmitters by neuronal and astrocytic OCT2 and OCT3 has also been identified as an essential regulatory mechanism for neurotransmission (Bacq et al. 2012; Nishijima and Tomiyama 2016). In studies performed using in vitro and murine models, brain parenchymal transport of dopamine, serotonin, epinephrine, norepinephrine, and histamine was shown to require functional expression of OCT2 while OCT3/Oct3 is involved in cellular uptake of dopamine and serotonin (Baganz et al. 2010; Kristufek et al. 2002; Yoshikawa et al. 2013). Immunohistochemical analysis of murine brain tissue confirmed localization of Oct2 expression primarily within neurons while Oct3 localization was detected in both neuronal and glial cells (Vialou et al. 2004). Localization of Oct3 in neurons is more controversial with some studies proposing expression of this transporter proximal to the synaptic cleft (Gasser et al. 2017) while others imply that Oct3 expression is exclusively found on astrocyte processes (Furihata and Anzai 2017). Such observations have led to the hypothesis that parenchymal Oct3 expression may vary depending upon brain region. In microglia, it has been proposed that OCTN1 expression and transport of homeostatic substrates such as ergothioneine can control pathological functions such as production of reactive oxygen species and release of proinflammatory cytokines (Ishimoto et al. 2018). In contrast, Oct2 or Oct3 mRNA was not detected in a mouse microglial cell line (BV_2) (Fan et al. 2018). In terms of pharmacotherapy, numerous therapeutics (i.e., amphetamines, anti-convulsant agents, neuroprotective drugs) require selective uptake by OCT isoforms so that they can access molecular targets in the brain (Table 1). Therefore, consideration of OCT and OCTN localization and functional expression in brain tissue is critical to understanding drug distribution within the brain as well as the ability of centrally acting cationic drugs to access their molecular targets and elicit a therapeutic effect.

5 Regulation of OCTs and OCTNs at Brain Barrier Sites

Targeting OCTs and OCTNs for optimized CNS delivery of centrally acting drugs requires an understanding of the "cellular machinery" involved in their regulation. Such pathways offer a unique opportunity to control OCT- and/or OCTN-mediated transport over a time course conducive to effective blood-to-brain drug uptake and, by extension, more efficacious pharmacotherapy. The regulation of OCT and OCTN isoforms that are expressed within the BBB and BCSFB is poorly understood; however, regulation in peripheral tissues (i.e., liver, kidney, and bronchial epithelial cells) can provide essential insights as to how these transporters may be regulated at brain barrier sites. Transcriptional regulation of OCTs/OCTNs involves binding of regulatory proteins to distinct binding sites in the gene promoter to modulate mRNA expression. Upstream binding factors that control de novo synthesis of these critical cation transporters include the upstream binding factor (USF)1, USF2, hepatic nuclear factor 4α (HNF-4α), and CCAAT/enhancer-binding proteins (Saborowski

Table 1 Key centrally-acting transport substrates for OCTs and OCTNs (adapted from Koepsell 2020)

Transporter	Endogenous substrates	Exogenous substrates
OCT1	5-HT, DA, NE, ACh, epinephrine, histamine, agmatine, salsolinol, tyramine, choline	Amantadine, amisulpride, berberine, butylscopolamine, O-desmethyltramadol, fluoxetine, haloperidol, ketamine, memantine, morphine, perphenazine, pramipexole, sumatriptan, sulpiride, varenicline, rizatriptan, naratriptan, sumatriptan, zolmitriptan
OCT2	5-HT, DA, NE, ACh, epinephrine, histamine, agmatine, salsolinol, choline, thiamine	Amisulpride, apomorphine, butylscopolamine, memantine, chlorprothixene, fampridine, ketamine, lappaconitine, methamphetamine, morphine, phenamil, selegiline, sulpiride, varenicline
OCT3	5-HT, DA, NE, ACh, epinephrine, histamine, agmatine, salsolinol, tyramine, thiamine	Amisulpride, butylscopolamine, ketamine, sulpiride
OCTN1	Cholines, ergothioneine, stachydrine	Amisulpride, bupropion
OCTN2	Cholines, acetyl-L-carnitine, D-carnitine, L-carnitine	Amisulpride

5-HT 5-hydroxytryptamine (serotonin), *DA* dopamine, *NE* norepinephrine, *ACh* acetylcholine

et al. 2006; Kajiwara et al. 2008; Rulcova et al. 2013). For example, treatment with dexamethasone, a glucocorticoid receptor agonist, has been shown to enhance cellular HNF-4α levels and subsequently increase expression of OCT1 within cultured human hepatocytes (Rulcova et al. 2013). HNF-4α has also been identified as a regulatory transcription factor in brain tissue (Wang et al. 2013; Niehof and Borlak 2009; Xu et al. 2011). Furthermore, evidence has been shown that regulation of numerous drug transporters at choroid plexus cells within the BCSFB is associated with HNF expression patterns (Wang et al. 2013; Niehof and Borlak 2009). Of particular note, cell-type specific and species-dependent variability of HNF-4α expression patterns can lead to variable OCT transporter expression (O'Brien et al. 2013; Lau et al. 2018). Indeed, upregulation of HNF-4α in brain microvessel endothelial cells and/or choroid plexus epithelial cells offers an opportunity to control OCT/Oct-mediated transport and optimize CNS disposition of centrally acting cationic drugs. Additionally, rodent Oct2 expression and transport activity is sensitive to exposure of sex-related hormones. Testosterone treatment has been shown to increase epithelial cell expression of Oct2 mRNA and to stimulate cellular uptake of the prototypical Oct transport substrate triethylammonium (TEA) (Urakami et al. 2000). In contrast, estradiol treatment caused an opposite response characterized by reduced Oct2 mRNA and decreased TEA transport (Urakami et al. 2000). Further evidence has implicated peroxisome proliferator-activated receptor (PPAR)-γ in the upregulation of OCTN2 expression within colonic epithelial cells and estrogen mediated upregulation within breast cancer cells (Maeda et al. 2008).

PPAR-γ signaling pathways may prove to be an important regulatory target for cation transporters because they are expressed at brain barrier sites and have been implicated in the regulation of various transporters that are involved in determining tissue drug permeation (More et al. 2017; Stopa et al. 2018). While more detailed molecular studies must be completed to confirm regulation of Oct isoforms by HNF-4α or steroid pathways at brain barrier sites, these data do provide a framework for experiments that can improve our understanding of cation transporter regulation at the BBB and/or BCSFB. The future implication of this work is an ability to control cation transport in brain barrier tissues in an effort to provide more effective treatment of neurological diseases.

Aside from pathways stimulated by steroid hormones, multiple other intracellular pathways have been identified as causal factors in altered cation transporter expression and decreased mRNA levels. For example, functional changes to human embryonic kidney (HEK) cellular uptake of a fluorescent OCT1 transport substrate (i.e., 4-(4-(dimethylamino)styryl)-N-methylpyridinium (ASP)) have identified specific regulatory mechanisms for this transporter that involve decreased activation of protein kinase A (PKA) via inhibition of calmodulin, calcium dependent CaM-kinase II, or selective $p56^{lck}$ tyrosine kinase (Ciarimboli et al. 2004). At the functional level, decreased PKA activity resulted in a reduction in ASP uptake (Ciarimboli et al. 2004). Further post-translational downregulation of Oct isoforms has been identified in ischemic models. Of particular note, these studies have identified the role of ischemia/reperfusion inducible protein (IRIP), a regulatory protein (RS1) pathway that co-regulates the expression and membrane translocation of several transporters including OCT1 and MATE1 (Li et al. 2013). The work of Li and colleagues demonstrated that overexpression of IRIP in transfected HEK293 cells resulted in reduced transport of the prototypical organic cation MPP^+ by both OCT1 and MATE1. IRIP-induced downregulation of OCT-mediated transport may result from altered trafficking of OCT1 from the Golgi to the cell membrane (Jiang et al. 2005). Of particular note, Jiang and colleagues showed that phosphorylation of the N-terminal RS1 domain on the *SLC22A1* gene following activation of IRIP led to decreased exocytosis of OCT1 from Golgi vesicles (Jiang et al. 2005). Post-translational modification of OCT isoforms has also been shown to play a role in the regulation of these transporters. Specifically, regulation of OCT transporter activity was observed to occur via changes in the phosphorylation state of tyrosine residues localized to intracellular loop domains, thereby altering transport kinetics and reducing OCT-mediated transport (Sprowl et al. 2016).

Studies involving regulation of OCTN are less abundant in the scientific literature. Transcriptional regulation of OCTN1 has been shown to be modulated by the Runt-related transcription factor 1(RUNX1), which binds to the first intron of the OCTN1 (*SLC22A4*) gene (Tokuhiro et al. 2003). Heat-shock factors have also been identified as critical regulators of OCTN1 and OCTN2 expression as binding of heat-shock protein 70 (Hsp70) to the promoter of the *SLC22A4* and *SLC22A5* genes can modulate transport of the cationic substrates TEA and carnitine (Peltekova et al. 2004). More recent studies have pointed towards epigenetic modifications as critical regulators of OCT and OCTN functional expression. For example, hypermethylation to the promoter region of the *SLC22A2* gene is associated with reduced OCT2

protein expression in human hepatocytes (Aoki et al. 2008). In contrast, the level of methylation of CpG sites in the *SLC22A2* promoter was lower in the kidney, an organ known to have much higher expression of OCT2 relative to the liver (Aoki et al. 2008). Similarly, previous studies on OCT2 localization at the BBB and BCSFB have shown higher levels of this cation transporter relative to OCT1; however, epigenetic modulation of OCT isoforms at brain barrier sites has not yet been determined. Such regulation is an important avenue to consider in an effort to fully understand both tissue-specific expression of OCTs and OCTNs and molecular regulation of these critical transport proteins.

5.1 CNS Delivery by OCTs and/or OCTNs of Centrally Acting Drugs for Treatment of Neurological Diseases

Targeting OCTs and OCTNs offers a unique opportunity to optimize CNS delivery of cationic therapeutic agents. Indeed, the potential for utilizing this family of transporters to improve CNS pharmacotherapy is supported by previous studies demonstrating blood-to-brain uptake of MPTP by Oct1 and Oct2 (Lin et al. 2010; Wu et al. 2015) as well as brain delivery of acetyl-L-carnitine that is mediated by OCTN2 at the BBB (Inano et al. 2003). Interestingly, current knowledge on localization of OCTs and OCTNs at brain barrier sites suggests that blood-to-brain transport of cationic substrates may occur primarily at the BBB. In contrast, cation transporters expressed at the BCSFB appear to be primarily involved in extrusion of potentially toxic organic cations from the CSF and/or maintenance of CSF carnitine concentrations. Therefore, this section will describe opportunities for improving CNS drug delivery that will primarily focus on targeting OCTs and OCTNs at the BBB. Below, we provide examples of neurological diseases that can be treated using cationic drugs and insights as to how OCTs and/or OCTNs can be targeted for optimized CNS delivery of such compounds. Examples of therapeutic drugs that are known transport substrates for OCTs and/or OCTNs are presented in Table 1.

5.2 Ischemic Stroke

Stroke is a neurological and vascular disease resulting from impairment of blood flow to a specific brain region. This restriction in cerebral blood flow results in decreased nutrient (i.e., glucose, oxygen) delivery to neurons and other cell types within the brain parenchyma. Additionally, the BBB is disrupted in ischemic stroke, an effect that contributes to development of vasogenic edema and/or hemorrhagic transformation (An et al. 2017; Abdullahi et al. 2018). Stroke is the third leading cause of death within developed nations following coronary disease and cancers and it affects a large subset of the population with progressively increasing incidence rates over the past decade (Gorelick 2019). The severity of stroke symptoms and the potential for post-stroke neurological recovery greatly depends upon physical factors (i.e., the region of the brain that is impacted) as well as environmental factors (i.e.,

mood and motivation), social factors (i.e., family support), and therapeutic factors (i.e., early start of pharmacotherapy and rehabilitation). Clinical signs that are utilized to indicate acute stroke onset follow the FAST acronym and include facial droop (F), arm weakness (A), and impaired speech (S), which indicate that it is time (T) to go to the hospital (Musuka et al. 2015).

Central to the pathophysiology of ischemic stroke is the NVU. Deprivation of oxygen and glucose leads to an irreversibly damaged ischemic core and potentially salvageable surrounding tissue known as the penumbra (Liu et al. 2010; Abdullahi et al. 2018). This process causes neuronal cell death in the core and substantial neuronal injury in the penumbra. Additionally, cell–cell interactions and signaling occur in a coordinated manner between the multiple NVU cell types and matrix constituents, events that lead to BBB dysfunction and further CNS injury. Indeed, BBB permeabilization enables blood-borne substances that are normally peripherally restricted, such as excitatory amino acids, kinins, prostaglandins, metals, and proteins, to enter the CNS and accelerate cell death in ischemic brain tissue (Thompson and Ronaldson 2014). Oxidative stress injury secondary to reperfusion is a critical process that leads to BBB dysfunction. Such mechanisms are accelerated by reperfusion (i.e., recanalization) and restoration of oxygen supply, which results in production of reactive oxygen and nitrogen species, such as superoxide, nitric oxide, and peroxynitrite, within the endothelium (Heo et al. 2005; Garcia-Bonilla et al. 2014). Oxidative stress in excess of the antioxidant capacity of the endothelial cell leads to alterations in organization and localization of tight junction protein complexes and contributes to endothelial dysfunction and increased BBB permeability (Lochhead et al. 2010, 2012). Such endothelial dysfunction permits movement of water and circulating proteins into brain parenchyma (Heo et al. 2005; Sandoval and Witt 2008; Brouns et al. 2011). In an in vivo global hypoxia-reoxygenation system, which models a component of stroke, oxidative stress due to reoxygenation caused changes in both structure and localization of occludin oligomeric assemblies at the tight junction and increased permeability of the BBB to [14C]-sucrose (Lochhead et al. 2010; McCaffrey et al. 2009; Witt et al. 2003). Sucrose is a vascular marker that does not permeate the BBB under normal physiological conditions (Lochhead et al. 2020). Clinically, such BBB changes are evident in patients with stroke 3–4 h following stroke onset (Giraud et al. 2015). Vasogenic edema following ischemia/reperfusion is a consequence of BBB disruption due to phasic tight junction disruption and MMP-9 activity, which leads to extravasation of fluid and plasma proteins into brain parenchyma. When fluid is permitted to accumulate in the extracellular space, brain volume is increased in concordance with intracranial pressure due to vasogenic edema (Michinaga and Koyama 2015; Witt et al. 2008). Consequences of increased BBB permeability following ischemic stroke are not limited to fluid extravasation. Blood–brain barrier dysfunction can lead to uncontrolled leak of exogenous xenobiotics, including drugs, into brain parenchyma. Pharmacologic interventions aimed at BBB protection can prevent this exacerbation of brain tissue damage and promote stroke recovery. Preservation of BBB integrity is critical to maximize stroke recovery and to provide

optimal CNS delivery of drugs with neuroprotective properties (Brzica et al. 2017; Williams et al. 2020).

As a stroke therapeutic, memantine functions as an antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors. During ischemia, decreased CNS concentrations of oxygen and glucose can trigger increased neuronal calcium influx, a process that results in enhanced release of the excitatory neurotransmitter glutamate. Excessive synaptic accumulation of glutamate is associated with neuronal cell death, a pathological condition known as excitotoxicity. Pharmacological blockade of NMDA receptors is known to protect against such neuronal cell death and it is on this basis that memantine has been developed as a neurotherapeutic with efficacy in the setting of stroke. Memantine is a small molecule that can cross biological membranes by passive transcellular diffusion; however, it is also predominantly positively charged at physiological pH as demarcated by a pKa of 10.27 (Mehta et al. 2013). The consensus is that memantine requires a specific transport mechanism to be taken up into target tissues. At present, transport properties of memantine at the BBB have not been fully elucidated; however, memantine has been reported to be a substrate for proton-coupled transport systems, such as OCT1 and OCT2 (Mehta et al. 2013). Studies in *Xenopus laevis* oocytes transfected with OCT2 showed saturable uptake transport ($K_m = 34 \pm 5$ μM) for memantine (Busch et al. 1998). More recently, memantine uptake via in situ transcardiac perfusion in Swiss outbred mice was shown to be independent of transmembrane electrochemical potential (i.e., changes in K+ concentration in the perfusate), which is an established characteristic of OCT1–3 mediated transport (Mehta et al. 2013). Additionally, memantine uptake was increased in the presence of an enhanced outwardly directed proton gradient, commonly observed in OCTN1 mediated transport (Mehta et al. 2013). In contrast, in studies using an immortalized human brain endothelial cell line, uptake of memantine was not inhibited by ergothioneine, an OCTN1 substrate (Higuchi et al. 2015). Indeed, the exact mechanism of memantine transport across the BBB requires more extensive research; however, therapeutic targeting of memantine to the CNS via OCT-dependent drug delivery may prove to be an effective mechanism to enhance the utility of this neuroprotective drug in ischemic stroke therapy. Furthermore, targeting of novel neuroprotective drugs to OCT or OCTN isoforms at the BBB is a viable strategy to ensure effective therapeutic delivery of such compounds to the ischemic brain.

Similar to memantine, several natural products that have been shown to elicit neuroprotective or vascular protective effects within the brain are also substrates for OCTN1 (Zhang et al. 2020; Koh et al. 2020). In human brain microvascular endothelial cells, ergothioneine exhibited vascular protection as demarcated by suppression of NADPH-1 oxidase and increased activity of glutathione reductase, catalase, and superoxide dismutase (Li et al. 2017). These beneficial effects were abolished when human brain endothelial cells were treated with an siRNA probe that selectively targeted OCTN1, suggesting the requirement of this transporter to facilitate protective effects associated with ergothioneine (Li et al. 2017). Indeed, ergothioneine has been reported to be a high-affinity transport substrate for OCTN1 (Gründemann et al. 2005; Engelhart et al. 2020). Recently, stachydrine, a

component of Japanese motherwort, was shown to protect against neurological deficits in male Sprague-Dawley rats subjected to experimental ischemic stroke (Li et al. 2020). Work by Grundemann and colleagues demonstrated that stachydrine is a transport substrate for OCTN1 (Gründemann et al. 2005), providing evidence for an endogenous mechanism that enables this natural product to traverse the BBB. As such, expression of OCTN1 in cerebral endothelial cells is a crucial mechanism that enables neuroprotective substrates to access brain parenchyma, thereby limiting pathology mediated degradation of neurons and, possibly, glial cells.

5.3 Parkinson's Disease

Parkinson's disease is a neurological disorder associated with progressive loss of voluntary motor control, increased muscle rigidity, and resting muscle tremors resulting from impaired striatal dopaminergic neurotransmission (DeMaagd and Philip 2015). Affecting more than 60,000 people per year, Parkinson's disease has become the second most common neurodegenerative disease to Alzheimer's disease with increased incidence rates observed in aging populations. The etiology of Parkinson's disease is associated with both genetic (i.e., inherited Parkinson's disease) and environmental factors (i.e., idiopathic or sporadic Parkinson's disease), which both result in elevation of alpha-synuclein accumulation within brain tissue and selective loss of dopaminergic neurons (Ball et al. 2019). Anatomically, dopaminergic neuronal loss is reflected by loss of pigmentation within the substantia nigra, which is observed in post-mortem brain tissue collected from patients with a positive diagnosis of Parkinson's disease. Evidence that dopaminergic cell loss is the root cause of Parkinson's disease comes from observations that the cationic neurotoxin MPP^+ induces symptoms of Parkinson's disease in animal models (Kopin 1992). MPP^+ is produced via a monoamine oxidase B (MAO-B)-mediated oxidation reaction of MPTP, a highly lipophilic substance that can readily cross the BBB. Additionally, MPP^+ is a known transport substrate of OCT isoforms, a mechanism that can contribute to its ability to permeate the neuronal plasma membrane and inhibit regeneration of ATP by oxidative phosphorylation and trigger neuronal apoptosis (Langston 2017; Hörmann et al. 2020). While the exact process of selective MPP^+ toxicity in dopaminergic neurons has yet to be fully elucidated, a previous in vitro study has discovered that disruption of intracellular dopamine homeostasis may be the "trigger" for cell death in this class of neurons (Choi et al. 2015). These results are critical to gaining a mechanistic understanding of neurodegeneration in Parkinson's disease.

Oral delivery of the dopamine prodrug, Levodopa (L-DOPA), is the gold standard of care for patients with Parkinson's disease; however, L-DOPA therapy is characterized by numerous side effects that require adjunct pharmacotherapy and/or limit its therapeutic use. As a result, multiple other drugs have been developed for treatment of Parkinson's disease. Interestingly, several anti-Parkinson's disease drugs including pramipexole (i.e., a dopamine D2 receptor agonist), selegiline (i.e., a selective MAO-B inhibitor), and amantadine (i.e., an antiviral drug that

promotes dopamine release) are transport substrates for OCT1 and/or OCT2 (Goralski et al. 2002; Ishiguro et al. 2005; Hendrickx et al. 2013). Clinical evidence for OCT-mediated transport of anti-Parkinson's disease drugs was provided by Becker and colleagues who showed that patients with a specific polymorphism in the *SLC22A1* gene required higher doses in order to achieve a pharmacological effect (Becker et al. 2011). As noted in this paper, the minor polymorphic C allele at rs622342 in the *SLC22A1* gene is associated with reduced transport function of OCT1 (Becker et al. 2011), an effect that may decrease brain uptake of substrate drugs. Additionally, both amantadine and selegiline have been reported to be OCT transport inhibitors (Lin et al. 2010). This is an important consideration given the multiple drugs commonly prescribed to patients with Parkinson's disease. Specifically, interactions with OCTs by amantadine or selegiline can alter the pharmacokinetics and tissue disposition of co-administered OCT substrate drugs, an effect that can lead to unwanted pharmacological effects and/or toxicity.

5.4 Schizophrenia

Psychoses such as schizophrenia are mental disorders in which thought and emotions are so impaired that contact is lost with external reality. The lifetime prevalence of schizophrenia and related disorders in the USA is approximately 1% (Dixon et al. 2018). The predominant symptoms of schizophrenia include delusions, hallucinations, incoherent or nonsensical speech, and inappropriate behavior for specific social situations. Schizophrenia is a multifactorial psychosis that is affected by environmental, maturational, neurological, and genetic/epigenetic factors. The general etiology of schizophrenia involves dysfunction in neurotransmitter systems associated with dopamine, serotonin, or glutamate. Specifically, dopaminergic overactivity in the mesolimbic tract is associated with delusions and hallucinations while hypoactivity in the mesocortical tract resulting from overactivation of presynaptic D2 receptors is associated with cognitive and emotional deficits (Brisch et al. 2014). Serotonergic overactivity in the frontal cortex, which is mediated via 5-HT_{2A} receptors, can also cause cognitive and emotional abnormalities in schizophrenia (Garcia-Bea et al. 2019; Puig and Gulledge 2011). Finally, reduced activity of NMDA glutamate receptors on inhibitory neurons leads to disinhibition of glutamatergic neurotransmission in the prefrontal cortex and subsequent cognitive and motor impairment (Stahl 2018). Indeed, neurotransmitter receptors associated with the pathogenesis of schizophrenia (i.e., D2 dopamine receptors, 5-HT_{2A} serotonin receptors, NMDA glutamate receptors) can only be effectively modified by drugs capable of permeating brain barriers. This requirement suggests that transporters such as OCT isoforms constitute an effective mechanism to facilitate blood-to-brain delivery of antipsychotic drugs.

Pharmacological treatment of schizophrenia requires chronic administration of antipsychotic drugs in order to manage symptoms of this disease. In order to achieve optimal symptom control, it is critical that these drugs permeate the BBB and attain effective free concentrations at their site of action. Indeed, several antipsychotic

drugs have been demonstrated to be OCT transport substrates. For example, studies in both a human brain endothelial cell line (hCMEC/d3) and a murine brain microvascular endothelial cell line (bEND.3) demonstrated that the commonly prescribed antipsychotic drugs (i.e., amisulpride, haloperidol) are transport substrates for OCT1/Oct1 (Sekhar et al. 2019). Involvement of OCT1 in the transport of both amisulpride and haloperidol was demonstrated using the OCT1/OCT3 inhibitor prazosin and the OCT1/OCT2 inhibitor amantadine (Sekhar et al. 2019). The atypical antipsychotic drug sulpiride has also been shown to be a transport substrate for both OCT1 and OCT2 (Dos Santos Pereira et al. 2014; Li et al. 2017; Takano et al. 2006). Of particular note, in vitro studies in transfected HEK293 cells (i.e., hOCT1-HEK293 and hOCT2-HEK293) demonstrated that sulpiride has greater affinity for OCT1 ($K_M = 2.6$ μM) than OCT2 ($K_M = 68$ μM) (Takano et al. 2006). Plasma concentrations of sulpiride following a pharmacological daily dose range between 70.1 ng/mL and 1,121.2 ng/mL (Tokunaga et al. 1997). These values correspond to molar concentrations of 0.21 μM and 3.28 μM, suggesting that OCT1 is more likely to contribute to CNS uptake of this compound. Since sulpiride is primarily charged at physiological pH 7.4, it is essential that a carrier-mediated transport process such as OCT1 be available at brain barrier sites to enable this compound to attain efficacious free concentrations at its site of action in the CNS (Li et al. 2017). Additional cation transporters that may be involved in sulpiride disposition include OCTN1, OCTN2, MATE1, and MATE2 (Watanabe et al. 2002; Li et al. 2017). Taken together, these studies provide strong evidence for the involvement of OCT isoforms in the delivery of antipsychotic drugs to therapeutic targets in the brain.

Polypharmacy in the treatment of schizophrenia implies a potential for transporter-mediated drug–drug interactions. Many such interactions can adversely affect delivery of drugs to molecular targets in the brain, thereby reducing the effectiveness of various treatment strategies. A good example is the interaction between lamotrigine, a commonly prescribed drug for treatment of bipolar disorder and seizures, and the antipsychotic drug quetiapine (Dickens et al. 2012). Lamotrigine is subject to active uptake in hCMEC/d3 cells that is mediated by OCT1 (Dickens et al. 2012). The K_M for OCT1-mediated lamotrigine transport (i.e., 68 μM) is higher than pharmacologically relevant concentrations between 4 and 42 μM (Dickens et al. 2012), suggesting that this transporter is likely to play a key role in lamotrigine delivery to the brain. Additionally, quetiapine is a potent inhibitor of OCT1-mediated transport (Dickens et al. 2012, 2018). In fact, the in vitro IC50 for quetiapine inhibition of OCT1-mediated lamotrigine transport (i.e., 1.9 μM) is slightly higher than reported average plasma concentrations of 1–1.6 μM (DeVane and Nemeroff 2001; Dickens et al. 2012); however, a standard quetiapine dosing regimen of 250 mg three times daily yields a C_{max} of 2.7 μM (Wong et al. 2001), indicating that concentrations necessary to inhibit OCT1-mediated lamotrigine transport can be achieved clinically. While this suggests a strong potential for a transporter-mediated drug–drug interaction, the effects of quetiapine-mediated inhibition of CNS delivery of lamotrigine require further study. Additionally, pharmacological inhibition of OCT-mediated antipsychotic drug transport at brain barrier

sites can have profound implications on treatment of other disorders such as brain infections. For example, haloperidol has been shown to inhibit OCT-mediated transport of the antimicrobial agent pentamidine at the BBB (Sekhar et al. 2017). While this interaction suggests that elevated concentrations of pentamidine are required to treat CNS effects of infections such as Human African trypanosomiasis in individuals that require haloperidol therapy for management of schizophrenic symptoms (Sekhar et al. 2017), it emphasizes an overall need to consider transporter-mediated interactions that can affect CNS drug delivery in patients receiving multiple drugs that interact with OCT isoforms.

6 Summary and Conclusion

OCTs and OCTNs are a compelling class of membrane transporters. It has been suggested that such transporters for organic cations may represent therapeutic targets in the brain, particularly in the setting of clinical depression where OCT2 is known to participate in clearance of monoamines such as norepinephrine and serotonin (Bacq et al. 2012). This is particularly relevant for antidepressant drugs such as bupropion where pharmacological inhibition of OCT-mediated transport may account for a component of its mechanism of action (Haenisch et al. 2012; Sandoval et al. 2018; Han et al. 2019). For other centrally-acting cationic drugs, the ability of OCTs and OCTNs to facilitate delivery across brain barriers is a critical mechanism that determines therapeutic effectiveness. This is certainly the case for currently marketed drugs used for treatment of ischemic stroke, Parkinson's disease, and schizophrenia. To date, localization, regulation, and functional expression of OCTs and OCTNs at the BBB and BCSFB have been understudied and many existing publications provide conflicting evidence of their mRNA and/or protein expression in these CNS barrier tissues. These discrepancies could be due to species differences, variable transporter expression in cell culture models, age-related expression differences, and/or environmental/epigenetic factors. Perhaps the strongest evidence for the role of cation transporters in CNS drug delivery comes from functional studies that have repeatedly shown selective uptake via OCTs and/or OCTNs of centrally acting cationic drugs into brain microvascular endothelial cells and/or choroid plexus epithelial cells as well as in animal models. Indeed, these studies highlight the potential for OCT- and/or OCTN-mediated drug delivery as an opportunity to optimize therapeutic effectiveness of novel compounds for treatment of neurological diseases. Central to this endeavor is knowledge derived from membrane topological studies of OCTs and OCTNs that have revealed specific amino acid residues responsible for substrate binding. Specifically, site-directed mutagenesis studies have determined key residues (i.e., Phe160, Trp218, Arg440, Leu447, and Asp475) as key contributors to substrate binding affinity, which have been demonstrated through modified cellular uptake of established transport substrates (Volk et al. 2009). Such information is crucial to rational design of novel brain penetrant cationic drugs. Indeed, optimized OCT- or OCTN-mediated transport of currently marketed drugs does not necessarily correlate to improved therapeutic

effectiveness. A good example is the case of anti-migraine triptan drugs (i.e., rizatriptan, naratriptan, zolmitriptan). While these compounds are established substrates for OCTs (Matthaei et al. 2016), it is questionable as to whether their blood-to-brain transport is necessary to confer an optimal therapeutic effect (Ahn and Basbaum, 2005). Therefore, it is critical to consider the molecular target of a drug before determining that optimized therapeutic delivery by a BBB transporter is required for compound advancement or for development of improved treatment paradigms for neurological diseases. While further research is needed to enhance our understanding of OCT and/or OCTN-mediated transport at the BBB and BCSFB, current data provides strong evidence for the utility of these transporters as targets for CNS drug delivery. Furthermore, the observation that numerous OCT/OCTN substrate drugs that are specific to CNS pathologies are commonly prescribed provides a critical framework and link for future discovery of new centrally acting drugs that are OCT and/or OCTN substrates.

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