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European Journal of Pharmacology 386 (1999) 145–153

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Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via σ receptors[1]

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Received 26 October 1999; accepted 29 October 1999

Abstract

Phencyclidine (PCP) binds to many sites in brain, including PCP receptors located within the *N*-methyl-D-aspartate (NMDA) receptor-operated cation channel and sigma (σ) receptors. In this study, we compare mechanisms by which PCP, dizocilpine (MK-801), the prototypical σ receptor agonist (+)-pentazocine, and the proposed endogenous σ receptor ligand neuropeptide Y regulate potassium (K^+)-stimulated [^{3}H]dopamine release from slices of rat nucleus accumbens. (+)-Pentazocine inhibits K^+-stimulated [^{3}H]dopamine release, and neuropeptide Y enhances it. Both effects are blocked by σ_1 and neuropeptide Y receptor antagonists, suggesting possible inverse agonism at a subpopulation of σ/neuropeptide Y receptors. In contrast, PCP and MK-801 both enhance K^+-stimulated [^{3}H]dopamine release via σ_1 and σ_2 receptor subtypes, as demonstrated by antagonist sensitivity. Regulation of release by both (+)-pentazocine and neuropeptide Y persists in the presence of tetrodotoxin suggests that the σ/neuropeptide Y receptors mediating the modulation are located presynaptically on dopaminergic nerve terminals, but tetrodotoxin eliminates regulation by PCP and MK-801, suggesting that receptors mediating their effects are located upstream from dopaminergic nerve terminals.

Keywords: σ Receptor; Dopamine release; PCP (phencyclidine); Neuropeptide Y; (+)-Pentazocine; MK-801

1. Introduction

Phencyclidine (PCP) is a drug of abuse that produces dysphoria, agitation, and motivation for continued ingestion due to feelings of power and invulnerability. In addition, speech disturbances and psychotic episodes that include hallucinations, delusions, and paranoid ideation are common. Despite its many dysphoric symptoms, PCP is a reinforcing drug. Elevation of dopamine levels in the nucleus accumbens is generally accepted as the mechanism of reinforcement for drugs of abuse, including PCP.

In rat striatal slices, PCP has been shown to inhibit *N*-methyl-D-aspartate (NMDA)-stimulated dopamine release at low concentrations by binding to its site within the NMDA receptor-operated cation channel. However, at higher concentrations PCP acts on at least one other binding site causing a net increase in dopamine release (Ohmori et al., 1992). In vivo studies have also shown that high concentrations of PCP increase extracellular dopamine levels in prefrontal cortex (Hondo et al., 1994), nucleus accumbens (McCullough and Salamone, 1992; Steinpreis and Salamone, 1993), and striatum (Marek et al., 1992), as does dizocilpine (MK-801) (Schmidt and Fadayel, 1996). The mechanism by which the dopamine elevation occurs has not been clearly identified, partly because PCP binds to multiple sites in the central nervous system. The primary location for PCP binding is its own site within the NMDA receptor-operated cation channel, where it acts as a noncompetitive antagonist to NMDA, preventing cation flow through the channel upon excitatory amino acid-

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[1] This work was supported by a grant from the National Institute on Drug Abuse (DA-06667) and a Faculty Research Enhancement Award from the George Washington University Medical Center to LLW. DTA is a predoctoral student in the Neuroscience Program, The George Washington Institute for Biomedical Sciences, and is supported by a predoctoral fellowship (DA-05711) from NIDA. This work is from a dissertation to be presented to the above program in partial fulfillment of the requirements for the PhD degree.

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PII: S0014-2999(99)00769-4

mediated activation (Kemp et al., 1987). An additional action of PCP is its ability to mediate a blockade of non-inactivating, voltage sensitive K^+ channels in rat brain synaptosomes (Bartschat and Blaustein, 1988). Another high affinity component of PCP binding appears to be the biogenic amine transporter site (Akunne et al., 1994). Finally, PCP also binds at sigma (σ) receptors (Su and Wu, 1990).

PCP and σ receptors were once considered to be identical, because of the similarity of physiological effects produced by PCP and the prototypical σ receptor agonist n-allylnormetazocine (SKF10,047) (Martin et al., 1976). Development of selective ligands for both PCP and σ binding sites helped to distinguish the identities and localization in brain of the two as discreet sets of receptors. Competition analysis has revealed that the PCP receptor within the NMDA receptor-gated cation channel has high affinity for PCP and lower affinity for SKF10,047 while the σ receptor has high affinity for SKF10,047 and lower affinity for PCP (Tam, 1983; Taylor and Dekleva, 1987). Of importance in separating the σ from the PCP binding sites has been the observation that σ receptors, but not PCP receptors, are sensitive to low concentrations of the dopamine D_2 receptor antagonist, haloperidol (Tam and Cook, 1984). Sigma receptors are mainly located in motor and limbic areas, including the nucleus accumbens, in rodents (Gundlach et al., 1986; McLean and Weber, 1988), non-human primates (Mash and Zabetian, 1992), and humans (Tam and Zhang, 1988). PCP sites are prevalent in cortex, striatum and thalamus (Contreras et al., 1986).

At least two σ receptor binding sites have been identified. The σ_1 site has a high affinity for (+)-isomers of benzomorphans and haloperidol. Recently, a mammalian binding site consistent pharmacologically with identification as a σ_1 receptor was purified from guinea pig liver, cloned, and expressed in yeast (Hanner et al., 1996). No functional role has yet been associated with the cloned protein, suggesting that additional subunits or proteins may be required for function of the binding site. The σ_2 site exhibits a reverse and lesser selectivity for the benzomorphans and also binds haloperidol with high affinity. PCP has low affinity for the σ_1 site ($K_i = 1.8$ μM) and moderate affinity for the σ_2 site ($K_i = 220$ nM) (Su and Wu, 1990).

Sigma receptors are located in areas of high dopaminergic innervation, and interaction of σ receptors with dopamine neurons has been demonstrated in many studies (Goldstein et al., 1989; Ceci et al., 1988). In our laboratory, we have demonstrated that σ receptors are capable of regulating dopamine release from rat striatal slices (Gonzalez-Alvear and Werling, 1994) and slices of guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996). In these studies, the prototypical σ receptor agonist (+)-pentazocine inhibits dopamine release through both σ_1 and σ_2 receptors, and these responses are reversed by σ receptor antagonists.

An impediment to understanding σ receptor function has been lack of identification of the endogenous ligand at these sites. Evidence exists suggesting that neuropeptide Y may be the endogenous ligand at a subpopulation of σ receptors. In addition to its own receptors, called Y receptors, neuropeptide Y binds to sites not considered classical neuropeptide Y receptors. In vivo competition has been demonstrated for [^{3}H]SKF10,047 to σ receptors by neuropeptide Y (Bouchard et al., 1993). In vitro experiments have yielded conflicting data on the ability of neuropeptide Y to compete for σ binding (Roman et al., 1989; Tam and Mitchell, 1991). Recently, a subtype of neuropeptide Y receptor has been suggested to be identical to the σ_1 receptor (Monnet et al., 1992a,b). Our own studies have shown that σ receptor antagonists can prevent an neuropeptide Y-mediated enhancement of NMDA-stimulated dopamine release from rat nucleus accumbal slices (Ault et al., 1998). A relationship between neuropeptide Y and PCP has also been demonstrated; PCP has been shown to decrease neuropeptide Y immunoreactivity in rat caudate-putamen, prefrontal cortex and nucleus accumbens (Midgley et al., 1992, 1993).

In the current study, we have sought to determine the mechanism by which PCP increases dopamine release in rat nucleus accumbens, and therefore become reinforcing. Because PCP has significant affinity for σ receptors, we have compared its effects on dopamine release to those of the σ receptor agonist (+)-pentazocine and to a proposed endogenous σ receptor ligand, neuropeptide Y. Furthermore, we have identified antagonists that block the enhancement in release produced by PCP, as well as the effects of σ receptor antagonists. Effects on dopamine release common to PCP and σ receptor ligands suggest that PCP may indeed exert some of its effects via σ receptors. Specifically, in this study, we compared the effects that each of these ligands has on K^+-stimulated [^{3}H]dopamine release in slices of rat nucleus accumbens. By characterizing the effects of neuropeptide Y, PCP and (+)-pentazocine on dopamine release, and determining the receptor population through which each has its effect, we may elucidate a possible means for correcting dopamine imbalance in PCP or other drug abuse.

2. Methods

2.1. Drugs and reagents

The following chemicals and reagents were kindly provided by or obtained from the following sources: domperidone, nomifensine, nisoxetine, (+)-MK-801 and tetrodotoxin, Research Biochemicals International (Natick, MA); neuropeptide Y (Human, Rat) and Ac-[3-(2,6-dichlorobenzyl)Tyr^{27},D-Thr^{32}]NPY-(27–36) amide (PYX-1), Peninsula Laboratories, (Belmont, CA); fluoxetine and

L-ascorbic acid, Sigma (St. Louis, MO); [^{3}H]dopamine, Amersham (Arlington Heights, IL); (+)-pentazocine and PCP, Research Technology Branch, National Institute on Drug Abuse (Rockville, MD); 1-(cyclopropylmethyl)-4-(2′(4″-fluorophenyl)-2′-oxoethyl)piperidine HBr (DuP734), Dr. Rob Zaczek, DuPont Merck Pharmaceutical (Wilmington, DE); *N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-pyrrolidinyl)ethylamine (BD1008), Dr. Wayne Bowen, National Institute of Digestive Disorders and Kidney (Bethesda, MD); and (endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1 *H*-benzimidazole-1-carboxyamidehydrochloride (BIMU-8), Dr. Doug Bonhaus, Roche Bioscience.

2.2. Measurement of stimulated [^{3}H]dopamine release from nucleus accumbal slices

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, Scottdale, PA) weighing 250–350 g were killed by decapitation, and brains 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 modified Krebs–HEPES buffer (MKB: 127 mM NaCl, 5 mM KCl, 1.3 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 15 mM HEPES, 10 mM glucose, pH adjusted to 7.4 with NaOH) by trituration through a plastic pipette. Buffers were oxygenated throughout the experiments. Following three washes in MKB, tissue was resuspended in 20 ml MKB and incubated for 30 min with 0.1 mM ascorbic acid and 15 nM [^{3}H]dopamine. Tissue was then washed twice in 20 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}H]dopamine. Due to 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 baseline release of approximately 0.9%/min was established over a 30 min period. Tissue was then stimulated by a 2 min exposure to 20 mM KCl (Stimulus 1; S1). Inflow was then returned to non-stimulating buffer during a 10 min interstimulus interval. 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 except 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 non-stimulating 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 at 2 min intervals in scintillation vials with the glass fiber filter discs and tissue collected into the final vials. Released radioactivity was determined by liquid scintillation spectroscopy.

All data were statistically analyzed as ratios (S2/S1). In this way, the change in responsiveness to the potassium stimulation could be controlled for when comparing to potassium stimulation in the presence of a test drug. The mean S2/S1 for potassium-stimulated [^{3}H]dopamine release in the absence of any test drug was 0.53 ± 0.15 ($N = 27$). An enhancement by test drug would result in a higher ratio and an inhibition in a lower ratio. In this way, 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 baseline 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 stimulated 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 analysis of variance (ANOVA) with post-hoc Dunnett's. Statistical significance is considered at $P < 0.05$.

3. Results

We first tested the prototypical σ agonist (+)-pentazocine, as well as the proposed endogenous agonist neu-

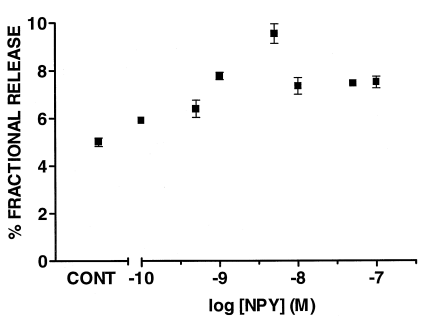

Fig. 1. Enhancement of K^+-stimulated [^{3}H]dopamine release by neuropeptideY (NPY) in rat nucleus accumbal slices. Release of preloaded [^{3}H]dopamine was stimulated by 20 mM K^+ in presence of indicated concentration of NPY. Data are expressed as radioactivity released above baseline during the collection interval as a fraction of the total radioactivity in the tissue at the beginning of the interval. Data were analyzed by two-way factorial ANOVA with post-hoc Dunnett's. NPY enhancement was significantly different from control at 0.3, 1, 3, 10, 30, and 100 nM. $N = 6$ for control and for 1, 3, and 10 nM concentrations of NPY. $N = 3$ for 0.1, 0.3, 30, and 100 nM concentrations of NPY.

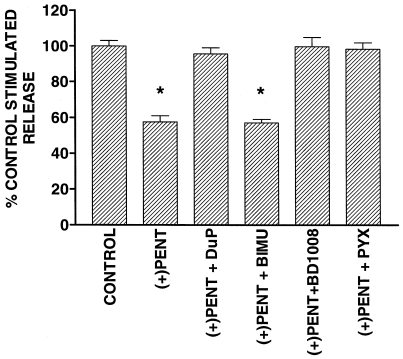

Fig. 2. Reversal of (+)-pentazocine-mediated inhibition of K^+-stimulated [^{3}H]dopamine release by σ_1 receptor antagonists and PYX-1. Release of preloaded [^{3}H]dopamine was stimulated by a 2 min exposure to 20 mM K^+ in presence of 500 nM (+)-pentazocine in the presence or absence of the antagonist 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 K^+-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. ($N = 3$).

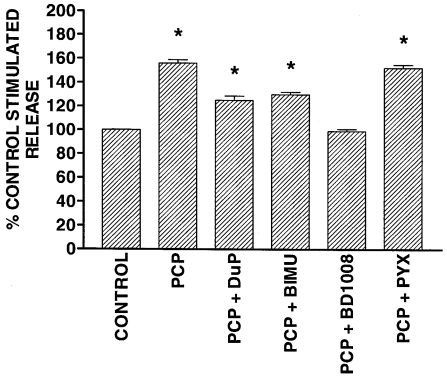

Fig. 4. Reversal of PCP-mediated enhancement of K^+-stimulated [^{3}H]dopamine release by σ receptor antagonists. Release of preloaded [^{3}H]dopamine was stimulated by a 2 min exposure to 20 mM K^+ in presence of 5 μM PCP in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008 (10 nM) and PYX 1 (500 nM). Data are expressed as % control K^+-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. ($N = 3$).

ropeptide Y against release of [^{3}H]dopamine stimulated by 20 mM K^+. We constructed a concentration–response curve for neuropeptide Y, and found that neuropeptide Y enhanced K^+-stimulated [^{3}H]dopamine release, with maximal enhancement (60% above continued K^+-stimulated release) achieved at 3 nM and lesser stimulation at higher concentrations (Fig. 1). This was similar to the enhancement we have seen previously when NMDA was used as a stimulator of [^{3}H]dopamine release. Also similar to results obtained with NMDA as a stimulator of release, (+)-pentazocine (500 nM) inhibited K^+-stimulated release by 40% (Fig. 2).

The σ_1 receptor antagonist DuP734 at 100 nM, as well as the non-subtype selective σ antagonist BD1008 at 10 nM both completely reversed the inhibition of release produced by (+)-pentazocine. However, the σ_2 receptor-selective antagonist BIMU-8 at 100 nM had no effect on the (+)-pentazocine-mediated inhibition. Interestingly, the non-subtype-selective neuropeptide Y receptor antagonist PYX-1 at 500 nM also reversed the inhibition of release produced by (+)-pentazocine (Fig. 2).

The enhancement of K^+-stimulated release by 3 nM neuropeptide Y was blocked by the same antagonists found to block the (+)-pentazocine-mediated inhibition of

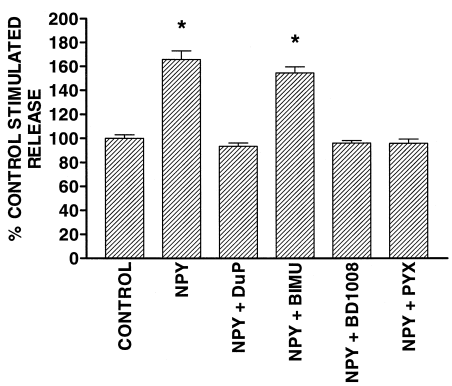

Fig. 3. Reversal of NPY-mediated enhancement of K^+-stimulated [^{3}H]dopamine release by σ_1 receptor antagonists and PYX-1. Release of preloaded [^{3}H]dopamine was stimulated by a 2 min exposure to 20 mM K^+ in presence of 3 nM NPY in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008 (10 nM) and PYX-1 (500 nM). Data are expressed as % control K^+-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. ($N = 3$).

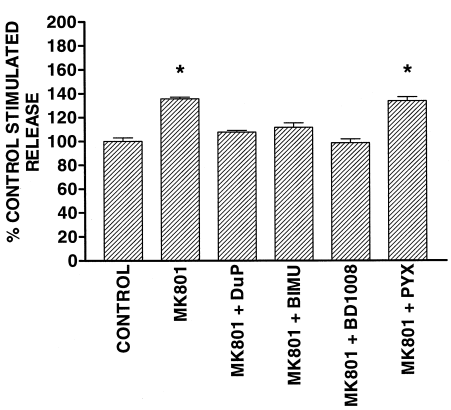

Fig. 5. Reversal of MK-801-mediated enhancement of K^+-stimulated [^{3}H]dopamine release by σ receptor antagonists. Release of preloaded [^{3}H]dopamine was stimulated by a 2 min exposure to 20 mM K^+ in presence of 1 μM MK-801 in the presence or absence of the antagonist indicated. Antagonists were tested at a concentration of 100 nM, except for BD1008 (10 nM) and PYX-1 (500 nM). Data are expressed as % control K^+-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. ($N = 3$).

stimulated release: DuP734, BD1008, and PYX-1 at the same concentrations used above all blocked the neuropeptide Y-mediated enhancement, while BIMU-8 did not (Fig. 3).

We next tested PCP and MK-801, which is more selective than PCP for the PCP binding site within the NMDA receptor-operated cation channel, against K^+-stimulated [^{3}H]dopamine release. In the presence of 500 nM PCP, release was not significantly different from control K^+-stimulated release (data not shown), but at 5 μM, PCP enhanced release by 60% (Fig. 4). The enhancement by 5 μM PCP was partially blocked by the σ_1 receptor-selective antagonist DuP734 at 100 nM, and also partially blocked by the σ_2 receptor-selective antagonist BIMU-8 at 100 nM. The non-selective σ receptor antagonist BD1008 (10 nM) blocked the enhancement by 5 μM PCP completely. In contrast, the neuropeptide Y receptor antagonist PYX-1 at 500 nM, a concentration that completely blocked the neuropeptide Y-mediated enhancement, had no effect on the enhancement of release produced by 5 μM PCP (Fig. 4).

The results produced by 1 μM MK-801 were similar to those produced by the 5 μM concentration of PCP, i.e., MK-801 enhanced release, and the enhancement was blocked by both σ_1 and σ_2 receptor antagonists, but unaffected by the neuropeptide Y receptor antagonist. The major difference between PCP and MK-801 was that each of the σ receptor subtype-selective antagonists almost completely blocked the MK-801-mediated enhancement of K^+-stimulated [^{3}H]dopamine release (Fig. 5).

Finally, we attempted to ascertain whether the various receptors identified as regulating [^{3}H]dopamine release in our system were localized on dopaminergic nerve terminals or on interneurons that impinged upon dopaminergic nerve terminals. Experiments testing neuropeptide Y, (+)-pentazocine, MK-801, and 5 μM PCP were repeated in the presence of 1 mM tetrodotoxin. Tetrodotoxin prevents action potential propagation by blocking sodium channels. Therefore, in the presence of tetrodotoxin, action potentials would not be propagated through interneurons. If the receptors regulating dopamine release were located on interneurons, they should not influence release in the presence of tetrodotoxin. The mean S2/S1 for K^+-stimulated release in the absence of any test drug was 0.535 ± 0.02 while that for K^+-stimulated release in the presence of tetrodotoxin was 0.531 ± 0.01. While both neuropeptide Y and (+)-pentazocine produced effects identical to those they produced in the absence of tetrodotoxin, neither MK-801 nor PCP had any effect in the presence of tetrodotoxin (Fig. 6).

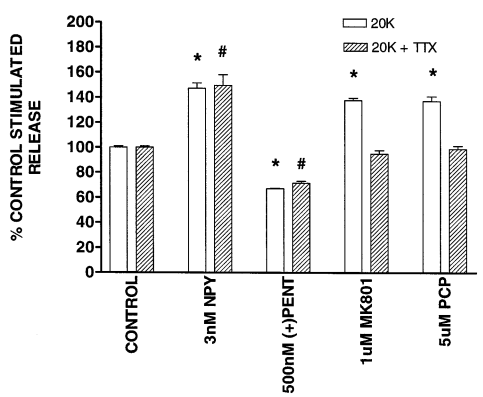

Fig. 6. Effects of NPY, (+)-Pentazocine, MK-801 and PCP on K^+-stimulated [^{3}H]dopamine release in the presence of tetrodotoxin (TTX). Release of preloaded [^{3}H]dopamine was stimulated by a 2 min exposure to 20 mM K^+ in presence of 3 nM NPY, 500 nM (+)-pentazocine, 1 μM MK-801, or 5 μM PCP with and without the addition of TTX. Data are expressed as % control K^+-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. # significantly different from control in the presence of TTX. $N = 6$ for control and for control in presence of TTX. $N = 3$ for all other treatments.

4. Discussion

Increased dopamine levels in the nucleus accumbens have been implicated in the reinforcing abilities of drugs of abuse such as PCP. Several pieces of information suggest that σ receptors may play a role in reinforcement by modulating dopamine levels in the nucleus accumbens. Sigma receptor binding sites are predominantly found in motor and limbic areas of many species (Gundlach et al., 1986), including humans, where they are especially dense in the nucleus accumbens (Weissman et al., 1988). The nucleus accumbens is an area particularly rich in dopamine, the neurotransmitter believed to modulate reinforcement. In addition, activation of σ receptors has been shown to modulate dopamine levels in several brain regions, including rat striatum (Gonzalez-Alvear and Werling, 1994; Gronier and Debonnel, 1999), rat nucleus accumbens (Gronier and Debonnel, 1999) and guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996). Elevated dopamine levels in reinforcement, therefore, may be exacerbated by inappropriate numbers of σ receptors or by σ receptors which are inappropriately activated. Investigation of the latter possibility would be facilitated if an endogenous ligand for σ receptors were known.

In previous studies, we presented results that are consistent with neuropeptide Y as a potential endogenous ligand at a subpopulation of σ receptors (Ault and Werling, 1997a,b; Ault et al., 1998). The current study, using tissue from nucleus accumbens, supports this hypothesis and further suggests that neuropeptide Y may be involved in σ mediation of reinforcement. Other evidence also points to the possible reinforcing ability of neuropeptide Y. For example, neuropeptide Y has been shown to increase food seeking behavior in both food-deprived and satiated rats,

regardless of the amount of food already eaten (Jewett et al., 1995). It is possible that neuropeptide Y may be involved in the reinforcing abilities of drugs of abuse much as it is involved in the reinforcing ability of food. In addition, neuropeptide Y has also been shown to generate place-preference behavior, an indication of rewarding effect, after its injection into the nucleus accumbens of rats (Josselyn and Beninger, 1993).

Roman et al. (1989) initially reported that neuropeptide Y could compete with [^{3}H]SKF10,047 for binding to σ receptors (IC_{50} = 10 nM). Although other binding studies could not replicate these findings (Quirion et al., 1991; Tam and Mitchell, 1991), supporting evidence has accumulated suggesting a commonality between subpopulations of σ and neuropeptide Y receptors (Junien et al., 1991; Monnet et al., 1992a,b; Bouchard et al., 1993; Pascaud et al., 1993; Riviere et al., 1993). In addition, we previously showed that neuropeptide Y enhances dopamine release in rat nucleus accumbal slices (Ault et al., 1998) through an effect reversed by both σ receptor antagonists and PYX-1, a non-specific Y receptor antagonist. The data showed that the enhancing ability of neuropeptide Y on dopamine release occurs through a σ_1-like receptor and not through neuropeptide Y_1, Y_2 or Y_3 receptor subtypes. The receptor through which neuropeptide Y acts appears to represent an overlap in populations of receptors characterized as σ and Y. In the previous study, NMDA was used as the stimulus for dopamine release.

In the current study, we investigated neuropeptide Y's effect on [^{3}H]dopamine release in slices of rat nucleus accumbens using K^+, instead of NMDA, as a stimulus. The stimulus was changed to facilitate comparison of the effects of neuropeptide Y with those of PCP, which noncompetitively binds to the NMDA receptor channel and, therefore, inhibits release stimulated by NMDA. Neuropeptide Y enhanced release similarly with either K^+ or NMDA as a stimulus, in a concentration-dependent manner. However, neuropeptide Y appears to enhance release at slightly lower concentrations when K^+ is used as a stimulus. Specifically, a 3 nM concentration of neuropeptide Y in the K^+-stimulated assay has an equivalent enhancing effect to a 10 nM concentration of neuropeptide Y in the NMDA-stimulated assay. The concentration of neuropeptide Y chosen to investigate antagonist reversal in the current studies was 3 nM. At this concentration, neuropeptide Y would be expected to occupy $>$ 80% of neuropeptide Y_1, Y_2 and Y_3 receptors (Dumont et al., 1995), but fewer than 50% of σ receptors based upon the IC_{50} of 10 nM reported by Roman et al. (1989). IC_{50} values for neuropeptide Y at neuropeptide Y receptors have been reported as 0.4 nM for Y_1, 0.07 nM for Y_2, and 1.8 nM for Y_3 (Higuchi et al., 1988; Dumont et al., 1995), although recently evidence for the existence of neuropeptide Y_3 receptors has been called into question (reviewed by Michel et al., 1998). Despite the expected lower occupancy of neuropeptide Y on σ receptors at a concentration of 3 nM, full reversal of the neuropeptide Y effect is achieved by σ receptor antagonists. Specifically, only those σ receptor antagonists that are non-selective for σ receptor subtype (BD1008), or are selective for the σ_1 receptor subtype (DuP734) completely reversed the neuropeptide Y-mediated enhancement of stimulated release. At concentrations above 3 nM, neuropeptide Y also enhanced release but did so to a lesser degree than the 3 nM concentration. This could be due to the increased inhibitory activity on dopamine release by neuropeptide Y acting at another population of receptors at concentrations greater than 3 nM. Activity of neuropeptide Y through these receptors would, therefore, act in physiological opposition to the enhancement produced at lower concentrations.

The effect of neuropeptide Y is opposite to that of the σ receptor agonist, (+)-pentazocine, which inhibited K^+-stimulated dopamine release. However, full reversal of the inhibitory effect is achieved by the same σ receptor antagonists (BD1008 and DuP734) that reverse the enhancing effect of neuropeptide Y. The non-selective σ receptor antagonist, BD1008, has a K_i of 1.2 nM at σ receptors (Vilner et al., 1995). The selective σ_1 receptor antagonist, DuP734, has a K_i for σ_1 receptors of 10 nM (Tam et al., 1992) and does not bind σ_2 receptors at concentrations up to 1 μM. BIMU-8, a σ_2 receptor selective antagonist with a K_i of 20 nM (Bonhaus et al., 1993) failed to reverse the effects of both neuropeptide Y and (+)-pentazocine suggesting neither drug is exerting its effects by σ_2 receptors in this system. Furthermore, PYX-1, a nonselective neuropeptide Y receptor antagonist with a K_D of approximately 500 nM (Tatemoto et al., 1992), reversed both the enhancing effect of neuropeptide Y as well as the inhibitory effect of (+)-pentazocine on K^+-stimulated dopamine release. Together, these results are consistent with our previous studies using NMDA as a stimulus (Ault and Werling, 1997a,b; Ault et al., 1998) and suggest that neuropeptide Y is acting through a σ_1 or a σ_1-like receptor.

One potential explanation for the opposite effects of neuropeptide Y and (+)-pentazocine on K^+-stimulated dopamine release is the involvement of multiple receptors. Neuropeptide Y may enhance release by acting at an unknown neuropeptide Y receptor subtype that is not Y_1, Y_2 nor Y_3. This receptor would not be the same as the σ receptor through which (+)-pentazocine acts. It is possible that one of the more recently-identified neuropeptide Y receptors, designated Y_4 (Bard et al., 1995; Gehlert et al., 1996), Y_5 (Gerald et al., 1996) and Y_6 (Gregor et al., 1996; Matsumoto et al., 1996; Weinberg et al., 1996), may be the one mediating our effects of neuropeptide Y and σ ligands on release. With this explanation, PYX-1 and the σ receptor antagonists used in this study would all have to be antagonists at both receptors involved.

Another explanation for the opposite effects of neuropeptide Y and (+)-pentazocine on K^+-stimulated dopamine release is that these compounds may act as

inverse agonists to one another (Milligan et al., 1995). Inverse agonism has previously been proposed for σ receptor ligands (Monnet et al., 1996). Our data suggest that the common receptor at which neuropeptide Y and (+)-pentazocine act to modulate dopamine release would represent an overlap in σ and neuropeptide Y receptors. This overlap would most likely be the σ_1 receptor or a receptor with σ_1-like pharmacology. Recently, a three-state receptor model has been proposed that helps explain inverse agonism (Leff et al., 1997). In this model, a receptor can exist in two active states, each having a different effect. It is possible that neuropeptide Y and (+)-pentazocine are binding to the same receptor, with each ligand preferring a different conformational state of that receptor. Both of these conformational states would be active forms of the receptor that each has a different and opposite effect. The antagonists used in our study would all bind to the receptor regardless of the conformational state. The observation that tetrodotoxin treatment did not alter the effect on release by either (+)-pentazocine or neuropeptide Y suggests that both are working at sites on the nerve terminal, consistent with, though not proving that both drugs act at a common receptor.

We next were interested in comparing PCP with neuropeptide Y and (+)-pentazocine as to its effects on stimulated dopamine release through σ receptor subtypes. We tested two concentrations of PCP, 500 nM and 5 μM. With a K_i of 220 nM at σ_2 receptors and a K_i of 1.8 μM at σ_1 receptors (Su and Wu, 1990), PCP would be expected to occupy 69% of σ_2 receptors and 25% of σ_1 receptors at a concentration of 500 nM. At a concentration of 5 μM 96% of σ_2 and 74% of σ_1 receptors should be occupied. The fact that the 500 nM concentration did not have an effect on K^+-stimulated dopamine release suggests that concentrations of PCP producing significant occupation of σ_1 receptors are necessary to enhance release. Like neuropeptide Y, the effect on dopamine release by PCP was an enhancement. However, unlike neuropeptide Y, *both* σ_1 (DuP734) and σ_2 (BIMU-8) receptor selective antagonists partially reversed the enhancing effect of PCP. The non-selective σ antagonist, BD1008, was able to reverse completely PCP's enhancing effect on dopamine release. This suggests that 5 μM PCP is acting either directly or indirectly through both σ_1 and σ_2 receptors, as predicted by the K_i values. Furthermore, PYX-1 had no effect on PCP enhancement of dopamine release. It is, therefore, unlikely that PCP is mediating dopamine release through the same σ_1-like receptor as neuropeptide Y and (+)-pentazocine.

In our study, MK-801 was able to enhance K^+-stimulated dopamine release like PCP. If release were stimulated through NMDA receptor activation, then both MK-801 and PCP would be expected to decrease dopamine release by an NMDA receptor-channel blockade. MK-801 has low affinity (K_i = 4800 nM) for σ receptor binding (Rothman et al., 1992). We tested a 5 μM concentration of MK-801 so that the potential activity of σ receptors could be investigated. Surprisingly, all of the σ receptor antagonists used in our study were able to reverse the enhancement produced by MK-801 suggesting that MK-801 could possibly have greater σ receptor activity than previously thought. However, since PYX-1 does not reverse MK-801 enhancement, then MK-801 is not acting through the same σ_1-like receptor as neuropeptide Y and (+)-pentazocine (σ/Y receptor).

Our study suggests that neuropeptide Y, (+)-pentazocine, PCP and MK-801 all have possible σ receptor activity. However, if this is true, PCP and MK-801 appear to act at a different population of σ receptors than do neuropeptide Y and (+)-pentazocine. This was shown by the different antagonist profiles, and also by the use of tetrodotoxin, which prevented the enhancement of dopamine release by PCP and MK-801 but not by neuropeptide Y and (+)-pentazocine. The lack of effect by tetrodotoxin on neuropeptide Y or (+)-pentazocine-mediated dopamine release points to a location on dopaminergic nerve terminals for the receptors mediating the enhancement or inhibition. On the other hand, receptors mediating the enhancement by PCP and MK-801 are most likely on interneurons since tetrodotoxin is able to prevent the effects.

Our favored explanation for the effects of PCP and MK-801, which incorporates interneurons, is as follows: interneurons that are tonically activated by NMDA-gated channels release an inhibitory neurotransmitter, such as γ-aminobutyric acid (GABA), onto dopaminergic nerve terminals. The presence of PCP or MK-801 blocks the NMDA receptor-channels, decreases the release of inhibitory neurotransmitter, and therefore, increases the release of transmitter from dopaminergic nerve terminals. With this explanation, PCP and MK-801 would *not* be acting directly on σ receptors but would be blocking the cation channel operated by an NMDA receptor. If MK-801 is acting to increase dopamine release via blocking the NMDA receptor-associated cation channel, then lower concentrations of MK-801 would be predicted to increase dopamine release as well. Furthermore, the σ_1-like receptor with neuropeptideY-like receptor properties mediating our neuropeptide Y and (+)-pentazocine effects does not have a regulatory effect on the interneuron-mediated dopamine release produced by PCP and MK-801, since PYX-1 does not prevent the effects of these ligands. Instead, both σ_1 and σ_2 receptors would have to be present on the GABAergic interneuron downstream of the NMDA receptor-channel where the antagonists, DuP734, BIMU-8 and BD1008, could reverse PCP and MK-801-mediated dopamine release. These σ_1 and σ_2 receptors do *not* have neuropeptide Y receptor properties. The receptor mediating the actions of both NPY and (+)-pentazocine would be located postsynaptically (likely on the dopaminergic neuron terminal) from the GABA interneuron and might appropriately be called the σ_1/Y receptor, while the

σ receptors that are indirectly mediating the upstream effects of PCP and MK-801 remained identified as separate receptors, consistent with classification as σ_1 and σ_2.

Alternatively, PCP and MK-801 could be acting on σ receptors that regulate K^+ channel rectifiers. This explanation would be in accordance with the data of Bartschat and Blaustein (1988), who report a σ_2 binding site associated with voltage-gated K^+ channels on nerve terminals. Specifically, PCP and MK-801 would be inhibiting these channels through a σ-mediated mechanism that is reversed with both σ_1 and σ_2 receptor antagonists. As in the previous explanation, the σ_1-like receptor with neuropeptide Y-like receptor properties does not have any regulatory effect on this mechanism since PYX-1 has no effect.

In summary, we have demonstrated that neuropeptide Y enhances K^+-stimulated dopamine release in slices of rat nucleus accumbens. This response likely occurs through the σ_1 or a σ_1-like receptor based on the ability of known σ_1 receptor antagonists to reverse the effect. Our data are consistent with the role of neuropeptide Y as an endogenous ligand for a subtype of σ receptor with characteristics different from neuropeptide Y_1, Y_2 or Y_3 receptors but sensitive to PYX-1. Furthermore, PCP, which can bind to σ receptors, does not increase dopamine release through the same population of σ receptors as neuropeptide Y. Nevertheless, σ receptor antagonists can reverse the dopamine elevation produced by PCP or MK-801, suggesting an indirect action of these drugs through σ receptors. If these findings can be extrapolated to humans, they suggest that σ receptor antagonists might be therapeutically useful in cases of PCP overdose.

Acknowledgements

We thank Dr. Wayne Bowen and Dr. Brian DeCosta for the gift of BD1008, Dr. Rob Zaczek for the gift of DuP734, and Dr. Doug Bonhaus for the gift of BIMU-8. We also thank Dr. David Perry for his critical evaluation of the manuscript.

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