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2-Aminoindan and its Ring-Substituted Derivatives Interact with Plasma Membrane Monoamine Transporters and α_2-Adrenergic Receptors

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ABSTRACT

Rationale: Over the last decade many new psychostimulant analogues have appeared on the recreational drug market and most are derivatives of amphetamine or cathinone. Another class of designer drugs is derived from the 2-aminoindan structural template. Several members of this class, including the parent compound 2-aminoindan (2-AI), have been sold as designer drugs. Another aminoindan derivative, 5-methoxy-2-aminoindan (5-MeO-AI or MEAI), is the active ingredient in a product marketed online as an alcohol substitute. *Methods:* Here we tested 2-AI and its ring-substituted derivatives 5-MeO-AI, 5-methoxy-6-methyl-2-aminoindan (MMAI), and 5,6-methylenedioxy-2-aminoindan (MDAI) for their abilities to interact with plasma membrane monoamine transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT). We also compared the binding affinities of the aminoindans at 29 receptor and transporter binding sites. *Results:* 2-AI was a selective substrate for NET and DAT. Ring substitution increased potency at SERT while reducing potency at DAT and NET. MDAI was moderately selective for SERT and NET, with 10-fold weaker effects on DAT. 5-MeO-AI exhibited some selectivity for SERT, having 6-fold lower potency at NET and 20-fold lower potency at DAT. MMAI was highly selective for SERT, with 100-fold lower potency at NET and DAT. The aminoindans had relatively high affinity for α_2-adrenoceptor subtypes. 2-AI had particularly high affinity for α_{2C} receptors (K_i = 41 nM) and slightly lower affinity for the α_{2A} (K_i = 134 nM) and α_{2B} (K_i = 211 nM) subtypes. 5-MeO-AI and MMAI also had moderate affinity for the 5-HT_{2B} receptor. *Conclusions:* 2-AI is predicted to have (+)-amphetamine-like effects and abuse potential whereas the ring-substituted derivatives may produce 3,4-methylenedioxymethamphetamine (MDMA)-like effects but with less abuse liability.

Keywords: dopamine, serotonin, norepinephrine, synaptosomes, binding, analgesia, stimulant, MEAI

1. INTRODUCTION

Although the phenomenon of designer drugs is not new, many novel controlled substance analogues have appeared on the recreational (i.e., non-medical) drug market over the last decade. New analogues are appearing at an alarming rate and the widespread availability and misuse of these substances is causing a significant public health problem (Baumann and Volkow 2016; Halberstadt 2017; Huestis et al. 2017). Some of these substances are amphetamine derivatives, for example 4-fluoroamphetamine and 4-methylamphetamine (Johansen and Hansen 2012; Elliott and Evans 2014; Linsen et al. 2015; Solis et al. 2017). Other substances are derived from cathinone (2-amino-1-phenylpropan-1-one), the β-keto analogue of amphetamine, which occurs naturally in the leaves of the Khat plant *Catha edulis*. Amphetamine and cathinone derivatives act as substrates for plasma membrane monoamine transporters and promote the non-exocytotic release of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) (Rothman et al. 2001; Baumann et al. 2012; Cozzi et al. 2013; Hutsell et al. 2016; Eshleman et al. 2017). The effects and abuse potential of monoamine releasers vary depending on their selectivity for NE, DA, and 5-HT transporters (NET/SLC6A2, DAT/SLC6A3, and SERT/SLC6A4, respectively). Substances that are relatively selective for NET and DAT, such as (+)-amphetamine and (+)-methamphetamine, act as psychostimulants, whereas 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") is nonselective for NET, DAT and SERT and is thought to produce "entactogenic" effects via 5-HT release (Liechti et al. 2000; Farre et al. 2007; Tancer and Johanson 2007). The abuse liability of monoamine-releasing drugs is correlated with their capacity to release NE and DA (Rothman et al. 2001). Conversely, non-selective or 5-HT-selective releasers have reduced abuse-potential, as evidenced by self-administration and

intracranial self-stimulation (ICSS) measures (Wee et al. 2005; Bauer et al. 2013; Schindler et al. 2016).

In addition to the cathinone derivatives, another class of designer drugs is derived from the 2-aminoindan structural template (see Figure 1). These substances can be viewed as cyclic analogues of amphetamines. The parent compound of this structural class, 2-aminoindan (2-AI, Su-8629), was likely first synthesized by Benedikt (1893) in low yield from 2-indanone via reduction of the oxime derivative. In terms of its human psychopharmacology, 2-AI reportedly produces mild stimulant effects, with a p.o. dose range of 50-100 mg (Anonymous 2017). 2-AI has been available in Europe as a designer drug (EMCDDA 2007; Brandt et al. 2013; Brunt et al. 2017).

Ring-substituted derivatives of 2-AI, such as 5-methoxy-2-aminoindan (5-MeO-AI, MEAI), 5-methoxy-6-methyl-2-aminoindan (MMAI), and 5,6-methylenedioxy-2-aminoindan (MDAI), have also been sold as designer drugs (Figure 1). Encounters with MDAI were reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2010 (EMCDDA 2011). MDAI and MMAI were synthesized by Nichols et al. as potentially non-neurotoxic entactogens (Nichols et al. 1990; Johnson et al. 1991b). Both of these substances produce MDMA-like behavioral effects in rats (Nichols et al. 1990; Johnson et al. 1991b; Gatch et al. 2016) and increase 5-HT release with some selectivity vs. NE and DA (Johnson et al. 1991a). Use of MDAI reportedly induces euphoria and feelings of empathy, with 150-200 mg p.o. being a typical recreational dose (Corkery et al. 2013). The third ring-substituted compound, 5-MeO-AI, appears to have been first synthesized in 1956 (Richter and Schenck 1956) and has been proposed as a potential alcohol substitute (Golan 2016; Shimshoni et al. 2017; Shimshoni et al. 2018). 5-MeO-AI reportedly produces mild psychoactive effects and

euphoria in recreational users (Slezak 2015). 5-MeO-AI is the active ingredient in a product called PaceDrink, which is marketed online as an alcohol-like intoxicant.

The goal of the present investigation was to assess the pharmacological properties of 2-AI derivatives. 2-AI, MMAI, and MDAI reportedly act as substrate-type monoamine releasers (Simmler et al. 2014b; Eshleman et al. 2017) but full dose-response data for releasing activity (i.e., EC_{50} values) are only available for the latter compound. Furthermore, much of what is known about the monoamine-releasing effects of aminoindans is based on assays conducted in non-neuronal cells overexpressing transporter proteins, which tend to underestimate the potency of substrate-type releasers by an order of magnitude or more compared to native tissues. In studies of HEK293 (HEK) cells expressing DAT, (+)-methamphetamine released preloaded [^{3}H]DA with EC_{50} = 435 nM (Eshleman et al. 2017) or EC_{50} = 1.56 μM (Simmler et al. 2013). By contrast, in rat brain synaptosomes, (+)-methamphetamine induced [^{3}H]substrate efflux through DAT with EC_{50} values ranging from 8.5 nM to 28.0 nM (Rothman et al. 2001; Nagai et al. 2007; Baumann et al. 2012). Although uptake assays have been used to assess interactions between aminoindans and monoamine transporters (Johnson et al. 1991a), those assays also tend to underestimate the potency of substrate releasers (Bhat et al. 2017). In the present studies, the monoamine-releasing properties of 2-AI, 5-MeO-AI, MMAI, and MDAI were compared using *in vitro* release assays for DAT, NET, and SERT in rat brain synaptosomes. In addition to their transporter interactions, aminoindans also bind to monoamine receptors (Marona-Lewicka and Nichols 1994; Iversen et al. 2013; Simmler et al. 2014b). Existing binding studies with 2-AI, MMAI and MDAI, however, have only focused on a small subset of 5-HT, DA, and NE receptor subtypes. Therefore, comprehensive binding studies were performed to assess the affinity of aminoindans at 5-HT, DA, and NE receptor subtypes. The aminoindans were found to act as

substrate-type monoamine releasers with differing patterns of selectivity for SERT, DAT, and NET. Consistent with previous reports indicating that certain 2-aminoindan derivatives bind to α_2-adrenoceptors (Iversen et al. 2013; Simmler et al. 2014b), 2-AI, 5-MeO-AI, MMAI and MDAI had moderate to high affinity for α_2-adrenoceptor subtypes.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley rats (300-400 g, Envigo, Frederick, MD, USA) were housed 2 per cage and maintained on a 12 h light-dark cycle. Food and water were provided *ad libitum*. Animal use procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Care and Use Committee of the Intramural Research Program of the National Institute on Drug Abuse (Baltimore, MD, USA).

2.2. Drugs

2-Aminoindan (2-AI) hydrochloride, 5-methoxy-6-methyl-2-aminoindan (MMAI) hydrochloride, and 5,6-methylenedioxy-2-aminoindan (MDAI) hydrochloride were obtained from Cayman Chemical (Ann Arbor, MI, USA). 5-Methoxy-2-aminoindan (2-MeO-AI) hydrochloride was obtained from Key Organics Ltd (Cornwall, UK).

2.3. Transporter Release Assays

Rats were euthanized by CO_2 narcosis and the brains were removed and processed to yield synaptosomes. Briefly, caudate tissue (for DAT assays) or whole brain minus cerebellum and caudate (for NET and SERT assays) was homogenized in ice-cold 10% sucrose containing 1 μM reserpine. After 12 strokes with a Potter-Elvehjem homogenizer, the homogenates were centrifuged at 1,000 × g at 4°C for 10 min and the supernatants (i.e., synaptosomal preparations) were retained on ice. Transporter assays were carried out as described previously (Baumann et al. 2013; Solis et al. 2017). For the release assays, 9 nM [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP$^+$) was used as the radiolabeled substrate for DAT and NET, whereas 5 nM [^{3}H]5-HT was used as the radiolabeled substrate for SERT. All buffers used in the release assays contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of release assays was optimized for a single transporter by including unlabeled blockers to prevent the uptake of [^{3}H]MPP$^+$ or [^{3}H]5-HT by competing transporters. Desipramine (100 nM) and citalopram (100 nM) were added to the buffer for DAT release experiments. Citalopram (100 nM) and GBR 12935 (50 nM) were added to the buffer for NET release experiments. GBR 12935 (50 nM) and nomifensine (100 nM) were added to the buffer for SERT release experiments. Synaptosomes were preloaded with radiolabelled substrate in Krebs-phosphate buffer for 1 h (steady state). Release assays were initiated by adding 850 μL of preloaded synaptosomes to 150 μL of test drug. The release assays were terminated by vacuum filtration and retained radioactivity was quantified by scintillation counting. Effects of test drugs on release were expressed as % maximum release, with maximum release (E_{max}) defined as the release produced by tyramine at doses that evoke the efflux of all 'releasable' tritium by synaptosomes (10 μM tyramine for DAT

and NET assay conditions, and 100 μM tyramine for SERT assay conditions; Rothman et al. 2001). Effects of test drugs on release were analyzed by nonlinear regression using GraphPad Prism 6 (GraphPad Software, San Diego, CA) to calculate EC_{50} values.

2.4. Radioligand Binding Assays

A screening at 29 receptor and transporter binding sites was performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). Most of these screenings were performed with cloned human receptors; exceptions are listed in Table 2. Test compounds were dissolved in DMSO and were tested at 10 μM in competition assays against radioactive probe compounds. Sites exhibiting > 50% inhibition at 10 μM were tested in secondary assays at the identified receptor or transporter using 12 concentrations of the drug (0.1 nM – 10 μM), measured in triplicate, to generate competition binding isotherms. K_i values were obtained from nonlinear regression of these binding isotherms from best-fit IC_{50} values using the Cheng-Prusoff equation (Cheng and Prusoff 1973). The radioligands used were as follows: [^{3}H]8-OH-DPAT (5-HT_{1A}), [^{3}H]GR125743 (5-$HT_{1B/1D}$), [^{3}H]ketanserin (5-HT_{2A}), [^{3}H]LSD (5-$HT_{2B/5A/6/7}$), [^{3}H]mesulergine (5-HT_{2C}), [^{3}H]citalopram (serotonin transporter), [^{3}H]prazosin ($\alpha_{1A/1B/1D}$), [^{3}H]rauwolscine ($\alpha_{2A/2B/2C}$), [^{125}I]pindolol (β_1), [^{3}H]CGP12177 (β_2, β_3), [^{3}H]nisoxetine (norepinephrine transporter), [^{3}H]SCH23390 (D_1, D_5), [^{3}H]*N*-methylspiperone ($D_{2/3/4}$), [^{3}H]WIN35428 (dopamine transporter), [^{3}H](+)-pentazocine (σ_1), and [^{3}H]DTG (σ_2). For more information, see: Besnard et al. (2012). The experimental protocols are available from the NIMH PDSP website (Roth 2013).

3. RESULTS

3.1. Effects on Monoamine Release

The aminoindans displayed efficacious releasing activity at DAT, NET, and SERT. As depicted in Figure 2, 2-AI, 5-MeO-AI, MMAI, and MDAI produced a dose-dependent increase in the efflux of [^{3}H]MPP$^+$ and [^{3}H]5-HT from preloaded synaptosomes. Table 1 summarizes the dose-response data for the 2-aminoindans, including the EC_{50} values and selectivity ratios for each compound. The unsubstituted parent compound 2-AI is a catecholamine-selective drug, with potent releasing actions at NET (EC_{50} = 86 nM) and DAT (EC_{50} = 439 nM) but not at SERT (EC_{50} > 10,000 nM). With regard to selectivity ratios, 2-AI displayed a DAT/NET ratio of 0.20 and a DAT/SERT ratio of > 22, confirming its selectivity toward catecholamine transporters. For comparative purposes, (+)-amphetamine showed a DAT/NET ratio of 0.29 and a DAT/SERT ratio of 71 in previous synaptosomal release experiments (Rothman et al. 2001). Ring-substitution markedly increases potency towards SERT, creating agents that are at least 10-fold selective for SERT over DAT. MDAI is equipotent at SERT and NET, with 10-fold weaker effects at DAT, yielding a DAT/SERT ratio of 0.08. Compared to MDAI, 5-MeO-AI had a lower DAT/SERT ratio (0.05), and was moderately selective for SERT vs. NET and DAT. MMAI is a potent and selective 5-HT releaser, displaying a DAT/SERT ratio of > 0.003.

3.2. Binding Affinities

The binding affinities of the aminoindans for 29 receptors and binding sites are shown in Table 2. All compounds bound to α_2 adrenoceptors with submicromolar or micromolar affinities but lacked appreciable affinity for α_1- and β-adrenergic receptors (< 50% displacement at 10 μM). 2-AI had high affinity for α_{2A} (K_i = 134 nM), α_{2B} (K_i = 211 nM), and α_{2C} (K_i = 41 nM) receptors. Compared to 2-AI, 5-MeO-AI and MMAI had 5-fold lower affinity for α_{2A} and α_{2B} and 30-fold lower affinity for α_{2C}, indicating that 5-methoxy substitution has a detrimental effect on α_2 binding. Similar reductions in affinity for α_2 adrenoceptor subtypes occurred with MDAI. 2-AI and MDAI lacked affinity for 5-HT receptors (< 50% displacement at 10 μM), whereas the 5-methoxy-substituted compounds 5-MeO-AI and MMAI had moderate affinity for 5-HT_{1A} and 5-HT_{2B} receptors. The presence of a 6-methyl-substituent apparently facilitates binding to 5-HT_{1B}, 5-HT_{1D}, and 5-HT_7 receptors because MMAI had moderate affinity for those sites whereas 5-MeO-AI had little or no affinity. With the exception of MDAI, which bound to SERT with a K_i of 4,822 nM, the aminoindans displayed low potency for inhibiting binding of high-affinity radioligands to or monoamine transporters (< 50% displacement at 10 μM). None of the compounds were active at dopaminergic receptors or σ binding sites.

4. DISCUSSION

All of the aminoindans tested stimulate monoamine efflux via transporters, albeit with varying degrees of selectivity for DAT, NET, and SERT. Based on the transporter data, the parent compound 2-AI is a selective substrate for NET and DAT, similar to (+)-amphetamine (Rothman et al. 2001). Ring substitution on 2-AI increased the potency of SERT-mediated release while reducing potency at DAT and NET. MDAI is a moderately selective releaser via

SERT and NET, with 10-fold weaker effects on DAT, meaning it increases 5-HT release in a manner similar to MDMA but has somewhat weaker effects on DA release (cf., Rothman et al. 2001). 5-MeO-AI exhibited some selectivity for SERT-mediated release, having 6-fold lower potency at NET and 20-fold lower potency at DAT. Consistent with previous reports (Johnson et al. 1991a; Marona-Lewicka and Nichols 1994; Luethi et al. 2017), MMAI is highly selective SERT releaser, with 100-fold lower potency at NET and DAT. In addition to their effects on monoamine release, the aminoindans had relatively high affinity for α_2-adrenoceptor subtypes. The 5-methoxy substituted compounds (5-MeO-AI and MMAI) also bind to 5-HT_{1A} and 5-HT_{2B} receptors with moderate affinity.

The release data for the aminoindans correlate well with data from previous studies. Liechti and colleagues examined the effects of 2-AI, MDAI, and MMAI on monoamine release from HEK cells expressing cloned transporters (Simmler et al. 2014b; Luethi et al. 2017). At 100 μM, 2-AI induced the release of preloaded [^{3}H]DA and [^{3}H]NE but not [^{3}H]5-HT, MDAI released [^{3}H]5-HT and [^{3}H]NE whereas [^{3}H]DA was not affected, and MMAI released [^{3}H]5-HT selectively. Although full dose-effect curves for [^{3}H]transmitter release were not reported by Liechti et al., we observed the same qualitative pattern of activity with 2-aminoindans in our synaptosomal release assays. Thus, data from human transporters expressed in non-neuronal cells agree with our data from rat transporters in native tissue preparations. According to another group (Eshleman et al. 2017), MDAI released preloaded [^{3}H]NE (EC_{50} = 0.57 μM), [^{3}H]5-HT (EC_{50} = 2.9 μM), and [^{3}H]DA (EC_{50} = 24 μM) from HEK cells expressing cloned transporters. These data confirm the ~10-fold selectivity of MDAI for 5-HT vs. DA release. In comparison, MDAI had 5- to 25-fold higher potency in our release assays, which is not surprising because HEK cells may not express critical elements of the protein machinery found in intact neurons

that are implicated in the monoamine-releasing effects of amphetamines. Finally, Johnson et al. (1991a) used synaptosomal uptake assays to characterize the interaction of MDAI and MMAI with DAT, NET and SERT. There was a narrow margin of separation between the effects of MDAI on [^{3}H]5-HT uptake (IC_{50} = 512 nM) and [^{3}H]NE uptake (IC_{50} = 1426 nM), whereas [^{3}H]DA uptake was inhibited with 10-fold lower potency (IC_{50} = 5,920 nM). Conversely, MMAI inhibited the uptake of [^{3}H]5-HT with an IC_{50} of 212 nM, which was 55-fold lower than the concentration required to inhibit [^{3}H]NE uptake (IC_{50} = 11,618 nM) and 93-fold lower than the concentration required to inhibit [^{3}H]DA uptake (IC_{50} = 19,793 nM). These data are consistent with the selectivity profile of MDAI and MMAI in our release assays.

Although we found little evidence of binding to DAT, NET, and SERT (in most cases there was < 50% displacement at 10 μM), this does not exclude the possibility that the aminoindans act as monoamine reuptake inhibitors. Indeed, as was noted above, MMAI inhibits synaptosomal [^{3}H]5-HT uptake at submicromolar concentrations (Johnson et al. 1991a). For substrate releasers, the concentration required to displace radioligand binding to the transporter is often 10- to 100-fold higher than the concentration required to inhibit neurotransmitter uptake (Simmler et al. 2013; Simmler et al. 2014a; Eshleman et al. 2017). For example, methamphetamine inhibits [^{3}H]DA uptake with about 70-fold higher potency than it displaces [^{125}I]RTI-55 binding to hDAT (IC_{50} = 0.0667 μM *vs.* K_i = 4.58 μM, respectively) (Eshleman et al. 2017). These potency differences likely occur because radiolabeled inhibitors stabilize monoamine transporters in the outward-facing conformation whereas substrate releasers shift transporters to the inward-facing conformation (Erreger et al. 2008; Sandtner et al. 2016; Bhat et al. 2017).

The release assays used in these experiments are based on the efflux of preloaded synaptosomal [^{3}H]neurotransmitter via a transporter-mediated exchange process thought to involve the reversal of normal transporter flux (i.e., "reverse" transport) (Rudnick and Clark 1993; Rothman and Baumann 2006b). Substrate-type drugs will deplete [^{3}H]neurotransmitter from synaptosomes via this reverse transport mechanism in a concentration-dependent manner. Synaptosomes are sealed vesicle-filled nerve endings with their plasma membrane leaflets oriented in a manner akin to neurons *in vivo* (Gray and Whittaker 1962; Wilhelm et al. 2014). In contrast to assay systems involving non-neuronal cells transfected with transporter proteins, synaptosomes possess all of the cellular machinery necessary for neurotransmitter synthesis, release, metabolism and reuptake. Synaptosomes, however, do not model all of the effects of amphetamine-type agents because the use of reserpine removes any contribution of the vesicular monoamine transporter VMAT2 (SLC18A2) to the release process. In addition to acting as a substrate for plasma membrane monoamine transporters, amphetamine also binds to VMAT, resulting in the redistribution of monoamines from vesicular stores to the cytoplasm (Sulzer et al. 1995; Partilla et al. 2006; Freyberg et al. 2016). Although transporter substrates can induce monoamine release in the absence of VMAT binding (Fon et al. 1997), it is important to recognize that 2-aminoindans may have effects in intact nerve terminals that are not fully replicated in synaptosomes. Follow-up studies will be conducted to evaluate whether 2-aminoindans are capable of interacting with VMAT. In addition to members of the solute carrier (SLC) family, several other presynaptic components are thought to contribute to the action of substrate releasers, for example monoamine oxidase (MAO), the trace amine-associated receptor TAAR1, and protein kinases (Sulzer et al. 2005; Sitte and Freissmuth 2015). It is important to

determine how those targets contribute to the effects of aminoindans and other monoamine-releasing compounds.

The subjective effects and abuse potential of substrate-type monoamine releasers vary depending on their transporter selectivity. Self-administration of monoamine releasers is driven primarily by DA efflux in the mesolimbic pathway (Wise 1996; Pierce and Kumaresan 2006) whereas the psychostimulant effects of amphetamines are mediated by their effects on NE release (Rothman et al. 2001; Sofuoglu et al. 2009; Hysek et al. 2011). By contrast, 5-HT release appears to produce MDMA-like entactogenic effects. The entactogenic effects of MDMA are blocked by pretreatment with the selective SERT inhibitors paroxetine and fluoxetine (Liechti et al. 2000; Farre et al. 2007; Tancer and Johanson 2007), which prevent carrier-mediated release of 5-HT without limiting access to catecholamine transporters or postsynaptic receptors. The relative catecholaminergic-serotonergic effects of monoamine releasing agents appear to be an important determinant of their abuse potential; catecholamine-selective drugs have the highest reinforcing potency in self-administration and ICSS studies, with stimulant and reinforcing effects declining as 5-HT releasing potency increases (Wee et al. 2005; Rothman and Baumann 2006a; Baumann et al. 2011; Bauer et al. 2013). Consistent with its reported amphetamine-like psychoactive effects in humans, 2-AI is a catecholamine-selective releaser, with minimal effect on 5-HT release. Based on the transporter data, MDAI and 5-MeO-AI may produce MDMA-like entactogenic and sympathomimetic effects but are likely to have less abuse liability than the latter agent. The effect of MMAI on monoamine release is reminiscent of *m*-trifluoromethylphenylpiperazine (TFMPP) and fenfluramine, which are highly selective for 5-HT release relative to DA and NE (Rothman et al. 2003; Baumann et al. 2005). Selective 5-HT releasers such as TFMPP and fenfluramine lack euphoric effects in humans and can produce

dysphoria at higher doses (Griffith et al. 1975; Foltin and Fischman 1991; Jan et al. 2010); therefore, MMAI may have unpleasant effects, limiting its abuse liability. Indeed, whereas MDMA (Bilsky et al. 1990; Marona-Lewicka et al. 1996) and MDAI (Gatch et al. 2016) produce conditioned place preference in rats, MMAI reportedly induces conditioned place aversion (Marona-Lewicka et al. 1996).

The effects of the aminoindans on monoamine release are consistent with their stimulus properties in rodents. 2-AI fully substituted for (+)-amphetamine, demonstrating that it produces an amphetamine-like interoceptive stimulus cue (Glennon et al. 1984). Another study reported only partial substitution by 2-AI in (+)-amphetamine-trained rats (Oberlender and Nichols, 1991); however, the range of 2-AI doses tested was limited by rate-depressant effects, and it cannot be excluded that 2-AI would have produced full substitution at higher doses. The discriminative stimulus cue evoked by MMAI appears to be mediated by 5-HT efflux; 5-HT releasers such as MDMA, *S*-(+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (*S*-(+)-MBDB), and (+)-fenfluramine fully substituted for MMAI, whereas (+)-amphetamine and cocaine did not substitute, and MMAI discrimination was antagonized by 5-HT uptake inhibitors as well as by depletion of endogenous 5-HT (Marona-Lewicka and Nichols 1994,1998). Likewise, MMAI fully substituted in rats trained to discriminate the 5-HT releasing drugs MDMA and *S*-(+)-MBDB, but did not substitute in (+)-amphetamine-trained rats (Johnson et al. 1991b). MDAI substituted for MDMA and *S*-(+)-MBDB (Nichols et al. 1990; Oberlender and Nichols 1990; Malmusi et al. 1996; Gatch et al. 2016), which is consistent with its effects on 5-HT efflux. Although MDAI did not substitute for (+)-amphetamine (Oberlender and Nichols 1991), it did produce full substitution in cocaine-trained rats and 75% drug-appropriate responding in (+)-methamphetamine-trained rats (Gatch et al. 2016); therefore, there may be

some overlap between the stimulus effects of MDAI and psychostimulants, as is the case with MDMA (Schechter 1986; Glennon 1989; Gatch et al. 2009). Data regarding the stimulus properties of 5-MeO-AI have not appeared in the literature.

All of the aminoindans tested in this study have moderate to high affinity for α_2-adrenoceptor subtypes. Consistent with our data, previous studies reported that 2-AI, MDAI, MMAI, and 5-iodo-2-aminoindan (5-IAI) bind to α_2 receptors with affinity in the submicromolar or low micromolar range (Marona-Lewicka and Nichols 1994; Iversen et al. 2013; Simmler et al. 2014b; Luethi et al. 2017). Activity in the series peaked with the unsubstituted compound 2-AI, which bound to the three subtypes with nanomolar affinity (α_{2A} K_i = 134 nM, α_{2B} K_i = 211 nM, and α_{2C} K_i = 41 nM). In comparison, (+)-amphetamine and (+)-methamphetamine have lower affinity for cloned human α_2-adrenoceptors labeled with [^{3}H]rauwolscine (K_i values of 2.8 μM and 6.1 μM, respectively; Simmler et al. 2013). MDMA also binds to α_2-adrenoceptors in frontal cortex homogenates with micromolar affinity (K_i = 3.2 μM vs. [^{3}H]*p*-aminoclonidine) (Battaglia et al. 1988). Although aminoindans have higher affinity for α_2-adrenoceptor subtypes compared to amphetamines, the significance of these interactions to the behavioral pharmacology of these compounds is unclear.

The interaction of aminoindans with 5-HT_{2B} receptors is noteworthy. It is apparent that aromatic ring substitution enhances the binding of aminoindans to 5-HT_{2B} receptor sites based on our finding that 5-MeO-AI and MMAI have higher affinity than 2-AI. Similarly, according to Iversen et al. (2013), 5-iodo-2-aminoindan (5-IAI) binds to the 5-HT_{2B} receptor with high affinity (K_i = 70 nM vs. [^{3}H]LSD). Although MDAI also displays ring-substitution at the 5 position, it was shown herein and in previous studies (Iversen et al. 2013) to have negligible affinity for the 5-HT_{2B} receptor (< 50% displacement at 10 μM). 5-HT_{2B} activation has been

linked to valvular heart disease induced by fenfluramine and ergot alkaloids such as methysergide, pergolide, cabergoline, and ergotamine (Fitzgerald et al. 2000; Rothman et al. 2000; Roth 2007; Huang et al. 2009). Additionally, the 5-HT_{2B} receptor may be responsible for the primary pulmonary hypertension observed in patients treated with fenfluramine or the anorectic aminorex (Rothman et al. 1999; Launay et al. 2002). Our study did not determine whether 5-MeO-AI and MMAI act as agonists or antagonists at the 5-HT_{2B} receptor but the fact that the drugs bind to this site raises the possibility that they may present some risk for cardiac and pulmonary toxicities. Indeed, abuse of substances with 5-HT_{2B} agonist activity has been linked to cardiac valvulopathy and pulmonary hypertension. MDMA (K_i = 500 nM vs. [^{3}H]LSD) and its *N*-demethylated metabolite MDA (K_i = 100 nM vs. [^{3}H]LSD) act as 5-HT_{2B} agonists (Setola et al. 2003); one study found an elevated incidence of valvular heart disease in a Belgian group of MDMA users (Droogmans et al. 2007). Abuse of 4-methylaminorex (McN-822, "U4Euh") has been associated with the development of pulmonary hypertension in case reports (Gaine et al. 2000).

In addition to their effects on cardiovascular physiology, 5-HT_{2B} receptors have also been shown to modulate the effects of psychostimulant and entactogenic drugs. 5-HT_{2B} receptor activation reportedly plays a permissive role in the activity of 5-HT neurons in the dorsal raphe nucleus (Belmer et al. 2018). Indeed, selective 5-HT_{2B} antagonists block MDMA-induced release of 5-HT and DA and inhibit the hyperlocomotor and reinforcing effects of MDMA in mice (Doly et al. 2008; Doly et al. 2009). According to another report, the ability of amphetamine to increase nucleus accumbens DA outflow and locomotor activity is significantly attenuated in animals pretreated with the selective 5-HT_{2B} antagonist LY 266097 (Auclair et al. 2010). Additionally, 5-HT_{2B} receptor gene variants have been linked to drug abuse (Lin et al.

2004; Tikkanen et al. 2015), which supports a potential role for this receptor in drug-induced rewarding effects. It is tempting to speculate that effects of 5-MeO-AI and MMAI on serotonergic and dopaminergic neurotransmission may be modulated by their interaction with the 5-HT_{2B} receptor.

In summary, aminoindans target plasma membrane monoamine transporters. The unsubstituted parent compound 2-AI increases DA and NE release in a manner analogous to (+)-amphetamine. 5-MeO-AI and MDAI, by contrast, produce MDMA-like effects on 5-HT and NE release, although they have less of an effect on DA release in comparison to the latter drug. MMAI increases 5-HT release selectively. Although these results are consistent with existing evidence indicating that 2-AI and MDAI have some abuse liability, self-administration studies are ultimately necessary to assess whether these substances produce reinforcing effects. It is especially important to perform these studies with 5-MeO-AI because it is the active ingredient in a product ("Pace") marketed online as a replacement for alcohol. Although some preliminary pharmacological data have been reported for 5-MeO-AI (Shimshoni et al. 2018), as far as we are aware the present studies are the first detailed investigation of this emerging drug. Additional studies with 5-MeO-AI are warranted given its ability to interact with monoamine transporters and 5-HT_{2B} receptors.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Table 1. Effects of 2-aminoindan analogues on the release of tritiated substrates via DAT, NET or SERT in rat brain synaptosomes

Drug	DAT-mediated release EC_{50} (nM) (E_{max})	NET-mediated release EC_{50} (nM) (E_{max})	SERT-mediated release EC_{50} (nM) (E_{max})	DAT/NET ratio[a]	DAT/SERT ratio[b]
2-AI	439 ± 38 (106%)	86 ± 13 (95%)	>10,000 n.d.	0.20	>22.78
5-MeO-AI	2,646 ± 565 (117%)	861 ± 118 (101%)	134 ± 13 (104%)	0.33	0.05
MMAI	>10,000 n.d.	3,101 ± 728 (105%)	31 ± 5 (99%)	>0.31	>0.003
MDAI	1,334 ± 226 (113%)	117 ± 17 (99%)	114 ± 15 (102%)	0.09	0.08

Data are mean ± S.D. for 3 independent experiments performed in triplicate

[a]DAT/NET ratio = $(\text{DAT } EC_{50})^{-1} \div (\text{NET } EC_{50})^{-1}$; a higher value indicates greater DAT selectivity

[b]DAT/SERT ratio = $(\text{DAT } EC_{50})^{-1} \div (\text{SERT } EC_{50})^{-1}$; a higher value indicates greater DAT selectivity

Table 2. Summary of radioligand binding data for the 2-aminoindan analogues

			2-AI	5-MeO-AI	MMAI	MDAI
Receptor	Species[a]	Radioligand	K_i (nM)[b]	K_i (nM)	K_i (nM)	K_i (nM)
5-HT_{1A}	Human	[^{3}H]8-OH-DPAT	> 10,000[c]	2,503 ± 1,867 (3)	1,077 ± 590 (4)	> 10,000
5-HT_{1B}	Human	[^{3}H]GR125743	> 10,000	> 10,000	2,777 ± 326 (3)	> 10,000
5-HT_{1D}	Human	[^{3}H]GR125743	> 10,000	> 10,000	2,559 ± 980 (3)	> 10,000
5-HT_{1E}	Human	[^{3}H]5-HT	> 10,000	> 10,000	> 10,000	> 10,000
5-HT_{2A}	Human	[^{3}H]ketanserin	> 10,000	> 10,000	> 10,000	> 10,000
5-HT_{2B}	Human	[^{3}H]LSD	> 10,000	4,793 ± 2,994 (3)	902 ± 445 (3)	> 10,000
5-HT_{2C}	Human	[^{3}H]mesulergine	> 10,000	> 10,000	> 10,000	> 10,000
5-HT_{5A}	Human	[^{3}H]LSD	> 10,000	> 10,000	> 10,000	> 10,000
5-HT_6	Human	[^{3}H]LSD	> 10,000	> 10,000	> 10,000	> 10,000
5-HT_7	Human	[^{3}H]LSD	> 10,000	> 10,000	1,008 ± 262 (3)	> 10,000
α_{1A}	Human	[^{3}H]prazosin	> 10,000	> 10,000	> 10,000	> 10,000
α_{1B}	Human	[^{3}H]prazosin	> 10,000	> 10,000	> 10,000	> 10,000
α_{1D}	Human	[^{3}H]prazosin	> 10,000	> 10,000	> 10,000	> 10,000
α_{2A}	Human	[^{3}H]rauwolscine	134 ± 31 (3)	751 ± 338 (3)	724 ± 477 (4)	322 ± 114 (3)
α_{2B}	Human	[^{3}H]rauwolscine	211 ± 81 (3)	1,555 ± 757 (3)	1,229 ± 483 (3)	1,121 ± 411 (3)
α_{2C}	Human	[^{3}H]rauwolscine	41 ± 26 (4)	1,224 ± 238 (3)	1,380 ± 769 (4)	363 ± 219 (4)
β_1	Human heart[d]	[^{125}I]pindolol	> 10,000	> 10,000	> 10,000	> 10,000
β_2	Human	[^{3}H]CGP12177	> 10,000	> 10,000	> 10,000	> 10,000
β_3	Human	[^{3}H]CGP12177	> 10,000	> 10,000	> 10,000	> 10,000
D_1	Human	[^{3}H]SCH23390	> 10,000	> 10,000	> 10,000	> 10,000
D_2	Human	[^{3}H]NMSP	> 10,000	> 10,000	> 10,000	> 10,000
D_3	Human	[^{3}H]NMSP	> 10,000	> 10,000	> 10,000	> 10,000
D_4	Human	[^{3}H]NMSP	> 10,000	> 10,000	> 10,000	> 10,000
D_5	Human	[^{3}H]SCH23390	> 10,000	> 10,000	> 10,000	> 10,000
DAT	Human	[^{3}H]WIN35,428	> 10,000	> 10,000	> 10,000	> 10,000
NET	Human	[^{3}H]nisoxetine	> 10,000	> 10,000	> 10,000	> 10,000
SERT	Human	[^{3}H]citalopram	> 10,000	> 10,000	4,822 ± 2,500 (3)	> 10,000
σ_1	Rat brain[d]	[^{3}H](+)pentazocine	> 10,000	> 10,000	> 10,000	> 10,000
σ_2	Rat PC12 cells[d]	[^{3}H]DTG	> 10,000	> 10,000	> 10,000	> 10,000

[a]The experiments were performed using cloned human receptors unless otherwise specified.

[b]Data represent mean and S.D. from 3–4 independent experiments performed in triplicate (the number of experiments is indicated in parentheses).

[c]< 50% displacement when tested at 10 μM in the primary binding assay.

[d]The experiment was performed using tissues or cells natively expressing the receptor.

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FIGURE CAPTIONS

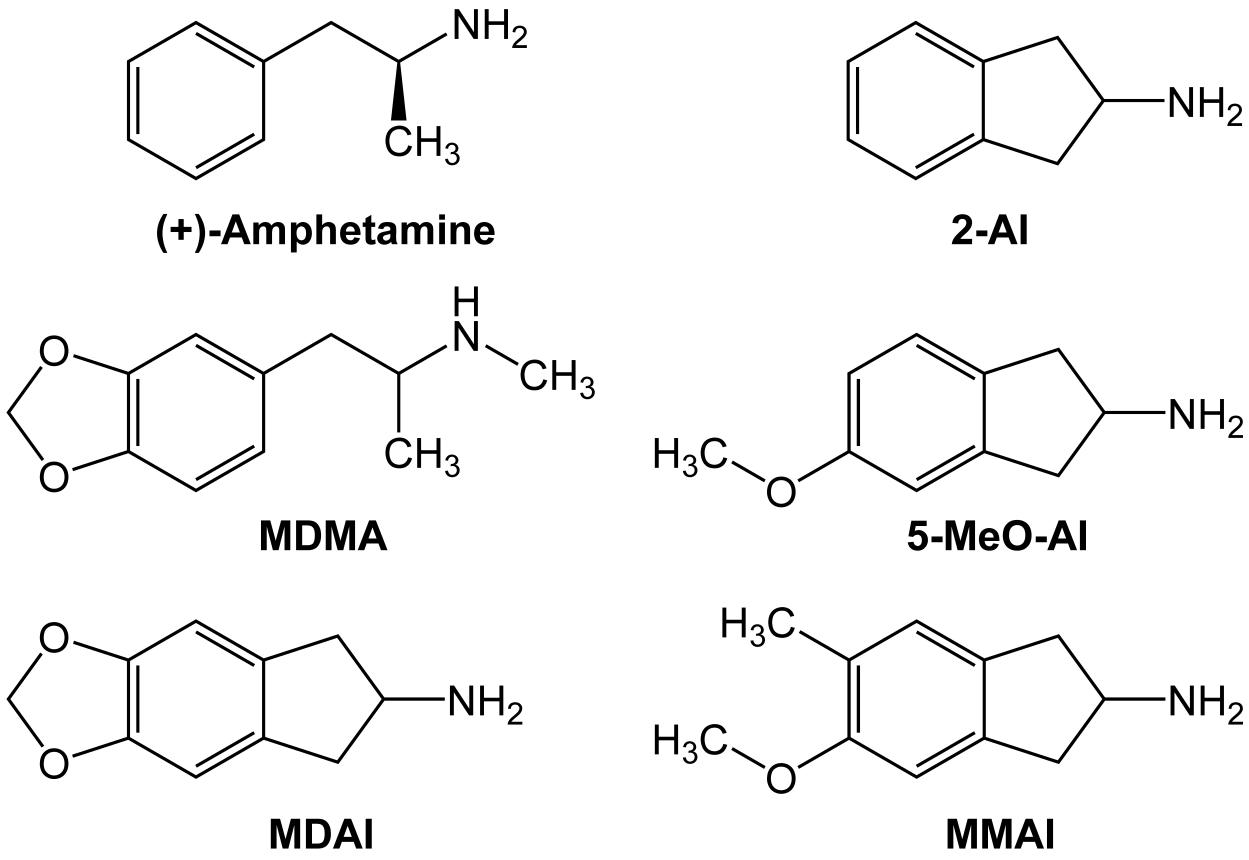

Figure 1. Chemical structures of aminoindans and related drugs. Abbreviations: *2-AI*, 2-aminoindan; *MDAI*, 5,6-methylenedioxy-2-aminoindan; *MDMA*, 3,4-methylenedioxymethamphetamine; *5-MeO-AI*, 5-methoxy-2-aminoindan; *MMAI*, 5-methoxy-6-methyl-2-aminoindan.

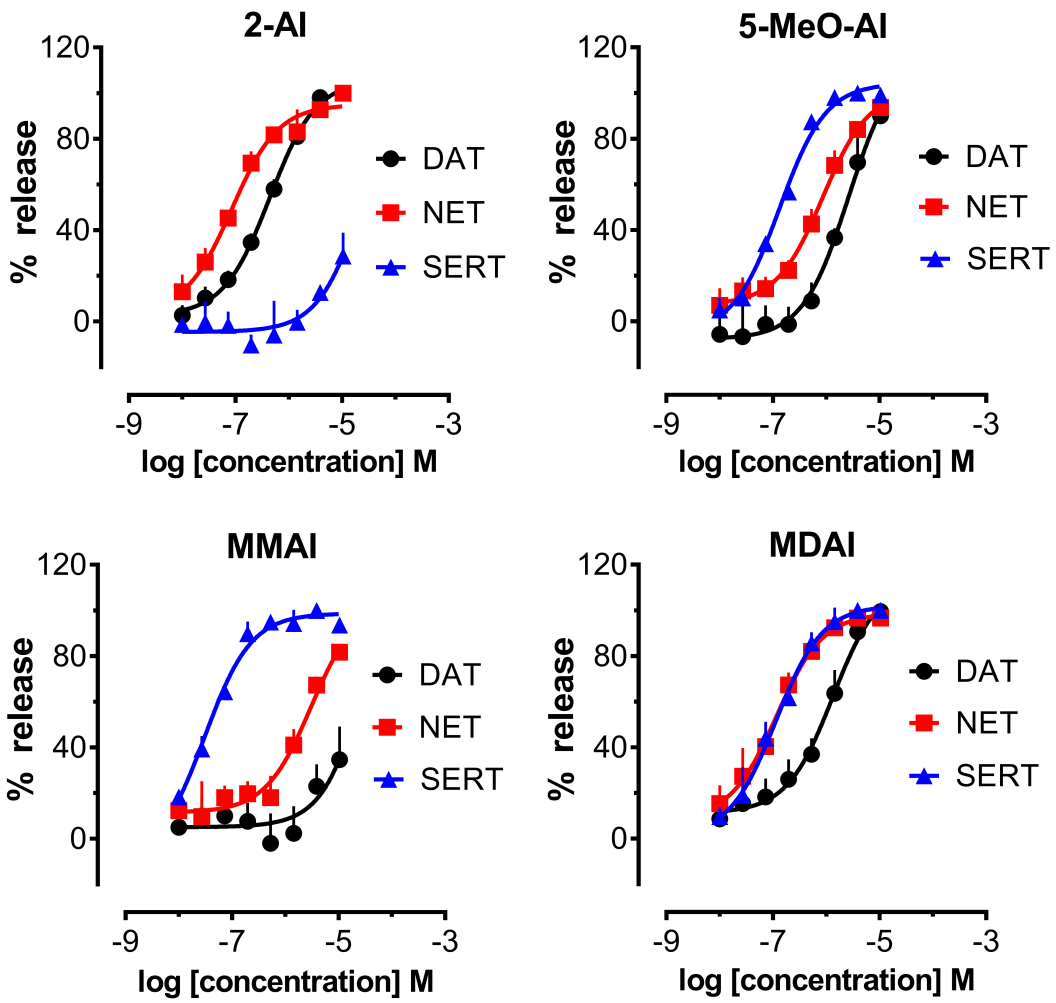

Figure 2. Dose-response effects of aminoindans on the release of [^{3}H]MPP$^+$ and [^{3}H]5-HT from rat brain synaptosomes *in vitro*, under conditions optimized for NET, DAT, and SERT. Dose-response curves were constructed by incubating various concentrations of each test drug with synaptosomes that had been preloaded with tritiated substrate ([^{3}H]MPP$^+$ for NET and DAT, [^{3}H]5-HT for SERT). Test drugs were 2-aminoindan (2-AI), 5-methoxy-2-aminoindan (5-MeO-AI), 5-methoxy-6-methyl-2-aminoindan (MMAI), and 5,6-methylenedioxy-2-aminoindan (MDAI). Data are mean±S.D. for 3 independent experiments performed in triplicate.