Modulation of $[^3H]$Dopamine Release from Rat Nucleus Accumbens by Neuropeptide Y May Involve a Sigma$_1$-like Receptor$^1$

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ABSTRACT
Sigma receptors are located in limbic areas, including the nucleus accumbens, where increased dopamine levels have been linked to psychosis and reinforcement. Neuropeptide Y (NPY) has been named as a possible endogenous ligand for a subpopulation of $\sigma$ receptors on the basis of its ability to compete for $\sigma$ receptor binding. Using a superfusion system, we found that NPY enhanced N-methyl-$\epsilon$-aspartate-stimulated $[^3H]$dopamine release in rat nucleus accumbens, whereas the prototypical $\sigma$ agonist (+)pentazocine inhibited release. However, four $\sigma$ antagonists, one of which is $\sigma_1$ selective, as well as a Y receptor antagonist, all reversed the enhancement by NPY and the inhibition by (+)pentazocine. A $\sigma_2$-selective antagonist had no effect on either NPY-mediated enhancement or (+)pentazocine-mediated inhibition. [Leu$^{21}$,Pro$^{34}$]NPY and NPY$_{13-36}$ also enhanced release, but the effects were not reversed by $\sigma$ antagonists. Peptide YY did not mimic the effect of NPY. Our findings are consistent with the potential role of NPY as an endogenous ligand for a subtype of $\sigma$ receptor with characteristics different from $\sigma_1$, $\sigma_2$ and $\sigma_3$ receptors but sensitive to Ac-[3-(2,6-dichlorobenzyl)Tyr$^{27}$,o-Thr$^{32}$]NPY-(27–36)amide. Our findings suggest a role for NPY, via $\sigma$ receptors, in the regulation of dopamine levels in areas of brain critical to psychosis and reinforcement.

Dopaminergic activity in the nucleus accumbens is believed to play a role in both the reinforcing abilities of drugs of abuse and the positive symptoms of schizophrenia. Therefore, receptors that modulate DA levels in the nucleus accumbens are potential targets for therapeutic management of drug abuse or schizophrenia. Sigma receptors have been localized to both motor and limbic areas containing high dopaminergic innervation, including the nucleus accumbens, in rodents (Gundlach et al., 1986; McLean and Weber, 1988), non-human primates (Leitner et al., 1994; Mash and Zabetian, 1992) and humans (Tam and Zhang, 1988). In the same motor and limbic brain regions, the interaction of $\sigma$ receptors with DA neurons has been demonstrated (Ceci et al., 1988; Goldstein et al., 1989). In our laboratory, we have shown that agonists at $\sigma$ receptors are capable of modulating DA release from rat striatal slices (Gonzalez-Alvear and Werling, 1994) and from nucleus accumbal and prefrontocortical slices of guinea pig brain (Weatherspoon et al., 1996).

However, the role of $\sigma$ receptors in physiological function is controversial. Originally identified by Martin et al. (1976), receptors were classified as a subtype of opioid receptors through which n-allylnormetazocine (SKF 10,047) produces psychomimetic effects in chronic spinal dogs. Later, because of the similarity in both physiological effects and common binding properties, $\sigma$ receptors were thought to be identical to PCP receptors. Since this initial classification, investigators have attempted to clarify the pharmacology and function of $\sigma$ receptors. Sigma receptors are now recognized as a distinct class of receptors, and they are neither PCP receptors nor members of the opioid receptor family (Vaupel, 1982; Tam and Cook, 1984). Recent evidence has shown that there are at least two subtypes of $\sigma$ receptors. The $\sigma_1$ site has a high affinity for (+)isomers of benzomorphans and haloperidol, whereas the $\sigma_2$ site shows a slight preference for the (−) isomers of benzomorphans and also has a high affinity for haloperidol. Furthermore, Hanner et al. (1996) have recently reported the purification from guinea pig liver, molecular cloning and expression of a mammalian $\sigma$ receptor in yeast. The pharmacology of the site is consistent

ABBREVIATIONS: ANOVA, analysis of variance; BD737, 1S,2R-(−)-N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)cyclohexylamine; BD1008, N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-pyrrolidinyl)ethylylamine; BIMU-8, (endo-N-[8-methyl-8-azabicyclo[3.2.1]oct-3-yl]-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazole-1-carboxamidylehydrochloride; DA, dopamine; DuP734, 1-(cyclopropylmethyl)-4-2’-4’-fluorophenyl)-2’-oxoethyl)piperidine HBr; MKB, modified Krebs-HEPES buffer; NMDA, N-methyl-$\epsilon$-aspartate; NPY, Neuropeptide Y; PYX-1, Ac-[3-(2,6-dichlorobenzyl)Tyr$^{27}$,o-Thr$^{32}$]NPY-(27–36)amide; PYY, peptide YY.
with its identification as \( \sigma_1 \), and it is a distinct receptor without significant sequence homology to any other identified receptor.

Although compounds acting as agonists or antagonists have been developed for \( \sigma \) receptors, lack of identification of the endogenous ligand(s) at these sites has limited our understanding of \( \sigma \) receptor function. It has been proposed that NPY may be the endogenous ligand at a subpopulation of \( \sigma \) receptors. NPY can compete, in vivo, for the binding of radio-labeled ligands at a subpopulation of \( \sigma \) receptors, although the identification of this subpopulation as \( \sigma_1 \) or \( \sigma_2 \)-like was not explored (Bouchard et al., 1993). However, in vitro experiments have yielded conflicting data on the ability of NPY to compete for \( \sigma \) binding (Roman et al., 1989; Tam and Mitchell, 1991). Furthermore, NPY activates a reasonably well characterized set of receptors called Y receptors. Autoradiographic studies suggest that the rat nucleus accumbens contains Y receptors mainly of the Y2 subtype (Dawdonow, 1993). However, the characterization of the Y3 receptor (Grundemar et al., 1991) and the more recent cloning of Y4 (Bard et al., 1995), Y5 (Gerald et al., 1996) and Y6 (Gregor et al., 1996; Matsumoto et al., 1996; Weinberg et al., 1996) receptor subtypes suggest that other receptor types sensitive to NPY may also be functional in the nucleus accumbens. An increase in NPY-like immunoreactivity has been found in the cerebrospinal fluid of drug-free schizophrenic patients (Peterson et al., 1990), which suggests that NPY may play a role in psychosis. In addition, microinjection of NPY into rat nucleus accumbens has been shown to generate place-preference behavior, a type of rewarding effect that is antagonized by the antipsychotic cis-flupenthixol (Josselyn and Beninger, 1993). NPY is also implicated in reinforcement of feeding behavior (Jewett et al., 1992).

A subtype of NPY receptor has been suggested, on the basis of electrophysiological studies in rat hippocampal slices, to be identical to the \( \sigma_1 \) receptor (Monnet et al., 1992a, b). In a previous study, we showed that in rat striatal slices, NPY enhanced dopamine release that was reversed by both \( \sigma \) receptor antagonists and PYX-1, a Y receptor antagonist, which suggests that there may be overlap in the population of receptors characterized as \( \sigma \) and the population of receptors characterized as Y (Ault and Werling, 1997). Our data showed that the enhancing effect of NPY on DA release occurred through a \( \sigma_1 \)-like receptor and not through Y1, Y2 or Y3 receptor subtypes. In the current study, we investigated the possible activity of NPY at \( \sigma \) receptors in the nucleus accumbens. We tested the ability of NPY to regulate NMDA-stimulated dopamine release in slices of rat nucleus accumbens. We then evaluated the abilities of various \( \sigma \) antagonists and PYX-1, a Y receptor antagonist, to reverse the effect of NPY. We also tested the ability of Y receptor subtype-selective NPY analogs to produce similar responses. By evaluating the effect of NPY on DA release and determining the receptor population through which it occurs in the nucleus accumbens, we may elucidate a possible means for improving DA imbalance in drug abuse and/or schizophrenia.

### Materials and Methods

The following chemicals and reagents were kindly provided by or obtained from the following sources: NMDA, domperidone, nomifensine, nisoxetine, haloperidol, and 1,3-di[2-tolyl]guanidine (DYG) (Research Biochemicals International, Natick, MA), NPY (human, rat), PYY (Human), PYX-1, [Leu\(^{31,Pro^{34}}\)]NPY (Porcine) and NPY\(_{13-36}\) (porcine) (Peninsula Laboratories, Inc., Belmont, CA), fluoxetine and L-aspartic acid (Sigma Chemical Co., St. Louis, MO), \(^{3} \)HDA (Amersham Corp., Arlington Heights, IL), (+)-pentazocine (Research Technology Branch, National Institute on Drug Abuse, Rockville, MD), DuP734 (Dr. William Tam and Dr. Rob Zacek, DuPont Merck Pharmaceutical Co., Wilmington, DE), BD737 and BD1008 (Dr. Wayne Bowen, National Institute of Digestive Disorders and Kidney, Bethesda, MD) and BIMU-8 (Dr. Doug Bonhaus, Roche Bioscience, Palo Alto, CA).

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 Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) weighing 250 to 350 g were killed by decapitation, and the brains were removed to ice. Nuclei accumbens were dissected, chopped in two planes at right angles into 250 × 250-μm strips with a Sorvall T-2 tissue sectioner and suspended in MKB (127 mM NaCl, 5 mM KCl, 1.3 mM NaH\(_{2}\)PO\(_4\), 2.5 mM CaCl\(_2\), 15 mM HEPES, 10 mM glucose, pH adjusted to 7.4 with NaOH) by trituration through a plastic 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. After three washes in MKB, tissue was resuspended in 20 ml of MKB and incubated for 30 min with 0.1 mM nomifensine and 15 nM \(^{3} \)HDA. Tissue was then washed twice in 2 ml MKB and once in MKB containing 10 μM nomifensine and 1 μM domperidone. These drugs were included in all subsequent steps to prevent reuptake of and feedback inhibition by the released \(^{3} \)HDA. Because of low selectivity among monoamine reuptake mechanisms, the 30-min incubation period also included reuptake blockers for other monoamines (100 nM fluoxetine to block the serotonin reuptake mechanism and 100 nM nisoxetine to block the norepinephrine reuptake mechanism). Tissue was suspended a final time in MKB and distributed in 275-μl aliquots between glass-fiber discs into chambers of a BRANDEL superfusion apparatus (Gaithersburg, MD). MKB was superfused over tissue at a rate of 0.6 ml/min. A low stable base-line release of approximately 0.9%/min was established after 25 min over a 30-min period. Tissue was then incubated by a 2-min exposure to 25 μM NMDA (Stimulus 1; S1). The concentration was chosen for its position on the ascending portion of the concentration-response curve. Inflow was then returned to nonstimulating buffer for a 10-min interstimulus interval (ISI). If an inhibitor of release was being tested, it was included at this time. Tissue was then exposed to a second stimulus (S2) identical to the first but in the presence of potential inhibitor, as appropriate. If a drug was being tested as an enhancer, it was introduced during the S2. Inflow was once again returned to nonstimulating buffer before extraction of the remaining radioactivity in the tissue by a 45-min exposure to 0.2 N HCl at a reduced flow rate. Superfusates were collected in 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 data were statistically analyzed as ratios (S2/S1). This made it possible to control for the change in responsiveness to the NMDA stimulation when comparing it with NMDA stimulation in the presence of a test drug. The mean S2/S1 for NMDA-stimulated \(^{3} \)HDA release in the absence of any test drug was 0.49 ± 0.02 (N = 30). An enhancement by test drug would result in a higher ratio; an inhibition would result in a lower ratio. In this way, the effects of desensitization at NMDA receptors after S1 (Sather et al., 1992; Zilberter et al., 1991), or other differences in responsivity between tissue samples, are taken into account and therefore do not affect the comparison of treatments. In the results, data are expressed as radioactivity released above base line during the collection interval as a fraction of the total radioactivity in the tissue at the beginning of the collection interval (fractional release, %) or as a percentage of the radioactivity released by the control stimulus (% control-stimu-
lated release). Data are presented as % control-stimulated release for facilitation of comparison across experiments. Under the experimental conditions used, the released radioactivity has been shown to be primarily dopamine (Werling et al., 1988). All statistical analyses were performed by two-way factorial ANOVA with post-hoc Dunnett’s. Statistical significance is considered to be achieved at P < .05.

Results

NPY, tested at a range of 0.1 to 100 nM, enhanced NMDA-stimulated [3H]DA release from rat striatal slices. The increase in release was concentration-dependent and exhibited a biphasic nature (fig. 1). A concentration of 10 nM NPY was chosen as the standard enhancement concentration for subsequent experiments. This concentration would be expected to produce >80% occupation of Y₁, Y₂ and Y₃ receptors.

The effects of several sigma antagonists on the enhancement of NMDA-stimulated [3H]DA release by 10 nM NPY were tested. We chose concentrations of antagonists that would produce >50% occupation of their preferred receptor. The five sigma antagonists chosen have Kᵣ values for sigma binding in the low nanomolar range. Haloperidol, DuP734, BD1008 and DTG each prevented the potentiation of release by NPY, whereas BIMU-8 had no effect (fig. 2). Neither NPY nor any of the antagonists had an effect on basal (nonstimulated) release.

The effect of the nonselective Y receptor antagonist PYX-1 was also tested. This compound, tested at 10, 100 and 500 nM, reversed the enhancing effect of NPY in a concentration-dependent manner, and complete reversal occurred at a PYX-1 concentration of 500 nM (fig. 3). PYX-1 had no effect on basal (nonstimulated) release.

The contribution of the Y₃ subtype can be determined by comparing the effect of PYY, which does not bind to Y₃ receptors, to that of NPY, which binds to Y₁, Y₂ and Y₃ receptors. In contrast to NPY, PYY had no effect on NMDA-stimulated [3H]DA release when tested at 1, 10 or 100 nM (fig. 4, only highest concentration shown). Two NPY analogs were also tested at 1, 10 and 100 nM to evaluate further the possible contribution of Y receptor subtypes. [Leu³¹,Pro³⁴]NPY binds to Y₁ and Y₃ receptors, and NPY₁₃₋₃₆ binds to Y₂ and Y₃ receptors. Both significantly enhanced NMDA-stimulated [3H]DA release at the 100 nM concentration (fig. 4, only highest concentration shown for each analog). Neither PYY nor the NPY analogs had an effect on basal release.

We also tested the actions of PYX-1 and three sigma antagonists, DuP734, BIMU-8 and BD1008, on the enhancing effects of [Leu³¹,Pro³⁴]NPY and NPY₁₃₋₃₆. Concentrations of DuP734, BIMU-8 and BD1008 were the same as those used against NPY-mediated enhancement. A concentration of 500 nM PYX-1 was chosen because this completely reversed the enhancing effect of NPY. None of the three sigma antagonists was able to block [Leu³¹,Pro³⁴]NPY- or NPY₁₃₋₃₆-mediated enhancement. However, the Y receptor antagonist prevented.
tazocine, concentrations that occupy >90% of σ₁ receptors, also inhibit NMDA-stimulated [³H]DA release in slices of rat nucleus accumbens (fig. 7). The abilities of PYX-1 and three sigma antagonists, DuP734, BIMU-8 and BD1008, to modulate the inhibition of NMDA-stimulated [³H]DA release by (+)pentazocine were also tested (fig. 7). Only the two sigma antagonists that act at the σ₁ subtype (DuP734 and BD1008) prevented the inhibition; BIMU-8, a σ₂-selective antagonist (Weatherspoon et al., 1996), had no effect. In addition, PYX-1 was able to prevent the inhibition by (+)pentazocine.

Discussion

Several observations suggest that σ receptors may play a role in psychosis and in the reinforcing abilities of drugs of abuse by modulating DA levels in the nucleus accumbens. First, the greatest density of σ receptor binding sites is found in motor and limbic areas of many species (Gundlach et al., 1986). In humans, σ receptors are also located in motor and limbic areas and are particularly prominent in the nucleus accumbens (Weissman et al., 1988), the brain region believed to be involved in psychosis and reinforcement. Second, the nucleus accumbens is an area rich in DA, the neurotransmitter believed to modulate psychosis and reinforcement. Third, σ receptors have been shown to modulate DA levels in several brain regions, including rat striatum (Gonzalez-Alvear and Werling, 1994) and guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996). Furthermore, some studies have shown changes in σ receptor density in post-mortem brain tissue of schizophrenics (Weissman et al., 1991; Shibuya et al., 1992). Thus a possible explanation for abnormal DA levels in psychosis and reinforcement is that σ receptors are present in inappropriate numbers or that they are inappropriately activated, leading to an increase in DA release. This explanation could be more easily investigated by an endogenous ligand for σ receptors were known.

In the current study, we present results that are consistent with NPY being a potential endogenous ligand at a subpopulation of σ receptors that may, therefore, be involved in σ receptor mediation of psychosis and reinforcement. An increase in NPY-like immunoreactivity found in cerebrospinal fluid of drug-free schizophrenics (Peters et al., 1990) supports involvement in psychosis. NPY has also been shown to generate place-preference behavior, a type of rewarding effect, after its injection into the nucleus accumbens of rats (Josselyn and Beninger, 1993). This effect was reversed by the antipsychotic cis-flupenthixol and supports the involvement of NPY in reinforcement. Furthermore, NPY has been shown to increase food-seeking behavior in rats under conditions of satiety (Jewett et al., 1992). The motivation in seeking food by satiated rats is comparable to that in food-deprived rats, regardless of the amount of food already eaten (Jewett et al., 1995). It is possible that NPY is involved in the reinforcing abilities of drugs of abuse much as it is involved in the reinforcing ability of food.

It was initially reported by Roman et al. (1989) that NPY, with an IC₅₀ of approximately 10 nM, could compete with [³H]SKF10,047 for binding to σ receptors. Other in vitro binding studies were unable to replicate these findings (Tam and Mitchell, 1991; Quirion et al., 1991). However, supporting evidence exists that suggests a commonality between σ and NPY receptors. These investigations include physiologi-

![Fig. 3. Reversal of NPY-mediated enhancement of NMDA-stimulated [³H]DA release by PYX-1. Release of preloaded [³H]DA was stimulated by a 2-min exposure to 25 μM NMDA in the presence of 10 nM NPY in the presence or absence of the indicated concentration of PYX-1. Data are expressed as % control NMDA-stimulated release above baseline. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett’s on untransformed data (S2/S1). *Significantly different from control. #Not significantly different from control. (N = 3) ]

![Fig. 4. Effects of PYY and NPY analogs on NMDA-stimulated [³H]DA release. Release of preloaded [³H]DA was stimulated by a 2-min exposure to 25 μM NMDA in the presence of 10 nM NPY, 100 nM PYY, 100 nM [Leu³¹-Pro³⁴]NPY or 100 nM NPY₁₃₋₃₆. Data are expressed as % control NMDA-stimulated release above baseline. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett’s on untransformed data (S2/S1). *Significantly different from control. N = 6 for control and NPY. N = 3 for other treatments. ]

the enhancement by both [Leu³¹-Pro³⁴]NPY and NPY₁₃₋₃₆. This suggests that [Leu³¹-Pro³⁴]NPY and NPY₁₃₋₃₆ are probably not acting through the same receptor as NPY to enhance stimulated release (figs. 5 and 6).

Previous studies in this laboratory have shown that various sigma antagonists can reverse BD737- and (+)pentazocine-mediated inhibition of NMDA-stimulated [³H]DA release in rat striatal slices (Gonzalez-Alvear and Werling, 1994) as well as in guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996). In the current study we confirmed that 100 nM BD737 and 500 nM (+)pentazocine, concentrations that occupy >90% of σ₁ receptors, also inhibit NMDA-stimulated [³H]DA release in slices of rat nucleus accumbens (fig. 7). The abilities of PYX-1 and three sigma antagonists, DuP734, BIMU-8 and BD1008, to modulate the inhibition of NMDA-stimulated [³H]DA release by (+)pentazocine were also tested (fig. 7). Only the two sigma antagonists that act at the σ₁ subtype (DuP734 and BD1008) prevented the inhibition; BIMU-8, a σ₂-selective antagonist (Weatherspoon et al., 1996), had no effect. In addition, PYX-1 was able to prevent the inhibition by (+)pentazocine.

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Fig. 5. Failure of sigma antagonists to reverse [Leu$^{31}$,Pro$^{34}$]NPY-mediated enhancement of NMDA-stimulated [3H]DA release. Release of preloaded [3H]DA was stimulated by a 2-min exposure to 25 μM NMDA in the presence of 100 nM [Leu$^{31}$,Pro$^{34}$]NPY in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008, which was tested at 10 nM, and PYX-1, which was tested at 500 nM. Data are expressed as % control NMDA-stimulated release above baseline. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett’s on untransformed data (S2/S1). *Significantly different from control. #Not significantly different from control. (N = 3)

Fig. 6. Failure of sigma antagonists to reverse NPY$^{13–36}$-mediated enhancement of NMDA-stimulated [3H]DA release. Release of preloaded [3H]DA was stimulated by a 2-min exposure to 25 μM NMDA in the presence of 100 nM NPY$^{13–36}$ in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008, which was tested at 10 nM, and PYX-1, which was tested at 500 nM. Data are expressed as % control NMDA-stimulated release above baseline. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett’s on untransformed data (S2/S1). *Significantly different from control. #Not significantly different from control. (N = 3)

Fig. 7. Reversal of (+)pentazocine-mediated inhibition of NMDA-stimulated [3H]DA release by sigma$_1$ antagonists and PYX-1. Release of preloaded [3H]DA was stimulated by a 2-min exposure to 25 μM NMDA in the presence of 100 nM BD737 or 500 nM (+)pentazocine in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008 (10 nM) and PYX-1 (500 nM). Data are expressed as % control NMDA-stimulated release above baseline. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett’s on untransformed data (S2/S1). *Significantly different from control. #Not significantly different from control. (N = 3)
eral studies on ion transport in jejunum (Riviere et al., 1993), on CRF-induced colonic motor activation (Junien et al., 1991) and on duodenal alkaline secretion (Pascaud et al., 1993) and electrophysiological studies showing that NPY can enhance NMDA-induced activation of CA3 pyramidal neurons and that this enhancement is reversible by haloperidol (Monnet et al., 1992a, b). Furthermore, we previously showed that NPY enhances DA release in rat striatal slices (Ault and Werling, 1997), an effect that is reversed by both σ receptor antagonists and PYX-1, a Y receptor antagonist. This suggests overlap in the population of receptors characterized as σ and the population of receptors characterized as Y. Our data showed that the enhancing ability of NPY on DA release occurs through a σ1-like receptor and not through Y1, Y2 or Y3 receptor subtypes. We now report similar results in slices of rat nucleus accumbens.

In the current study, NPY enhanced NMDA-stimulated DA release in a concentration-dependent manner. This enhancement has a biphasic nature, consistent with its action at more than one receptor type. Because both the Y1-selective peptide [Leu31,Pro34]NPY and the Y2-selective peptide NPY13–36 also enhanced release, one might expect that a portion of the NPY-mediated enhancement was via these Y receptor subtypes. The concentration of NPY chosen for antagonist studies was 10 nM, which would have activated all receptor populations contributing to the biphasic response. At this concentration, NPY would be expected, on the basis of the IC50 of 10 nM reported by Roman et al. (1989), to occupy >80% of Y1, Y2 and Y3 receptors (Dumont et al., 1995) and 50% of σ receptors. IC50 values for NPY at Y receptors have been reported as 0.4 nM for Y1, 0.07 nM for Y2 and 1.8 nM for Y3 (Dumont et al., 1995; Higuchi et al., 1988). However, NPY-mediated, [Leu31,Pro34]NPY-mediated and NPY13–36-mediated enhancements were differentially affected by σ antagonists; whereas some σ antagonists completely reversed NPY-mediated effects, they had no effect on either [Leu31,Pro34]NPY-mediated or NPY13–36-mediated enhancement. Only those σ antagonists that are nonselective for σ subtype (BD1008, DTG and haloperidol) or are selective for the σ1 subtype (DuP734) completely reversed the NPY-mediated enhancement of stimulated release. Therefore, two receptor populations may participate in the enhancement by NPY. However, because full reversal is achieved by σ antagonists, these two receptor populations cannot be divided as σ and non-σ. In addition, they cannot be divided as σ1 and non-σ1, because full blockade of enhancement of release was achieved by the selective σ1 antagonist DuP734.

The effect of NPY on NMDA-stimulated DA release is opposite to the effect seen in our previous studies with the σ agonists. BD737, which acts as a selective σ1 agonist at concentrations ≤100 nM, and (+)pentazocine both inhibited NMDA-stimulated DA release in rat striatum (Gonzalez-Alvear and Werling, 1994) and guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996). Despite the opposite effect of NPY and σ agonists on NMDA-stimulated DA release, in the current experiments the enhancement of release by NPY was reversed by the same σ antagonists that reversed the inhibition of release by BD737 and (+)pentazocine. Four σ antagonists were able to reverse the enhancement of NMDA-stimulated DA release by NPY. The non-subtype-selective antagonists have Ks values as follows: BD1008, unspecified for subtype, 1.24 nM (Vilner et al., 1995); DTG, 12 nM at σ1 and 38 nM at σ2 (Walker et al., 1990); haloperidol, 1.9 nM at σ1 and 80 nM at σ2 (Vilner et al., 1992). The selective σ1 antagonist DuP734 has a Ks for σ1 receptors of 10 nM (Tam et al., 1992) and no binding at σ2 receptors at concentrations up to 1 μM. All four antagonists fully reversed NPY-mediated enhancement of NMDA-stimulated [3H]DA release. The ability of DuP734 to reverse the enhancing effect of NPY to the same extent as the σ1/σ2 antagonists suggests that NPY is acting through a σ1 or σ1-like receptor. This interpretation is supported by the inability of BIMU-8, a σ2-selective antagonist with a Ks of 20 nM (Bonhaus et al., 1993), to reverse the enhancing effect of NPY. Monnet et al. (1996), using different conditions from ours, have shown that in their hands, some σ ligands can enhance [3H]norepinephrine release from rat hippocampal slices.

Our data also show that NPY is not acting through any of the three best-characterized Y receptor subtypes to enhance DA release. NPY analogs most specific for Y1, Y2 and Y3 receptor subtypes were used at concentrations expected to occupy their preferred receptor types maximally and to occupy 20% or fewer of the nonpreferred receptors, on the basis of IC50 values previously reported (0.3 ± 0.1 nM for [Leu31,Pro34]NPY and 0.24 ± 0.1 nM for NPY13–36 (Dumont et al., 1995). [Leu31,Pro34]NPY binds to Y1 and Y3 receptors; whereas NPY13–36 binds to Y2 and Y3 receptors (Dumont et al., 1993; Fuhlendorff et al., 1990; Wahlestedt et al., 1990). Both of these peptide analogs had an enhancing effect on NMDA-stimulated DA release, which suggests that NPY could be acting through any of the three best-characterized Y receptor subtypes. However, our results indicate that the enhancing effects of NPY, [Leu31,Pro34]NPY and NPY13–36 occur through different receptors and that NPY is not acting through Y1, Y2 or Y3. First, the four σ antagonists that reverse the enhancing effect of NPY do not reverse the enhancing effect of either [Leu31,Pro34]NPY or NPY13–36. Second, the use of PYY in our system had no effect on DA release. PYY differs from NPY in that it does not bind to the Y1 receptor (Grundemar et al., 1991; Wahlestedt et al., 1992). PYY binds to Y2 and Y3 with a greater affinity than NPY analogs and does not enhance DA release as NPY does, so PYY cannot be acting through the Y1 or Y2 receptor. These data support the conclusion that although the responses of NPY and NPY analogs are the same, the receptors involved are different. NPY acting through a σ1 or σ1-like receptor and PYY analogs acting through Y receptors.

The use of PYX-1 further shows that NPY may be acting via a σ1 or σ1-like receptor. PYX-1 is a nonselective Y receptor antagonist with a Ks of approximately 500 nM (Tatemoto et al., 1992). We found that PYX-1 reversed the enhancing effect of NPY as well as the inhibitory effects of (+)pentazocine on NMDA-stimulated DA release. Because our results with NPY analogs and PYX suggest that NPY is acting through a σ receptor and not a currently identified Y receptor, PYX-1 may have σ antagonist properties in addition to its nonselective Y receptor antagonist properties.

One potential explanation for the opposite effects of NPY and (+)pentazocine on NMDA-stimulated DA release is that these compounds may act as inverse agonists to one another (Milligan et al., 1995). Inverse agonism has been proposed for σ ligands (Monnet et al., 1996). Our data, in the current and previous (Ault and Werling, 1997) studies, suggest that the
common receptor at which NPY and (+)-pentazocine act to modulate DA release would represent an overlap between σ and Y receptors. This overlap would probably be the σ1 receptor or a receptor with σ1-like pharmacology.

Another explanation is the involvement of multiple receptors in the regulation of DA release. NPY may enhance release by acting at an unknown Y receptor subtype that is not Y1, Y2 or Y5. This receptor would not be the same as the σ receptor through which (+)-pentazocine acts. Recent data from both cloning and functional studies demonstrate the existence of newly identified receptors sensitive to NPY and designated Y4 (Bard et al., 1995; Gehlert et al., 1996), Y9 (Gerald et al., 1996) and Y6 (Gregor et al., 1996; Matsumoto et al., 1993; Weinberg et al., 1996). It is possible one of these new Y receptors, Y4, Y9 or Y6, may be mediating the effects of NPY and σ ligands on DA release. This explanation would require PXY-1 to be an antagonist at the σ receptor in addition to the Y receptors. Similarly, the σ antagonists used in this study would have to be antagonists at this new Y receptor. Currently, we cannot eliminate either possible explanation.

In summary, we have demonstrated that NPY enhances NMDA-stimulated DA release in slices of rat nucleus accumbens. This response probably occurs through a σ1 or σ2-like receptor; known σ1 antagonists reverse the effect. Furthermore, the response does not appear to occur through a Y1, Y2 or Y5 receptor; PYY does not produce the same effect, and σ antagonists do not reverse the enhancement of DA release by [Leu²⁵,Pro⁶⁴]NPY or NPY₁₃–₃₆. Our data are consistent with NPY acting as an endogenous ligand for a subtype of σ receptor with characteristics different from Y1, Y2 or Y5 receptors but sensitive to PXY-1.

Many potential therapeutic applications of NPY receptor ligands have been proposed (Wahlestedt et al., 1993). Our data suggest an additional possibility: a role in therapy of dopaminergic disorders localized to nucleus accumbens, such as psychosis and drug abuse. Haloperidol, a clinically effective agent in both the treatment of psychosis and drug abuse. Haloperidol, a clinically effective agent in both the treatment of psychosis and drug abuse.