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Neuropharmacology of Synthetic Cathinones

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Abstract

Synthetic cathinones are derivatives of the naturally occurring compound cathinone, the main psychoactive ingredient in the khat plant *Catha edulis*. Cathinone is the β -keto analog of amphetamine, and all synthetic cathinones display a β -keto moiety in their structure. Several synthetic cathinones are widely prescribed medications (e.g., bupropion, Wellbutrin®), while others are problematic drugs of abuse (e.g., 4-methylmethcathinone, mephedrone). Similar to amphetamines, synthetic cathinones are psychomotor stimulants that exert their effects by impairing the normal function of plasma membrane transporters for dopamine (DAT), norepinephrine (NET), and 5-HT (SERT). Ring-substituted cathinones like mephedrone are transporter substrates that evoke neurotransmitter release by reversing the normal direction of transporter flux (i.e., releasers), whereas pyrrolidine-containing cathinones like 3,4-methylenedioxypyrovalerone (MDPV) are potent transporter inhibitors that block neurotransmitter uptake (i.e., blockers). Regardless of molecular mechanism, all synthetic cathinones increase extracellular monoamine concentrations in the brain, thereby enhancing cell-to-cell monoamine signaling. Here, we briefly review the mechanisms of action, structure-activity relationships, and in vivo pharmacology of synthetic cathinones. Overall, the findings show that certain synthetic cathinones are powerful drugs of abuse that could pose significant risk to users.

Keywords

Cathinone; Dopamine; Monoamine; Serotonin; Stimulant; Transporter

1 Introduction

1.1 Synthetic Cathinones as Medications and Drugs of Abuse

Synthetic cathinones are chemical analogs of the naturally occurring compound cathinone, the main psychoactive ingredient in the khat plant *Catha edulis*. From a structural perspective, cathinone is the β -keto analog of amphetamine, and synthetic cathinones are often referred to as “bk-amphetamines” (see Fig. 1 for chemical structures). The stimulant effects of khat have been known for centuries, and the practice of chewing khat leaves is still popular today in many countries of East Africa and the Arabian Peninsula (Al-Hebshi and Skaugh 2005; Engidawork 2017). However, it was not until the 1970s that cathinone, specifically the (*S*)-(–) stereoisomer of cathinone, was isolated from khat leaves and identified as the primary psychoactive compound in the plant (Kalix 1990). Many years before the discovery of cathinone in khat, the compound was synthesized by medicinal chemists (e.g., Van der Schoot et al. 1962), and various derivatives have been investigated for therapeutic potential.

Figure 1 depicts the chemical structures of cathinone-related compounds that are prescribed medications approved by the Food and Drug Administration (FDA) in the USA.

Diethylpropion, or amfepramone, is the *N,N*-diethyl analog of cathinone. Diethylpropion was developed as an anorectic agent in the early 1960s by the German pharmaceutical company Temmler-Werke (Schütte 1961) and is still prescribed today as Tenuate®, an efficacious adjunct for weight loss (Cercato et al. 2009; Suplicy et al. 2014). The pyrrolidine-containing cathinone analog, pyrovalerone, was investigated as an anti-fatigue agent in the 1960s (Wander 1963; Thomae 1963; Seeger 1967; Goldberg et al. 1973). Although pyrovalerone is an approved medication in the USA, it is rarely prescribed. Finally, bupropion is an *N-tert*-butyl analog of cathinone that was initially investigated as an antidepressant in the 1970s by Burroughs Wellcome (now GlaxoSmithKline) and subsequently approved for clinical use as Wellbutrin® (Mehta 1974; Dhillon et al. 2008). Bupropion was also approved as the smoking cessation aid Zyban® in 1997 (Dwoskin et al. 2006). In 2016, bupropion was the fifth most prescribed psychiatric medication in the USA (Grohol 2017).

While the cathinone-related compounds described above are used as efficacious medications, other synthetic analogs are misused as drugs of abuse. The *N*-methylated analog of cathinone, methcathinone, was a popular drug of abuse known as ephedrone or “Jeff” in Russia during the 1980s (Emerson and Cisek 1993). Given that methcathinone is the β -keto analog of methamphetamine (see Fig. 1), it is not surprising that methcathinone induces powerful psychomotor stimulant effects and is known to show dependency-producing properties in animals and humans (Goldstone 1993; Kaminski and Griffiths 1994). An epidemic of methcathinone misuse occurred in the USA during the 1990s but quickly subsided. It is noteworthy that the chronic use of methcathinone was associated with the development of an irreversible Parkinsonian syndrome due to manganese toxicity (Stepens et al. 2008; Sikk and Taba 2015), secondary to the use of potassium permanganate as an oxidizing agent in the clandestine synthesis of the drug. In more recent times, a variety

of “designer” synthetic cathinones have appeared in the nonmedical (i.e., recreational) drug market as substances of abuse (Baumann 2014; De Felice et al. 2014).

1.2 New Psychoactive Substances (NPS) and “Bath Salts” Cathinones

The abuse of psychomotor stimulants like methamphetamine and cocaine is a wide-spread public health problem that continues to plague modern society (Degenhardt et al. 2014). In this regard, a disturbing new trend is the increased recreational use of so-called designer drugs, legal highs, or research chemicals (Baumann et al. 2014a; Madras 2017; Huestis et al. 2017). These drugs, collectively known as “new psychoactive substances” (NPS), are synthetic alternatives to more traditional drugs of abuse. NPS can be more formally defined as individual drugs in pure form or complex preparations that are not scheduled under the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but which might pose a public health threat (Madras 2017). Synthetic cathinones represent a predominant class of NPS (Baumann 2014). The first cathinone-related NPS in the USA were found in so-called “bath salts” products which were available in the recreational drug market during late 2010 (Prosser and Nelson 2012). By 2011, there were increasing reports of bath salts intoxications to poison control centers and emergency departments nationwide (Centers for Disease Control and Prevention 2011; Spiller et al. 2011; Warrick et al. 2013). Bath salts consist of powders or crystals that are administered intranasally, intravenously, or orally to produce their psychoactive effects. Low doses of bath salts produce typical stimulant effects such as increased energy, elevated mood and euphoria, but high doses or repeated use can induce serious symptoms including hallucinations, psychosis, tachycardia, hypertension, and hyperthermia, often accompanied by aggressive or violent behaviors (Banks et al. 2014; Karch 2015).

Forensic analysis of bath salts products in 2010 and 2011 identified three synthetic compounds: 4-methyl-*N*-methylcathinone (4-MMC, mephedrone), 3,4-methylenedioxy-*N*-methylcathinone (MDMC, methylone), and 3,4-methylene dioxypyrovalerone (MDPV) (Spiller et al. 2011; Shanks et al. 2012). Figure 1 depicts the chemical structures of the principal bath salts cathinones. Legislation passed in 2013 placed mephedrone, methylone, and MDPV into permanent Schedule I control, making the drugs illegal in the USA (DEA 2013). However, new cathinone derivatives are constantly being developed to circumvent legislative control, and by 2017 more than 100 cathinones had been identified worldwide (UNODC 2017). Most synthetic cathinones are manufactured by Asian chemical companies and sold over the Internet. Synthetic chemists consult the biomedical and patent literature for lead compounds to create novel analogs for recreational use. Given the increasing variety of synthetic cathinones in the recreational drug market, and the renewed interest in these compounds, the purpose of this chapter is to briefly review the molecular mechanisms of action, structure-activity relationships (SAR), and in vivo biological effects of synthetic cathinones.

2 Molecular Mechanisms of Action

2.1 Stimulant Drugs Target SLC6 Plasma Membrane Transporters

As noted above, synthetic cathinones are β -keto analogs of amphetamine (see Fig. 1). The bath salts drugs, mephedrone and methylone, have functional groups attached to their phenyl rings and are considered ring-substituted cathinones. MDPV has a more complex structure, with a bulky nitrogen-containing pyrrolidine ring and a flexible propyl chain extending from its α -carbon. Like other stimulant drugs, bath salts cathinones exert their effects by interacting with plasma membrane transporter proteins expressed on nerve cells that synthesize the monoamine neurotransmitters dopamine, norepinephrine, and serotonin (5-HT) (Baumann et al. 2013; Eshleman et al. 2013; Simmler et al. 2013). In order to understand the molecular mechanism of action for cathinone analogs, it is essential to first consider the normal physiological role of monoamine transporters and the types of drugs targeting these proteins.

The solute carrier 6 (SLC6) transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT) are responsible for translocating previously released neurotransmitter molecules from the extracellular space back into the neuronal cytoplasm, a process known as neurotransmitter “uptake” (Kristensen et al. 2011; Alexander et al. 2017). The uptake mechanism is a complex active-transport process dependent upon ionic gradients across neuronal membranes. Importantly, transporter-mediated neurotransmitter uptake is the principal mechanism for terminating the action of monoamine signaling, so drugs targeting these transporter proteins can have profound effects on cell-to-cell monoamine transmission. Monoamine transporters are the principal sites of action for medications used to treat a range of psychiatric diseases such as major depression, anxiety, and attention-deficit hyperactivity disorder (Iverson 2006; Sanchez et al. 2014; Faraone 2018). Drugs which preferentially interact at SERT, or 5-HT-selective reuptake inhibitors (SSRIs), are widely prescribed as treatments for major depression and anxiety disorders. By contrast, drugs which preferentially act at DAT and NET, such as amphetamine and methamphetamine, can have powerful psychomotor stimulant and dependence-producing properties (Rothman and Baumann 2003; Howell and Kimmel 2008; Espana and Jones 2013).

2.2 Transporter Blockers Versus Substrates

Drugs that bind to monoamine transporters can be divided into two types based on their precise mode of action: (1) cocaine-like “blockers” bind to the orthosteric site on the transporter and inhibit uptake of neurotransmitters from the extracellular space, whereas (2) amphetamine-like “substrates” also bind to the orthosteric site but are subsequently translocated through the transporter channel into the neuronal cytoplasm and trigger efflux of intracellular neurotransmitter molecules by reverse transport (i.e., transporter-mediated release) (Reith et al. 2015; Sitte and Freissmuth 2015). Drugs that act as transporter substrates are sometimes called transporter “releasers” because they induce non-exocytotic transporter-mediated release of neurotransmitters from neurons. Regardless of molecular mechanism, all drugs which interact with transporters can dramatically increase extracellular concentrations of monoamines in vivo, amplifying cell-to-cell chemical signaling throughout the central nervous system.

It is important to distinguish between transporter blockers and substrates because substrates display a number of unique properties: they are translocated into cells along with sodium ions, they induce inward depolarizing currents (Sonders et al. 1997; Sitte et al. 1998), and they reverse the normal direction of transporter flux to trigger non-exocytotic release of neurotransmitters (i.e., reverse transport) (Hilber et al. 2005; Robertson et al. 2009). Finally, because substrate-type drugs are transported into cells, they can accumulate in the cytoplasm and interact with neuronal proteins to inhibit neurotransmitter synthesis and disrupt vesicular storage, leading to long-term neurotransmitter deficits (Fleckenstein et al. 2007; Baumann et al. 2014b). Table 1 summarizes some fundamental differences between transporter blockers versus substrates.

3 Methods to Study Transporter Function

The existence of monoamine transport mechanisms in cells was postulated long ago based on the ability of native tissues to take up and accumulate radiolabeled monoamines. For example, early studies characterized the accumulation of systemically administered [³H]norepinephrine into mammalian tissues such as the heart, spleen, and liver (Axelrod et al. 1961). Other investigations showed that certain drugs, like cocaine and amphetamine, were able to inhibit the uptake of [³H] neurotransmitters into nervous tissue, providing evidence for specific binding sites associated with transport mechanisms (Heikkila et al. 1975). The use of tissue homogenates, like synaptosomes, allowed for the possibility of studying transport mechanisms in situ in a high-throughput manner. Synaptosomes consist of sealed nerve endings with their plasma membrane leaflets oriented in a manner akin to neurons in vivo. Importantly, synaptosomes contain the full complement of protein machinery required for synthesis, metabolism, uptake, and exocytotic release of neurotransmitters.

3.1 Transporter Assays in Synaptosomes

Rothman and colleagues developed in vitro functional assays to assess the ability of test drugs to act as transporter blockers or substrates at DAT, NET, and SERT (Rothman et al. 2001, 2003a, b). We have adapted these methods in our laboratories and perform two types of assays: (1) uptake inhibition and (2) release stimulation. The assays are carried out in synaptosomes prepared from rat brain tissue and are designed to assess potency and efficacy of drugs at all three transporters under similar physiologically relevant conditions. For the uptake inhibition assays, radiolabeled substrate (i.e., [³H]neurotransmitter) and test drug are co-incubated with synaptosomes for a brief period of time, and the reaction is stopped by vacuum filtration. If test drugs are transporter blockers, the accumulation of [³H]neurotransmitter into synaptosomes (i.e., uptake) is reduced because the test drug and neurotransmitter compete for the same orthosteric site on the transporter protein. It is noteworthy that uptake inhibition assays cannot distinguish between blockers and substrates because both types of drugs will effectively inhibit the accumulation of [³H]neurotransmitter into synaptosomes.

To identify substrate-type drugs, we use release stimulation assays. For the release assays, synaptosomes are first incubated with radiolabeled substrate molecules in order to fill or

“preload” the interior of the synaptosomes. [^3H]1-Methyl-4-phenylpyridinium ([^3H]MPP $^+$) is used as the radiolabeled substrate for DAT and NET release assays, whereas [^3H]5-HT is used for SERT release assays. Once synaptosomes are preloaded, test drug is added for a brief incubation period, and the reaction is stopped by vacuum filtration. Drugs that act as transportable substrates will evoke efflux of [^3H]MPP $^+$ or [^3H]5-HT out of the synaptosomes (i.e., release) by reversal of the normal direction of transporter flux. Drugs that act as non-transportable blockers will not cause substantial release of [^3H]MPP $^+$ or [^3H]5-HT from preloaded synaptosomes. Thus, by testing drugs in the combined uptake inhibition and release assay procedures, the precise molecular mechanism of drug action can be ascertained. The data in Fig. 2 illustrate that amphetamine and cocaine both inhibit [^3H]dopamine uptake in synaptosomes, whereas only the substrate amphetamine is able to induce fully efficacious release of [^3H]MPP $^+$ via DAT.

3.2 Transporter Assays in Transfected Cells

The cloning of human isoforms of DAT, NET, and SERT in the 1990s (Pacholczyk et al. 1991; Giros et al. 1991; Shimada et al. 1991; Kilty et al. 1991) initiated a new era for evaluating the effects of psychostimulants and other drugs in heterologous expression systems (Eshleman et al. 1994; Pifl et al. 1995). The expression of cloned transporters in cells enabled the investigation of pure populations of a single transporter type in the absence of the synaptic protein machinery normally present in synaptosomes. Using cells transfected with DAT, NET, or SERT, it is possible to examine the effects of drugs on uptake and release of [^3H]neurotransmitters in a controlled and detailed manner. We have compared the pharmacological effects of many compounds in synaptosomes and human embryonic kidney 293 (HEK) cells stably expressing human monoamine transporters, to address possible differences in the results from these two approaches (Baumann et al. 2014b; Saha et al. 2015; Sandtner et al. 2016; Mayer et al. 2016). Overall, the findings demonstrate excellent agreement between synaptosomes and transporter-expressing cells in terms of identifying drugs as either transporter blockers or substrates. However, there are often discrepancies in absolute potency estimates for drugs (e.g., IC $_{50}$ or EC $_{50}$ values) in synaptosomes versus transfected cells. For example, the EC $_{50}$ values for substrate-type drugs to evoke transporter-mediated release are often tenfold lower in synaptosomes when compared to cells expressing transporters. It also must be noted that the absolute amount of [^3H]neurotransmitter release can differ substantially across various assays, depending on the expression system used and specific transporter under examination.

3.3 Transporter-Associated Ionic Currents

From a mechanistic perspective, the transporter-mediated uptake of substrate molecules is best described by the “alternating access” model originally proposed by Jardetzky (1966) more than 50 years ago. The model posits that transporter proteins alternate between two distinct conformations: (1) an “outward-facing” conformation which has binding sites for substrate (e.g., dopamine) and co-substrate ions (e.g., Na $^+$, Cl $^-$) on the extracellular side of the protein and (2) an “inward-facing” conformation which has binding sites on the intracellular side and allows detachment of the substrate into the cytoplasm. The transition from outward-facing to inward-facing conformation is causally linked to movement of substrate molecules through the transporter. Additionally, the process of translocating

substrates and their co-transported ions generates measurable ionic currents (Sonders and Amara 1996). Transporter-associated currents are a distinct property of the proteins that resemble ion channel function, though the currents generated by transporters are much smaller than those generated by true ion channel proteins.

Transporter-mediated uptake of substrates is an active process that is fueled by the coupling of substrate flux to the movement of co-substrate ions down their electrochemical gradients. In particular, substrate translocation is dependent upon intact sodium gradients across cell membranes. The binding of substrate and co-substrate ions occurs in a fixed ratio, determined by the specific binding site topology of each transporter. Hence, the ion/substrate stoichiometry predicts the movement of a fixed number of electrical charges during every translocation cycle, whereby uptake of substrate will result in a net transmembrane current. Thus far, all transporters examined elicit inward positive current when translocating substrates, so they are considered electrogenic. It is noteworthy that SERT uses a counter-transported potassium ion to facilitate its return from the inward-facing to outward-facing conformation. The counter-transported potassium ion should render the transport cycle of SERT electroneutral, since 1 net-positive charge in (i.e., 1 Na⁺, 1 5-HT⁺ and 1 Cl⁻) is canceled by the 1 positive K⁺ charge out (Rudnick 1998). In contrast to this prediction, several studies show that SERT generates a positive inward current upon administration of 5-HT or other substrates (Mager et al. 1994; Adams and DeFelice 2003; Quick 2003; Hilber et al. 2005).

DAT and NET do not counter-transport potassium and, therefore, work in an electrogenic manner. Importantly, DAT, NET, and SERT display channel-like properties, since they allow the passage of ions “in excess” of the stoichiometric prediction, generally termed uncoupled conductance (Sonders et al. 1997; Sitte et al. 1998). Using voltage-clamp techniques in cells expressing transporter proteins, the ionic currents generated by cognate substrates (e.g., dopamine) and substrate-type drugs (e.g., amphetamine) can be accurately measured. It is now established that generation of transporter-associated currents is an inherent property of transporter substrates only, thus distinguishing transportable substrates from non-transportable blockers which do not induce currents (Schicker et al. 2012).

3.4 Effects of the Ionophore Monensin

As noted above, the transporter-mediated movement of substrate molecules is an energy-requiring process dependent upon intact ionic gradients across cell membranes. We have conducted experiments to examine transporter function under conditions where intracellular sodium concentrations are elevated by the addition of ouabain or monensin (Scholze et al. 2000; Sitte et al. 2000). Ouabain inhibits the activity of Na⁺/K⁺-ATPase to disrupt sodium gradients across cells, whereas monensin is an ionophore which facilitates transmembrane exchange of sodium ions for protons (Mollenhauer et al. 1990). It is noteworthy that a rise in intracellular sodium concentration greatly increases the propensity for outward transport and [³H]neurotransmitter efflux via the transporter (Raiteri et al. 1978; Liang and Rutledge 1982; Bönisch 1986). We have used monensin as a tool to discriminate transporter substrates from blockers in release assays carried out in cells expressing transporter proteins. Cells preloaded with [³H]MPP⁺ are incubated with transporter ligands in the presence or absence

of monensin. Under these conditions, the efflux of [^3H]MPP $^+$ induced by transporter substrates is greatly enhanced in the presence of monensin, whereas the effects of transporter blockers are unaltered (Baumann et al. 2013; Mayer et al. 2016).

4 Structure-Activity Relationships

Structure-activity relationship (SAR) investigations examine the effects of altering the chemical structure of a given drug molecule on biological responses. In the simplest approach to SAR, one specific substituent on a drug molecule is altered, while the remainder of the molecule is “locked-in” and stays constant (Glennon and Dukat 2017). Employing SAR studies, it is possible to determine the role of a given chemical group in modulating the functional activity of candidate medications or drugs of abuse. Prior to the appearance of bath salts cathinones in 2010–2011, few scientific studies had examined the SAR for cathinone-related compounds. Evidence from the 1980s showed that cathinone and methcathinone release dopamine from rat brain tissue by an amphetamine-like mechanism (Kalix and Glennon 1986; Glennon et al. 1987), and subsequent reports demonstrated that methcathinone acted as a potent substrate at DAT and NET but not SERT (Cozzi et al. 1999; Rothman et al. 2003b). Cozzi et al. first reported that methylone acts as an uptake blocker at monoamine transporters (Cozzi et al. 1999), while other investigations showed the drug is a transporter substrate capable of releasing dopamine, norepinephrine, and 5-HT from rat brain tissue (Nagai et al. 2007). A number of more recent studies have characterized the SAR for ring-substituted and pyrrolidine-containing cathinones.

4.1 Ring-Substituted Cathinones Are Transporter Substrates

Hadlock et al. (2011) carried out the first comprehensive investigation of the pharmacology of the bath salts cathinone, mephedrone. These investigators found that mephedrone inhibits dopamine uptake and stimulates dopamine release from rat brain synaptosomes. López-Arnau et al. (2012) reported that mephedrone and methylone both inhibit uptake at DAT and SERT in synaptosomes, but no transporter release data were reported in their study. Our laboratory extended the findings of López-Arnau and coworkers by showing that mephedrone and methylone act as transporter substrates in rat brain synaptosomes, thereby evoking the release of [^3H] MPP $^+$ from DAT and NET and release of [^3H]5-HT from SERT (Baumann et al. 2012). The nonselective substrate activity of mephedrone and methylone at monoamine transporters is similar to the molecular mechanism of action for the club drug MDMA (Baumann et al. 2007; Sandtner et al. 2016).

In assay systems using human transporters expressed in HEK cells, mephedrone and methylone inhibit neurotransmitter uptake and act as substrates at DAT, NET, and SERT (Eshleman et al. 2013; Simmler et al. 2013; Mayer et al. 2016; Piffl et al. 2015), consistent with findings in synaptosomes. Importantly, voltage-clamp experiments carried out in *Xenopus* oocytes expressing either DAT or SERT reveal that mephedrone induces robust inward sodium currents, whereas the pyrrolidine-containing cathinone MDPV does not (Cameron et al. 2013; Solis 2017). Other studies show that monensin treatment markedly enhances transporter-mediated release of [^3H]MPP $^+$ evoked by mephedrone in HEK cells (Mayer et al. 2016). The unpublished data depicted in Fig. 3 show that DAT-mediated efflux

of [^3H]MPP $^+$ produced by mephedrone is significantly augmented in the presence of monensin, whereas the modest effects of MDPV are unaffected. Taken together, the results from studies using rat and human transporters agree that ring-substituted cathinones like mephedrone and methylone are transporter substrates capable of inducing transmitter release via DAT, NET, and SERT.

Older studies examining the pharmacology of amphetamine analogs demonstrated that adding bulky substituents to the phenyl ring enhances potency at SERT relative to DAT. For example, the 3-trifluoromethyl analog of amphetamine, norfenfluramine, has much greater potency as a SERT substrate when compared to amphetamine itself (Rothman et al. 2003a). In a similar manner, Cozzi et al. demonstrated that 4-trifluoromethyl-*N*-methylcathinone is a much more potent substrate at SERT than DAT, whereas the parent compound methcathinone displays the opposite selectivity (Cozzi et al. 2013). It is noteworthy that Cozzi et al. also showed that methcathinone is a powerful locomotor stimulant in rats, whereas its 4-trifluoromethyl analog is not, suggesting an increase in potency at SERT is inhibitory to motor stimulant actions. Bonano et al. carried out the first detailed SAR studies to investigate the role of *para*-position (i.e., 4-position) ring substitution on the biological activity of methcathinone analogs (Bonano et al. 2015; Sakloth et al. 2015). In their work, substituents of increasing size (i.e., increasing steric volume) were added to the 4-position of methcathinone, and substrate activity was examined at DAT and SERT in rat brain synaptosomes. The findings summarized in Table 2 reveal that increasing steric bulk at the 4-position enhances potency at SERT relative to DAT, thereby decreasing the DAT/SERT ratio of the compounds. The same compounds were tested in the rat intracranial self-stimulation (ICSS) paradigm which can identify abuse-related and abuse-limiting effects of drugs (Negus and Miller 2014). It was found that increasing steric bulk on the 4-position is associated with reduced abuse-related effects, and enhanced abuse-limiting effects, of the analogs. A high positive correlation was shown between DAT/SERT ratio and abuse potential of the analogs. The summed findings predict that drugs with a high DAT/SERT ratio will have high abuse potential, whereas those with lower DAT/SERT ratio will have low abuse liability (Negus and Banks 2017).

Investigations carried out in HEK cells transfected with human transporters also showed that adding substituents to the 4-position of cathinone-related compounds increases activity at SERT relative to DAT. Rickli et al. showed that 4-methyl, 4-ethyl, and 4-bromo analogs of methcathinone have enhanced potency to inhibit [^3H]neurotransmitter uptake at SERT when compared to methcathinone (Rickli et al. 2015). Eshleman et al. examined transporter-mediated uptake inhibition and [^3H]neurotransmitter release for a series of methcathinone analogs and found that 4-chloro and 4-bromo analogs display greater potency at SERT when compared to methcathinone (Eshleman et al. 2017). Molecular modeling studies based on the crystal structure of *Drosophila* DAT provide evidence that a subregion of the substrate-binding pocket of SERT is slightly larger than that of DAT, allowing for accommodation of larger phenyl ring substituents in SERT (Sakloth et al. 2015). More research investigations using molecular docking approaches and dynamic simulations are warranted to address the precise underpinnings of transporter selectivity for ring-substituted cathinones (Zdrazil et al. 2016; Seddik et al. 2017).

4.2 Pyrrolidine-Containing Cathinones Are Transporter Inhibitors

Investigations from the 1990s revealed that pyrovalerone, a structural analog of MDPV (see Fig. 1), is a potent dopamine uptake blocker which produces psychomotor stimulant effects when administered to rodents (Vaugois et al. 1993; Héron et al. 1994). A seminal study by Meltzer et al. (2006) examined the monoamine transporter activities for several pyrovalerone analogs and showed these agents are potent inhibitors of DAT and NET, with minimal activity at SERT. Importantly, the study of Meltzer and colleagues did not address whether pyrovalerone analogs might act as transporter substrates, and no assessment of MDPV pharmacology was included. To this end, we examined the in vitro transporter activity of MDPV in rat brain synaptosomes and showed the drug displays potent uptake inhibition at DAT and NET, with much weaker activity at SERT (Baumann et al. 2013). The in vitro results with MDPV are consistent with prior data of Meltzer et al. showing that pyrovalerone analogs are potent blockers of DAT and NET. When compared to the prototypical uptake inhibitor cocaine, MDPV is 50-fold more potent as an inhibitor at DAT, tenfold more potent at NET, and tenfold less potent at SERT (see Table 3).

In assays using HEK cells expressing human transporters, Eshleman et al. (2013) and Simmler et al. (2013) confirmed that MDPV is a potent blocker at DAT and NET, but not SERT, and the drug does not evoke transporter-mediated release. These same investigators examined the potency of MDPV at various G protein-coupled receptor subtypes and found no significant affinity of the drug for non-transporter sites of action (Eshleman et al. 2013; Simmler et al. 2013). One recent study using single-cell amperometric methods reported that low concentrations of MDPV cause reverse transport of dopamine via DAT, suggestive of substrate activity (Shekar et al. 2017). However, as noted previously, MDPV does not induce transporter-associated inward currents in DAT-expressing *Xenopus* oocytes (Cameron et al. 2013; Solis 2017), and effects of the drug in HEK cells are not affected by monensin treatment. Thus, findings from a variety of different assay methods in native tissues and transporter-expressing cells indicate that MDPV is a potent blocker at DAT and NET, with minimal substrate activity.

Kolanos et al. (2013) performed an SAR study which “deconstructed” the MDPV molecule piece-by-piece to determine which structural features govern its ability to inhibit [³H]dopamine uptake in cells transfected with human DAT. It was found that the bulky pyrrolidine ring and the flexible α -carbon chain were critical attributes for potent uptake inhibition at DAT, whereas the 3,4-methylenedioxy ring moiety was of minor consequence. Marusich et al. (2014) followed up this study and confirmed that removing the 3,4-methylenedioxy ring substituent of MDPV, to form α -pyrrolidinovalerophenone (α -PVP), has little influence on potency to inhibit DAT or NET in rat brain synaptosomes. These investigators also examined the effects of altering α -carbon chain length on potency to inhibit [³H]neurotransmitter uptake at DAT, NET, and SERT. The data summarized in Table 3 show that decreasing the α -carbon chain length of α -PVP to form α -pyrrolidinobutylphenone (α -PBP) and α -pyrrolidinopropylphenone (α -PPP) produces a stepwise reduction in potency to inhibit uptake at DAT and NET. Nevertheless, all of the pyrrolidine-containing compounds maintain high selectivity at DAT and NET over SERT, with DAT/SERT ratios ranging from 50 to 800. It is noteworthy that α -PPP, the weakest

compound tested, is similar in potency to cocaine at DAT and NET. Increasing the α -carbon chain length to a butyl group to form α -pyrrolidinohexiophenone (α -PHP) engenders similar potency to α -PVP. Indeed, analogs of α -PVP with large bulky groups attached at the α -carbon position (e.g., cyclohexyl) retain high potency as blockers of DAT and NET (Kolanos et al. 2015a).

The formulation of MDPV available in the recreational drug marketplace is a racemic mixture of (*S*) and (*R*) stereoisomers. Meltzer et al. (2006) showed that (*S*)-pyrovalerone is much more potent as a blocker at DAT and NET when compared to (*R*)-pyrovalerone, indicating MDPV stereoisomers might exhibit a similar degree of transporter selectivity. Kolanos et al. (2015b) reported the stereoselective synthesis of MDPV enantiomers using (*S*)- and (*R*)-norvaline as starting materials, whereas Suzuki et al. (2015) resolved MDPV enantiomers from the racemic mixture. In the study of Kolanos et al., (*S*)-MDPV was 100 times more potent at inhibiting DAT when compared to (*R*)-MDPV. Therefore, similar to the findings reported for pyrovalerone, the biological activity of racemic MDPV resides primarily with the (*S*)-isomer. In agreement with the in vitro transporter results, (*S*)-MDPV is much more potent than (*R*)-MDPV in eliciting locomotor stimulant and reinforcing effects in both rats and mice (Kolanos et al. 2015b; Gannon et al. 2016, 2017).

5 In Vivo Pharmacology

5.1 In Vivo Microdialysis

Drugs acting as inhibitors or substrates at DAT, NET, and SERT increase the extracellular concentrations of dopamine, norepinephrine, and 5-HT in the brain to enhance monoamine signaling (Rothman and Baumann 2003; Howell and Kimmel 2008). We use in vivo methods to simultaneously examine neurochemical and behavioral effects of transporter ligands in rats (Rothman et al. 2005; Baumann et al. 2011). Specifically, in vivo microdialysis is used to collect samples of extracellular fluid (i.e., dialysate samples) from the brains of conscious freely behaving rats. The microdialysis probes are placed into the nucleus accumbens, a brain region implicated in the locomotor stimulant and rewarding effects of abused drugs (Willuhn et al. 2010; Ikemoto and Bonci 2014), and dialysate samples are analyzed for concentrations of dopamine and 5-HT using high-performance liquid chromatography coupled to electrochemical detection. Rats undergoing microdialysis are housed in arenas equipped with photo beams sensitive to locomotor activity in the horizontal plane (i.e., ambulation) and repetitive back-and-forth movements of the head, trunk, and limbs (i.e., stereotypy). Our methods allow for the assessment of relationships between extracellular monoamines and behavior. In previous studies, we found a significant positive correlation between the amount of dialysate dopamine in the nucleus accumbens and the extent of locomotor activation produced by transporter ligands (Zolkowska et al. 2009; Baumann et al. 2011). Furthermore, data reveal that elevations in dialysate 5-HT alone are not sufficient to produce locomotor activation (Cozzi et al. 2013), but elevations in extracellular 5-HT can dampen the motor stimulant effects mediated by concurrent elevations in extracellular dopamine (Rothman et al. 2005; Baumann et al. 2011).

Kehr et al. first reported that subcutaneous (s.c.) administration of mephedrone to rats evokes elevations in extracellular dopamine and 5-HT in the nucleus accumbens (Kehr et al.

2011), and other research groups confirmed these findings in rats receiving either s.c. or intraperitoneal (i.p.) mephedrone injections (Wright et al. 2012; Shortall et al. 2016; Suyama et al. 2016). We found that intravenous (i.v.) administration of mephedrone or methylone produces dose-related increases in extracellular dopamine and 5-HT in rat nucleus accumbens, with mephedrone being slightly more potent than methylone (Baumann et al. 2012; Mayer et al. 2016; Elmore et al. 2017). Rats repeatedly exposed to mephedrone during adolescence and re-exposed later in life show a potentiation of dopamine and 5-HT release in the nucleus accumbens and prefrontal cortex, indicating the development of neurochemical sensitization (Kaminska et al. 2018). Interestingly, most microdialysis studies testing the acute effects of mephedrone and methylone have found the magnitude of increase in dialysate 5-HT exceeds the accompanying increase in dialysate dopamine. This profile of in vivo neurochemical effects produced by mephedrone and methylone is consistent with the nonselective substrate activity of these drugs and mimics the known neurochemical effects of MDMA (Baumann et al. 2008, 2012; Kehr et al. 2011). We first reported that i.v. administration of MDPV to rats produces dose-related increases in extracellular dopamine but not 5-HT, and MDPV is tenfold more potent than cocaine in this regard (Baumann et al. 2013). The ability of MDPV to increase extracellular dopamine has been confirmed in rats and mice (Johnson et al. 2018; Wojcieszak et al. 2018). The rise in extracellular dopamine produced by MDPV is fully consistent with the potent inhibition of dopamine uptake produced by the drug in vitro.

Microdialysis methods have been used to elucidate mechanisms underlying the elevations in extracellular neurotransmitters produced by transporter ligands in vivo (Nomikos et al. 1990; Chen and Reith 1994; Gundlach et al. 1997). In particular, reserpine has been used as a pharmacological tool to distinguish between the effects of transporter blockers versus substrates. Reserpine is a naturally occurring indole alkaloid that induces long-lasting depletion of monoamine neurotransmitters from synaptic vesicles in the brain and periphery (Arbuthnott et al. 1990). A number of microdialysis investigations have shown that reserpine pretreatment blocks cocaine-induced increases in extracellular dopamine and norepinephrine in rat brain, without affecting amphetamine-induced neurotransmitter increases (Butcher et al. 1988; Callaway et al. 1989; Florin et al. 1995). These data demonstrate that transporter blockers like cocaine increase dialysate neurotransmitter concentrations via a vesicular pool linked to exocytosis, while transporter substrates like amphetamine can increase dialysate neurotransmitter concentrations from a non-vesicular pool. We recently carried out a microdialysis investigation comparing the effects of MDPV and mephedrone in rats pretreated with reserpine. Male Sprague-Dawley rats were pretreated with 5 mg/kg s.c. reserpine 24 h before being subjected to microdialysis testing. The unpublished data in Fig. 4 show that MDPV-induced dopamine elevations are significantly blunted by reserpine pretreatment, but the effects of mephedrone are not altered. The findings from reserpinized rats indicate that MDPV acts as a transporter blocker in vivo, with dopamine responses dependent upon ongoing exocytotic transmitter release. By contrast, mephedrone acts as a transporter substrate in vivo that can release dopamine from a non-vesicular pool. Future microdialysis studies should address the in vivo mechanisms of action for ring-substituted and pyrrolidine-containing cathinones.

5.2 Locomotor Activation

Studies from the 1980s revealed that cathinone and methcathinone display powerful locomotor stimulant effects in mice and rats (Kalix 1980; Glennon et al. 1987). Accordingly, most of the synthetic cathinones found in the recreational drug marketplace are reported to stimulate locomotor activity when administered to rats (Baumann et al. 2012; Huang et al. 2012; Aarde et al. 2013a; Shortall et al. 2013) or mice (López-Arnau et al. 2012; Marusich et al. 2012; Fantegrossi et al. 2013; Gatch et al. 2013). In one of the first studies to examine the behavioral effects of bath salts cathinones, Marusich et al. showed that mephedrone, methylone, and MDPV produce dose-dependent increases in ambulation in mice, and MDPV is somewhat more potent in this regard (Marusich et al. 2012). We found that MDPV is about tenfold more potent than cocaine as a locomotor stimulant in rats, and MDPV is also more efficacious than cocaine, stimulating an overall greater magnitude of motor activation (Baumann et al. 2013). When MDPV and other synthetic cathinones are administered across a broad range of doses, the dose-response relationship for ambulation is an inverted U-shaped function (Aarde et al. 2013a; Gatch et al. 2013); the reduction in forward locomotion at higher drug doses is due to the emergence of focused stereotypies, such as in-place perseverative sniffing and head bobbing, as dose increases. Recent studies demonstrate that MDPV and its unsubstituted phenyl ring analogs α -PVP, α -PBP, and α -PPP induce dose-related stimulation of locomotor activity in mice, and in vivo potency is correlated with in vitro DAT activity (Marusich et al. 2014). Specifically, as the α -carbon alkyl chain length decreases across the series of compounds, potency to induce motor activation and inhibit uptake at DAT decrease in parallel (see Table 3). In mice, the locomotor stimulant effects of MDPV and α -PVP were significantly blunted by pretreatment with antagonists for either D₁ or D₂ dopamine receptors (Kaizaki et al. 2014; Marusich et al. 2014). Taken together with the microdialysis data, the available evidence indicates that MDPV, and perhaps other synthetic cathinones, elevate extracellular dopamine in critical brain circuits via DAT inhibition, and subsequent activation of D₁ and D₂ receptors by endogenous dopamine underlies locomotor stimulant effects of the drugs.

The role of extracellular 5-HT in modulating the dopaminergic effects of synthetic cathinones is a topic of great interest. As mentioned already, most ring-substituted cathinones act as nonselective transporter substrates, which can increase extracellular concentrations of both dopamine and 5-HT. A recent investigation compared the neurochemical and locomotor effects of MDPV and methylone in rats to examine potential interactions between dopaminergic and serotonergic effects of these drugs (Schindler et al. 2016). It was found that i.v. doses of 0.3 mg/kg MDPV and 3.0 mg/kg methylone produce nearly identical threefold elevations in extracellular dopamine, whereas only methylone produces a dramatic tenfold elevation in extracellular 5-HT. At these same doses, MDPV elicits a much greater stimulation of ambulation and stereotypy when compared to methylone. The data comparing MDPV and methylone are reminiscent of the data comparing methcathinone and its 4-trifluoromethyl analog; methcathinone is a potent DAT substrate with robust motor stimulant effects, but 4-trifluoromethyl-*N*-methcathinone (4-TFM MCAT) is a SERT-preferring substrate with minimal stimulant effects (Cozzi et al. 2013). One interpretation of these findings is that elevations in extracellular 5-HT tend to reduce locomotor stimulant effects mediated by extracellular dopamine. Indeed, substantial

evidence indicates that high-affinity 5-HT_{2C} receptor sites in the brain provide a strong inhibitory influence over dopamine-mediated behavioral effects of cocaine and other psychomotor stimulant drugs of abuse (Devroye et al. 2013; Howell and Cunningham 2015). Future studies are warranted to examine the role of 5-HT receptor subtypes in modulating the motor stimulant effects of new synthetic cathinones as they appear in the recreational drug marketplace.

5.3 Rewarding Effects

The acute rewarding effects of abused drugs are related to their ability to increase extracellular dopamine concentrations in the nucleus accumbens, by acting directly or indirectly with mesocorticolimbic neuronal pathways (Di Chiara and Imperato 1988; Volkow and Morales 2015). Psychomotor stimulant drugs are capable of directly activating mesolimbic dopamine neurons because they act as DAT blockers or substrates which increase extracellular dopamine concentrations. Despite many differences in complex behaviors between humans and animal species, the self-administration of abused drugs is largely conserved among humans (Henningfield et al. 1991), nonhuman primates (Beveridge et al. 2006), rats (Deroche-Gamonet et al. 2004), and mice (Yan et al. 2014). In the case of synthetic cathinones, animal studies are critical for determining the rewarding effects of these drugs because no controlled laboratory studies in humans have been carried out. Moreover, results from animal studies are needed to assess risk and inform legislative decisions for banning specific drugs as they emerge in the recreational drug marketplace. Two experimental paradigms that have been used to characterize the abuse liability of drugs in rats are drug self-administration and ICSS.

Drug self-administration is often considered the “gold standard” behavioral test for determining the abuse potential of drugs (Watterson and Olive 2017). In the rat drug self-administration paradigm, animals with surgically implanted i.v. catheters are trained to lever-press or nose-poke to obtain i.v. drug injections which are delivered via a computer-controlled infusion pump. The data reviewed thus far predict that DAT/NET-selective drugs like MDPV would engender potent rewarding effects. Indeed, rats rapidly learn to self-administer MDPV under fixed ratio conditions and maintain high rates of responding during 1 or 2 h self-administration sessions (Aarde et al. 2013a; Watterson et al. 2014; Schindler et al. 2016). When tested in the progressive ratio test, MDPV shows breakpoints similar to methamphetamine and amphetamine, confirming the efficacious reinforcing properties of MDPV. In addition, when access to MDPV is extended to 6 h, MDPV shows escalation of drug intake for doses between 0.1 and 0.5 mg/kg i.v. (Watterson et al. 2014). More recent studies show that MDPV analogs like α -PVP are also readily self-administered, with potency and efficacy comparable to MDPV itself (Aarde et al. 2015; Huskinson et al. 2017; Gannon et al. 2018). Taken together, the self-administration data with pyrrolidine-containing cathinones indicate these compounds are highly rewarding and possess risk for compulsive use.

The neurochemical effects of mephedrone and methylone are similar to the effects of MDMA, the prototypical “entactogen-type” drug of abuse. The term entactogen is used to describe agents like MDMA, which engender feelings of emotional communion, oneness, or

relatedness in human users (Aarde and Taffe 2017). The unique subjective effects of MDMA are presumably related to elevations in extracellular 5-HT, which subsequently activates 5-HT receptor subtypes, particularly 5-HT_{2A} sites, in the brain (Liechti and Vollenweider 2001; Farre et al. 2007; Hysek et al. 2012). The serotonergic activity of ring-substituted cathinones might be predicted to reduce their abuse liability since preclinical evidence supports a role for 5-HT in dampening reinforcing effects of stimulant drugs (Wee et al. 2005; Wee and Woolverton 2006). However, a number of studies in rats demonstrate that mephedrone and methylone are readily self-administered and maintain high rates of drug-appropriate responding (Hadlock et al. 2011; Watterson et al. 2012; Aarde et al. 2013b; Motbey et al. 2013; Schindler et al. 2016). In particular, the pattern of mephedrone self-administration under fixed ratio schedules seems closer to highly dependence-producing drugs like methamphetamine rather than MDMA, though mephedrone is about tenfold less potent than methamphetamine (Aarde et al. 2013; Motbey et al. 2013). Taffe and colleagues directly compared self-administration behavior for various entactogens and found mephedrone-trained rats show much higher levels of responding than either methylone- or MDMA-trained rats (Creehan et al. 2015; Vandewater et al. 2015), pointing to higher abuse liability for mephedrone. Importantly, when the duration of self-administration sessions is extended from 2 to 6 h, mephedrone, methylone, and MDMA all display escalation of drug intake consistent with compulsive use (Vandewater et al. 2015). Collectively, the self-administration data published thus far indicate that mephedrone and methylone display substantial abuse liability. These findings suggest that rewarding effects of synthetic cathinones are complex and may involve neurotransmitter systems in addition to dopamine and 5-HT (e.g., norepinephrine). More studies are needed to clarify the role of specific neurotransmitter systems, and their receptor subtypes, in modulating rewarding effects of ring-synthetic cathinones.

ICSS is a technique that can be used to characterize the rewarding effects of drugs. ICSS involves the electrical stimulation of the medial forebrain bundle, a collection of nerve fibers including axons of ascending dopaminergic projections from the ventral tegmental area to the nucleus accumbens, which sustains reward in different species (Negus and Miller 2014). One important feature of the ICSS paradigm is the ability to discern abuse-related and abuse-limiting effects of drugs. For example, prior ICSS studies show that most monoamine transporter ligands induce facilitation of ICSS responding, which is an abuse-related effect mediated by extracellular dopamine. By contrast, the SERT-selective substrate fenfluramine induces suppression of ICSS responding, an abuse-limiting effect (Bauer et al. 2013). Bonano et al. compared ICSS responses following administration of MDPV, mephedrone, or methylone and showed all three compounds facilitate ICSS responding, consistent with abuse-related and rewarding effects of the drugs. However, there were important differences across the compounds tested. MDPV engenders robust ICSS facilitation which is comparable to the effects of methcathinone and has a long duration of action. Methylone showed comparable effects to MDPV in terms of ICSS facilitation and duration of action, but it is tenfold less potent than MDPV. Surprisingly, mephedrone was the weakest of the compounds tested to facilitate ICSS responding (Bonano et al. 2014). The finding that methylone induces greater facilitation of ICSS responding when compared to mephedrone contrasts with the self-administration data discussed previously, which clearly show

mephedrone is a more potent and efficacious reinforcer. It seems possible that drug self-administration and ICSS responding may involve distinct yet overlapping circuitries which can yield disparate results.

Bonano et al. carried out a study to examine the SAR of ring-substituted cathinones with different substituents at the *para*-position of the phenyl ring (Bonano et al. 2015). By modifying the same position of the methcathinone scaffold, it was possible to obtain transporter substrates ranging from 300-fold selective for DAT over SERT (e.g., methcathinone) to 20-fold selective for SERT over DAT (e.g., 4-trifluoromethyl-*N*-methcathinone). As discussed above, abuse-related effects of the compounds were positively correlated with DAT/SERT ratio, and highly DAT-selective analogs strongly facilitated ICSS responding. Nonselective analogs produced mild and variable facilitation of ICSS, and highly SERT-selective analogs strongly depressed ICSS responding.

6 Summary

The research investigations reviewed in this chapter show that synthetic cathinones interact with monoamine transporter proteins as either blockers or substrates. Pyrrolidine-containing cathinones like MDPV and α -PVP are potent uptake blockers at DAT and NET, with much less potent effects at SERT. The bulky pyrrolidine ring and α -carbon alkyl chain are critical determinants of DAT/NET activity, and shorter α -carbon chain length is associated with decreased potency. Most evidence indicates that pyrrolidine-containing cathinones are devoid of substrate activity, perhaps because they are sterically too large to fit through the transporter permeation pore. Ring-substituted cathinones like mephedrone and methylone are nonselective transporter substrates, which induce non-exocytotic release of dopamine, norepinephrine, and 5-HT by reverse transport. Due to their substrate activity, mephedrone and methylone are capable of inducing transporter-associated ionic currents. Ring-substituted cathinones with bulky *para* substituents have enhanced activity at SERT, and DAT/SERT selectivity decreases as substituent size increases.

Regardless of whether synthetic cathinones act as blockers or substrates, they all increase extracellular concentrations of monoamines in brain reward pathways. Drug-induced elevations in extracellular dopamine in the nucleus accumbens appear to underlie locomotor and rewarding effects in vivo, consistent with the effects of other stimulant drugs. Importantly, the precise molecular mechanism of drug action is less important than overall selectivity across DAT, NET, and SERT. In general, synthetic cathinones with high DAT selectivity are potent and efficacious reinforcers, whereas those with high SERT selectivity are less reinforcing. However, there are many caveats to this simplistic view. A number of synthetic cathinones with mixed DAT/SERT activity display greater abuse liability than MDMA in animal models. The in vivo data with mephedrone are most intriguing, since this compound acts as an efficacious reinforcer in self-administration assays but is weak in its ability to facilitate ICSS responding. Despite the increasing knowledge about the neuropharmacology of synthetic cathinones, many questions remain unanswered, including the poorly understood role of non-transporter sites of action, drug pharmacokinetics, and drug metabolism. More research is warranted to examine the biological effects of synthetic

cathinones in rodent models, especially investigations aimed at determining the mechanisms underlying motor stimulant and rewarding effects of the drugs.

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Acronyms of the Discussed New Psychoactive Substances (NPS)

4-Bromo MCAT	1-(4-Bromophenyl)-2-(methylamino)propan-1-one (brepheдрone)
4-Chloro MCAT	1-(4-Chlorophenyl)-2-(methylamino)propan-1-one (clepheдрone)
4-Fluoro MCAT	1-(4-Fluorophenyl)-2-(methylamino)propan-1-one (flepheдрone)
4-Methyl MCAT	(4-MMC) 2-(Methylamino)-1-(4-methylphenyl)propan-1-one (mephedrone)
4-Methoxy MCAT	1-(4-Methoxyphenyl)-2-(methylamino)propan-1-one (methedrone)
4-TFM MCAT	2-(Methylamino)-1-[4-(trifluoromethyl)phenyl] propan-1-one
MCAT	2-(Methylamino)-1-phenylpropan-1-one (methcathinone)
MDMA	1-(2 <i>H</i> -1,3-Benzodioxol-5-yl)- <i>N</i> -methylpropan-2-amine
MDMC	1-(2 <i>H</i> -1,3-Benzodioxol-5-yl)-2-(methylamino) propan-1-one (methylone)
MDPV	1-(2 <i>H</i> -1,3-Benzodioxol-5-yl)-2-(pyrrolidin-1-yl) pentan-1-one
α-PBP	1-Phenyl-2-(pyrrolidin-1-yl)butan-1-one
α-PHP	1-Phenyl-2-(pyrrolidin-1-yl)hexan-1-one
α-PPP	1-Phenyl-2-(pyrrolidin-1-yl)propan-1-one
α-PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one

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Fig. 1. Chemical structures of synthetic cathinones. Synthetic cathinones are β -keto amphetamines. Diethylpropion, bupropion, and pyrovalerone are FDA-approved medications in the USA, whereas mephedrone, methyone, and MDPV are abused drugs that were first encountered in so-called “bath salts” products

Fig. 2.

Dose-response effects for cocaine and amphetamine in DAT uptake and release assays in rat brain synaptosomes. Data are depicted as mean $\pm$ SD for $N=3$ experiments performed in triplicate. Note that cocaine and amphetamine both fully inhibit [^3H]dopamine uptake (left panel), whereas only the transporter substrate amphetamine evokes fully efficacious release of [^3H]MPP $^{+}$ (right panel)

Fig. 3.

Effects of monensin on $[^3\text{H}]\text{MPP}^+$ efflux induced by MDPV or mephedrone in HEK cells expressing human DAT. Vehicle (VEH, physiological buffer) or 10 μM monensin (MON) was added to the perfusion solution at 4 min, whereas 10 μM MDPV or 10 μM mephedrone (MEPH) was added at 12 min. Data are mean $\pm$ SD for $N=3$ experiments performed in triplicate. Note that MON has no effect on efflux produced by MDPV (left panel) but markedly enhances efflux produced by the substrate MEPH (right panel). * denotes significance with respect to VEH + MEPH group, $P < 0.05$

Fig. 4.

Effects of reserpine pretreatment on extracellular dopamine responses produced by MDPV or mephedrone in rats undergoing microdialysis in nucleus accumbens. Rats received 5 mg/kg i.p. reserpine 24 h prior to microdialysis testing. Rats received i.v. injections of MDPV or mephedrone (MEPH) at time zero and 60 min later. Data are mean $\pm$ SEM for $N=6$ rats/group, depicted as % basal calculated from three preinjection samples. Basal dopamine concentrations in control and reserpinized rats were 3.98 ± 0.88 and 1.44 ± 0.22 pg/ μ L, respectively. Note that reserpine reduces dialysate dopamine responses produced by the blocker MDPV (left panel), without altering effects of the substrate MEPH (right panel). * denotes significant difference from vehicle-treated group, $P < 0.05$

Table 1

Comparison between the effects of monoamine transporter blockers versus substrates

Parameter	Monoamine transporter blockers	Monoamine transporter substrates (i.e., releasers)
Inhibit neurotransmitter uptake	Yes	Yes
Enter into neurons	No	Yes
Induce inward depolarizing Na ⁺ currents	No	Yes
Trigger reverse transport (transporter-mediated release)	No	Yes
Increase extracellular concentrations of transmitters	Yes	Yes
Neurochemical effects impulse- and TTX-sensitive	Yes	No
Neurochemical effects Ca ⁺⁺ - and reserpine-sensitive	Yes	No
Long-term neurotoxic deficits in monoamine neurons	No	Yes

Table 2

Effects of *para*-position (i.e., 4-position) ring substitution on potency to release [³H]MPP+ via DAT and [³H]5-HT via SERT in rat brain synaptosomes

<i>Para</i> group	Drug	Steric volume (cubic Å)	DAT release EC ₅₀ (nM)	SERT release EC ₅₀ (nM)	DAT/SERT ratio
H	Methcathinone (MCAT)	150.4	12.5	3,860	309
F	4-Fluoro MCAT (flephedrone)	153.8	83.4	1,290	15.4
Cl	4-Chloro MCAT (clephedrone)	164.4	42.2	144	3.40
CH ₃	4-Methyl MCAT (mephedrone)	166.9	49.1	118	2.41
Br	4-Bromo MCAT (brephedrone)	169.9	59.4	60.2	1.01
OCH ₃	4-Methoxy MCAT (methedrone)	175.0	506	120	0.24
CF ₃	4-Trifluoromethyl (4-TFM MCAT)	178.4	2,700	190	0.07

Data are mean EC₅₀ values for *N* = 3 experiments performed in triplicate, adapted from Bonano et al. (2015). DAT/SERT ratio = (DAT EC₅₀)⁻¹ / (SERT EC₅₀)⁻¹, where higher value indicates greater DAT selectivity.

Table 3

Effect of α -carbon alkyl chain length on potency to inhibit uptake of [^3H]neurotransmitters via DAT, NET, and SERT in rat brain synaptosomes

Alpha-carbon Chain length	Drug	DAT uptake inhibition IC_{50} (nM)	NET uptake inhibition IC_{50} (nM)	SERT uptake inhibition IC_{50} (nM)	DAT/SERT ratio
	Cocaine	211	292	313	1.5
3C (Propyl)	MDPV	4.1	25.9	3,305	806
4C (Butyl)	α -PHP	11.4	26.3	>10,000	>877
3C (Propyl)	α -PVP	12.8	14.2	>10,000	>781
2C (Ethyl)	α -PBP	63.3	91.5	>10,000	>159
1C (Methyl)	α -PPP	196	445	>10,000	>51

Data are mean IC_{50} values for $N=3$ experiments, adapted from Marusich et al. (2014), except for α -PHP data which are unpublished. DAT/SERT ratio = $(\text{DAT } \text{IC}_{50})^{-1}/(\text{SERT } \text{IC}_{50})^{-1}$, where higher value indicates greater DAT selectivity