Pharmacology of MDMA- and Amphetamine-Like New Psychoactive Substances

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Abstract

New psychoactive substances (NPS) with amphetamine-, aminoindan-, and benzofuran basic chemical structures have recently emerged for recreational drug use. Detailed information about their psychotropic effects and health risks is often limited. At the same time, it emerged that the pharmacological profiles of these NPS resemble those of amphetamine or MDMA. Amphetamine-like NPS induce psychostimulation and euphoria mediated predominantly by norepinephrine (NE)- and dopamine (DA) transporter (NET and DAT) inhibition and transporter-mediated release of NE and DA, thus, showing a more catecholamine-selective profile. MDMA-like NPS frequently induce well-being, empathy, and prosocial effects and have only moderate psychostimulant properties. These MDMA-like substances primarily act by inhibiting the serotonin (5-HT) transporter (SERT) and NET, also inducing 5-HT and NE release. Monoamine receptor interactions vary considerably among amphetamine- and MDMA-like NPS. Clinically, amphetamine- and MDMA-like NPS can induce sympathomimetic toxicity. The aim of this chapter is to review the state of knowledge

regarding these substances with a focus on the description of the *in vitro* pharmacology of selected amphetamine- and MDMA-like NPS. In addition, it is aimed to provide links between pharmacological profiles and *in vivo* effects and toxicity, which leads to the conclusion that abuse liability for amphetamine-like NPS may be higher than for MDMA-like NPS, but that the risk for developing the life-threatening serotonin syndrome may be increased for MDMA-like NPS.

Keywords
Aminoindans; amphetamine, benzofurans; DAT; dopamine; 4-fluoroamphetamine; 4-FA; 5-IT; NET; noradrenaline; NPS; MDMA; monoamines; release; serotonin; SERT; uptake

Acronyms of the Discussed New Psychoactive Substances (NPS)

2-AI	2-Aminoindane
3-MMC	3-Methyl-*N*-methylcathinone
4-APB	4-(2-Aminopropyl)benzofuran
5-APB	5-(2-Aminopropyl)benzofuran
6-APB	6-(2-Aminopropyl)benzofuran
7-APB	7-(2-Aminopropyl)benzofuran
5-APDB	5-(2-Aminopropyl)-2,3-dihydrobenzofuran
6-APDB	6-(2-Aminopropyl)-2,3-dihydrobenzofuran
5-EAPB	5-(2-Ethylaminopropyl)benzofuran
4-FA	4-Fluoroamphetamine
5-IAI	5-Iodoaminoindan
5-IT, 5-API	5-(2-Aminopropyl)indole
4-MA	4-Methylamphetamine
5-MAPDB	1-(2,3-Dihydrobenzofuran-5-yl)-*N*-methylpropan-2-amine
MBDB	3,4-Methylenedioxyphenyl-*N*-methyl-2-butanamine
MDA	3,4-Methylenedioxyamphetamine
MDAI	3,4-Methylenedioxyaminoindan
MDEA	3,4-Methylenedioxy-*N*-ethylamphetamine
MMAI	5-Methoxy-6-methyl-2-aminoindan
4-MTA	4-Methylthioamphetamine
PMA	*para*-Methoxyamphetamine
PMMA	*para*-Methoxymethamphetamine

1. Introduction

Amphetamine and its derivative 3,4-methylenedioxymethamphetamine (MDMA) are substances that have been abused for decades. Amphetamine, and alternatively methamphetamine are typically sold under the street name “speed”, and MDMA is the substance typically associated with “ecstasy” pills. Although MDMA is a 3,4-methylenedioxy derivative of amphetamine, the subjective effects as well as the pharmacological profiles of MDMA and amphetamine are distinct. Psychostimulation and euphoria are commonly described acute subjective effects of amphetamine consumption (Dolder et al. 2017). MDMA is the prototypical entactogenic/empathogenic drug (Nichols 1986) and induces fewer psychostimulant effects than amphetamine (Bershad et al. 2016). Enhancement of feelings of love, happiness, and closeness to others are typical entactogenic effects (Hysek et al. 2014a; Hysek et al. 2014b; Liechti et al. 2001). Amphetamine and MDMA act on monoamine re-uptake transporters (Simmler et al. 2013; Verrico et al. 2007). By blocking the serotonin (5-HT), dopamine (DA), and norepinephrine (NE) transporters (SERT, DAT, and NET, respectively), re-uptake of the respective neurotransmitters is prevented, causing increased neurotransmitter concentrations in the synaptic cleft (Kehr et al. 2011; Torres et al. 2003). Amphetamine derivatives typically also induce transporter-mediated release of neurotransmitters (Blakely et al. 2005; Hysek et al. 2012c). Since the neurotransmitters 5-HT, DA, and NE are differentially involved in modulating behavior and subjective effects, distinct pharmacological profiles of drugs of abuse can be linked to specific psychotropic effects and intoxication (Dolder et al. 2017; Liechti 2015; Schmid et al. 2014). As such, amphetamine with preference for human DAT and NET is experienced differently than MDMA, which, in contrast, preferentially acts at human SERT and NET vs. DAT (Simmler et al. 2013).

The chemical diversity found among substances commonly referred to as new psychoactive substances (NPS) is quite pronounced. Over 600 different NPS have emerged on the illicit drug market since the beginning of this century (EMCDDA 2016). The pharmacology and psychotropic effects range widely among amphetamine-based NPS. For example, subjective effects induced by NPS based on the amphetamine template may range from hallucinogenic *via* entactogenic to stimulant properties predominantly depending on the nature and location of substituents on the phenyl ring (Hill and Thomas 2011; Liechti 2015; Zwartsen et al. 2017; Nichols 2017) In addition, many amphetamine-based NPS carry a keto group on the ß-position of the carbon side chain, thus, giving rise to the cathinone template (Figure 1) (Prosser and Nelson 2012), Cathinone NPS are discussed in detail in the preceding chapter of this book. The present chapter will focus on non-ß-keto NPS that resemble amphetamine and MDMA in their pharmacology and subjective effects. The main focus of this chapter is the description of the *in vitro* pharmacology of selected amphetamine- and MDMA-like NPS, with the additional aim to provide links between pharmacological profiles and *in vivo* effects and toxicity.

By using the terms amphetamine- and MDMA-like NPS, we refer mostly to pharmacological profiles that are comparable to amphetamine and/or MDMA. Pivotal are the relative potencies for inhibition of the human SERT, DAT, and NET. Furthermore, characteristic for amphetamine- and MDMA-like substances is that they induce transporter-mediated release of monoamines. Release is typical for amphetamine and MDMA and distinguishes amphetamine-derivatives from pure uptake blockers such as cocaine or the NPS 3,4-methylenedioxypyrovalerone (MDPV; Baumann et al. 2013). Our classification of NPS as amphetamine- or MDMA-like is

mainly based on the relative activity for uptake inhibition at DAT *vs.* SERT, referred to as DAT/SERT ratio. DAT/SERT ratios are calculated as IC_{50} value for SERT divided by IC_{50} value for DAT (also as 1/(DAT IC_{50}) divided by 1/(SERT IC_{50})). DAT/SERT ratios can also be calculated for EC_{50} values for transporter-mediated release (Baumann et al. 2012; Marusich et al. 2016). MDMA acts preferentially on SERT, reflected in a low DAT/SERT ratio. In contrast, amphetamine acts preferentially on DAT, reflected in a high DAT/SERT ratio (Simmler et al. 2013). Below we will discuss the pharmacology and psychotropic effects of amphetamine-like and MDMA-like NPS separately.

2. MDMA- and amphetamine-like NPS

The amphetamine-derivatives discussed in this chapter are *N*-ethylamphetamine, 4-fluoroamphetamine (4-FA), 4-fluoromethamphetamine, 5-(2-aminopropyl)indole (5-IT, also known as 5-API), 4-methylamphetamine (4-MA), 3,4-methylenedioxyphenyl-*N*-methyl-2-butanamine (MBDB), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 4-methylthioamphetamine (4-MTA), *para*-methoxyamphetamine (PMA), and *para*-methoxymethamphetamine (PMMA). Aminoindans discussed are 2-aminoindane (2-AI), 5-iodoaminoindan (5-IAI), 3,4-methylenedioxyaminoindan (MDAI), and 5-methoxy-6-methyl-2-aminoindan (MMAI). Benzofurans discussed are 4-(2-aminopropyl)benzofuran (4-APB), 5-(2-aminopropyl)benzofuran (5-APB), 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB), 5-(2-ethylaminopropyl)benzofuran (5-EAPB), 1-(2,3-dihydrobenzofuran-5-yl)-*N*-methylpropan-2-amine (5-MAPDB), 6-(2-aminopropyl)benzofuran (6-APB), 6-(2-aminopropyl)-2,3-dihydrobenzofuran (6-APDB), and 7-(2-aminopropyl)benzofuran (7-APB).

If not otherwise noted, we refer to the racemic mixtures of compounds, except for D- or (*S*)-(+)-amphetamine. The isomers of psychoactive drugs with asymmetric centers can have different biological activity (Baumann et al. 2007). However, since street drugs are produced as racemic mixtures, pharmacological *in vitro*- or animal studies that use the racemic mixtures of the compounds are representative reflections of street drug activity. Chemical structures of amphetamine, MDMA and selected NPS are shown in Figure 1. Cathinone-derivatives are discussed in the preceding chapter of this book, but the structure of cathinone is displayed in Figure 1 to illustrate the ß-keto substituent typical for cathinone-based NPS. 2-AI forms the basic structure of aminoindan-derived NPS, and 5-APB is shown as example for benzofuran NPS.

3. MDMA-like NPS

3.1. Pharmacology of MDMA-like substances

Several specific ring-substituted amphetamine-based NPS that lack the ß-keto substituent resemble MDMA in their pharmacological profile. MDMA-like NPS potently inhibit the NET and SERT with lower potency for DAT inhibition. Accordingly, their DAT/SERT ratio is low, comparable to MDMA, for which our laboratory reported a DAT/SERT ratio of 0.08 (Simmler et al. 2013). DAT/SERT ratios for MDMA below 1 were also reported for release (Eshleman et al. 2017; Marusich et al. 2016; Baumann

et al. 2012). As for uptake inhibition (Simmler et al. 2013), these studies also report DAT/SERT ratios for amphetamine- or methamphetamine-induced release that are >150 times higher than the DAT/SERT ratio of MDMA. A low DAT/SERT ratio predicts MDMA-like subjective effects and a lower dependence potential compared to amphetamine (Suyama et al. 2016; Liechti 2015). MDMA-like pharmacological properties can be found among benzofurans, aminoindans, and amphetamines.

MDMA is a prototypical entactogenic/empathogenic drug that retains some psychostimulant effects. It increases empathy, sociability, closeness to others, but also happiness and self-esteem (Hysek et al. 2014a; Hysek et al. 2014b; Liechti et al. 2001). Cardiostimulant effects are common and include increased blood pressure, increased heart rate, and hyperthermia (Vizeli and Liechti 2017). Bruxism resulting from increased muscle tension is also experienced frequently (Cole and Sumnall 2003). The psychotropic and cardiostimulant effects have been attributed foremost to the 5-HT and NE release properties of MDMA (Hysek et al. 2011; Hysek et al. 2012c). Concomitant to inducing transporter-mediated 5-HT and NE release, MDMA inhibits the re-uptake of the respective neurotransmitters at SERT and NET. MDMA inhibits DAT with significant lower potency than it inhibits NET and SERT. Its low DAT/SERT ratio (0.08; Simmler et al. 2013) is a representative measure for selectivity of SERT over DAT and is used here to compare NPS to MDMA.

MDMA is also a low-potency partial agonist of the 5-HT_{2A} receptor. Although not frequent, mild hallucinogen-like effects of MDMA have been reported, which may be attributable to 5-HT_{2A} agonism (Nichols 2004; Liechti et al. 2000). MDA, the active metabolite of MDMA (Hysek et al. 2011), shows a 10-fold higher potency for 5-HT_{2A} agonism than MDMA (Rickli et al. 2015c). MDA likely contributes to the mode of action of MDMA and might contribute to the mild hallucinogenic effects of MDMA.

Binding affinity of MDMA for adrenergic receptors is low, but since MDMA increases NE levels *via* transporter-mediated NE release and NET uptake inhibition, indirectly NE-mediated effects at adrenergic receptors clearly contribute to MDMA action (Hysek et al. 2011). β-Adrenoceptors are involved in MDMA-induced increase of heart rate (Hysek et al. 2010). The α_1- and β-adrenoceptors have been implicated in hyperthermia and drug-induced vasoconstriction (Hysek et al. 2012a). α_{2A}-Adrenocepors are associated with sympathomimetic toxicity and augmented NE release (Hysek et al. 2012b). Potent transporter-mediated NE release or even NET inhibition seems sufficient to induce cardiostimulant effects mediated through the different adrenergic receptors (Hysek et al. 2011). NPS with potent effects at NET thus likely induce psychostimulation and sympathomimetic toxicity.

3.1.1. Serotonergic toxicity

Increased levels of 5-HT can lead to serotonergic toxicity and, in extreme cases, can result in precipitation of the serotonin syndrome. Typical symptoms of the serotonin syndrome include neuromuscular hyperactivity, clonus, autonomous hyperactivity (including hyperthermia), sweating, agitation and confusion (Gillman 2005; Liechti 2015). MDMA-like drugs typically induce symptoms of serotonergic intoxication, potentially leading to a severe serotonin syndrome including hyperthermia but also a syndrome of inadequate diuretic hormone (SIADH) resulting in hyponatremia (Simmler et al. 2011; Hartung et al. 2002; Holden and Jackson 1996). Hyperthermia, followed by life-threatening complications such as rhabdomyolysis, intravascular coagulation, and organ failure, is commonly involved in fatal intoxications with psychostimulants (Cole and Sumnall 2003). Although hyperthermia is a well-described unwanted effect

of MDMA (Liechti 2014), the past has shown that certain psychoactive substances induce hyperthermia more readily than MDMA, which has been associated with fatal complications. For example, the more traditional substances PMA, PMMA, and 4-MTA show a selectivity for SERT over DAT similar to MDMA (Table 1) but the morbidity and mortality linked to these particular substances has been observed to be greater and more distinct compared to MDMA (Lurie et al. 2012; Vevelstad et al. 2012). Inhibition of monoamine oxidase (MAO)-A by PMA, PMMA and 4-MTA (Matsumoto et al. 2014), might play a major role in the induction of severe hyperthermia. Increased levels of cytosolic 5-HT, resulting from MAO inhibition, might augment drug-induced release of 5-HT *via* SERT. The combination of MAO inhibition and 5-HT release, as reported for PMA, PMMA and 4-MTA (Matsumoto et al. 2014), is therefore particularly prone to induce potentially life-threatening serotonergic intoxication. MAO inhibition has been implicated in hyperthermia and the life-threatening serotonin syndrome (Carmo et al. 2003; Gillman 2005). From the use of selective SERT inhibitors as antidepressants, it is well known that the combination of SERT inhibition together with MAO inhibition can cause life-threatening serotoninergic intoxication, including hyperthermia. Testing NPS for the potential of MAO inhibition might therefore warrant further investigation.

3.1.2. Noradrenergic toxicity

Sympathomimetic toxicity results from increased activation of the NE system, either *via* direct activation of adrenergic receptors or indirectly *via* receptor activation due to increased NE levels (Hysek et al. 2012a; Hysek et al. 2011; Hysek et al. 2010). Hyperthermia, hypertension, tachycardia and agitation are typical symptoms of stimulant-induced noradrenergic toxicity (Cruickshank and Dyer 2009; Cole and Sumnall 2003). Cardiovascular sympathomimetic toxicity is typically associated with amphetamine but also occurs following MDMA administration *via* induction of NE release and increases in plasma NE levels. NE-mediated hyperthermia involves stimulation of α_1- and $ß_3$-adrenoceptors (Sprague et al. 2004; Hysek et al. 2012a). Mechanistically, hyperthermia occurs by activation of α_1-adrenoceptors via increased vasoconstriction, leading to decreased heat dissipation. $ß_3$-Adrenoceptor activation causes mitochondrial uncoupling, which increases heat generation (Liechti 2014).

3.2. MDMA-like amphetamine derivatives

The amphetamine-derivatives MBDB, 4-MA, MDEA, 4-MTA, PMA, and PMMA show uptake inhibition profiles and DAT/SERT ratios similar to MDMA (Table 1). *In vivo* drug discrimination experiments in rats suggest that PMA, PMMA and 4-MTA show MDMA-like properties (Dukat et al. 2002; Glennon et al. 2007). However, these substances did not substitute for amphetamine in the drug discrimination experiments, which predicts that PMA, PMMA and 4-MTA have less stimulant-like properties than MDMA. The (*S*)-(+)-isomer of MDMA showed stimulant properties in rats, reflected in hyperlocomotion and substitution for amphetamine in drug discrimination tests (Glennon et al. 1988). 4-MTA, PMA and PMMA have been associated with severe serotonergic toxicity (Liechti 2015). The combination of MAO inhibition and potent 5-HT release properties are likely the cause for the high morbidity and mortality reported for these substances. Similarly, 4-MA is a potent 5-HT releaser (Baumann et al. 2011) and inhibits MAO, and has been associated with fatal intoxications (Blanckaert et al. 2013). Furthermore, 4-MA is a potent partial agonist at the 5-HT_{2B} receptor (Luethi et

al. 2017). This receptor has been implicated in endocardial fibrosis (Roth 2007) but whether chronic substance use indeed causes such cardiac complications remains to be established.

MDEA, although equal to MDMA for SERT inhibition potency (Table 1), induces hyperthermia in rats less potently than MDMA (Colado et al. 1999). The lower potency at NET compared to MDMA (Table 1) might account for this difference, since NE release plays a crucial role in the induction of drug-induced hyperthermia (Sprague et al. 2004; Hysek et al. 2012a; Liechti 2014). Similarly, MBDB is less potent at NET inhibition than MDMA (Table 1; or in rat synaptosomes $IC_{50(MDMA)}$ = 405 nM, $IC_{50(MBDB)}$ = 1233 (Johnson et al. 1991)). MBDB is considered to share a range of psychopharmacological effects also observed with MDMA and drug discrimination studies revealed that MBDB substitutes for MDMA (Aerts et al. 2000). To the best of our knowledge, no studies on the effect of MBDB and MDEA on MAO exist to date.

3.3. MDMA-like aminoindans

Aminoindans were originally developed as potential therapeutic bronchodilators, but have emerged in the recent years as NPS among recreational drug users, although with relatively low prevalence (Brandt et al. 2013; Sainsbury et al. 2011). Among the relatively few NPS falling into the aminoindan class, differences in the pharmacological profiles have been described. The aminoindans 5-IAI, MDAI, and MMAI show an MDMA-like uptake inhibition profile with DAT/SERT ratios smaller or equal to 0.2 (Table 1). In contrast, 2-aminoindan (2-AI) and *N*-methyl-2-AI are selective NET inhibitors and NE releasers, with 2-AI also releasing DA (Simmler et al. 2014b; Luethi et al. 2017). MDAI induces transporter-mediated release of 5-HT and NE, similar to MDMA, but, unlike MDMA, MDAI does not induce DA release through the human DAT under the conditions investigated (Simmler et al. 2014b; Eshleman et al. 2017). 5-IAI causes 5-HT and DA release, but not NE release, although it acts as a potent NET inhibitor (Iversen et al. 2013; Simmler et al. 2014b). According to user reports, MDAI and 5-IAI cause euphoria and have entactogenic properties (Pinterova et al. 2017). Interestingly, MMAI was shown to be selective for SERT over DAT and NET when inducing uptake inhibition and transporter-mediated release (Johnson et al. 1991; Luethi et al. 2017). The lack of NET activity predicts that MMAI might not be truly experienced as MDMA-like, since NE-mediated psychostimulation is likely absent or weak in acute MMAI effects, whereas MDMA induces pronounced NE-mediated psychostimulation (Hysek et al. 2012a; Hysek et al. 2011; Hysek et al. 2010). The selective serotonergic activity could imply a high risk for serotonin syndrome, similar to 4-MTA. However, unlike 4-MTA, MMAI does not significantly inhibit MAO (Scorza et al. 1999), which might be relevant when considering the potential for severe adverse effects.

Some aminoindans were developed as potential non-neurotoxic alternatives for MDMA. MDAI was reported to substitute for MDMA, but not LSD, in drug discrimination studies in rats. However, in contrast to MDMA, MDAI did not cause 5-HT depletion (Nichols et al. 1990). Similarly, no indication for 5-HT toxicity was found for 5-IAI (Nichols et al. 1991). Non-neurotoxic effects of MDAI and 5-IAI suggested that these aminoindans might display a safer risk profile compared to MDMA but recent animal studies indicated that MDAI can induce potentially life-threatening toxicity related to the serotonin syndrome (Gatch et al. 2016; Palenicek et al. 2016). Three fatal intoxications involving MDAI and other substances have been described and from

ante-mortem information available in one case, the involvement of serotonin toxicity was considered likely (Corkery et al. 2013).

The receptor interaction profiles of MDMA-like aminoindans show that 5-IAI exhibits nanomolar affinity at the 5-HT_{1A}-, 5-HT_{2A}- (Simmler et al. 2014b) and the 5-HT_{2B} receptors (Iversen et al. 2013). Furthermore, affinity (K_i = 1.2 µM) for 5-IAI was also observed at the 5-HT_{2C} receptor (Simmler et al. 2014b). This is in contrast to MDAI, which did not exhibit affinity to these 5-HT receptors, and to MMAI, which only had activity in the micromolar range (Luethi et al. 2017).

3.4. MDMA-like benzofurans

The pharmacology and toxicology of benzofurans is relatively poorly explored to date, but fatal, analytically confirmed intoxication with the benzofuran 5-APB alone or in combination with 3-MMC have been reported (Adamowicz et al. 2014; McIntyre et al. 2015). Benzofurans were described by users as substances inducing entactogenic and stimulant effects, but also sympathomimetic toxicity, including hyperthermia (Welter-Luedeke and Maurer 2016). In drug discrimination studies in rats, the 2,3-dihydrobenzofurans 5-APDB and 6-APDB substituted for MDMA-like entactogens, but not for amphetamine (Monte et al. 1993).

Many benzofurans show MDMA-like pharmacological profiles. Rickli *et al.* (Rickli et al. 2015b) have characterized a set of benzofurans, which were all potent NET inhibitors and showed DAT/SERT ratios < 1 (Table 1). 5-APDB, 5-MAPDB, 5-APB, and 6-APDB showed high selectivity for SERT over DAT inhibition with DAT/SERT ratios of 0.01-0.07. 5-EAPB and 6-APB have DAT/SERT ratios of 0.15 and 0.29, respectively. The least selective compounds for SERT over DAT were 4-APB and 7-APB with DAT/SERT ratios of 0.46 and 0.65. All benzofurans characterized in this study induced transporter-mediated release of one, two, or all three monoamines.

Interestingly, most benzofurans were partial agonists at the 5-HT_{2A} and the 5-HT_{2B} receptors. 5-HT_{2A} agonism of benzofurans is comparable to MDMA (activation potency of 6 µM and 55% efficacy), but MDMA does not have functional activity at the 5-HT_{2B} receptor (Rickli et al. 2015b). Since activation of the 5-HT_{2B} receptor has been associated with heart valve fibrosis (Roth 2007), chronic consumption of benzofurans might pose a risk for long-term cardiotoxicity (Dawson et al. 2014). 4-APB, 6-APB, 6-APBP, and 7-APB showed affinity for the α_{2A}-adrenoceptor in the nanomolar range (K_i values of 140 – 870 nM; (Rickli et al. 2015b), which might contribute to the sympathomimetic toxicity by augmenting vesicular NE release (Hysek et al. 2012b).

7-APB is a moderately potent human trace amine-associated receptor 1 (TAAR1) receptor agonist with an EC_{50} in the nanomolar range (630 nM), similar to the endogenous ligands ß-PEA (260 nM) and *p*-tyramine (990 nM) and more than 10-fold more potent than other benzofurans (Simmler et al. 2016). The activation of human TAAR1 might diminish the effects of psychostimulation and intoxication arising from 7-APB effects on monoamine transporters (see 4.1.3. for more details). Affinity to mouse and rat TAAR1 has been shown for many psychostimulants, but species differences are common (Simmler et al. 2016). For example, 5-IT and 4-MA bind and activate TAAR1 in the nanomolar range, but do not activate human TAAR1.

3.5. Conclusions

Although MDMA-like substances have low abuse liability due to their selectivity for SERT over DAT, the risk for potentially life-threatening intoxication appears high

considering serotonergic toxicity that, for some MDMA-like NPS, might be augmented due to inhibition of MAO. Sympathomimetic toxicity arising from NE action is also common. The classification into MDMA-like NPS is approximately based on DAT/SERT ratios and does not account for subtle differences in the pharmacology of NPS, such as receptor interactions or increased prevalence of 5-HT toxicity. Consequently, collecting clinical and pre-clinical information for each MDMA-like NPS is helpful for contextualizing the clinical features seen in acute toxicity cases.

4. Amphetamine-like NPS

4.1. Pharmacology of amphetamine-like substances

Subjective effects of amphetamine involve psychostimulation, euphoria, and increased arousal. Other clinical features include acute cardiotoxicity, such as hypertension, increased blood pressure, heart rate, and body temperature (Dolder et al. 2017). Regular amphetamine consumption bears a considerable risk for abuse and dependence. Amphetamine increases DA and NE levels by DAT and NET inhibition and induction of transporter-mediated release (Heal et al. 2013). Amphetamine further shows moderate affinity for the α_{2A} adrenoceptor and the 5-HT_{1A} receptor (Simmler et al. 2013) and is a TAAR1 ligand (Bunzow et al. 2001) with potent full agonist properties at the human TAAR1 (Simmler et al. 2016).

Non-ß-keto NPS described here as amphetamine-like show relatively good uptake inhibition potencies at SERT with DAT/SERT ratios between 1 and 6 (Table 1). In comparison to amphetamine or methamphetamine, 4-fluoroamphetamine, 4-fluoromethamphetamine, *N*-ethylamphetamine, and 5-IT are less selective at DAT and NET and rather non-selective for all monoamine transporters, although about 10-fold more potent at NET than DAT and SERT. Like amphetamine, these amphetamine-like NPS also act as monoamine releasers (Simmler et al. 2014a; Rickli et al. 2015a; Luethi et al. 2017). In contrast, there are several cathinone-derivatives with DAT/SERT ratios > 10, such as cathinone, methcathinone, or 3-fluoromethcathinone (Simmler et al. 2013; Simmler et al. 2014a), which resemble amphetamine more closely in their transporter inhibition profile.

4.1.1. Acute dopaminergic toxicity

Induction of rapid increase of DA level in the mesolimbic DA system is a typical acute effect of many drugs of abuse (Kehr et al. 2011), including amphetamine and cocaine, but also opioids, which have indirect effects onto the DA system (Luscher and Malenka 2011). Drug-induced increase of DA levels activates the reward system and causes euphoria (Heal et al. 2013; Sulzer 2011). Unwanted drug effects such as psychotic states and aggression can also be attributed to excessive/chronic stimulation of dopaminergic action (Harro 2015). Life-threatening excited delirium syndrome has been associated with acute dopaminergic toxicity (Mash et al. 2009).

4.1.2. Abuse liability

The dopamine system is crucially involved in plasticity underlying drug dependence and compulsive drug use (Luscher and Malenka 2011; Pascoli et al. 2015; Koob and Volkow 2016). Substances acting to increase DA levels *via* DAT inhibition and/or DA reverse transport might therefore cause dependence, possibly progressing to addiction. However, the 5-HT system can oppose dopaminergic effects (Daw et al. 2002; Alex and Pehek 2007) and serotonergic properties can lower the abuse liability of psychostimulants (Simmler et al. 2017; Suyama et al. 2016; Liechti 2015; Bauer et al. 2013; Rothman and Baumann 2006; Wee et al. 2005). Accordingly, the relative action at DAT *vs.* SERT is crucial for assessing abuse liability of psychostimulants (Baumann et al. 2012). From a pre-clinical perspective, high DAT/SERT ratios are generally considered indicative of a significant potential for abuse and dependence. The *in vitro* selectivity for DAT vs. SERT predicts the DA vs. 5-HT release as measured using *in vivo* microdialysis and also correlates with measures of reward such as intracranial self-stimulation thresholds as shown for a series of *para*-ring-substituted cathinones (Suyama et al. 2016). In addition, cathinones with a predominant action on the DA system are self-administered more readily than substances with a more 5-HT activating profile (Bonano et al. 2014; Schindler et al. 2016; Gannon et al. 2018). MDMA shows a relatively low abuse liability and has high selectivity of SERT over DAT. In the assays carried out in the authors' laboratory, the abuse dependence liability of cocaine is associated with a DAT/SERT ratio of 3.1 and amphetamine and methamphetamine have an even higher DAT/SERT ratio of > 10 (Simmler et al. 2013). The amphetamine-like NPS discussed here show DAT/SERT ratios of 1-5. From the perspective of these studies, these amphetamine-like NPS might show lower abuse liability than amphetamine. However, other factors such as drug kinetics, receptor interactions, routes of administration and social context also play important roles for the clinical picture.

4.1.3. Activation of TAAR1

TAAR1 is a target of amphetamine and many amphetamine-derivatives. TAAR1 is involved in the regulation of DA activity (Bradaia et al. 2009) and activation of TAAR1 reduces the abuse liability of drugs such as cocaine (Pei et al. 2014; Pei et al. 2015). Amphetamine and MDMA induce more pronounced psychostimulant effects in rodents not expressing TAAR1 (Lindemann et al. 2008; Di Cara et al. 2011). Psychostimulants, which act directly on TAAR1, can therefore induce negative modulation of psychotropic effects. TAAR1 activation might have protective effect with respect to drug toxicity and abuse liability. However, species differences between the rodent TAAR1 and human TAAR1 are frequent (Simmler et al. 2016). MDMA activates rat and mouse TAAR1 with low micromolar potencies, but its activation potency for the human TAAR1 is very low. Amphetamine, in contrast, activates human TAAR1 with an EC_{50} of 2.8 μM (Simmler et al. 2016). For the present chapter, available evidence on the activity of NPS at the human TAAR1are presented.

4.2. 4-Fluoroamphetamine, 4-fluoromethamphetamine and *N*-ethylamphetamine

4-FA is a popular NPS and described by users to induce a mixture of amphetamine- and MDMA-like effects, which include stimulation, euphoria and empathy (Linsen et al. 2015; Hondebrink et al. 2017). Despite the entactogenic properties reported for 4-

FA, which are typical for MDMA-like substances, 4-FA has been included in this section due to its DAT/SERT ratio above a value of 1 (Wee et al. 2005; Rickli et al. 2015a; Eshleman et al. 2017). 4-FA inhibits monoamine transporters with potencies in the rank order NET > DAT > SERT (Table 1, Eshleman et al. 2017). The same rank order has also been described for the potency of 4-FA to induce monoamine release (EC_{50} values of 28 nM (NE), 52 nM (DA), and 939 nM (5-HT) (Wee et al. 2005). Cardiotoxicity and hyperthermia are typical symptoms of sympathomimetic intoxication associated with 4-FA use, and severe headache has been increasingly reported (Hondebrink et al. 2017). Cerebral hemorrhage and severe cardiovascular toxicity were diagnosed in several cases of severe or fatal intoxications (Wijers et al. 2017; Hondebrink et al. 2017). 4-FA exhibits moderate affinity for the α_{2A} adrenoceptor and shows weak to moderate binding affinity or activation potency at the 5-HT_{1A}-, 5-HT_{2A}-, 5-HT_{2B}-, and 5-HT_{2C} receptors. 4-FA is also a partial agonist at the human TAAR1 (Rickli et al. 2015a).

4-Fluoromethamphetamine is less frequently reported, but resembles 4-FA in its pharmacological profile at monoamine transporters and receptors (Table 1; Rickli et al. 2015a). The *in vitro* data suggest that clinical effects and toxicity might be similar to those reported for 4-FA. *N*-Ethylamphetamine induces hyperlocomotion in mice (Tessel et al. 1975) and substitutes for amphetamine in drug-discrimination tests in rhesus monkeys (Woolverton and English 1997), which indicates stimulant-like properties of *N*-ethylamphetamine. Furthermore, *N*-ethylamphetamine showed reinforcing properties in rhesus monkeys (Tessel and Woods 1975). *N*-Ethylamphetamine was found to show a comparable uptake inhibition profile as 4-fluoromethamphetamine (Table 1) and functioned as a releaser of DA, NE, and 5-HT (EC_{50} values of 2.6 µM (NE), 93 µM (DA), and 43 µM (5-HT); Tessel and Rutledge 1976). *N*-Ethylamphetamine has moderate binding affinity for the α_{2A}-, 5-HT_{2A}-, and 5-HT_{2C}-receptors (Simmler et al. 2014a), but does not activate human TAAR1 (Simmler et al. 2016). Similar to 4-fluoromethamphetamine, the pharmacological profile for *N*-ethylamphetamine predict effects and clinical features to be similar to 4-FA.

4.3. 5-(2-Aminopropyl)indole (5-IT)

5-IT (5-API) has caused a considerable number of fatal intoxications since its emergence in 2012 (EMCDDA 2014). The clinical cases presented with sympathomimetic and serotonergic toxicity, including hyperthermia, cardiotoxicity, and organ failure (Bäckberg et al. 2014). 5-IT inhibits NET, DAT and SERT and 5-IT induces transporter-mediated release of NE, DA and 5-HT (Marusich et al. 2016; Luethi et al. 2017). Although 5-IT acts more potently at NET and DAT than at SERT, serotonergic toxicity has been implicated in the intoxication cases. In addition, 5-IT inhibits MAO-A (Herraiz and Brandt 2014), which can augment the rise of 5-HT levels and poses the risk for resulting in the development of the serotonin syndrome. 5-IT has affinity for the α_{1A}- and α_{2A} adrenoceptors (K_i of 5.4 and 1.7 µM, respectively; (Luethi et al. 2017), which might contribute to sympathomimetic toxicity. 5-IT is also a potent partial agonist at 5-HT_{2A} and 5-HT_{2B} receptors (EC_{50} of 0.5 and 1.5 µM, respectively; Luethi et al. 2017), which are important for mediating hallucinogenic effects *via* 5-HT_{2A} activity (Nichols 2004) and, in the long-term use, might result in heart valve fibrosis mediated *via* 5-HT_{2B} activity (Roth 2007).

4.4. 2-Aminoindan

The use and reported fatalities of 2-AI are relatively rare (Elliott and Evans 2014), and not much more recent information about its *in vivo* pharmacology and toxicology could be identified. It is worth noting that the *in vitro* pharmacological profile of 2-AI is distinct from the MDMA-like aminoindans 5-IAI, MDAI and MMAI (see Section 3.3.). In contrast to the MDMA-like aminoindans, 2-AI did not inhibit SERT (Table 1; Simmler et al. 2014b). 2-AI selectively inhibits NET, also inducing NE release, and at higher concentrations, also acts as a DAT inhibitor and releaser (Simmler et al. 2014b). The selectivity of 2-AI for NET over DAT at the human transporters implies that it causes psychotropic effects that may be distinct from amphetamine, since DA-mediated euphoria might be low or absent for 2-AI. However, in rat synaptosomes, 2-AI showed DAT and NET inhibition (Horn and Snyder 1972). Amphetamine-like properties of 2-AI were also indicated behaviourally in rats by induction of hyperlocomotion (Mrongovius et al. 1978) and by substitution for amphetamine in drug discrimination experiments (Glennon et al. 1984). Sympathomimetic effects of 2-AI can arise from increased NE levels due to NET inhibition and NE release, and high-affinity (K_i = 450 nM) binding of 2-AI to the α_{2A} adrenoceptor (Simmler et al. 2014b) likely contributes to the sympathomimetic effect of 2-AI. Interestingly, 2-AI was a full agonist at the human TAAR1 with similar potency (EC_{50} of 1.5 µM) similar to amphetamine (EC_{50} of 2.8 µM), which also has full-agonist properties (Simmler et al. 2016).

4.5. Conclusions

Amphetamine-like NPS preferentially inhibit DAT and NET and act as releasers. The NPS discussed here show preference for DAT over SERT inhibition, but with lower DAT/SERT ratios than amphetamine. The receptor interaction profiles of amphetamine-like NPS vary and might contribute to drug-specific psychotropic effects and toxicity. MAO inhibition has been shown for 5-IT and suggests particular risk for fatal intoxication.

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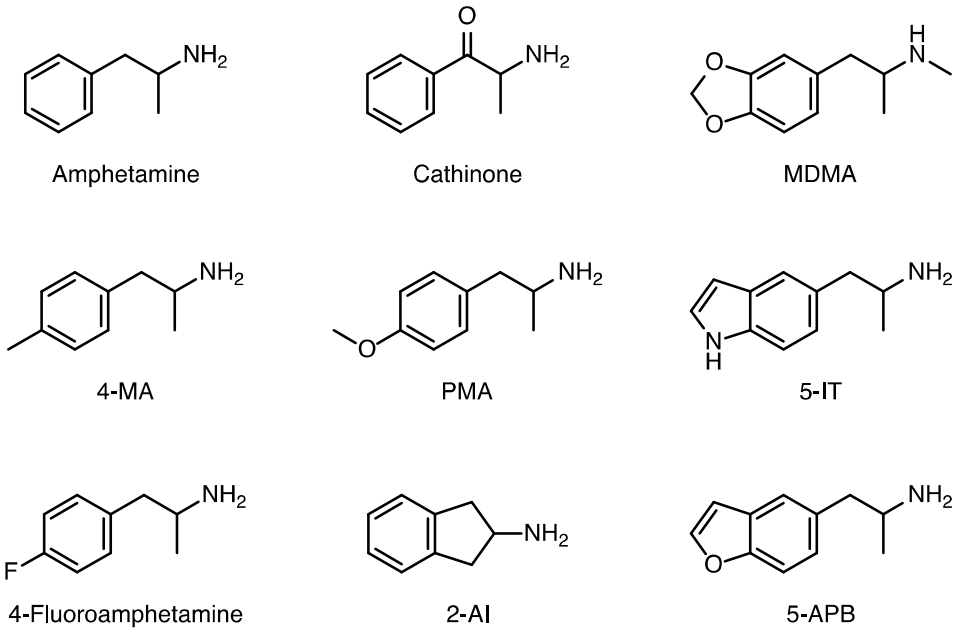

Figure 1: Chemical structures of amphetamine, MDMA and selected NPS.

Table 1: Uptake inhibition potencies of MDMA, amphetamine, methamphetamine, and selected MDMA- and amphetamine-like NPS in alphabetical order. Experiments were conducted *in vitro* with cultured cells that express the human NET, DAT, or SERT. All substances were tested as racemic mixtures except for D-amphetamine.

	NET	**DAT**	**SERT**		
	IC_{50} (µM) (95% CI)	**IC_{50} (µM) (95% CI)**	**IC_{50} (µM) (95% CI)**	**DAT/SERT ratios**	**Values from**
2-AI	0.54 (0.42-0.69)	58 (4-905)	>100	>1	(3)
D-Amphetamine	0.094 (0.06-0.14)	1.30 (0.83-2.0)	>10	>10	(1)
4-APB	0.24 (0.2-0.3)	12 (9-16)	5.5 (3.4-8.7)	0.46	(5)
5-APB	0.16 (0.08-0.3)	6.1 (4-9)	0.29 (0.17-0.5)	0.05	(5)
6-APB	0.19 (0.1-0.3)	3.3 (2.4-4.5)	0.93 (0.7-1.3)	0.29	(5)
7-APB	0.27 (0.2-0.3)	20 (16-26)	13 (9-18)	0.65	(5)
5-APDB	0.29 (0.2-0.5)	49 (33-73)	0.58 (0.4-0.9)	0.01	(5)
6-APDB	0.56 (0.4-0.8)	33 (25-43)	2.3 (1.4-3.9)	0.07	(5)
5-EAPB	0.56 (0.4-0.7)	4.9 (3-8)	0.72 (0.5-1.1)	0.15	(5)
N-Ethylamphetamine	0.20 (0.15-0.27)	5.86 (4.8-7.1)	8.77 (6-13)	1.5	(2)
4-Fluoroamphetamine	0.20 (0.14-0.28)	3.7 (2.4-5.7)	19 (11-33)	5.1	(4)
4-Fluoromethamphetamine	0.22 (0.14-0.35)	7.7 (2.5-24)	8.7 (3.8-20)	1.1	(4)
5-IAI	0.76 (0.60-0.98)	23 (15-35)	2.5 (1.9-3.4)	0.11	(3)
5-IT	0.04 (0.03-0.06)	0.68 (0.55-0.85)	1.3 (0.9-1.7)	1.9	(6)
5-MAPDB	0.96 (0.5-1.7)	77 (62-96)	1.2 (0.7-2)	0.02	(5)
MBDB	2.80 (1.9-4.1)	22 (20-26)	2.04 (1.4-3.0)	0.09	(1)
MDA	0.42 (0.3-0.6)	20.5 (20.3-20.6)	4.9 (3.5-6.8)	0.24	(5)
MDAI	0.65 (0.50-0.84)	31 (23-41)	8.3 (3.2-22)	0.2	(3)
MDEA	1.02 (0.78-1.3)	9.3 (8.0-11)	1.27 (0.93-1.7)	0.14	(1)
MDMA	0.447 (0.33-0.60)	17 (12-24)	1.36 (1.0-2.0)	0.08	(1)
D-Methamphetamine	0.064 (0.04-0.09)	1.05 (0.74-1.5)	>10	>10	(1)
4-Methylamphetamine	0.31 (0.24-0.42)	5.6 (4.5-6.9)	0.82 (0.64-1.05)	0.15	(6)
MMAI	3.6 (2.5-5.3)	193 (167-225)	0.68 (0.50-0.92)	0.004	(6)
4-MTA	1.52 (1.3-1.9)	22 (15-32)	0.54 (0.37-0.80)	0.02	(2)
PMA	0.80 (0.50-1.0)	71 (60-83)	2.37 (2.0-2.9)	0.03	(2)
PMMA	1.20 (0.75-1.8)	49 (18-135)	1.77 (1.1-2.9)	0.04	(2)

(1) Simmler *et al.*, 2013
(2) Simmler *et al.*, 2014a
(3) Simmler *et al.,* 2014b
(4) Rickli *et al.,* 2015a
(5) Rickli *et al.*, 2015b
(6) Luethi *et al.,* 2017