ELSEVIER

European Journal of Pharmacology 294 (1995) 713–719

ejp

σ_1 Receptors in rat striatum regulate NMDA-stimulated [^{3}H]dopamine release via a presynaptic mechanism

Grace M. Gonzalez-Alvear, Linda L. Werling *

Department of Pharmacology, The George Washington University Medical Center, 2300 Eye Street, N.W., Washington, DC 20037, USA

Received 17 August 1995; accepted 26 September 1995

Abstract

The role of the σ_1 receptor in the regulation of *N*-methyl-D-aspartate (NMDA)-stimulated [^{3}H]dopamine release from rat striatal slices was examined. The σ receptor agonist 1*S*,2*R*-(−)-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine (BD737) inhibited stimulated release in a concentration-dependent manner. The σ_1 receptor antagonist, 1-(cyclopropylmethyl)-4-(2′-(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr (DuP 734), reversed inhibition of release by BD737. Haloperidol, di-*o*-tolylguanidine (DTG) and *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (BD1008) reversed the BD737-mediated inhibition of release. Haloperidol and DTG also antagonized inhibition of stimulated release by (+)-pentazocine. Furthermore, BD737 and (+)-pentazocine inhibited stimulated release in the presence of tetrodotoxin, suggesting that σ_1 receptors regulating dopamine release are located on dopaminergic nerve terminals. These data suggest that σ_1 receptors may be important in the regulation of glutamate-stimulated dopamine release.

Keywords: σ Receptor; Dopamine release; (+)-Pentazocine; BD737 (1*S*,2*R*-(−)-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine); DuP 734 (1-(cyclopropylmethyl)-4-(2′-(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr); BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-pyrrodinyl)cyclohexylamine)

1. Introduction

Although the functional role of σ receptors in the central nervous system remains unclear, recent studies indicate the existence of multiple σ receptor subtypes. σ Receptors have been classified into two subtypes, σ_1 and σ_2, primarily based upon binding profile differences (Hellewell and Bowen, 1990; Quirion et al., 1992; Rothman et al., 1991). A reverse stereoselectivity for benzomorphans is observed with the two σ receptor subtypes (Di Paolo et al., 1991; Hellewell and Bowen, 1990). σ_1 Receptors bind (+)-isomers of benzomorphans, such as *N*-allylnormetazocine (SKF10,047) and (+)-pentazocine, with greater affinity than σ_2 receptors. The (−)-isomers of benzomorphans are slightly more potent at σ_2 receptors than the (+)-isomers, yet they do not differentiate the two sites. The σ ligand di-*o*-tolylguanidine (DTG) does not have selectivity for one subtype over the other. However, haloperidol, which has high affinity for σ receptors, has approximately a 40-fold preference for σ_1 sites. Recent data on regulation of receptor binding also support the existence of at least two distinct σ sites. σ_1 Receptor binding appears to exhibit sensitivity to GTP or a stable GTP analog (Beart et al., 1989; Itzhak, 1989; Itzhak and Stein, 1992) whereas σ_2 receptor binding remains unaffected. Furthermore, phenytoin allosterically modulates σ_1 sites, yet does not appear to affect σ_2 sites (McCann and Su, 1992; De Haven-Hudkins et al., 1993). In addition, Basile et al. (1992) have demonstrated that σ_1 receptor binding is sensitive to divalent cations, suggesting a possible involvement of σ_1 receptors in the regulation of cation channels. Little direct evidence for physiological roles of σ receptor subtypes has been reported, since the discrimination is releatively recent. In our laboratory, we have demonstrated σ_1 receptor regulation of [^{3}H]arachidonic acid release from rat cerebellar granule cells (Starr and Werling, 1994).

Both anatomical and physiological evidence support a role for σ receptors in motor function. σ Receptors

* Corresponding author. Tel.: (202) 994-2918; fax: (202) 994-2870.

SSDI 0014-2999(95)00617-6

appear to be highly localized in dopaminergic brain areas which are essential for motor control (Walker et al., 1990). Both the striatum and the substantia nigra (pars compacta), important dopaminergic areas which are critical in the regulation of movement, are enriched with σ receptors (Graybiel et al., 1989; Gundlach et al., 1986; McLean and Weber, 1988; Heroux et al., 1992). Lesions of dopaminergic neurons in the striatum and substantia nigra with 6-hydroxydopamine were found to produce a significant decrease in the number of σ receptors in these areas (Gundlach et al., 1986). σ Receptors have also been implicated in the regulation of dopaminergic neuronal firing patterns in the substantia nigra (Engberg and Wikström, 1991). In addition, the σ_2 subtype appears to be involved in the modulation of circling behavior induced by intranigral microinjections of σ ligands (Walker et al., 1993). Injection of σ ligands into the red nucleus of rats produced dystonic-like symptoms (Walker et al., 1988). An apparent increased number of σ receptors as well as increased affinity for the σ ligand DTG has been observed in genetically dystonic rats (Bowen et al., 1988), although in a recent study, no changes in DTG binding to σ_1 or σ_2 sites in a dystonic strain were found (Weissman et al., 1993). In addition, there have been numerous electrophysiological and biochemical studies which have reported modulation of striatal dopamine activity by several compounds with reported affinity for σ receptors (Freeman and Zhang, 1992; French and Ceci, 1990; Steinfels et al., 1989; Iyengar et al., 1990). These initial studies, however, did not produce consistent effects, most likely due to affinities of many of the compounds tested for multiple receptor types.

We have previously reported the inhibition of *N*-methyl-D-aspartate (NMDA)-stimulated [^{3}H]dopamine release from rat striatum by σ receptor ligands (Gonzalez-Alvear and Werling, 1994). (+)-Pentazocine was found to produce a biphasic inhibition of [^{3}H]dopamine release, suggesting its effects were mediated through multiple receptor types, including σ_1, σ_2 and PCP receptors. Inhibition of release by nanomolar concentrations of (+)-pentazocine produced an inhibition of stimulated release that was reversible by the selective σ_1 receptor antagonist 1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr (DuP 734), suggesting σ_1 receptor involvement in the regulation of dopamine release. In the current study, we have further examined the role of the σ_1 receptor in the regulation of dopamine release in rat striatum by investigating the effects of the novel σ receptor agonist, 1*S*,2*R*-(−)-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine (BD737), which discriminates well between σ_1 and σ_2 receptors, either alone or in combination with the σ_1 receptor antagonist DuP 734. We have also performed preliminary experiments investigating the localization of the σ_1 receptor and have found evidence of its location of dopaminergic nerve terminals.

2. Materials and methods

2.1. [^{3}H]Dopamine release experiments

Methods are essentially as described in Gonzalez-Alvear and Werling (1994). All experiments were carried out in accordance with the guidelines and the approval of The George Washington University Institutional Animal Use and Care Committee. Male or female Sprague-Dawley rats (Hilltop, Scottdale, PA, USA) weighing approximately 250–350 g were killed by decapitation and their brains were removed to ice. Striata were dissected and chopped into 250 μm by 250 μm strips with a Sorvall (Wilmington, DE, USA) T-2 tissue sectioner. Striatal slices were suspended in oxygenated modified Krebs-Hepes buffer (MKB: in mM, 127 NaCl, 5 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 15 Hepes, 10 glucose; pH adjusted to 7.4 with NaOH) by trituration through a plastic transfer pipette. Magnesium was always omitted from the buffer because of its physiological antagonism at the NMDA receptor/channel complex. Buffers were oxygenated throughout the experiments. Following three washes in MKB, tissue was resuspended in 20 ml MKB and incubated in 15 nM [^{3}H]dopamine and 0.1 mM ascorbic acid for 30 min. Tissue was then washed twice in 20 ml MKB and once in MKB containing 10 μM nomifensine and 1 μM domperidone. Nomifensine and domperidone were included in all subsequent steps of the experiment to prevent reuptake of released [^{3}H]dopamine and prevent feedback inhibition via D_2 receptors, respectively. 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, USA) 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%/min was established over a 30 min period. Tissue was then stimulated to release [^{3}H]dopamine by a 2 min exposure to 25 μM NMDA (stimulus 1, S1). A 100 μM concentration of NMDA was used as a standard stimulus in release experiments in the presence of tetrodotoxin. The inflow was then returned to a non-stimulating buffer (interstimulus interval, ISI) for a period of 10 min. If a potential inhibitor of release was to be tested, it was introduced during this time. The tissue was then stimulated a second time for 2 min in the presence or absence of inhibitor as appropriate (stimulus 2, S2). Inflow was again returned to non-stimulating buffer to allow return to baseline release before final extraction of radioactivity remaining in tissue by a 45 min expo-

sure to 0.2 N HCl. Superfusates were collected at 2 min intervals in scintillation vials and radioactivity determined by liquid scintillation spectroscopy.

2.2. Materials

Chemicals and reagents were obtained from the following sources: *N*-methyl-D-aspartate (NMDA), di-*o*-tolylguanidine (DTG), haloperidol, naloxone, nomifensine and domperidone, Research Biochemicals (Natick, MA, USA); (+)-pentazocine, Research Technology Branch, NIDA (Rockville, MD, USA); tetrodotoxin, Sigma Chemical Co. (St. Louis, MO, USA); [^{3}H]dopamine (specific activity 50 Ci/mmol), Amersham Corp. (Arlington Heights, IL, USA). The following compounds were generous gifts: BD737 and BD1008, Dr. Wayne Bowen and Dr. Brian De Costa, NIDDK, National Institutes of Health (Bethesda, MD, USA); DuP 734, Dr. William Tam, Du Pont Merck Pharmaceutical Co. (Wilmington, DE, USA).

2.3. Statistical analysis

Data were expressed as radioactivity released above baseline during the collection interval (fractional release, %) or as a percentage of radioactivity released by the control stimulus (% control stimulated release). In experiments on inhibition of release by various drugs, all data were statistically analyzed as ratios (S2/S1) before transformation of data into % control stimulated release. Data are presented as % control stimulated release for facility in comparison across experiments. All statistical analyses were performed by two-way factorial analysis of variance (ANOVA). Post-hoc Dunnett's test was also performed as indicated. Statistical significance was considered at P values < 0.05.

3. Results

3.1. σ_1 Receptors regulate dopamine release

Release stimulated by a 2 min exposure to 25 μM NMDA was 8.5 ± 0.96% ($n = 34$) of total radiolabeled dopamine at the onset of the initial stimulation interval. The σ receptor agonist BD737 inhibited NMDA-stimulated [^{3}H]dopamine release from rat striatal slices in a concentration-dependent manner (Fig. 1). BD737 inhibited approximately 40% of NMDA-stimulated [^{3}H]dopamine release, with an apparent IC_{50} of approximately 1.0 nM, over the concentration range tested (0.1–100 nM). In some experiments, concentrations of BD737 above 100 nM were also tested and produced a similar inhibition of stimulated release (500 nM BD737, 54 ± 3.7% of stimulated release ($n = 3$); 1 μM BD737, 59 ± 3.7% of stimulated release, ($n = 11$)). The effects of the σ_1 receptor antagonist DuP 734 on BD737-mediated inhibition of stimulated release were also examined. Although DuP 734 has no effect on stimulated release in the absence of BD737 (Gonzalez-Alvear and Werling, 1994), it completely antagonized inhibition of NMDA-stimulated [^{3}H]dopamine release by all concentrations of BD737 tested (Fig. 1).

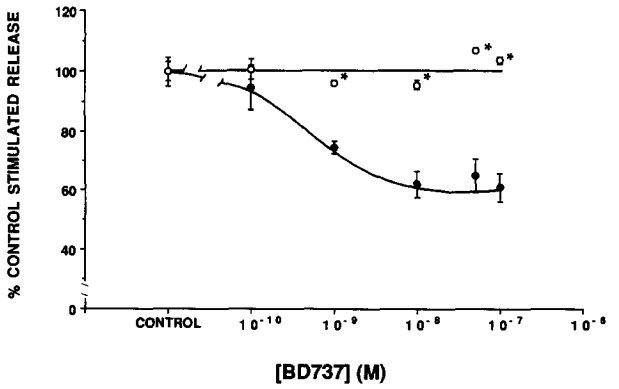

Fig. 1. Effects of BD737 on NMDA-stimulated [^{3}H]dopamine release from rat striatal slices in the absence and presence of DuP 734. Data are expressed as % control stimulated release above baseline. Release of [^{3}H]dopamine was stimulated by 25 μM NMDA in the presence of increasing concentrations of BD737 in the absence (●) or in the presence of 100 nM DuP 734 (○). Statistical analyses were performed on untransformed data (S2/S1). S2/S1 for control was 0.57 (S.E.M. = 0.020). ANOVA indicated a significant inhibition of NMDA-stimulated [^{3}H]dopamine release by increasing concentrations of BD737 ($P = 0.0001$). Inhibition of stimulated release was significant by Dunnett's at BD737 concentrations of 1 nM ($q'_{0.05,(2),38,3} = 2.593$), 10 nM ($q'_{0.01,(2),38,4} = 6.228$), 50 nM ($q'_{0.01,(2)38,5} = 6.024$) and 100 nM ($q'_{0.01,(2),38,6} = 6.016$) ($n = 4$–6). * Post-hoc t-tests indicated significant differences between control and DuP 734 addition at BD737 concentrations of 1 nM, 10 nM, 50 nM and 100 nM (* $P < 0.05$) ($n = 3$).

κ-Opioid receptor agonists have been shown to inhibit NMDA-stimulated [^{3}H]dopamine release from rat striatum (Werling et al., 1990). Since BD737 is a *cis* isomer of the κ-opioid receptor agonist *trans*-3,4-dichloro-*N*-methyl-*N*[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate hydrate (U50,488), the ability of the opioid receptor antagonist naloxone to antagonize the BD737-mediated inhibition of release was also investigated in order to evaluate any potential opioid receptor contribution. A 1 μM concentration of naloxone failed to reverse the inhibition of release produced by 1 μM BD737 (BD737, 56 ± 8.2% of stimulated release; BD737 and naloxone, 64 ± 5.7% of stimulated release ($n = 4$)).

3.2. Reversal of σ_1 receptor-mediated inhibition of dopamine release

σ Ligands that have been identified as σ receptor antagonists in other systems were assessed for antago-

Table 1
Lack of effect of potential σ receptor antagonists on NMDA-stimulated release of [^{3}H]dopamine from rat striatal slices

Stimulus	[σ drug]	% Control stimulated releaes	n
NMDA (25 μM)	–	100 ± 7.5	4
NMDA (25 μM)	DTG (100 nM)	100 ± 6.6	3
NMDA (25 μM)	Halperidol (100 nM)	100 ± 8.1	4

Release was stimulated by NMDA in the presence or absence of σ receptor ligand as indicated. Results are mean values for triplicate determinations in each n experiments. See Materials and methods for additional details.

nist properties in our bioassay. Concentrations tested for each potential antagonist were as follows: haloperidol, 100 nM; DTG, 100 nM; BD1008, 10 nM. Concentrations were chosen to occupy greater than 50% of receptors based on their respective reported affinities for σ receptors (haloperidol: K_i for $\sigma_1 = 1.9$ nM, K_i for $\sigma_2 = 80$ nM, Vilner and Bowen, 1992; DTG: K_i for $\sigma_1 = 12$ nM, K_i for $\sigma_2 = 38$ nM, Walker et al., 1990; BD1008, $K_i = 1.24$ nM (unspecified for subtype), Vilner et al., 1995). Neither haloperidol nor DTG had any effect on NMDA-stimulated [^{3}H]dopamine release in the absence of a σ receptor agonist (Table 1). Haloperidol, DTG and BD1008 completely antagonized the inhibition of stimulated release produced by 50 nM concentration of BD737 (Fig. 2).

We further evaluated the antagonist properties of these compounds by examining their effects on the inhibition of NMDA-stimulated [^{3}H]dopamine release by (+)-pentazocine. A 500 nM concentration of (+)-pentazocine inhibited approximately 40% of stimulated release (Fig. 3). Haloperidol (100 nM) and DTG (100 nM) completely reversed the inhibition of NMDA-stimulated [^{3}H]dopamine release by (+)-pentazocine (Fig. 3). We have previously shown that BD1008 produced complete reversal of (+)-pentazocine-mediated inhibition of release (Gonzalez-Alvear and Werling, 1994).

3.3. σ_1 Receptor localization on dopaminergic nerve terminals

The inhibitory effects of BD737 and (+)-pentazocine on NMDA-stimulated [^{3}H]dopamine release from rat striatal slices were examined in the presence of tetrodotoxin, an inhibitor of action potential propagation (Narahashi et al., 1964). A 100 μM concentration of NMDA was chosen as a standard stimulus in these experiments in order to produce reliable stimulation of dopamine release, since a fraction of NMDA-stimulated release is tetrodotoxin-sensitive (Jacocks and Cox, 1992). Release evoked by a 2 min exposure to 100 μM NMDA in the presence of tetrodotoxin was 7.8 ± 2.2% ($n = 3$). In our study, tetrodotoxin (1 μM) failed to affect the inhibitory actions of BD737 (1 μM) and (+)-pentazocine (1 μM) on NMDA-stimulated [^{3}H]dopamine release from rat striatal slices (Fig. 4),

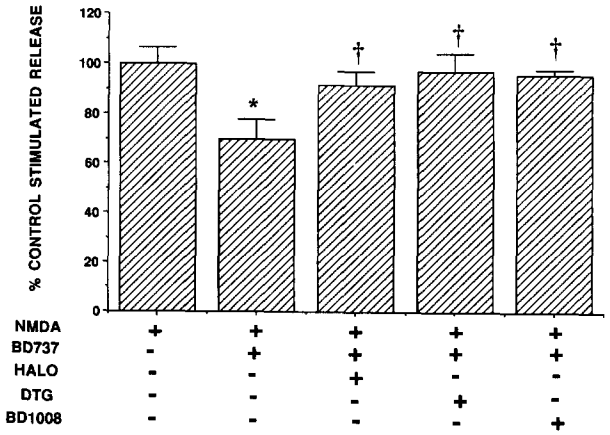

Fig. 2. Effects of putative σ receptor antagonists on inhibition of NMDA-stimulated [^{3}H]dopamine release by BD737. Release was stimulated by 25 μM NMDA in the presence (+) or absence (−) of BD737 (50 nM), haloperidol (100 nM), DTG (100 nM) or BD1008 (10 nM) as indicated. Data are expressed as % control stimulated release above baseline. Statistical analysis was performed on untransformed data (S2/S1). Treatment groups were found to differ by ANOVA ($P < 0.0005$). * Inhibition of stimulated release significantly different from control stimulated release by Dunnett's ($q'_{0.05,(2),7,2} = 3.313$). † Significantly different from inhibition by BD737 by Dunnett's: haloperidol, $q'_{0.05,(2),7,2} = 2.508$; DTG, $q'_{0.05,(2),7,2} = 2.82$; BD1008, $q'_{0.01,(2),7,2} = 3.518$ ($n = 4$).

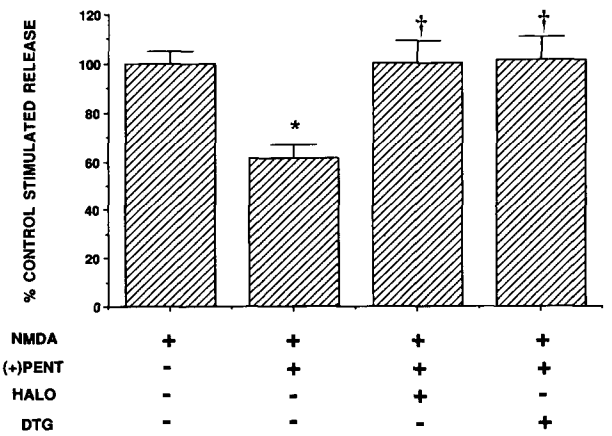

Fig. 3. Effects of putative σ antagonists on inhibition of NMDA-stimulated [^{3}H]dopamine release by (+)-pentazocine. Release was stimulated by 25 μM NMDA in the presence (+) or absence (−) of (+)-pentazocine (500 nM), haloperidol (100 nM) or DTG (100 nM) as indicated. Data are expressed as % control stimulated release above baseline. Statistical analysis was performed on untransformed data (S2/S1). Treatment groups were found to differ by ANOVA ($P = 0.0001$). * Significantly different from no inhibitor by Dunnett's ($q'_{0.01,(2),11,2} = 5.676$). † Significantly different from (+)-pentazocine inhibition of stimulated release by Dunnett's: haloperidol, $q'_{0.01,(2),11,2} = 4.036$; DTG, $q'_{0.01,(2),8,2} = 4.504$ ($n = 6$).

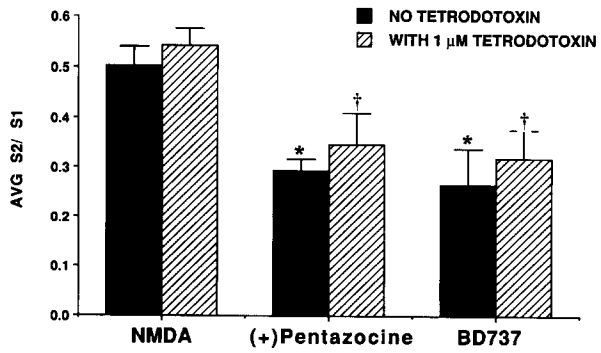

Fig. 4. Lack of effect of tetrodotoxin on inhibition of NMDA-stimulated [^{3}H]dopamine release from striatal slices by BD737 and (+)-pentazocine. Data are expressed as ratio of [^{3}H]dopamine released during S2 over that released during S1 (Avg S2/S1 ± S.E.M.). Release was stimulated by 100 μM NMDA in the presence or absence of BD737 (1 μM) or (+)-pentazocine (1 μM) and/or tetrodotoxin (1 μM) as indicated. Treatment groups were found to differ by ANOVA ($P < 0.05$). * Significantly different from control stimulated release by Dunnett's: (+)-pentazocine, $q'_{0.01,(2),8,2} = 3.616$; BD737, $q'_{0.01,(2),8,3} = 4.125$. † Significantly different from matched control by Dunnett's: (+)-pentazocine, $q'_{0.01,(2),8,2} = 3.387$; BD737, $q'_{0.05,(2),8,3} = 3.45$ ($n = 3$).

suggesting that σ_1 receptors are located on dopaminergic nerve terminals.

4. Discussion

The σ receptor agonist BD737 inhibited NMDA-stimulated [^{3}H]dopamine release in a concentration-dependent, monophasic manner. BD737, a *cis* diastereomer of the κ-opioid receptor agonist U50,488, binds to both σ_1 and σ_2 receptors, with K_i values of approximately 1.5 nM (Bowen et al., 1992) and 502 nM (Rothman et al., 1990) respectively. *Cis* diastereomers of U50,488 display high affinities for σ receptors with little affinity at other receptor types (De Costa et al., 1989). At the highest concentration tested (100 nM), BD737 should produce total occupancy (99%) of σ_1 receptors and only about 16% occupancy of σ_2 sites. The IC_{50} observed for BD737-mediated inhibition of NMDA-stimulated dopamine release was approximately 1.0 nM. Therefore, over the concentration range tested, BD737 inhibition of NMDA-stimulated dopamine release is most likely mediated via σ_1 receptors. Furthermore, BD737-mediated inhibition of dopamine release was fully reversible by the σ_1 receptor antagonist DuP 734. A 100 nM concentration of DuP 734 was chosen based on its affinity for the σ_1 receptor ($K_i = 10$ nM)(Culp et al., 1992; Tam et al., 1992). At this concentration, DuP 734 should produce almost total occupancy (91%) of σ_1 receptors. DuP 734 has no effect on NMDA-stimulated [^{3}H]dopamine release in the absence of a σ receptor agonist (Gonzalez-Alvear and Werling, 1994). These findings demonstrate regulation of NMDA-stimulated dopamine release in striatum by σ_1 receptors.

We also tested the ability of the putative non-subtype-selective σ receptor antagonists haloperidol, DTG and BD1008 to reverse the σ receptor agonist-mediated inhibition of stimulated dopamine release. Neither haloperidol nor DTG had any effect on NMDA-stimulated [^{3}H]dopamine release, suggesting that neither acted as a σ receptor agonist in our system. We have previously shown that BD1008 has no effect on NMDA-stimulated [^{3}H]dopamine release in the absence of a σ receptor agonist (Gonzalez-Alvear and Werling, 1994). Haloperidol, DTG and BD1008 completely antagonized inhibition of dopamine release by BD737. The effects of these compounds on (+)-pentazocine-mediated inhibition of dopamine release were also investigated. We have previously shown full concentration curves for inhibition of NMDA-stimulated [^{3}H]dopamine release from rat striatal slices by (+)-pentazocine (Gonzalez-Alvear and Werling, 1994). Concentrations of (+)-pentazocine below 1 μM produce approximately a 40% inhibition of dopamine release which appears to be σ_1 receptor mediated. At concentrations above 1 μM, (+)-pentazocine also binds to the phencyclidine receptor. Therefore, a 500 nM concentration of (+)-pentazocine was chosen in order to minimize any possible phencyclidine receptor involvement as well as produce maximal inhibition of stimulated release through σ receptors. At this concentration, (+)-pentazocine should produce approximately 99% occupancy of σ_1 receptors and approximately 50% occupancy of σ_2 receptors. It has previously been shown that (+)-pentazocine-mediated inhibition of dopamine release is reversible by BD1008 (Gonzalez-Alvear and Werling, 1994). Both haloperidol and DTG fully antagonized the inhibition of release by (+)-pentazocine. Since haloperidol, DTG, and BD1008 have affinity for both σ receptor subtypes, the antagonism of σ receptor agonist-mediated inhibition of release by these compounds may be through actions at both σ_1 and σ_2 receptors. However, since the inhibition of NMDA-stimulated [^{3}H]dopamine release appears to be primarily σ_1 receptor-mediated, the action of these σ ligands is most likely via antagonism at σ_1 sites.

Since BD737 is a derivative of κ-opioid receptor agonist and opioid receptor agonists have been previously shown to inhibit NMDA-stimulated [^{3}H]dopamine release from rat striatum (Werling et al., 1990), we examined the ability of the opioid receptor antagonist naloxone to reverse the BD737-mediated inhibition of dopamine release. Naloxone failed to reverse the inhibition of dopamine release by BD737. (+)-Pentazocine inhibition of NMDA-stimulated dopamine release in striatal slices is also not affected by naloxone (Gonzalez-Alvear and Werling, 1994). The lack of an-

tagonism by naloxone of the inhibition of NMDA-stimulated [^{3}H]dopamine release by these σ agonists suggests a lack of opioid receptor involvement.

The cellular localization of σ_1 receptors regulating dopamine release was also investigated. We repeated experiments testing inhibition by both BD737 and (+)-pentazocine in the presence of tetrodotoxin. Action potential propagation is prevented by tetrodotoxin through blockade of sodium channels (Narahashi et al., 1964). If a σ receptor agonist produces an inhibitory effect on NMDA-stimulated dopamine release in the presence of tetrodotoxin, this would suggest that action potential propagation through a functional interneuron was unnecessary for its actions. Therefore, the receptor could be localized to the dopaminergic nerve terminal. Both σ receptor agonists BD737 and (+)-pentazocine were capable of inhibiting dopamine release in the presence of tetrodotoxin, implying that action potential propagation was not essential for σ_1 receptor activation to inhibit dopamine release from striatal dopaminergic neurons. Thus, σ_1 receptors involved in the regulation of dopamine release from striatum appear to be localized to dopaminergic nerve terminals. This assertion is supported by Gundlach et al. (1986) who demonstrated that 6-hydroxydopamine lesions of striatal dopaminergic neurons produced a decrease in σ receptor density. Approximately 20% of the total number of σ receptors were localized on dopaminergic nerve terminals.

In summary, we have demonstrated a functional role for σ_1 receptors, namely to regulate NMDA-stimulated dopamine release in striatum. This is one of the first assignments of a defined role for subtypes of σ receptors. Furthermore, we have localized these sites to dopaminergic nerve terminals in the striatum. Since striatal dopaminergic activity is crucial for proper motor function, σ_1 receptors are potential therapeutic targets in motor disorders due to striatal dopamine imbalance.

Acknowledgements

This work was supported by a grant from NIDA to L.L.W. and a MARC predoctoral fellowship from the NIGMS to G.M.G.-A. This work is from a dissertation to be presented to the Department of Pharmacology at The George Washington University Medical Center, in partial fulfillment of the requirements for the Ph.D. degree.

References

Basile, A.S., I.A. Paul, A. Mirchevich, G. Kuijpers and B. DeCosta, 1992, Modulation of (+)-[^{3}H]pentazocine binding to guinea pig cerebellum by divalent cations, J. Pharmacol. Exp. Ther. 42, 882.

Beart, P.M., R.D. O'Shea and D.T. Mallanack, 1989, Regulation of σ receptors; high and low affinity agonist states, GTP shifts and up-regulation by rimcazole and 1,3-di(2- tolyl)guanidine, J. Neurochem. 53, 779.

Bowen, W.D., B.N. Kirschner, A.H. Newman and K.C. Rice, 1988, σ Receptors negatively modulate agonist-stimulated phosphoinositide metabolism in rat brain, Eur. J. Pharmacol. 149, 399.

Bowen, W.D., J.M. Walker, B.R. DeCosta, R. Wu, P.J. Tolentino, D. Finn, R.B. Rothman and K.C. Rice, 1992, Characterization of the enantiomers of *cis-N*-[2-(3,4- dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexyl-amine (BD737 and BD738): novel compounds with high affinity, selectivity and biological efficacy at sigma receptors, J. Pharmacol. Exp. Ther. 262, 32.

Culp, S.G., D. Rominger, S.W. Tam and E.B. De Souza, 1992, [^{3}H]DuP 734 [1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr]: a receptor binding profile of a high affinity novel sigma receptor ligand in guinea pig brain, J. Pharmacol. Exp. Ther. 263, 1175.

De Costa, B.R., W.D. Bowen, S.B. Hellewell, C. George, R.B. Rothman, A.A. Reid, J.M. Walker, A.E. Jacobson and K.R. Rice, 1989, Alterations in the stereochemistry of the kappa selective opioid agonist U50,488 result in high affinity sigma ligands, J. Med Chem. 32, 1996.

De Haven-Hudkins, D.L., F.Y. Ford-Rice, J.T. Allen and R.L. Hudkins, 1993, Allosteric modulation of ligand binding to [^{3}H](+)pentazocine σ recognition sites by phenytoin, Life Sci. 53, 41.

Di Paolo, L., F.F. Carroll, P. Abraham, X. Bai, K. Parham, S.W. Mascarella, X. Zhang, P. Wallace, J.M. Walker and W.D. Bowen, 1991, *N*-Substituted derivatives of normetazocine: differentiation of sigma-1 and sigma-2 receptors, Soc. Neurosci. Abstr. 17, 814.

Engberg, G. and H. Wikström, 1991, Sigma receptors: implication for the control of neuronal activity of nigral dopamine-containing neurons, Eur. J. Pharmacol. 201, 199.

Freeman, A.S. and J. Zhang, 1992, In vivo electrophysiological effects of ligands for PCP and sigma receptors on midbrain dopaminergic neurons, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection?, eds. J.-M. Kamenka and E.F. Domino (NPP Books, Ann Arbor, MI) p. 227.

French, E.D. and A. Ceci, 1990, Non-competitive *N*-methyl-D-aspartate antagonists are potent activators of ventral tegmental A_{10} dopaminergic neurons, Neurosci. Lett. 119, 159.

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

Graybiel, A.M., M.-J. Besson and E. Weber, 1989, Neuroleptic-sensitive binding sites in the nigrostriatal system: evidence for differential distribution of sigma sites in the substantia nigra pars compacta of cat, J. Neurosci. 9, 326.

Gundlach, A.L., B.L. Largent and S.H. Snyder, 1986, Autoradiographic localization of σ-receptor binding sites in guinea pig and rat central nervous system with (+)-^{3}H-3-(3- hydroxyphenyl)-*N*-(1-propyl)-piperidine, J. Neurosci. 6, 1757.

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

Heroux, J.A., S.W. Tam and E.B. De Souza, 1992, Autoradiographic identification and characterization of sigma receptors in guinea pig brain using [^{3}H]1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)piperidine ([^{3}H]DuP 734), a novel sigma receptor ligand, Brain Res. 598, 76.

Itzhak, Y., 1989, Multiple affinity binding states of the sigma receptor: effect of GTP binding protein modifying agents, Mol. Pharmacol. 36, 512.

Itzhak, Y. and I. Stein, 1992, Pharmacological properties of sigma 1 and sigma 2 receptor sites: allosteric interactions between sigma 1 and monoamine oxidase (MAO) in the brain, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection?, eds. J.-M. Kamenka and E.F. Domino (NPP Books, Ann Arbor, MI) p. 241.

Iyengar, S., W.M. Dilworth, S.J. Mick, P.C. Contreras, J.B. Monahan, T.S. Rao and P.L. Wood, 1990, Sigma receptors modulate both A9 and A10 dopaminergic neurons in the rat brain: functional interaction with NMDA receptors, Brain Res. 524, 322.

Jacocks III, H.M. and B.M. Cox, 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, glutamic acid and D-amphetamine, J. Pharmacol. Exp. Ther. 262, 356.

McCann, D.J. and T.-P. Su, 1992, Stimulation of σ ligand binding by phenytoin: apparent binding site and ligand specificity, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection?, eds. J.-M. Kamenka and E.F. Domino (NPP Books, Ann Arbor, MI) p. 295.

McLean, S. and E. Weber, 1988, Autoradiographic visualization of haloperidol-sensitive sigma receptors in guinea-pig brain, Neuroscience 25, 259.

Narahashi, T., J.W. Moor and W.R. Scott, 1964, Tetrodotoxin blockage of sodium conductances increase in lobster giant axons, J. Gen. Physiol. 47, 965.

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

Rothman, R.B., A. Mahboubi, A.A. Reid, B.R. DeCosta. A.E. Jacobson and K.R. Rice, 1990, Interaction of calcium channel blockers with multiple sigma binding sites in guinea pig brain: Proceedings of the Committee on Drug Dependence, NIDA Monograph Series.

Rothman, R.B., A. Reid, A. Mahboubi, C.-H. Kim, B.R. DeCosta, A.E. Jacobson and K.C. Rice, 1991, Labeling by [^{3}H]1,3-di(2-tolyl)guanidine of two high affinity binding sites in guinea pig brain: evidence for allosteric regulation by calcium channel antagonists and pseudoallosteric modulation of σ ligands, Mol. Pharmacol. 39, 222.

Starr, J.B. and L.L. Werling, 1994, σ-Receptor regulation of [^{3}H]arachidonic acid release from rat neonatal cerebellar granule cells in culture, J. Neurochem. 63, 1311.

Steinfels, G.F., B. Wolfson, L. Cook and S.W. Tam, 1989, Electrophysiological interactions between sigma and dopamine receptor ligands on midbrain dopamine neurons, Soc. Neurosci. Abstr. 15, 453.5.

Tam, S.W., G.F. Steinfels, P.J., Gilligan, W.K. Schmidt and L. Cook, 1992, DuP 734 [1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr], a potential antipsychotic agent: preclinical behavioural effects, J. Pharmacol. Exp. Ther. 263, 1167.

Vilner, B.J. and W.D. Bowen, 1992, Characterization of sigma-like binding sites of NB41A3, S-20Y, and N1E-115 neuroblastomas, C6 glioma, and NG108-15 neuroblastoma-glioma hybrid cells: further evidence for sigma$_2$ receptors, in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection?, eds. J.-M. Kamenka and E.F. Domino (NPP Books, Ann Arbor, MI) p. 341.

Vilner, B.J., B.R. DeCosta and W.D. Bowen, 1995, Cytotoxic effects of sigma ligands: sigma receptor-mediated alterations in cellular morphology, J. Neurosci. 15, 117.

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

Walker, J.M., R.R. Matsumoto, W.D. Bowen, D.L. Gans, K.D. Jones and F.O. Walker, 1988, Evidence for a role of haloperidol-sensitive σ-'opiate' receptors in the motor effects of antipsychotic drugs, Neurology 38, 961.

Walker, J.M., W.D. Bowen, S.L. Patrick, W.E. Williams, S.W. Mascarella, X. Bai and F.I. Carroll, 1993, A comparison of (−)deoxybenzomorphans devoid of opiate activity with their dextrorotatory phenolic counterparts suggests a role for sigma$_2$ receptors in motor function, Eur. J. Pharmacol. 231, 61.

Weissman, A.D., D.J. McCann, J.F. Lorden and T.P. Su, 1993, An absence of changes in sigma receptor subtypes in the brains of genetically dystonic (*dt*) rats, Eur. J. Pharmacol. 250, 329.

Werling, L.L., H.M. Jacocks and P.N. McMahon, 1990, Regulation of [^{3}H]dopamine release from guinea pig striatum by NMDA receptor/channel activators and inhibitors, J. Pharmacol. Exp. Ther. 255, 40.