ELSEVIER

European Journal of Pharmacology 346 (1998) 189–196

ejp

Modulation of amphetamine-stimulated (transporter mediated) dopamine release in vitro by σ_2 receptor agonists and antagonists

Sari Izenwasser [a,*], DeDeene Thompson-Montgomery [b], Sophia E. Deben [b], Iffat N. Chowdhury [b], Linda L. Werling [b]

[a] *Psychobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, P.O. Box 5180, Baltimore, MD 21224, USA*

[b] *Department of Pharmacology, George Washington University Medical Center, Washington, DC, USA*

Received 23 October 1997; revised 15 December 1997; accepted 16 January 1998

Abstract

Some σ receptor ligands have been shown to bind with low affinity to the dopamine transporter and to inhibit [^{3}H]dopamine uptake. It has not previously been shown whether any of these compounds influence release of dopamine via facilitated exchange diffusion. To further examine the nature of the interaction between σ receptor ligands and the dopamine transporter, the effects of σ receptor ligands on amphetamine-stimulated [^{3}H]dopamine release were examined in slices prepared from rat caudate putamen. In the absence of exogenous Ca^{2+}, both (+)-pentazocine and (−)-pentazocine potentiated amphetamine-stimulated [^{3}H]dopamine release at concentrations consistent with their affinities for σ_2 receptors. In contrast, BD737 (1*S*,2*R*-(−)-*cis*-*N*-{2-(3,4-dichlorophenyl)ethyl}-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine), a σ_1 receptor agonist, had no effect on amphetamine-stimulated release. Neither isomer of pentazocine alone had any effect on basal [^{3}H]dopamine release under these conditions. Three antagonists at σ receptors, one of which is non-selective for subtypes, and two of which are σ_2-selective, all blocked the enhancement of stimulated release produced by (+)-pentazocine. Enhancement of stimulated release by (−)-pentazocine was similarly blocked by σ_2 receptor antagonists. Our data support the contention that it is possible to regulate transporter-mediated events with drugs that act at a subpopulation of σ receptors pharmacologically identified as the σ_2 subtype. © 1998 Elsevier Science B.V.

Keywords: Caudate putamen; Cocaine; Dopamine transporter; Dopamine uptake

1. Introduction

Both amphetamine and cocaine are thought to produce their behavioral and reinforcing effects via actions at the dopamine transporter. Cocaine binds to the dopamine transporter and inhibits reuptake of dopamine into presynaptic terminals, resulting in an increase in extracellular dopamine. Amphetamine also acts via the dopamine transporter: it is transported into the terminals where it causes release of vesicular pools of dopamine into the cytoplasm and ultimately into the extracellular fluid (Schuldiner et al., 1993; Sulzer et al., 1993, 1995). Because of these actions, much interest has been focused on mechanisms by which transporter function can be regulated, in the hope that this information will lead to the development of agents that may be of use in the treatment of drug abuse.

There are few studies showing receptor regulation of dopamine transporter function. There is some evidence that dopamine D_2 receptors might modulate dopamine reuptake. When inward transport of dopamine is mathematically modeled from studies using rotating disk electrode voltammetry to measure dopamine levels it appears that the dopamine D_2 receptor agonist quinpirole increases the rate of removal of dopamine from the synapse (Meiergerd et al., 1993), while haloperidol (McElvain and Schenk, 1992), and raclopride (Cass and Gerhardt, 1994), both dopamine D_2 receptor antagonists, decrease dopamine reuptake. These effects are not robust, and alterations in dopamine uptake in the presence of dopamine D_2 agonists are not apparent when in vitro measurements of dopamine uptake into synaptosomes are used (Dean and Copolov,

* Corresponding author. Current address: Department of Neurology, University of Miami School of Medicine, 1501 NW 9th Avenue, Room #4061, Miami, FL 33136 USA. Tel.: +1-305-243-2032; fax: +1-305-243-3649.

1989). Other studies have shown that nicotine inhibits dopamine uptake, but does not bind to the dopamine transporter (Izenwasser et al., 1991). In addition, the inhibition by nicotine is blocked by nicotinic receptor antagonists, suggesting a regulation of the transporter by nicotine receptors (Izenwasser et al., 1991; Yamashita et al., 1995).

Recently it was shown that some σ receptor ligands bind with low affinity to the cocaine binding site on the dopamine transporter (Izenwasser et al., 1993) and inhibit dopamine uptake (Woodward and Harms, 1992; Izenwasser et al., 1993). The mechanism for the inhibition of uptake appears to be noncompetitive, in that the compounds are binding to a regulatory site on the transporter (as evidenced by their competition against binding to the cocaine binding site). (+)-Pentazocine produces no inhibition of uptake at concentrations up to 10 μM. Previously, it has also been shown that cocaine interacts with σ receptors (Sharkey et al., 1988).

Another mechanism by which σ receptor ligands could regulate synaptic dopamine levels would be to facilitate reversal of transport. In the current study, we tested (+)-pentazocine, (−)- pentazocine, and BD737 (1*S*,2*R*-(−)-*cis*-*N*-{2-(3,4-dichlorophenyl)ethyl}-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine), all widely held to be σ receptor agonists, for their effects on dopamine release stimulated by amphetamine. Although (+)-pentazocine can inhibit dopamine uptake at high concentrations, we used only concentrations consistent with activation of the two σ receptor subtypes, but too low to have effects on uptake (Izenwasser et al., 1993).

We have previously found that σ receptor agonists such as (+)-pentazocine, (−)-pentazocine and BD737 inhibit NMDA-stimulated release of dopamine from the striatum (Gonzalez-Alvear and Werling, 1994; Gonzalez-Alvear and Werling, 1995), nucleus accumbens, and prefrontal cortex (Weatherspoon et al., 1996). These studies were done under conditions favorable for NMDA-receptor stimulation (i.e., in the presence of Ca^{2+} and absence of Mg^{2+}). In addition, a dopamine uptake inhibitor was used, so as to prevent the reuptake of released dopamine, thus precluding any conclusions about the effects of these compounds on transporter-mediated release. Under these conditions, the σ receptor agonists do not have any effect on basal dopamine release.

The present study was undertaken in order to further investigate the nature of the interaction between σ receptor ligands and the dopamine transporter. Since amphetamine causes the release of dopamine via reversal of the dopamine transporter (Liang and Rutledge, 1982; Jacocks and Cox, 1992; Giros et al., 1996), the effects of σ receptor agonists and antagonists on amphetamine-stimulated [^{3}H]dopamine release were examined. Additionally, the effects of Mg^{2+} and Ca^{2+} were considered in these interactions. Since the presence of Mg^{2+} blocks the activation of the NMDA-operated cation channel, it was possible to rule out a contribution of NMDA-mediated stimulation of dopamine release by measuring release in the presence of Mg^{2+}. Similarly, since extracellular Ca^{2+} is necessary for exocytotic release to occur, and NMDA-stimulated release is exocytotic, removal of Ca^{2+} ensured that any observed release was likely to be via the dopamine transporter.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were obtained from the following sources: [^{3}H]dopamine (specific activity ranged from 47–50 Ci/mmol) from Amersham (Arlington Heights, IL); (+)- and (−)-pentazocine, and amphetamine from the National Institute on Drug Abuse, Rockville, MD; DTG (1,3-di-*o*-tolylguanidine) from Research Biochemicals, (Natick, MA); BD737 (1*S*,2*R*-(−)-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine) and BD 1008 (*N*-{2-(3,4-dichlorylphenyl)ethyl}*N*-methyl-2-pyrrolidinyl)ethylamine) from Wayne Bowen at NIDDK, NIH; BIMU-8 (*endo*-*N*-(8-methyl-8-azabicyclo{3.2.1.}oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamidehydrochloride) from Doug Bonhaus at Roche Bioscience; DuP734 (1-(cyclopropylmethyl]-4-(2′4″-fluorophenyl)-2′-oxoethyl)piperidine HBr) from Robert Zaczek at Dupont Merck; Lu28-179 (1′-[4-[1-94-fluorophenyl)-1*H*-indol-3-yl]-1-butyl]spiro[isobenzofuran-1(3*H*), 4′-piperidine]) from Connie Sanchez at H. Lundbeck.

2.2. Dopamine release assays

Dopamine release was measured essentially as previously described (Werling et al., 1988; Gonzalez-Alvear and Werling, 1994). Male Sprague–Dawley rats (350–500 g, Hilltop, Scottdale, PA) were killed by decapitation and their brains removed to an ice-cooled dish for dissection of the striatum. The tissue was weighed and chopped into 250 μm slices on a Sorvall TC2 tissue slicer with two successive cuts at an angle of 90°. The strips of tissue were suspended in oxygenated modified Krebs–HEPES buffer (MKB) consisting of 127 mM NaCl; 5 mM KCl; 1.3 mM NaH_2PO_4; 1.2 mM $MgSO_4$ (where indicated); 15 mM HEPES acid; 10 mM glucose; (adjusted to pH 7.4 with NaOH) which was presaturated with 95% O_2/5% CO_2 and warmed to 37°C. The tissue was rinsed three times, each in approximately 20 volumes of buffer. The tissue slice suspensions were then incubated in 20 ml buffer containing [^{3}H]dopamine (final concentration 15 nM) and 0.1 mM ascorbic acid at 37°C for 30 min. Tissue was then washed twice in 20 ml MKB and once in MKB containing domperidone, which was included in all subsequent steps of the experiment to prevent feedback inhibition by released [^{3}H]dopamine. Tissue was suspended a final time in

MKB and distributed in 275 μl aliquots between glass-fiber filter discs into chambers of a BRANDEL (Gaithersburg, MD) superfusion apparatus. MKB was superfused over the tissue at a flow rate of 0.6 ml/min. A low, stable baseline release, of approximately 1.1%/collection interval of two minutes, was established over a 30 min period. Tissue was then stimulated to release [^{3}H]dopamine by a 2 min exposure to amphetamine (Stimulus 1, S1). The inflow was then returned to a non-stimulating buffer (interstimulus interval) for a period of 10 min. When the effect of a test drug on basal release was examined, the drug was introduced at the beginning of the interstimulus interval. The tissue was then stimulated a second time for 2 min with amphetamine in the presence or absence of the drug being tested, as appropriate (Stimulus 2, S2). Inflow was again returned to non-stimulating 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. After concentration-response curves for amphetamine had been established, a standard stimulus of 10 μM amphetamine was chosen for subsequent experiments. Superfusates were collected at 2 min intervals in scintillation vials, and the glass-fiber filter discs and tissue were collected into the final vials. Released radioactivity was determined by liquid scintillation spectroscopy.

All experiments were carried out in accordance with the guidelines of, and with the approval of, The George Washington University Institutional Animal Use and Care Committee. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Division of Intramural Research, National Institute on Drug Abuse, National Institutes of Health, and the Guide for Care and Use of Laboratory Animals, National Research Council, Department of Health, Education and Welfare, NIH Publication 85-23, revised 1985.

2.3. Data analysis

Data were expressed as radioactivity released above baseline during the collection interval as a fraction of total radioactivity in the tissue at the beginning of the collection interval (percent fractional release) to allow visualization of absolute amounts of release, or as a percentage of radioactivity released by the control stimulus (percent control stimulated release) to facilitate comparison across agonist/antagonist treatments. In experiments on the effects of σ receptor ligands on amphetamine-stimulated release, all data were statistically analyzed as ratios (S2/S1) before transformation of data into percent control stimulated release. Data were analyzed as ratios so that each tissue sample could serve as its own control. Since the test drug is added after the first and before the second stimulus, a ratio higher than the control ratio indicates facilitation of release, while that lower than the control indicates an inhibition. In drug treatment experiments, the control S2/S1 ratio (typically 0.8) was set to 100%, and the drug treatments were expressed relative to this control. This transformation facilitates comparison across treatments which may have been used in different experiments. Under the experimental conditions used, the released radioactivity has been shown to be primarily dopamine (Werling et al., 1988). Data were analyzed using a two-way factorial analysis of variance (ANOVA). Post-hoc Dunnett's tests were also performed as indicated. Results were considered to be significantly different when $P < 0.05$.

3. Results

Amphetamine stimulated the release of preloaded radiolabeled dopamine in a concentration-dependent manner (Fig. 1). In the presence of Mg^{2+}, slightly less [^{3}H]dopamine was released by amphetamine. The IC_{50} for stimulation of release by amphetamine was 1 μM in the absence of Mg^{2+} and 1.5 μM in its presence. For subsequent experiments, a concentration of 10 μM amphetamine was chosen to stimulate release. To limit a potential contribution of NMDA-receptor-mediated stimulation to release, as well as to enhance membrane stability in the absence of added Ca^{2+}, all subsequent experiments were performed in the presence of Mg^{2+}.

In three experiments, (+)-pentazocine was tested as a potential stimulator of basal release by exposing the tissue to various concentrations of this drug for 2 min, as would be done for any stimulating drug. As seen in Table 1, at all concentrations tested (10 nM–1 μM) release by (+)-pentazocine was not significantly different from unstimulated basal release. In contrast, (+)-pentazocine enhanced amphetamine-stimulated [^{3}H]dopamine release in a concentration-dependent manner, with an IC_{50} above 100 nM (Fig. 2). When endogenous Ca^{2+} was chelated by the inclusion

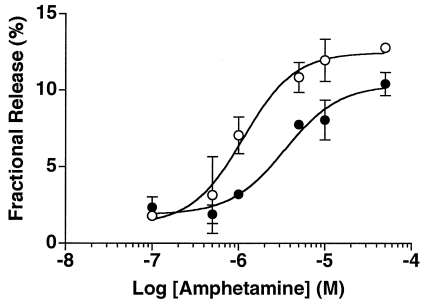

Fig. 1. Stimulation of [^{3}H]dopamine release by amphetamine (S1). Data are expressed as percent fractional release ± S.E.M. for increasing concentrations of amphetamine. Closed circles show the effect of amphetamine in the presence of Mg^{2+} (1.2 mM), while open circles represent stimulation in the absence of Mg^{2+}. ANOVA revealed a significant effect of increasing amphetamine concentration. The presence or absence of Mg^{2+} produced no significant differences. Release produced by the second stimulation (S2) was approximately 80% of that measured in S1. $n = 3$.

Table 1
Effect of (+)-pentazocine on basal release

(+)-Pentazocine (nM)	[^{3}H]Dopamine release (fractional release (%)/2 min ± S.E.M.)
None	1.10 ± 0.24
10	1.39 ± 0.54
50	1.00 ± 0.55
100	1.04 ± 0.68
500	1.08 ± 0.33
1000	1.26 ± 0.66

(+)-Pentazocine had no effect on basal release over a range of concentrations. Release was measured as described in Section 2, except that instead of amphetamine in S2, the indicated concentration of (+)-pentazocine was added after the interstimulus interval. Data are expressed as the mean % fractional release per 2 min collection interval. $n = 3$.

of 1 mM EGTA, the stimulated release of [^{3}H]dopamine by amphetamine alone was not significantly affected, but the enhancement of release by (+)-pentazocine was abolished.

The effects of (+)-pentazocine on amphetamine-stimulated [^{3}H]dopamine release in the presence of several reported σ receptor antagonists were also measured (Fig. 3). The antagonists themselves had no significant effects on either basal or amphetamine-stimulated release. The lowest concentration of (+)-pentazocine (100 nM) that had produced significant enhancement (140%) over amphetamine alone was chosen to test for reversal by σ receptor antagonists. At 100 nM, (+)-pentazocine should occupy 95% of σ_1 receptors ($K_i = 5$ nM) and 18% of σ_2 receptors ($K_i = 440$ nM, Connor and Chavkin, 1992) so that the contribution of σ_1 and σ_2 receptors to the response could be evaluated. The enhancement produced by 100 nM (+)-pentazocine in this set of experiments was about 150% of control amphetamine-stimulated release. The (+)-pentazocine-mediated enhancement of release was significantly reversed by the non-subtype selective antagonist BD 1008 (10 nM), and by the selective σ_2 receptor

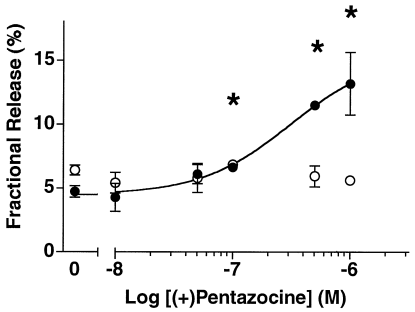

Fig. 2. Enhancement of amphetamine-stimulated [^{3}H]dopamine release by (+)-pentazocine. Data are expressed as percent fractional release ± S.E.M. for increasing concentrations of (+)-pentazocine during the second stimulation (S2) by 10 μM amphetamine. Release measured in the absence (closed circles) or the presence of EGTA (1 mM; open circles). ANOVA detected an effect of increasing (+)-pentazocine concentrations. * significantly different from control amphetamine-stimulated release by post-hoc Dunnett's at $P < 0.05$. $n = 6$.

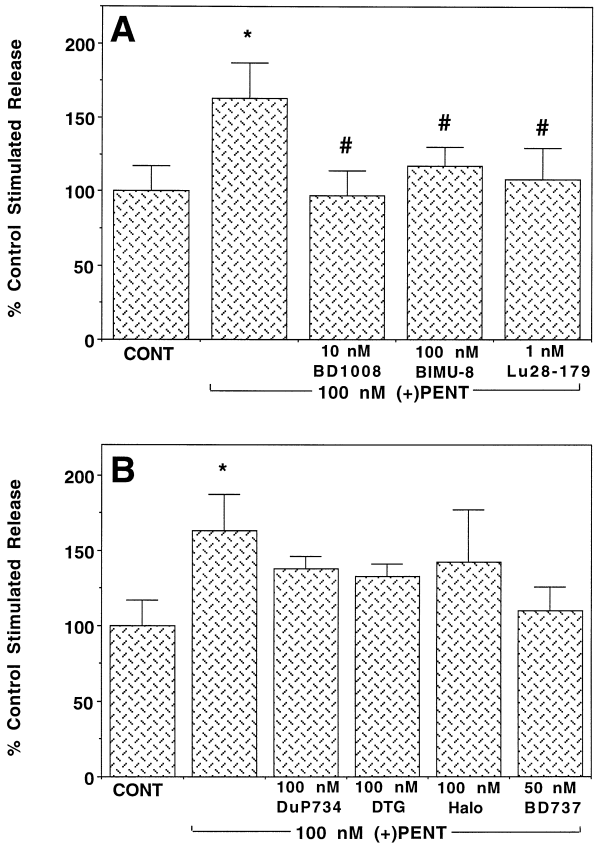

Fig. 3. Effects of σ receptor agonists and antagonists on (+)-pentazocine (100 nM) enhancement of amphetamine-stimulated [^{3}H]dopamine release. Release stimulated by 10 μM amphetamine alone is indicated as CONT. (A) Antagonists producing full reversal of (+)-pentazocine-mediated enhancement. BD1008 was tested at 10 nM, BIMU-8 was tested at 100 nM, and Lu28-179 was tested at 1 nM. (B) Antagonists that did not significantly reverse enhancement, and lack of effects of the sigma$_1$ receptor agonist BD737. Dup734, DTG, and haloperidol were tested at 100 nM. BD737 was tested at 50 nM. Data are expressed as percent control release ± S.E.M. stimulated by 10 μM amphetamine, set at 100%. ANOVA revealed significant treatment differences. * significantly different from control amphetamine-stimulated release by post-hoc Dunnett's at $P < 0.05$. # reversal by antagonists to values not significantly different from control amphetamine-stimulated release. $n = 4$.

antagonists BIMU-8 (100 nM), and Lu28-179 (1 nM) (Fig. 3A). The σ_1-selective receptor antagonist DuP734 (100 nM), and the purported antagonists DTG (100 nM) and haloperidol (100 nM) did not produce a significant reversal of (+)-pentazocine-mediated enhancement (Fig. 3B). Additionally, BD737, which is an agonist selective for σ_1 sites at concentrations below 100 nM was also tested at 10 and 50 nM, and found to have no effect on amphetamine-stimulated release (Fig. 3B, 50 nM concentration only shown).

In the absence of a selective σ_2 receptor agonist, to confirm that enhancement was mediated by σ_2 receptors, we also tested (−)-pentazocine at 100 nM on amphetamine-stimulated release. The (−)-isomer of pentazocine has similar K_i values at both σ_1 (87 nM) and σ_2 receptors (82 nM), with slightly greater affinity at σ_2 sites

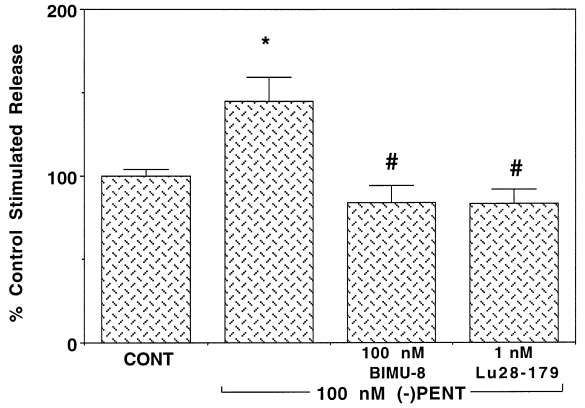

Fig. 4. Enhancement of amphetamine-stimulated [^{3}H]dopamine release by (−)-pentazocine (100 nM), and reversal of enhancement by σ_2 receptor antagonists. BIMU-8 was tested at 100 nM and Lu28-179 was tested at 1 nM. Data are expressed as percent control release ± S.E.M. stimulated by 10 μM amphetamine, set at 100%. Release stimulated by 10 μM amphetamine alone is indicated as CONT. ANOVA revealed significant differences among treatments. * significantly different from control stimulated release at $P < 0.05$ as compared to amphetamine alone by post-hoc Dunnett's. # not significantly different from control stimulated release by post-hoc Dunnett's. $n = 3$.

(Hellewell and Bowen, 1990). At 100 nM, (−)- pentazocine should occupy about 50% of σ_2 receptors. (−)-Pentazocine produced 140% of control stimulated release, and both selective σ_2 receptor antagonists, BIMU-8 and Lu28-179 blocked the enhancement (Fig. 4).

4. Discussion

Amphetamine stimulated [^{3}H]dopamine release from slices of rat caudate putamen in a concentration-dependent manner. The stimulation was slightly diminished in the presence of Mg^{2+}. Since dopamine release can also be stimulated by activation of NMDA receptors, but only in the absence of Mg^{2+} (and the presence of Ca^{2+}), all further studies were conducted in the presence of Mg^{2+} to eliminate a potential contribution of NMDA receptor- mediated stimulation to the release measured. The release of dopamine by amphetamine is primarily non-exocytotic in nature, since no exogenous Ca^{2+} was added in any of these assays. Secondly, it has previously been shown that σ receptor ligands such as (+)-pentazocine inhibit NMDA-stimulated dopamine release (Gonzalez-Alvear and Werling, 1994, 1995; Weatherspoon et al., 1996). The enhancing effect of (+)-pentazocine on amphetamine-stimulated release are in direct contrast to its inhibiting effects on NMDA-stimulated release previously reported (Gonzalez-Alvear and Werling, 1994, 1995). In those studies, the release stimulated by NMDA was Ca^{2+}-dependent, vesicular release.

The present findings show that amphetamine-stimulated dopamine release can be modulated by several drugs identified as agonists and antagonists at σ receptors. If the effects of drugs used in this study are mediated via σ receptors, identification of the subtypes involved must be considered. (+)-Pentazocine is generally held to be a σ_1 and σ_2 receptor agonist (reviewed by Walker et al., 1990), with higher affinity for σ_1 receptors, and moderate affinity for σ_2 receptors. (+)-Pentazocine also has the ability to inhibit dopamine uptake, but only at concentrations well above those required to bind to σ_1 or σ_2 receptors (Izenwasser et al., 1993). In the current study, (+)-pentazocine was tested for its ability to modulate amphetamine-stimulated release over a concentration range at which both σ_1 and σ_2 receptors would be activated, but lower than necessary for inhibition of uptake. We do not have a selective σ_2 receptor agonist, but (−)-pentazocine has similar affinity for the σ_2 and σ_1 receptors, so if the σ_2 receptor is responsible for the enhancement of amphetamine-stimulated release, then (−)-pentazocine would be expected to enhance stimulated release similarly to (+)-pentazocine. Finally, at concentrations below 100 nM, BD737 is a σ_1-selective receptor agonist. BD737 can be used to confirm or rule out σ_1 receptor involvement in the observed effects of (+)-pentazocine. Although most research has focused on σ_1 and σ_2 subtypes of σ receptors, additional binding sites have been proposed (Zhou and Musacchio, 1991; Quirion et al., 1992). A *trans*-1-phenyl-3-aminotetralin-sensitive site called σ_3 has been described (Owens et al., 1995), as has a site termed σ_4 (Bowen et al., 1995). Monnet and colleagues have described effects of steroid-sensitive σ-like receptors pharmacologically different from σ_1 and σ_2 that regulate NMDA-stimulated norepinephrine release (Monnet et al., 1995, 1996).

(+)-Pentazocine has high affinity for σ_1 sites (K_i = 1–5 nM; Carroll et al., 1992; Connor and Chavkin, 1992) but also has good affinity for σ_2 sites (K_i = 400–500 nM; Walker et al., 1990; Connor and Chavkin, 1992), and for the newly identified σ_4 sites ($K_i < 100$ nM; Bowen et al., 1995). The σ_3 binding site is not (+)-pentazocine-sensitive (Owens et al., 1995). In the present study, the enhancement of amphetamine-stimulated dopamine release by (+)-pentazocine did not occur at concentrations below 100 nM, suggesting that the majority of the stimulation was unlikely to be mediated via σ_1 receptors. Additionally, the selective σ_1 receptor agonist BD737 produced no significant enhancement of amphetamine-stimulated [^{3}H]dopamine release, supporting the assertion that σ_1 receptors do not participate in the enhancement by (+)-pentazocine. At concentrations of (+)-pentazocine that produce significant occupation of σ_2 receptors (18% fractional occupancy at 100 nM, increasing to 67% at 1 μM), an appreciable enhancement of release was observed. The effects of 100 nM (+)-pentazocine were completely antagonized by the non-selective σ receptor antagonist BD 1008 (Gonzalez-Alvear and Werling, 1994), and by BIMU-8 and Lu28-179, both selective σ_2 receptor antago-

nists (Perregard et al., 1995; Weatherspoon et al., 1996). Antagonists were tested at approximately 1 log unit above their K_i values for the σ_2 receptor. The K_i values for antagonists are: BD1008, 1.2 nM, unspecified for subtype (De Costa et al., 1989); BIMU-8, 20 nM at σ_2, >1 μM at σ_1 (Bonhaus et al., 1993); Lu28-179, 0.12 nM at σ_2, 17 nM at σ_1 (Moltzen et al., 1995). These observations suggest that (+)-pentazocine is more likely to enhance release via σ_2 than σ_1 receptors in the current studies. Since little is known about σ_4 receptors, it is not possible to exclude their participation in regulation of release with the current evidence. At 100 nM, (−)-pentazocine occupies about 50% of σ_2 receptors, and significantly enhanced amphetamine-stimulated [^{3}H]dopamine release. Although (−)-pentazocine is also a κ opioid receptor agonist, the complete reversal of the effects of (−)-pentazocine by the selective σ_2 receptor antagonists suggest that enhancement by (−)-pentazocine is not mediated by opioid receptors. (−)-Pentazocine, at 100 nM, enhanced amphetamine-stimulated release by almost 150%, while (+)- pentazocine, at the same concentration, enhanced release by 160%. Thus, a lower fractional occupancy of σ_2 receptors by (+)-pentazocine produces a relatively greater effect. This may be due to differing efficacies of the two agonists. This possibility was not explored in the current study. The reversal of both isomers' release-enhancing effects by σ_2 selective receptor antagonists argues that both isomers produce their enhancement via σ_2 receptors.

In the current study, both DTG and DuP734, at the concentrations tested, appear to slightly antagonize (+)-pentazocine-mediated enhancement of amphetamine-stimulated dopamine release, but neither attained statistical significance. DTG is considered a σ receptor agonist by some investigators and a σ receptor antagonist by others. It has a K_i at $\sigma_1 = 12$ nM and at $\sigma_2 = 38$ nM (Walker et al., 1990). We have previously shown that DTG behaves as a σ_1 receptor antagonist against the σ receptor agonists (+)-pentazocine and BD737 in modulating [^{3}H]norepinephrine release from hippocampal slices (Gonzalez and Werling, 1995). DuP734 is a highly selective σ_1 receptor antagonist (K_i at $\sigma_1 = 10$ nM; K_i at $\sigma_2 > 1$ μM; Culp et al., 1992; Gonzalez-Alvear and Werling, 1994, 1995; Gonzalez and Werling, 1995).

It is interesting to note that (+)-pentazocine had no effect on dopamine release in the absence of amphetamine. The lack of effect on basal release suggests that the σ_2 ligands do not facilitate release via the same mechanisms that amphetamine stimulates release, or the effects of the combination of test drug and amphetamine would be expected to be additive. Since the mechanism by which amphetamine releases dopamine is complex, it is difficult to predict the exact cellular location mediating the σ receptor drug effect.

There are several possible explanations for the apparent increase in the ability of amphetamine to stimulate dopamine release in the presence of (+)-pentazocine.

(1) (+)-Pentazocine may increase the uptake of amphetamine into the cells via the membrane transporter. This explanation is unlikely since we have previously shown that the uptake of dopamine via the membrane transporter is decreased, not increased by some σ receptor ligands (Izenwasser et al., 1993). In addition, (+)-pentazocine, which at the highest concentration tested, 1 μM, produced approximately a 300% increase in dopamine release when combined with amphetamine, has no effect on dopamine uptake under identical conditions to those used in the present experiment.

(2) The σ_2 agonists might facilitate the reversal of the membrane transporter such that a greater amount of dopamine is transported out of the cell upon exposure to amphetamine. If this were occurring, there might not be an effect of σ_2 agonists alone on basal release, since presumably the cytoplasmic concentrations of dopamine are relatively low, and increase only following the entrance of amphetamine into the storage vesicles. Facilitation of reversal of the membrane transporter by σ_2 agonists seems unlikely, however, because a reversal of the transporter would produce an apparent inhibition of dopamine uptake (i.e., if the transporter was reversed, less dopamine would be entering the cell in this manner). In fact, (+)-pentazocine has previously been shown to have no effect on dopamine uptake in the absence of amphetamine (Izenwasser et al., 1993).

(3) Perhaps the most likely suggestion is that σ receptor agonists may increase the uptake of amphetamine into the intracellular storage vesicles. If this is happening, it suggests that σ receptor agonists can regulate uptake into the vesicle. This would have to occur either by (a) penetration of the cells by the σ receptor agonists and subsequent action at intracellular sites; or (b) regulation of vesicular uptake secondary to binding to the cell membrane σ receptor binding sites. If (a) occurs, it further suggests that there might be σ receptors that regulate the vesicular transporter residing on the vesicle. It has been shown that there is a subcellular distribution of σ receptors in both rat brain (McCann and Su, 1990) and liver (Samovilova and Vinogradov, 1992). σ receptors have been proposed as targets for steroids (Su, 1982; Monnet et al., 1995, 1996). Steroid receptors are known to be intracellular. Furthermore, penetration of the cells by the σ receptor ligands is possible. The log P value for pentazocine is 4.45, suggesting that both isomers are quite lipophilic, and may enter the cell to their exert their effects. If these compounds are indeed acting at intracellular binding sites, it is not clear whether those sites might reside on, or be able to regulate the storage vesicles. Further studies to examine these possibilities are underway.

It is important to point out that if indeed the compounds having effects in the current system are acting intracellularly, the ability of each compound (both agonists and antagonists) to enter the cell could greatly influence the results. It is reasonable to speculate that due to the polar

guanidino function of DTG, it is probably charged at physiological pH, and cell penetrability may be compromised. Haloperidol, a putative σ receptor antagonist, has previously been shown to antagonize NMDA-stimulated norepinephrine release in a manner consistent with a σ_2 receptor antagonist (Gonzalez and Werling, 1995). Haloperidol is quite lipid soluble, so should be capable of acting at intracellular sites. Only a single concentration of haloperidol was tested here, so the lack of significant reversibility may be due to the concentration tested.

The mechanism by which regulation of vesicular uptake by cell membrane binding sites (b) might occur is unclear, but the lack of potentiation by high concentrations of (+)-pentazocine in the presence of EGTA (i.e., removal of endogenous Ca^{2+}) suggests that a Ca^{2+} pool might be involved. It has previously been shown that σ receptor ligands acting on σ_2 receptors release Ca^{2+} from intracellular stores, suggesting that there are σ receptors that are capable of regulating intracellular Ca^{2+} release (Vilner and Bowen, 1995). Thus, it is possible that either extracellular membrane-bound σ receptors, or receptors located on a subcellular fraction, regulate a Ca^{2+} pool that can in turn modulate vesicular uptake. We have not addressed the source of the Ca^{2+} utilized in the (+)-pentazocine-mediated enhancement of release. While EGTA is usually used to deplete external Ca^{2+}, the extent to which it can also chelate intracellular Ca^{2+} is dependent upon the incubation time, since at longer times, EGTA depletes intracellular stores as well.

Collectively, our data suggest that σ_2 receptor ligands can regulate release of dopamine stimulated by reversing the transporter. This is based upon the concentration range over which (+)-pentazocine enhanced amphetamine-stimulated release, a similar enhancement by (−)-pentazocine, and the complete reversal of the enhancement by two antagonists at σ_2 receptors. Additionally, the σ_1 receptor agonist BD737 did not enhance stimulated release, nor did the σ_1-selective receptor antagonist DuP734 reverse (+)-pentazocine-mediated enhancement. In addition, σ_2 receptors have been associated with the regulation of intracellular Ca^{2+} levels (Vilner and Bowen, 1995), and the (+)-pentazocine effect at high concentrations on release was dependent on endogenous Ca^{2+}. Nevertheless, participation of other σ receptor subtypes cannot be ruled out, due to the identification of the σ_4 subtype, which has a pharmacological profile consistent with the (+)-pentazocine and BD 1008 effects. Too little is known about the σ_4 site to speculate further at this point.

Although much remains to be learned about the mechanisms responsible for these findings, they do support the belief that it is possible to regulate transporter-mediated events with drugs that act at receptors. Specifically, these findings suggest that σ receptors are a potential target for the development of therapeutics for drug abuse, as well as other for other diseases that disrupt dopaminergic function such as Parkinson's disease or schizophrenia.

Acknowledgements

We thank the following persons for their valuable contributions to this manuscript: Dr. Wayne Bowen (NIDDK, NIH) for BD737 and BD1008; Dr. Rob Zaczek (Dupont Merck) for DuP734; Dr. Connie Sanchez (H. Lundbeck) for Lu28-179; Dr. Doug Bonhaus (Roche Bioscience) for BIMU-8; Dr. Amy Newman for help and comments on a previous version of this manuscript. This research was supported by a grant from the National Institute on Drug Abuse (LLW) and the National Institute on Drug Abuse Intramural Research Program.

References

Bonhaus, D.W., Loury, D.N., Jakeman, L.B., To, Z., DeSouza, A., Eglen, R.M., Wong, E.H.F., 1993. [^{3}H]BIMU-1, a $5HT_3$ receptor ligand in NG108 cells, selectively labels a σ_2 binding site in guinea pig hippocampus. J. Pharmacol. Exp. Ther. 267, 961–970.

Bowen, W.D., Vilner, B.J., Lee, K.S., He, X.-S., deCosta, B.R., Weinberger, D.R., 1995. A haloperidol-insensitive binding site for sigma-active aryl ethylene diamine-related compounds: A novel σ receptor subtype?. Soc. Neurosci. Abst. 21, 526.

Carroll, F.I., Abraham, P., Parham, K., Bai, X., Zhang, Z., Brine, G.A., Mascarella, S.W., Martin, B.R., May, E.L., Sauss, C., DiPaolo, L., Wallace, P., Walker, J.M., Bowen, W.D., 1992. Enantiomeric *N*-substituted *n*-normetazocines: a comparative study of affinities at σ, PCP, and μ opioid receptors. J. Med. Chem. 35, 2812–2818.

Cass, W.A., Gerhardt, G.A., 1994. Direct in vivo evidence that dopamine D_2 receptors can modulate dopamine uptake. Neurosci. Lett. 176, 259–263.

Connor, M.A., Chavkin, C., 1992. Ionic zinc may function as an endogenous ligand for the haloperidol-sensitive σ_2 receptor in rat brain. Mol. Pharmacol. 42, 471–479.

Culp, S.G., Rominger, D., Tam, S.W., DeSouza, E.B., 1992. [^{3}H]DuP734 [1-(cyclopropylmethyl]-4-(2′4″-flourophenyl)-2′-oxoethyl)piperidine HBr]: A receptor binding profile of a high affinity novel σ receptor ligand in guinea pig brain. J. Pharmacol. Exp. Ther. 263, 1175–1187.

De Costa, B.R., Bowen, W.D., Hellewel, S.B., Walker, J.M., Thurkauf, A., Jacobson, A.E., Rice, K.C., 1989. Synthesis and evaluation of optically pure [^{3}H](+)-pentazocine, a highly potent and selective radioligand for σ receptors. FEBS Lett. 251, 53–58.

Dean, B., Copolov, D.L., 1989. Dopamine uptake by platelets is selective, temperature dependent and not influenced by the dopamine-D_1 or dopamine-D_2 receptor. Life Sci. 45, 401–411.

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M., Caron, M.G., 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606–612.

Gonzalez, A., Werling, L.L., 1995. Sigma receptor regulation of norepinephrine release from rat hippocampal slices. Brain Res. 673, 61–69.

Gonzalez-Alvear, G.M., Werling, L.M., 1994. Regulation of [^{3}H]dopamine release from rat striatal slices by σ receptor ligands. J. Pharmacol. Exp. Ther. 271, 212–219.

Gonzalez-Alvear, G., Werling, L.L., 1995. σ_1 receptors in rat striatum regulate NMDA-stimulated [^{3}H]dopamine release via a presynaptic mechanism. Eur. J. Pharmacol. 294, 713–719.

Hellewell, S.B., Bowen, W.D., 1990. A sigma-like binding site in rat pheochromocytoma (PC12) cells: decreased affinity for (+)-benzomorphans and lower molecular weight suggest a different sigma receptor from that of guinea pig brain. Brain Res. 527, 244–253.

Izenwasser, S., Jacocks, H.M., Rosenberger, J.G., Cox, B.M., 1991. Nicotine indirectly inhibits [^{3}H]dopamine uptake at concentrations which do not directly promote [^{3}H]dopamine release in rat striatum. J. Neurochem. 56, 603–610.

Izenwasser, S., Newman, A.H., Katz, J.L., 1993. Cocaine and several sigma receptor ligands inhibit dopamine uptake in rat caudate-putamen. Eur. J. Pharmacol. 243, 201–205.

Jacocks, H.M., Cox, B.M., 1992. Serotonin-stimulated release of [^{3}H]dopamine via reversal of the dopamine transporter in rat striatum and nucleus accumbens: A comparison with release elicited by potassium, *N*-methyl-D-aspartic acid and D-amphetamine. J. Pharmacol. Exp. Ther. 262, 356–364.

Liang, N.Y., Rutledge, C.O., 1982. Evidence for carrier-mediated efflux of dopamine from corpus striatum. Biochem. Pharmacol. 31, 2484–2497.

McCann, D.J., Su, T.-P., 1990. Haloperidol-sensitive (+) [^{3}H]SKF-10,047 binding sites (σ sites) exhibit a unique distribution in rat brain subcellular fractions. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 188, 211–218.

McElvain, J.S., Schenk, J.O., 1992. Blockade of dopamine autoreceptors by haloperidol and the apparent dynamics of potassium-stimulated endogenous release of dopamine from and reuptake into striatal suspensions in the rat. Neuropharmacology 31, 649–659.

Meiergerd, S.M., Patterson, T.A., Schenk, J.O., 1993. D_2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. J. Neurochem. 61, 764–767.

Moltzen, E.K., Perregard, J., Meier, E., 1995. σ ligands with subnanomolar affinity and preference for the σ_2 binding site 2. Spiro-joined benzofuran, isobenzofuran, and benzopyran piperidines. J. Med. Chem. 38, 2009–2017.

Monnet, F.P., Mahé, V., Robel, P., Baulieu, E.E., 1995. Neurosteroids, via σ receptors modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. Proc. Natl. Acad. Sci. USA 92, 3774–3778.

Monnet, F.P., deCosta, B.R., Bowen, W.D., 1996. Differentiation of σ ligand-activated receptor subtypes that modulate NMDA-evoked [^{3}H]noradrenaline release in rat hippocampal slices. Br. J. Pharmacol. 119, 65–72.

Owens, C.E., Bucholtz, E.C., Hooper, P.C., Meyers, A.M., Kula, N.S., Baldessarini, R.J., Mailman, R.B., Wyrick, S.D., Booth, R.G., 1995. Structure-activity relationships for phenylaminotetralins at [^{3}H]PAT labeled σ_3 sites in rodent brain. Soc. Neurosci. Abst. 21, 1610.

Perregard, J., Moltzen, E.K., Meier, E., Sánchez, C., 1995. σ ligands with subnanomolar affinity and preference for the σ_2 binding site 1. 3-(ω-aminoalkyl)-1*H*-indoles. J. Med. Chem. 38, 1998–2007.

Quirion, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.-P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Samovilova, N.N., Vinogradov, V.A., 1992. Subcellular distribution of (+)-[^{3}H]SKF 10,047 binding sites in rat liver. Eur. J. Pharmacol. Mol. Pharmacol. Sec. 225, 69–74.

Schuldiner, S., Steiner-Mordoch, S., Yelin, R., Wall, S.C., Rudnick, G., 1993. Amphetamine derivatives interact with both plasma membrane and secretory vesicle biogenic amine transporters. J. Pharmacol. Exp. Ther. 44, 1227–1231.

Sharkey, J., Glen, K.A., Wolfe, S., Kuhar, M.J., 1988. Cocaine binding at sigma receptors. Eur. J. Pharmacol. 149, 171–174.

Su, T.-P., 1982. Evidence for sigma opioid receptor: binding of [^{3}H]SKF-10047 to etorphine-inaccessible sites in guinea pig brain. J. Pharmacol. Exp. Ther. 223, 284–290.

Sulzer, D., Maidment, N.T., Rayport, S., 1993. Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. J. Neurochem. 60, 527–535.

Sulzer, D., Chen, T.-K., Lau, Y.Y., Kristensen, H., Rayport, S., Ewing, A., 1995. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J. Neurosci. 15, 4102–4108.

Vilner, B.J., Bowen, W.D., 1995. Dual modulation of cellular calcium by sigma receptor ligands: release from intracellular stores and blockade of voltage-dependent influx. Soc. Neurosci. Abstr. 21, 1608.

Walker, J.M., Bowen, W.D., Walker, F.O., Matsumoto, R.R., Costa, B.D., Rice, K.C., 1990. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402.

Weatherspoon, J.K., Gonzalez-Alvear, G.M., Frank, A.R., Werling, L.L., 1996. Regulation of [^{3}H]dopamine release from mesolimbic and mesocortical areas of guinea pig brain by sigma receptors. Schizophr. Res. 21, 51–62.

Werling, L.L., Frattali, A., Portoghese, P.S., Takemori, A.E., Cox, B.M., 1988. Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. J. Pharmacol. Exp. Ther. 246, 282–286.

Woodward, J.J., Harms, J., 1992. The putative polyamine antagonists ifenprodil and SL 82.0715 enhance dopamine efflux from rat striatal slices independent of NMDA receptor activation. Eur. J. Pharmacol. 210, 265–270.

Yamashita, H., Kitayam, S., Zhang, Y.-X., Takahashi, T., Dohi, T., Nakamura, S., 1995. Effect of nicotine on dopamine uptake in COS cells possessing the rat dopamine transporter and in PC12 cells. Biochem. Pharmacol. 49, 742–745.

Zhou, G.-Z., Musacchio, J.M., 1991. Computer-assisted modeling of multiple dextromethorphan and sigma binding sites in guinea pig brain. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 206, 261–269.