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Receptor interaction profiles of novel *N*-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs)

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ABSTRACT

Background: *N*-2-methoxybenzyl-phenethylamines (NBOMe drugs) are newly used psychoactive substances with poorly defined pharmacological properties. The aim of the present study was to characterize the receptor binding profiles of a series of NBOMe drugs compared with their 2,5-dimethoxy-phenethylamine analogs (2C drugs) and lysergic acid diethylamide (LSD) *in vitro*.
Methods: We investigated the binding affinities of 2C drugs (2C-B, 2C-C, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-4, 2C-T-7, and mescaline), their NBOMe analogs, and LSD at monoamine receptors and determined functional 5-hydroxytryptamine-2A (5-HT_{2A}) and 5-HT_{2B} receptor activation. Binding at and the inhibition of monoamine uptake transporters were also determined. Human cells that were transfected with the respective human receptors or transporters were used (with the exception of trace amine-associated receptor-1 [$TAAR_1$], in which rat/mouse receptors were used).
Results: All of the compounds potently interacted with serotonergic 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors and rat $TAAR_1$ (most K_i and EC_{50}: <1 μM). The *N*-2-methoxybenzyl substitution of 2C drugs increased the binding affinity at serotonergic 5-HT_{2A}, 5-HT_{2C}, adrenergic α_1, dopaminergic $D_{1\text{-}3}$, and histaminergic H_1 receptors and monoamine transporters but reduced binding to 5-HT_{1A} receptors and $TAAR_1$. As a result, NBOMe drugs were very potent 5-HT_{2A} receptor agonists (EC_{50}: 0.04–0.5 μM) with high $5\text{-HT}_{2A}/5\text{-HT}_{1A}$ selectivity and affinity for adrenergic α_1 receptors (K_i: 0.3–0.9 μM) and $TAAR_1$ (K_i: 0.06–2.2 μM), similar to LSD, but not dopaminergic D_{1-3} receptors (most K_i: > 1 μM), unlike LSD.
Conclusion: The binding profile of NBOMe drugs predicts strong hallucinogenic effects, similar to LSD, but possibly more stimulant properties because of α_1 receptor interactions.

Abbreviations: 25B-NBOMe, 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25C-NBOMe, 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25D-NBOMe, 2-(4-methyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25E-NBOMe, 2-(4-ethyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25H-NBOMe, 2-(2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25I-NBOMe, 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25N-NBOMe, 2-(4-nitro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25P-NBOMe, 2-(4-propyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T2-NBOMe, 2-(2,5-dimethoxy-4-ethylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T4-NBOMe, 2-(2,5-dimethoxy-4-isopropylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T7-NBOMe, 2-(2,5-dimethoxy-4-*n*-propylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 2C-B, 4-bromo-2,5-dimethoxyphenethylamine; 2C-C, 2-(4-chloro-2,5-dimethoxy)ethanamine; 2C-D, 2-(2,5-dimethoxy-4-methyl) ethanamine; 2C-E, 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminoethane; 2C-H, 2,5-dimethoxyphenethylamine; 2C-I, 4-iodo-2,5-dimethoxyphenethylamine; 2C-N, 2-(2,5-dimethoxy-4-nitro)ethanamine; 2C-P, 2-(2,5-dimethoxy-4-propylphenyl)ethanamine; 25CN-NBOH, 2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]-methyl)phenol; 2C-T-2, 2-[2,5-dimethoxy-4-(ethylthio)phenyl]ethanamine; 2C-T-4, 2,5-dimethoxy-4-isopropylthiophenethylamine; 2C-T-7, 2-[2,5-dimethoxy-4-(propylthio)phenyl]ethanamine; 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; DAT, dopamine transporter; mescaline, 2-(3,4,5-trimethoxyphenyl)ethanamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; NBOMe, *N*-(2-methoxy)benzyl; NE, norepinephrine; NET, norepinephrine transporter; SERT, serotonin transporter; TAAR, trace amine-associated receptor; LSD, lysergic acid diethylamide.

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1. Introduction

New psychoactive substances are constantly emerging on the illicit drug market and typically sold via the Internet. Of particular interest are *N*-2-methoxybenzyl-phenethylamines (NBOMe drugs), which are novel and reportedly very potent hallucinogens that have been increasingly used recreationally (Forrester, 2014; Hill et al., 2013; Ninnemann and Stuart, 2013; Rose et al., 2013; Walterscheid et al., 2014; Wood et al., 2015; Zuba, 2012), with additional potential use as radiotracers (Ettrup et al., 2011, 2010). Recreationally used NBOMe drugs include 25I-NBOMe, 25C-NBOMe, 25B-NBOMe, and 25D-NBOMe (Armenian and Gerona, 2014; Poklis et al., 2014; Rose et al., 2013), which are derivatives of 2,5-dimethoxy-4-substituted phenethylamines (2C drugs; Dean et al., 2013; Hill and Thomas, 2011; Shulgin and Shulgin, 1991) (see Fig. 1). *N*-2-methoxybenzyl substitution enhances the potency of 2C drugs at serotonergic 5-hydroxytryptamine-2A (5-HT_{2A}) receptors, resulting in exceptionally potent 5-HT_{2A} receptor agonists (Braden et al., 2006; Heim, 2004; Nichols et al., 2015) with strong hallucinogenic properties in animals and humans (Halberstadt and Geyer, 2014; Srisuma et al., 2015). Pharmacological interactions between NBOMe drugs and 5-HT_2 receptors have been well characterized for some compounds of this novel drug family (Blaazer et al., 2008; Braden et al., 2006; Ettrup et al., 2011, 2010; Hansen et al., 2014; Nichols et al., 2008). However, systematic characterizations of the effects of a larger series of NBOMe drugs at a wider range of relevant human receptors and comparisons with their 2C parent drugs are lacking. Importantly, NBOMe drugs have been reported to produce psycho- and cardiovascular stimulant effects, in addition to hallucinations. Specifically, sympathomimetic toxicity, including tachycardia, hypertension, mydriasis, agitation, and hyperthermia, is commonly reported in cases of acute NBOMe drug intoxication (Hill et al., 2013; Rose et al., 2013; Srisuma et al., 2015; Stellpflug et al., 2014; Wood et al., 2015). Pharmacologically, compounds of the 2C series, including 2C-C, 2C-E, and 2C-I, inhibit the norepinephrine (NE) and serotonin transporters (NET and SERT, respectively), similar to amphetamines, although with only very low potency (Eshleman et al., 2014; Nagai et al., 2007). These findings raise the question of whether NBOMe drugs may have similar but more potent stimulant-type pharmacological properties, including inhibition of the NET, dopamine (DA) transporter (DAT), and SERT, or interactions with adrenergic α_1 receptors that lead to vasoconstriction.

We assessed the *in vitro* pharmacology of a series of NBOMe drugs compared with their 2C parent drugs. We characterized the binding affinity profiles at monoamine receptors and DAT, NET, and SERT inhibition potencies. We also determined the functional 5-HT_{2A} receptor activation potencies because 5-HT_{2A} receptors mediate hallucinogenic effects (Nichols, 2004). The prototypical serotonergic hallucinogen lysergic acid diethylamide (LSD) was included as a comparator drug (Nichols, 2004; Passie et al., 2008).

2. Methods

2.1. Drugs

2C-B, 2C-C, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-4, 2C-T-7, mescaline, 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25H-NBOMe, 25I-NBOMe, 25N-NBOMe, 25P-NBOMe, 25T2-NBOMe, 25T4-NBOMe, 25T7-NBOMe, and mescaline-NBOMe were synthesized by Lipomed (Arlesheim, Switzerland) for this study at no cost. All of the compounds were used as hydrochloride salts. Purity was >98% for all of the substances. [^{3}H]NE and [^{3}H]DA were obtained from Perkin–Elmer (Schwerzenbach, Switzerland), and [^{3}H]5-HT was obtained from Anawa (Zürich, Switzerland).

2.2. Radioligand receptor and transporter binding assays

The radioligand binding assays were performed as described previously (Hysek et al., 2012; Simmler et al., 2013). Briefly, membrane preparations of human embryonic kidney (HEK) 293 cells (Invitrogen, Zug, Switzerland) that overexpress the respective transporters (Tatsumi et al., 1997) or receptors (human genes, with the exception of rat and mouse genes for trace amine-association receptor 1 [$TAAR_1$]; (Revel et al., 2011)) were incubated with the radiolabeled selective ligands at concentrations equal to K_d, and ligand displacement by the compounds was measured. Specific binding of the radioligand to the target receptor was defined as the difference between the total binding and nonspecific binding that was determined in the presence of selected competitors in excess. The following radioligands and competitors, respectively, were used: *N*-methyl-[^{3}H]-nisoxetine and indatraline (NET), [^{3}H]citalopram and indatraline (SERT), [^{3}H]WIN35,428 and indatraline (DAT), [^{3}H]8-hydroxy-2-(di-*n*-propylamine)tetralin and indatraline (5-HT_{1A} receptor), [^{3}H]ketanserin and spiperone (5-HT_{2A} receptor), [^{3}H]mesulgerine and mianserin (5-HT_{2C} receptor), [^{3}H]prazosin and risperidone (adrenergic α_1 receptor), [^{3}H]rauwolscine and phentolamine (adrenergic α_2 receptor), [^{3}H]SCH 23390 and butaclamol (D_1 receptor), [^{3}H]spiperone and spiperone (D_2 and D_3 receptors), [^{3}H]pyrilamine and clozapine, (histaminergic H_1 receptor), and [^{3}H]RO5166017 and RO5166017 ($TAAR_1$). IC_{50} values were determined by calculating non-linear regression curves for a one-site model using three to five independent 10-point concentration–response curves for each compound. K_i (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation.

2.3. Activity at serotonin 5-HT_{2A} receptor

Human 5-HT_{2A} receptor-expressing NIH-3T3 cells were incubated in HEPES- Hank's Balanced Salt Solution (HBSS) buffer (70′000 cells/100 μl) for 1 h at 37 °C in 96-well poly-D-lysine-coated plates. To each well 100 μl of Dye solution (FLIPR calcium 5 assay kit; Molecular Devices, Sunnyvale, CA, USA) was added and plates were incubated for 1 h at 37 °C. The plates were then placed in a fluorescence imaging plate reader (FLIPR), and 25 μl of the test substances diluted in HEPES-HBSS buffer containing 250 mM probenicid were added online. The increase in fluorescence was then measured. EC_{50} values were derived from the concentration–response curves using nonlinear regression. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

2.4. Activity at serotonin 5-HT_{2B} receptor

Human 5-HT_{2B} receptor-expressing HEK293 cells were incubated in growth medium (DMEM high glucose [Invitrogen, Zug, Switzerland], 10 ml/l PenStrep [Gibco, Life Technologies, Zug, Switzerland]), 10% FCS non dialyzed heat inactivated and 250 mg/l geneticin) at a density of 50′000 cells/well at 37 °C in 96-well poly-D-lysine-coated plates over-night. On the next day the growth medium was removed by snap inversion, and 100 μl of Fluo-4 solution (calcium indicator; Molecular Probes, Eugene, OR, USA) was added to each well. The plates were incubated for 45 min at 31 °C. The Fluo-4 solution was removed by snap inversion, and 100 μl of Fluo-4 solution was added a second time. The cells were then incubated for another 45 min at 31 °C. Immediately before testing, the cells were washed with HBSS (Gibco) and 20 mM HEPES (assay buffer; Gibco) using an EMBLA cell washer, and 100 μl assay buffer was added. The plate was placed in a fluorescence imaging plate reader (FLIPR), and 25 μl of the test substances diluted in assay