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Trishomocubanes: novel σ-receptor ligands modulate amphetamine-stimulated [^{3}H]dopamine release

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Abstract

Several trishomocubane analogues of the type 4-azahexacyclo [5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecane exhibited moderate to high affinity at σ-receptor subtypes and low or negligible affinity at dopamine and serotonin transporters (SERT). Selected compounds were examined for their effects on amphetamine-stimulated [^{3}H]dopamine release from striatal slices in vitro. Compounds 1, 2, 3 and 4 significantly enhanced amphetamine-stimulated release in a concentration-dependent manner. Compound 4, with the highest affinity and selectivity for the σ_2-receptor subtype, displayed the greatest potency. The enhancement produced by 1 and 2 was fully reversed by the selective σ_2 antagonists 1′-[4-[1-(4-fluorophenyl)-1-*H*-indol-3-yl]-1-butyl]spiro[*iso*-benzofuran-1(3H), 4′piperidine] (Lu28-179), endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1-*H*-benzimidazole-1-carboxyamidehydrochloride (BIMU-8) and the non-subtype selective antagonist *N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-pyrrolidinyl)ethylamine (BD1008). These data suggested a potential role for compounds 1 through 4 as σ_2-receptor agonists in functional studies. In addition, a D_3-trishomocubane compound 5 displayed low affinity at σ receptors ($K_i = 3$ μM) and moderate affinity at dopamine transporters ($K_i = 623$ nM). Compound 5 significantly inhibited the potentiation mediated by compound 2, presumably through σ_2-receptor antagonism, or a direct action on dopamine transporters.

Keywords: σ Receptor; Trishomocubane; Azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}] dodecane; Dopamine release; Amphetamine

1. Introduction

σ Receptors were first postulated by Martin et al. (1976) to describe the unique psychotomimetic effects of the prototypic (±)-SKF-10,047 (*N*-allylnormetazocine). Two types of σ receptors have so far been identified, termed σ_1 and σ_2 receptors (Hellewell and Bowen, 1990; Quirion et al., 1992). Both σ-receptor subtypes have been found highly enriched in dopaminergic brain areas, including striatum and nucleus accumbens, supported by autoradiographic localization (Gundlach et al., 1986; McLean and Weber, 1988; Walker et al., 1990). A number of behavioral and electrophysiological studies followed, also suggesting that both σ-receptor subtypes may be involved in motor functions regulated by the striatal dopaminergic system (Goldstein et al., 1989; Iyengar et al., 1990; Walker et al., 1988).

Gonzalez-Alvear and Werling first demonstrated the regulation of *N*-methyl-D-aspartate (NMDA)-stimulated [^{3}H]dopamine release from rat striatum by σ-receptor ligands, including (+)-pentazocine, (+) SKF-10,047 and 1S,2R-(−)-*cis*-*N*-[2-(3,4-dichlophenyl)ethyl]-*N*-methly-2-(1-pyrrolidinyl)cyclohexylamine (BD737). The inhibition produced by low concentrations of these ligands was fully reversed by the selective σ_1-receptor antagonist 1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxethyl)piperidine HBr (DuP734), suggesting a possible functional role for σ_1 receptors in regulation of NMDA-stimulated dopamine release in striatum (Gonzalez-Alvear and Werling, 1994, 1995).

Research on the functional roles of σ_2 receptors has been limited largely due to the lack of selective σ_2 lig-

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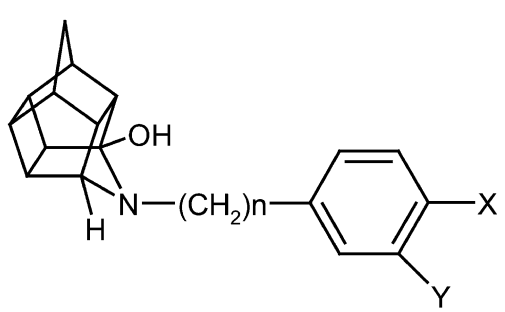
ands. It has been shown that some σ-receptor ligands, including rimcazole, *N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-pyrrolidinyl)ethylamine (BD1008), *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methly-2(dimethlyamino)ethylamine (BD1047) and *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine (LR172), attenuate the stimulant effects induced by cocaine (Izenwasser et al., 1993; McCracken et al., 1999a,b), suggesting the σ receptor as a potential target for treatment of drug abuse. Functional studies by Izenwasser et al. revealed that amphetamine-stimulated (transporter-mediated) dopamine release can be modulated by the σ_2-receptor agonists and antagonists in vitro. The σ agonist, (+)-pentazocine, potentiated amphetamine-stimulated dopamine release in a concentration-dependent manner. The enhancement produced by (+)-pentazocine and (−)-pentazocine was fully blocked by the selective σ_2 antagonists endo-*N*-(8-methyl--azabicyclo[3.2.1]oct-3-yl)- 2, 3- dihydro - (1 - methyl)ethyl - - oxo- 1 - *H*- benzimidazole - 1 - carboxyamidehydrochloride (BIMU-8) and 1′-[4-[1-(4-fluorophenyl)-1-*H*-indol-3-yl]-1-butyl]spiro[*iso*-benzofuran-1(3H), 4′ piperidine] (Lu28-179) and non-subtype selective σ antagonist, *N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-pyrrolidinyl)ethylamine (BD1008) and not by the σ_1 antagonist, DuP734, indicating a σ_2-receptor-mediated mechanism (Izenwasser et al., 1998). Further studies in PC12 cells demonstrated that the enhancement of amphetamine-stimulated [^{3}H]dopamine release by (+)-pentazocine was dependent upon Ca^{2+}/calmodulin-dependent protein kinase II (Ca^{2+}/CaM kinase II) by showing that two different inhibitors of the enzyme block the enhancing effects of (+)-pentazocine (Weatherspoon and Werling, 1999), while in the brain slice model, there appeared to be an involvement of protein kinase C (PKC) instead of Ca^{2+}/CaM kinase II as the second messenger system activated by σ_2-receptor agonists (Derbez et al., 1999; Derbez and Werling, in press).



1: n=2, X=Cl, Y=Cl
2: n=2, X=H, Y=F
3: n=4, X=H, Y=H
4: n=1, X=H, Y=F

Fig. 1. Trishomocubanes of the type 4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}] dodecane.

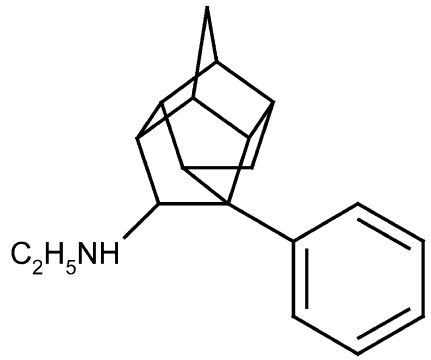


Fig. 2. D_3-Trishomocubane analogue 5: 3-phenyl-D_3-trishomocubyl-4-(*N*-ethyl)amine.

We have recently reported the synthesis and binding of a novel series of trishomocubanes of the type 4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecane (Fig. 1), which show moderate to high affinity for σ binding sites and no cross-reactivity with other receptor subtypes, such as those for dopamine, opioids, phencyclidine (PCP), NMDA and serotonin (Kassiou et al., 1996; Nguyen et al., 1996; Liu et al., 1999).

In the current study, the compounds 1, 2, 3 and 4 (Fig. 1), four trishomocubane analogues with high affinity for σ_1 or σ_2 receptors, were firstly examined for their affinities at dopamine (DAT) and serotonin transporters (SERT), then evaluated for their effects on the dopamine release stimulated by amphetamine from striatal slices in vitro. Compound 5, a D_3-trishomocubane analogue (Fig. 2) that was reported to demonstrate promising anti-Parkinsonian properties in vivo (Oliver et al., 1991) was also tested in the assay with a view to elucidating its possible mode of action.

2. Materials and methods

2.1. Materials for DAT and SERT binding

All of compounds tested were synthesized in Aldrien Albert Medicinal Chemistry Laboratory, Department of Pharmacology, The University of Sydney. [^{3}H]WIN 35,428, [*N*-Methyl-^{3}H] (2β-carbomethoxy-3β(4-fluoro-phenyl)tropane, 84.5 Ci/mmol) and [^{3}H]citalopram, [*N*-Methyl-^{3}H] (82 Ci/mmol) were purchased from Dupont/New England Nuclear (Boston, MA, USA). GBR-12909, clomipramine hydrochloride and imipramine were purchased from Research Biochemicals (Natick, MA, USA). Cocaine was from Australian Pharmaceutical Industry (Sydney, Australia). The scintillant used was Emulsifier-Safe and was purchased from Packard Instruments-Chemical Operations (Groningen, Netherlands). Whatman GF/B filters were purchased from Whatman International (Maidstone, England). Binding assays were performed in polycarbon P3 tubes purchased from John's Medical Supplies

(Sydney, Australia). Liquid scintillation spectroscopy was carried out using a Packard 1500 Tri-Carb Liquid Scintillation Analyser (Packard Instrument, Downers Grove, IL, USA).

2.2. DAT binding assay

2.2.1. Tissue preparation

Brain tissue was harvested within 30 min of death from adult male and female guinea pigs and stored at −85 °C until dissection. At the time of dissection, the caudate-putamen was removed from coronal slices and yielded about 1 g of wet tissue. The tissue was homogenized with a polytron in 10 volumes (w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4 at 4 °C). The homogenate was centrifuged at 38,700 × *g* (~17,850 rpm) for 20 min at 0–4 °C and the pellet was resuspended in 40 volumes of buffer. The wash procedure was repeated twice. After washing, the membrane suspension (25 mg original wet weight of tissue per ml) was stored in small aliquots at −85 °C until use, generally within 2 weeks.

Immediately before assay, the suspension was thawed, diluted to 7.5 mg wet weight/ml in buffer and dispersed with polytron. The final concentration of tissue suspension in the tubes was 3.75 mg wet weight/ml in a total volume of 1 ml.

2.2.2. DAT binding assay

[^{3}H]WIN 35,428 (2 nM) was used to label DAT binding sites. Nonspecific binding was determined using 40 μM cocaine. In brief, each assay tube contained [^{3}H](+)-pentazocine at a final concentration of 2 nM, tissue suspension (approximately 7.5 mg wet weight per tube), various concentrations of test compounds (1 nM–100 μM) and 50 mM Tris–HCl (pH 7.4) assay buffer containing 120 mM NaCl in a final volume of 1 ml. After incubation at 0–4 °C for 120 min, the reaction was terminated by rapid filtration using a BRANDEL 24-well cell harvester over Whatman GF/B glass fiber filters that were presoaked in a solution of 0.5% polyethylenimine at room temperature for at least 2 h prior to use. Following addition of scintillation cocktail, samples were allowed to equilibrate overnight. The amount of bound radioactivity was determined by liquid scintillation spectroscopy using a Packard Liquid Scintillation Analyser. Each concentration of compound was tested in triplicate. Binding data were fitted using KaleidaGraph, Version 3.0, Abelbeck Software (Javitch et al., 1984; Madras et al., 1989).

2.3. SERT binding assays

2.3.1. Tissue preparation

Forebrain tissue was harvested within 30 min of death from adult male rats and stored in 0.32 M sucrose at −85 °C until preparation. At the time of preparation, the tissue was homogenized with a Brinkmann polytron in 25 volumes (w/v) of ice-cold 50 mM Tris–HCl buffer (pH 7.4 at 25 °C) containing 120 mM NaCl and 5 mM KCl (incubation buffer) and centrifuged at 45,000 × *g* (~19,300 rpm) for 10 min at 4 °C. Pellets were resuspended in 25 volumes of buffer and recentrifuged. The wash procedure was repeated once more before the membrane suspension (40 mg original wet weight of tissue per ml) was stored in 10 ml aliquots at −85 °C until use, generally within 2 weeks.

Immediately before assay, two aliquots (20 ml) of the suspension were thawed, diluted to 72 ml in incubation buffer and dispersed with the polytron. The final assay concentration of tissue suspension was about 5.6 mg wet weight/ml in a total volume of 1 ml each tube.

2.3.2. SERT binding assay

The affinity for SERT binding sites was measured by labeling with [^{3}H]citalopram. Nonspecific binding was determined using clomipramine. Briefly, each assay tube contained 7 nM [^{3}H]citalopram, tissue suspension, various concentrations of test compounds and the assay buffer in a final volume of 1 ml. Following incubation at 25 °C for 1 h in the assay, the subsequent procedure was as described in the DAT assay (Brown et al., 1986; D'Amato et al., 1987).

2.4. Functional assays of dopamine release

Modulation of amphetamine-stimulated dopamine release by trishomocubane analogues was measured according to the procedure described previously (Izenwasser et al., 1998). Briefly, male Sprague–Dawley rats were killed and striatum dissected. The tissue was sliced, suspended and rinsed in oxygenated modified Krebs-Hepes buffer (MKB). The tissue slice suspensions were then incubated in 20 ml buffer containing [^{3}H]dopamine (47–50 Ci/mmol; Amersham Arlington Heights, IL, USA) and ascorbic acid at 37 °C for 30 min. The tissue was washed three times and suspended a final time in MKB containing 100 nM nisoxetine (RBI, Natick, MA, USA) to prohibit reuptake of [^{3}H]dopamine into noradrenergic terminals and 1 μM domperidone (RBI) to prevent feedback effects of D2 receptors and, subsequently, distributed in 275 μl aliquots between glass-fiber filter discs into chambers of a BRANDEL (Gaithersburg, MD, USA) superfusion apparatus. After a low and stable baseline was achieved, tissue was stimulated to release [^{3}H] dopamine by a 2-min exposure to 10 μM amphetamine (*S*1). The inflow was then returned to a nonstimulating buffer for 10 min. The tissue was then stimulated a second time for 2 min with 10 μM amphetamine in the presence or absence of the drug being tested as appropriate (*S*2). Inflow was again returned to nonstimulating buffer to allow a return to baseline release before the final extraction of radioactivity remaining in the tissue by a 45-min exposure to 0.2 M HCl. Superfusates were collected at 2-min intervals into the scintillation vials

Table 1
Binding affinities of selected compounds for σ-receptor subtypes, dopamine transporter (DAT) and serotonin transporter (SERT)

Compound	K_i (nM)			
	σ_1	σ_2	DAT	SERT
1	10	233	1 μM	750
2	11	370	–	–
3	9	171	8 μM	3 μM
4	152	20	–	–
5	3 μM	–	623	3 μM
4-IBP	1.9	95	–	–

'–' Indicates that $K_i > 10$ μM.

and the glass-fiber filter discs and the tissue were collected into the final vials. Released radioactivity was determined by liquid scintillation spectroscopy.

All data were statistically analyzed as ratios ($S2/S1$) so that each tissue sample could serve as its own control. Data were analyzed using a two-way factorial analysis of variance (ANOVA) followed by post hoc Dunnett's. Results were considered to be significantly different when $P < 0.05$.

3. Results

The affinities of selected compounds at σ-receptor subtypes, DAT and SERT are presented in Table 1. Compounds 1, 2 and 3 all displayed nanomolar K_i values for σ_1 binding sites and also good affinity ($K_i = 150–310$ nM) for σ_2 sites, while compound 4 exhibited the highest affinity for σ_2 sites ($K_{i\sigma_1} = 152$ nM, $K_{i\sigma_2} = 20$ nM). In contrast, compound 5, a D_3-trishomocubane analogue showed relatively low σ affinity ($K_{i\sigma_1} = 3$ μM, $K_{i\sigma_2} > 10$ μM). To examine the potential interaction of these compounds with monamine transporter systems, the binding affinity of these compounds at dopamine and serotonin transporter were also measured. All of the compounds demonstrated markedly lower affinity at DAT or SERT ($K_i = 750$ nM–8 μM) than those at σ receptors except that compound 5 displayed moderate affinity ($K_i = 623$ nM) for the DAT. Additionally, the σ-receptor ligand 4-[125I]-*N*-(*N*-benzylpiperidin-4-yl)-4-idobenzamide (4-IBP) showed great potency at both σ sites; however, no appreciable affinity at either the DAT or SERT site.

In the initial dopamine release experiments, compounds 1, 2, 3 and 4 demonstrated significant enhancement over amphetamine-stimulated [^{3}H]dopamine release at concentrations of 1 μM. The enhancement produced by these compounds at the concentration of 1 μM ranged from 30% to 45% over the amphetamine control, which was roughly equivalent to that produced by 1 μM of (+)-pentazocine. We then constructed full dose–response curves for all four compounds. Compounds 1, 2 and 3 potentiated amphetamine-stimulated [^{3}H]dopamine release in a concentration-dependent manner, with an IC_{50} of around 1 μM,

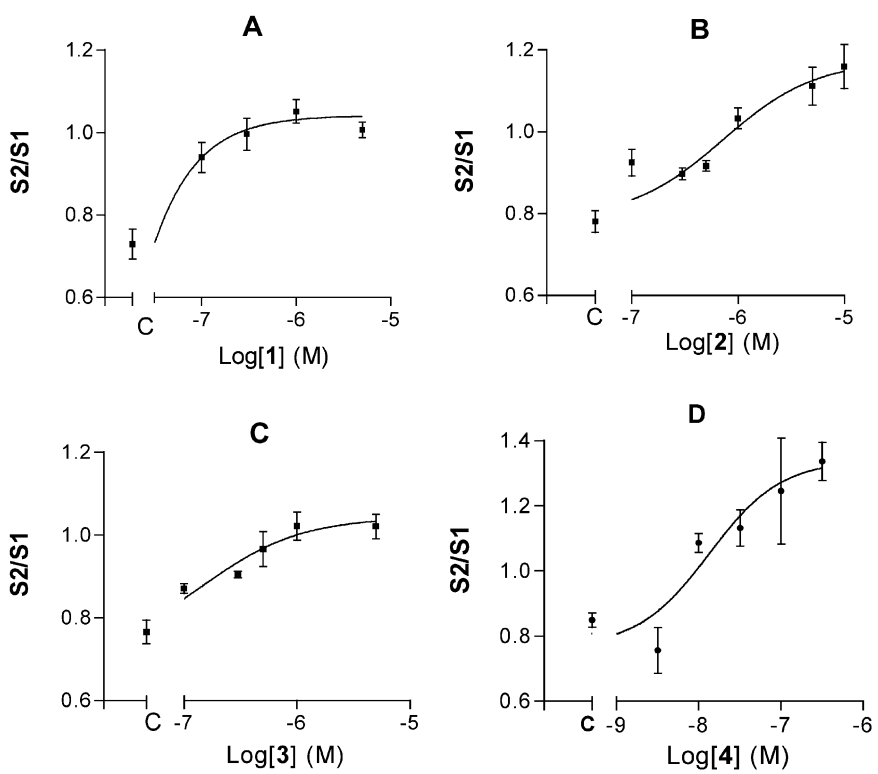


Fig. 3. Dose–response curves for compounds (A) 1, (B) 2, (C) 3 and (D) 4 on amphetamine (10 μM)-stimulated [^{3}H]dopamine release. Data are expressed as ratios ($S2/S1$) of release of [^{3}H]DA produced by the first and the second stimulus. $n > 3$. Note: The *Y*-axis starts at 0.6.

while compound 4, with the highest affinity at the σ_2-receptor subtype, exhibited the greatest potency ($IC_{50} < 100$ nM) (Fig. 3).

The effects of the tested trishomocubane analogues on amphetamine-stimulated release in the presence of several σ-receptor compounds identified as antagonists were also measured (Fig. 4). Compound 1 (1 μM), which had produced significant enhancement over amphetamine alone, was chosen to test for reversal by σ-receptor antagonists. In these experiments, compound 1 produced over 130% of control amphetamine-stimulated release. The enhancement of release was significantly reversed by the selective σ_2-receptor antagonists Lu28-179 (1 nM), BIMU-8 (100 nM), non-subtype selective σ-receptor antagonists BD1008 (10 nM) and selective σ_1 antagonist DuP734 (100 nM) (Panel A, Fig. 4). In a similar pattern, the potentiation of control release produced by a 1 μM concentration of compound 2

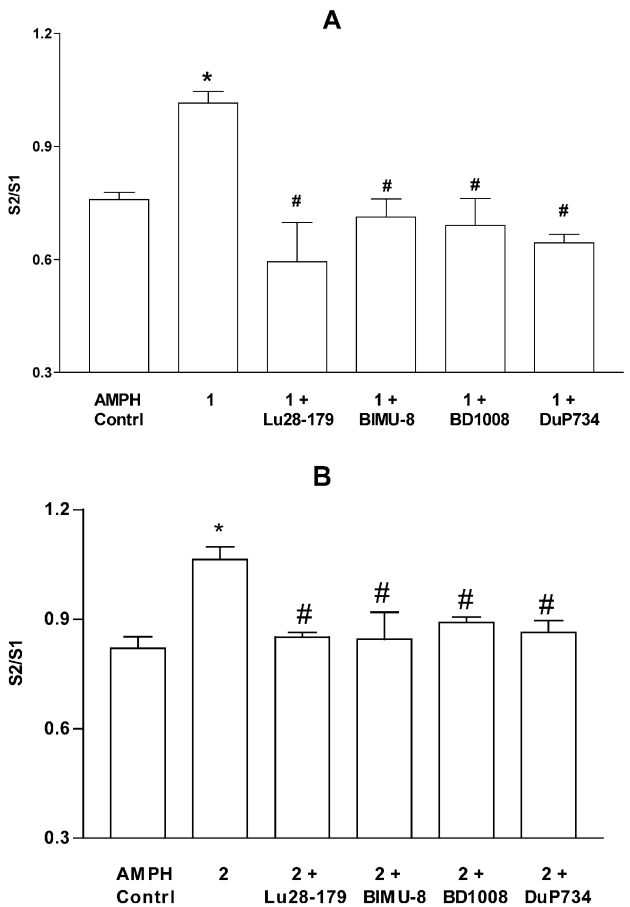


Fig. 4. Effects of σ-receptor antagonists on enhancement of amphetamine (10 μM)-stimulated [^{3}H]dopamine release by compounds 1 and 2 (1 μM). (A) Antagonists produce full reversal of compound 1 (1 μM) mediated enhancement. Lu28-179 was tested at 1 nM, BIMU-8 at 100 nM, BD1008 at 10 nM, and DuP734 at 100 nM. (B) Antagonists above block the enhancement produced by compound 2. Data are expressed as ratio ($S2/S1$). * Significantly different compare to 10 μM amphetamine (control), with $P < 0.05$, $n > 4$. #Significantly different compared to 1 μM compound 1 or 2, with $P < 0.05$, $n = 3$. Note: The *Y*-axis starts at 0.3.

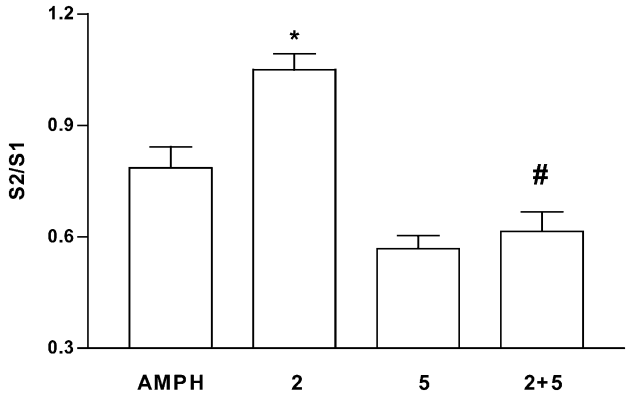


Fig. 5. Effects of compound 5 (10 μM) on amphetamine control (10 μM) or compound 2 (1 μM) enhancement over the control. * Significantly different compare to 10 μM of amphetamine (control), with $P < 0.05$, $n > 4$. #Significantly different compared to 1 μM of compound 2, with $P < 0.05$, $n = 3$. Note: The *Y*-axis starts at 0.3.

was antagonized by the σ antagonists above (Panel B, Fig. 4). In addition, compound 3 ($K_{i\sigma_1} = 10$ nM, $K_{i\sigma_2} = 171$ nM) alone produced about 45% enhancement over control-stimulated release. It was then tested in combination with compound 1 and found no additional effect on the potentiation produced by 1, nor any reversal of the effect of compound 1, therefore, confirming its agonist-like effect at σ_2-receptors (Data not shown). Interestingly, as seen in Fig. 4, DuP734, a selective σ_1 antagonist, also reversed the potentiation of compounds 1 and 2, which was not observed for (+)-pentazocine in the previous study (Izenwasser et al., 1998).

As demonstrated in Fig. 5, the D_3-trishomocubane analogue, compound 5, at a concentration up to 10 μM, a concentration which should have produced full occupation of σ_1 and σ_2 receptors, very effectively antagonized the enhancing effect of compound 2, suggesting that it could behave as a σ_2 antagonist. When tested alone, it appeared to slightly inhibit amphetamine-stimulated release; however, values for these conditions were not significantly different from one another. However, the slight decrease could indicate the presence of endogenous σ_2 tone that was antagonized by compound 5.

We also tested the σ-imaging compound 4-IBP. This ligand has a high affinity at both σ_1 ($K_i = 1.9$ nM) and σ_2 ($K_i = 95$ nM). At concentrations up to 1 μM, 4-IBP had no significant effect on amphetamine-stimulated [^{3}H]dopamine release. At 5 μM, 4-IBP produced about 30% enhancement over amphetamine-stimulated release. We also found no evidence for antagonist action at σ_2 receptors, as 4-IBP at 5 μM had no effect on the enhancement produced by compound 2.

4. Discussion

It has previously been shown that amphetamine stimulates [^{3}H]dopamine release from slices of rat caudate puta-

men in a concentration-dependent manner and the stimulation can be modulated by several σ-receptor agonists and antagonists. Moreover, the enhancement of this action of amphetamine by σ agonists, such as (+)-pentazocine and (−)-pentazocine, was mainly mediated via σ_2 receptors, supported by the concentration range over which (+)-pentazocine-enhanced amphetamine-stimulated release, lack of effect of σ_1-receptor agonist BD737 and the complete block of the potentiation by σ_2-receptor antagonists (Izenwasser et al., 1998).

(+)-Pentazocine and (−)-pentazocine were used as the release stimulators in the above functional assays due to the lack of σ_2-selective agonists. In experiments testing trishomocubane analogues, compounds 1, 2 and 3, of which have high affinity for σ_1 receptors and also good affinity for σ_2 subtype (see Table 1), potentiated [^{3}H]-dopamine release stimulated by amphetamine from rat caudate putamen in a concentration-dependent manner (Fig. 3). At 1 μM, compounds 1, 2 and 3 increase amphetamine-stimulated release by 35–45%, which was about equipotent to that of (+)-pentazocine at the same concentration, suggesting that these three compounds act as σ_2-receptor agonists in this functional assay. The similar potency of the enhancing effect of these three analogues was consistent with their similar K_i values at the σ_2-receptor subtype (171–370 nM). Compound 4, which showed the highest affinity at σ_2 receptors ($K_i = 20$ nM), exhibited about 10-fold greater potency than that of compounds 1, 2 and 3 in enhancing amphetamine-stimulated release (D, Fig. 3). These data indicated that the trishomocubane analogue-mediated enhancement was likely to be related to the activation of σ_2-receptor subtype.

The action of σ_2-receptor antagonists generally confirmed that these compounds are σ_2-receptor agonists. The potentiation produced by 1 μM of compounds 1 and 2 was fully reversed by the selective σ_2-receptor antagonist Lu28-179 ($K_{i\sigma_2} = 0.12$ nM, $K_{i\sigma_1} = 17$ nM) (Moltzen et al., 1995) at a concentration of 1 nM, BIMU-8 ($K_{i\sigma_2} = 20$ nM, $K_{i\sigma_1} > 1$ μM) (Bonhaus et al., 1993) at 100 nM and a non-subtype selective σ-receptor ligand BD1008 ($K_{i\sigma_2} =$ 1.2 nM, $K_{i\sigma_2} = 8.2$ nM) (De Costa et al., 1989) at 10 nM, supporting the identification of the receptor involved as σ_2 subtype, as well as compounds 1 and 2 as σ_2-receptor agonists. However, unlike (+)-pentazocine, which was not affected by DuP734, a selective σ_1 antagonist (Izenwasser et al., 1998), the potentiation mediated by both compounds 1 and 2 was significantly reversed by DuP734 (Fig. 4). It is possible that DuP734, compounds 1 and 2 (but not (+)-pentazocine) may interact with certain unidentified receptor subtypes other than those we currently appreciate. This receptor could be a σ-receptor subtype or a non-σ receptor. Further investigation is underway to elucidate this interaction.

Interestingly, compound 4-IBP, with a K_i value of 95 nM at σ_2 site and 1.9 nM at σ_1 sites, only produced significant enhancement over amphetamine-stimulated release at a concentration of 5 μM and had no effect on the potentiation produced by compound 2. Since both populations of σ receptors would have been completely occupied at the 1 μM concentration, we believe the enhancing action at 5 μM to be via some other mechanism than an agonist effects at σ receptors.

The D_3-trishomocubane compound 5, which exhibited great potency in reversing reserpine-induced catalepsy in vivo (Oliver et al., 1991), lacked affinities for a range of neurotransmitter receptor subtypes, including dopamine, opioid, 5-HT, muscarinic, NMDA and phencyclidine (PCP) binding sites ($K_i > 10$ μM, data not shown). In this functional assay, when tested alone, compound 5 at 10 μM slightly inhibited amphetamine-stimulated [^{3}H]dopamine release, however, significantly blocked the enhancing effect produced by compound 2 at the same concentration. The opposite action of compound 5 to that of the other trishomocubanes identified as σ_2-receptor agonists, including compounds 1, 2, 3 and 4, suggested that it could be a σ_2-receptor antagonist. Nonetheless, considering the moderate affinity of compound 5 at the DAT ($K_i = 623$ nM), the inhibition could have resulted from its direct action on dopamine transporters. Further studies are needed to clarify whether or not the inhibitory effect of D_3-trishomocubane 5 in this assay may contribute to its anticataleptic property in vivo as reported previously (Oliver et al., 1991).

In conclusion, trishomocubane analogues 1, 2, 3 and 4 have been identified as σ_2-receptor agonists in modulation of amphetamine-stimulated [^{3}H]dopamine release assays in vitro. There has been a shortage of σ_2-receptor agonists, compared with a number of high affinity and selective σ_1-receptor agonists and antagonists to date. As a result, the trishomocubane analogues identified as σ_2-receptor agonists in the current work will greatly aid the research into σ_2-receptor-mediated activities. Moreover, based on these compounds, it may be possible to develop more potent σ_2-receptor agonists for future functional studies.

Acknowledgements

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