ABSTRACT

Neuropeptide Y (NPY), an apparent neuromodulating neuropeptide, has been linked to dopamine systems and dopamine-related psychotic disorders. Because of this association, we determined and compared the effects of psychomimetic drugs on extrapyramidal and limbic NPY systems. We observed that phencyclidine, methamphetamine (METH), (+)-methylendioxymethamphetamine (MDMA), and cocaine, but not (-)-MDMA, similarly reduced the striatal content of NPY-like immunoreactivity from 54% (phencyclidine) to 74% ([+]-MDMA) of control. The effects of METH on NPY levels in the nucleus accumbens, caudate nucleus, globus pallidus, and substantia nigra were characterized in greater detail. We observed that METH decreased NPY levels in specific regions of the nucleus accumbens and the caudate, but had no effect on NPY in the globus pallidus or the substantia nigra. The dopamine D1 receptor antagonist SCH-23390 blocked these effects of METH, suggesting that NPY levels throughout the nucleus accumbens and the caudate are regulated through D1 pathways. The D2 receptor antagonist eticlopride did not appear to alter the METH effect, but this was difficult to determine because eticlopride decreased NPY levels by itself. A single dose of METH was sufficient to lower NPY levels in some, but not all, regions examined. The effects on NPY levels after multiple METH administrations were substantially greater and persisted up to 48 h after treatment; this suggests that synthesis of this neuropeptide may be suppressed even after the drug is gone. These findings suggest that NPY systems may contribute to the D1 receptor-mediated effects of the psychostimulants.

Neuropeptide Y (NPY) is a 36-amino acid peptide first isolated from porcine brain by Tatemoto et al. (1982). It is now known to be found in high concentrations in several brain regions and in various mammalian species. NPY satisfies many of the criteria for a neurotransmitter or neuromodulator (Allen et al., 1983; Martel et al., 1987) and it has important effects in regulating neuroendocrine function, circadian rhythms, consumatory behavior, central autonomic control, memory processing, and in regulating contraction and relaxation of cerebral blood vessels (for a review, see Heilig and Widerlov, 1990). NPY also has been shown to interact with neurotransmitter systems such as dopamine (DA), γ-aminobutyric acid (GABA), and norepinephrine (Kubota et al., 1988; Salin et al., 1990; Wahlestedt et al., 1991). NPY colocalizes with several other neurotransmitters such as GABA, somatostatin, and norepinephrine (Beal et al., 1986; Kawaguchi et al., 1995). In the striatum, NPY, somatostatin and GABA are always colocalized and found in interneurons (Kawaguchi et al., 1995). Specifically, the glutamate NMDA antagonists, MK801, and phencyclidine (PCP) lower extrapyramidal and limbic NPY tissue levels, and GABA agonists reverse the reduction of NPY content due to PCP administration (Midgley et al., 1992, 1993). In addition, the D1 receptor agonist SKF 38393 and the D2 receptor agonist quinpirole decrease striatal and nucleus accumbens NPY levels (Midgley et al., 1994).

In the striatum, these NPY interneurons receive input from the thalamus and the cortex and have reciprocal synaptic interaction with cholinergic interneurons and the direct (D1) DA pathways (for a review, see Kawaguchi et al., 1995). No D2 mRNA is found in NPY interneurons, indicating that the indirect (D2) DA pathways do not synapse on these neurons (Le Moine et al., 1991). NPY has also been implicated in neuropsychiatric illnesses (Widerlov et al., 1988; Heilig and Widerlov, 1990; Frederiksen et al., 1991). The above studies demonstrating that DA and NPY systems are interrelated suggest that NPY may be influenced by conditions, such as schizophrenia, in which normal dopaminergic transmission is altered. In support of this suggestion, Peters et al. (1990) reported increased NPY content in the CSF of drug-free schizophrenics, and in another study, reduced NPY concentrations were observed in the temporal cortex of a subpopulation of schizophrenic patients (Heilig and Widerlov, 1990). More recently, Ault and Werling (1998) suggested that lack of NPY or low numbers of its receptors in the prefrontal cortex may be responsible for...
DA hypoactivity in this region and thus the corresponding negative symptoms of schizophrenia.

If NPY systems are altered in DA-associated psychotic disorders, it is likely that treatment with DA-enhancing drugs, which mimic psychotic states, would also alter NPY activity. Such an effect was recently reported after administration of the psychomimetic PCP, a drug which can induce schizophrenic behavior (Dodd, 1996); thus, single or multiple injections of PCP dramatically reduce the levels of NPY in the caudate nucleus, nucleus accumbens, and frontal cortex (Midgley et al., 1992, 1994).

The present study had two objectives: 1) to establish further the role of NPY in psychiatric disorders by determining the effects of several psychogenic stimulants of abuse on NPY systems and 2) to characterize the effect of methamphetamine (METH) on NPY systems to identify the features of these stimulant-evoked responses.

Materials and Methods

Male Sprague-Dawley rats (180–270 g) were housed in a temperature-controlled environment with a 12-h light/dark cycle. The animals were allowed free access to food and water. For the multiple-dose experiments, the animals received five drug administrations (6-h interval between injections) either s.c. (PCP, National Institute on Drug Abuse, Rockville MD; (+) or (−)methyleneoxydymethamphetamine (MDMA; National Institute on Drug Abuse) or i.p. (METH; National Institute on Drug Abuse; cocaine, National Institute on Drug Abuse; SCH-23390, Research Biochemicals Inc., Natick, MA; or eticlopride, Research Biochemicals Inc.). All drugs were delivered in 0.9% saline, and control animals received injections at the same intervals as treated animals, but of vehicle only. For the single-dose experiment, 15 mg/kg METH was delivered, whereas for multiple drug administrations, PCP, MDMA, METH, and cocaine were administered as 10, 10, 5 to 10, or 30 mg/kg per administration, respectively. The DA D1 (SCH-23390) or D2 (eticlopride) receptor antagonists were administered as 0.5 mg/kg doses 15 min before the METH injection in experiments represented by Fig. 8.

After the rats were sacrificed, brains were rapidly removed, frozen on dry ice, and stored at −80°C; specific regions were dissected out later. The areas analyzed were identified using the atlas of Konig and Klippel (1963). One-millimeter slices were made at 2.5 mm anterior to bregma for the anterior nucleus accumbens and 1.5 mm anterior to bregma for the posterior nucleus accumbens and lateral and medial anterior caudate regions. The lateral and medial posterior caudate areas were dissected at 0.5 mm anterior to bregma and the globus pallidus was taken at 0.5 mm posterior to bregma (Fig. 1). The substantia nigra was dissected at 5.5 mm posterior to bregma. The tissue samples were frozen at −80°C until assayed for NPY-like immunoreactivity (NPYLI) using a radioimmunoassay technique described in detail by Midgley et al. (1992).

Results are expressed as a percentage of the respective controls and represent the means ± S.E.M. for each group. Statistical analysis was conducted using ANOVA followed by Fisher-protected least
significant difference multiple comparisons test. The level of significance was set at $p < .05$.

### Results

**Effects of PCP, METH, (+) or (−)MDMA, or Cocaine on the Content of Caudate NPYLI Content.** Eighteen hours after five injections of the psychomimetic drugs of abuse PCP, METH, (+)MDMA, and cocaine, striatal levels of NPYLI were significantly reduced to 54%, 55%, 74%, and 63% of control. In contrast, a similar treatment with the less active (−)MDMA isomer did not significantly alter striatal NPY systems (Fig. 2).

**Dose-Response of Multiple Administrations of METH on NPYLI Content in the Nucleus Accumbens, Caudate Nucleus, Globus Pallidus, and Substantia Nigra.** To characterize the effects of the stimulants on NPY systems, METH was selected for study because it appeared to have the greatest impact of the central nervous system neurostimulants on this neuropeptide system. The anterior and posterior nucleus accumbens, the lateral and medial anterior caudate, the lateral and medial posterior caudate, the globus pallidus, and the substantia nigra were dissected out (see Fig. 1) and tested for NPYLI concentrations after treatments with multiple administrations of METH. NPYLI in all caudate and accumbens regions was significantly decreased after treatment with 10 mg/kg METH (Figs. 3, 4, and 5): the lower dose of 5 mg/kg significantly reduced NPYLI levels in all of these same regions except the posterior nucleus accumbens (Fig. 3B) and the lateral anterior caudate (Fig. 4A). In contrast, no changes in NPYLI were observed in the globus pallidus and substantia nigra regardless of METH treatment (not shown).

Because all of the affected caudate and accumbens regions showed a similar NPY response after METH treatment at 10 mg/kg, we characterized the effect only in the posterior nucleus accumbens and the medial posterior caudate.

**Effect of a Single Dose of METH on NPYLI Levels after 12, 18, and 24 h.** To determine the sensitivity of the NPY responses, the effect of a single dose of METH was determined various times after treatment. One administration of 15 mg/kg METH was sufficient to lower NPYLI content in the posterior nucleus accumbens to 65% of control by 12 h but levels were no longer significantly different from control at 18 or 24 h after treatment (Fig. 6A). The single dose of METH did not alter NPYLI levels in the medial posterior caudate at any of the times examined (Fig. 6B).

**Effect of Multiple Doses of METH on NPYLI Levels after 18, 24, and 48 h in the Posterior Nucleus Accumbens and the Medial Posterior Caudate.** The time-response of NPY systems to multiple administrations of METH was determined 18, 24, and 48 h after drug treatments. Multiple injections of METH (10 mg/kg at 6-h intervals) reduced NPY levels in the posterior nucleus accumbens to 44% of control at 18 and 24 h and 61% of control at 48 h (Fig. 7A). In the medial posterior caudate, tissue NPYLI content was lowered to 38% of control at 18 h, 58% at 24 h, and 54% at 48 h (Fig. 7B).
Blockade of METH Effect by Pretreatment with a DA D1, but not a D2, Receptor Antagonist. To determine the mechanism whereby METH causes changes in NPY systems, animals were pretreated with a selective DA D1 or D2 receptor antagonist. METH-induced reduction of NPYLI content in the posterior nucleus accumbens and medial posterior caudate was completely prevented by D1 receptor blockade with SCH-23390, whereas the D1 antagonist had no significant effect on NPY levels when administered alone. The D2 antagonist eticlopride did not appear to alter the METH effect; however, the actual effect of blocking D2 receptors on METH-induced changes in NPY systems was difficult to assess because eticlopride significantly lowered NPYLI levels in the posterior nucleus accumbens and in the medial posterior caudate when administered alone (Fig. 8).

Discussion

The results from this study demonstrate that several potent central nervous system stimulants with psychotomimetic activity similarly affect striatal levels of NPYLI (Fig. 2). Consistent with the hypothesis that NPY systems contribute to the psychosis-inducing properties of these drugs, there was no change in NPY levels after similar treatment by the relatively inactive (−)isomer MDMA (Hiramatsu and Cho, 1990). It is noteworthy that other striatal neuropeptide systems are also altered by these stimulant agents. For example, striatal levels of neurotensin (Hanson et al., 1992) and dynorphin (Hanson et al., 1995) are dramatically increased after similar treatments with these drugs. However, because the striatal content increases for neurotensin and dynorphin, but decreases for NPY, the responses by these neuropeptide systems to these drugs are likely quite distinct. Although the precise mechanisms for these opposite effects is unclear, it may be relevant that in the cerebrospinal fluid of subpopulations of schizophrenic patients there are also opposite responses of these peptide systems with a decrease in neurotensin (Lindstrom et al., 1988) and an increase in NPY (Peters et al., 1990). Thus, it appears that such changes in neuropeptide activity might be important factors in the expression of naturally occurring or drug-related psychoses such as schizophrenia.
We further characterized the effect of METH on NPYLI in several extrapyramidal and limbic brain regions, including the anterior and posterior nucleus accumbens, four regions in the caudate according to their anterior-posterior and medial-lateral location, the globus pallidus, and substantia nigra. Multiple doses of 5 mg/kg were sufficient to alter NPYLI in most of the regions examined; the neuropeptide content was reduced to 39 to 73% of the respective controls. Administration of 10 mg/kg METH caused reductions in NPYLI of 35 to 66% of controls (Figs. 3–5). In contrast, no changes in NPYLI were observed in the globus pallidus and substantia nigra regardless of METH treatments (not shown). This absence of effect in the substantia nigra correlates with previous studies using PCP (Midgley et al., 1994).

The decrease in NPY tissue content after METH administration might reflect an increase in release. However, in the case of neurotensin, similar METH treatment increases tissue levels of NT and release of neurotensin (Wagstaff et al., 1996). Similarly, dynorphin release has been shown to increase in response to treatment with the D1 agonist SKF 38393 (You et al., 1994). If the effects of METH on NPY and NT are truly opposite, then drug-induced decreases in NPY would be due to decreased synthesis of the neuropeptide. This is supported by studies from Wahlestedt et al. (1991), which have shown that NPYLI content and NPY mRNA are reduced in response to cocaine administration. Because cocaine also elevates extracellular DA levels, we expect METH would cause a similar reduction in NPY mRNA. Such a change in synthesis alone, or in combination with increased release of this peptide, could lead to an eventual neuropeptide depletion and a decrease in NPYLI tissue levels. Additional studies are required to test these possibilities.

Another possibility is that this dosing regimen with METH (10 mg/kg, 5 doses at 6-h intervals) is neurotoxic to NPY-containing neurons. However, there are findings that argue against this possibility. GABA has been shown to be colocalized with NPY in the striatum (Kawaguchi et al., 1995). Hotchkiss et al. (1979) reported that multiple high doses of METH had no effect on the levels of the synthesizing enzyme for GABA, glutamate decarboxylase, in the striatum, suggesting GABA-containing neurons are not damaged by this drug. In addition, striatal dynorphin and neurotensin levels return to normal after METH treatment (Hanson et al., 1988;
The primary effect of METH administration on the accumbens and the striatum is to dramatically increase the levels of extracellular DA in these brain regions. We would therefore assume that the majority of METH pharmacological effects including reduction in NPYLI are DA-mediated. Previous studies have shown that D1 receptors are involved in the effects of psychostimulants on NPY levels, and D1 agonists have been shown to cause a reduction in striatal and accumbens NPYLI tissue content (Midgley et al., 1992 and 1994). In this study, we demonstrated that the METH-induced changes in NPYLI are blocked by SCH-23390 in both the accumbens and the striatum (Fig. 8).

In contrast to the effects of the D1 antagonists, we were unable to block the METH-induced reduction of NPYLI by administering the D2 antagonist eticlopride. In both the nucleus accumbens and the striatum the combination of eticlopride and METH was not significantly different from the effect of METH alone (Fig. 8). Eticlopride administered separately lowered NPY levels significantly in the striatum to the same extent of METH’s effect in the accumbens. This is consistent with previous studies that reported a reduction in striatal and accumbens NPY levels in response to D2 agonists (Kerkerian et al., 1989; Midgley et al., 1994). Interestingly, the D2 agonist quinpirole has also been reported to decrease NPYLI (Midgley et al., 1994). The reason that D2 agonists and antagonists have similar effects on NPYLI is unclear, but it is probably due to indirect effects. Le Moine et al. (1991) tested somatostatin interneurons for D2 receptor mRNA and found none present. Because NPY is colocalized with somatostatin in the accumbens and the striatum, it is likely that there are no D2 receptors on NPY-containing neurons. Midgley et al. (1992) reported that PCP- and MK-801-induced decreases in NPYLI are blocked by GABA agonists, and Maura et al. (1988) showed that D2 agonists block glutamate release; thus, it is possible that D2 agonists and antagonists may act on GABA and/or glutamate systems to indirectly affect NPY levels. Cholinergic interneurons may also be involved as they have been shown to have reciprocal interactions with NPY-containing neurons and are innervated by dopaminergic systems (Kerkerian et al., 1991).

We demonstrated that NPY levels were reduced by the administration of stimulant drugs of abuse, especially METH. We have also shown that METH produces these effects through a DA D1 receptor. As mentioned above, this is similar to previous data implicating a D1 mechanism in the reduction of NPY levels in response to PCP (Midgley et al., 1992, 1994). METH treatment also increases neurotensin and dynorphin tissue levels through D1 receptor activation (Hanson et al., 1992, 1995). This would suggest that stimulant-induced changes in NPY, neurotensin, and dynorphin levels may be mediated through similar DA pathways. However, there are significant differences between the neuropeptide systems; the neurotensin and dynorphin levels return to normal within 48 h of drug treatment (Merchant et al., 1988; Hanson et al., 1988), whereas the NPY system did not, indicating that there could be a persistent decrease in the synthesis of NPY long after the drug is eliminated. One differ-

![Fig. 8. Effect of D1 and D2 antagonists on METH-induced changes in NPYLI content in the posterior nucleus accumbens (A) and the medial posterior caudate (B). Animals were pretreated with either SCH-23390 or eticlopride 15 min before each of the multiple METH administrations. Animals were sacrificed 18 h after treatment. Control values of NPYLI were 1964 pg/mg protein and 1070 pg/mg protein respectively. The results are expressed as a percentage of respective controls ± S.E.M. (n = 8–10). *p < .05 versus saline, **p < .05 versus eticlopride.](image-url)
ence in the neuropeptide response is that the NPY found in the accumbens and the caudate is associated with interneurons (Kawaguchi et al., 1995), whereas the neurotensin and dynorphin in this region are found in the projection neurons (Sugimoto and Mizuno, 1987; Gerfen and Young, 1988). Thus, the distinct responses may reflect very different contributions by the interneuronal and efferent projections to the effects of psychostimulants.

Finally, because both PCP (Midgley et al., 1993) and METH have been demonstrated to involve D1 pathways in reducing of NPY, we hypothesize that the other drugs of abuse shown in Fig. 2, namely, MDMA and cocaine, also reduce NPY through D1 mediated pathways. Further studies are needed to test this hypothesis.

References