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Differential neurotensin responses to low and high doses of methamphetamine in the terminal regions of striatal efferents

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Abstract

Neurotensin is a neuropeptide associated with basal ganglia dopaminergic neurons. Because levels of neurotensin in striatal tissue are differentially affected by low or high doses of methamphetamine, we employed microdialysis to assess the dose-dependent effects of methamphetamine on neurotensin release from the terminals of striatonigral and striatopallidal neurons. A low (0.5 mg/kg), but not high (10 mg/kg), dose of methamphetamine significantly increased nigral extracellular levels of neurotensin. The low-dose effect on extracellular nigral neurotensin levels was blocked by pretreatment with either a dopamine D1 or D2 receptor antagonist. In the globus pallidus, only half of the animals demonstrated increased neurotensin release after the low dose of methamphetamine. These findings suggest that low and high doses of methamphetamine differentially affect the release of neurotensin from the terminals of striatonigral neurons and that both dopamine D1 and D2 receptor activation contributes to the low-dose methamphetamine effects in the substantia nigra.

Keywords: Dopamine; Eticlopride; Globus pallidus; Microdialysis; Neuropeptide; SCH 23390; Striatum; Substantia nigra

1. Introduction

Neurotensin is a tridecapeptide with neurotransmitter/neuromodulatory activity in the central nervous system (CNS) (Tyler-McMahon et al., 2000) that is linked with basal ganglia structures (Binder et al., 2001). Within the basal ganglia, neurotensin receptors (NT1 and NT2) are located on striatal neurons, the cell bodies and terminals of nigrostriatal dopamine neurons, and on neurons of the substantia nigra and globus pallidus receiving projections from striatal efferent neurons (Fassio et al., 2000; Sarret et al., 2003; Tyler-McMahon et al., 2000).

Several lines of evidence suggest that neurotensin systems play an important role in mediating basal ganglia-dependent behaviors. First, in the basal ganglia, neurotensin is found in spiny efferent neurons of striatum that project to both the substantia nigra and globus pallidus (Castel et al., 1993, 1994b). Second, this neuropeptide has been shown to have direct excitatory properties, as administration of neurotensin into either the striatum or the substantia nigra typically increases neural firing as measured electrophysiologically or by transmitter release (Ferraro et al., 2001; Legault et al., 2002). Finally, the neurotensin NT1 receptor has been shown to co-localize with dopamine D2 receptors in axon terminals in the basal ganglia (Delle Donne et al., 2004), where neurotensin, via the neurotensin NT1 receptor (Diaz-Cabiale et al., 2002; Legault et al., 2002), is thought to antagonize dopamine D2 receptor-mediated inhibition of transmitter release. Neurotensin is therefore likely to play a critical role in regulating basal ganglia function.

Studies have also suggested that neurotensin systems contribute to the activity of the nigrostriatal dopamine pathway. However, the significance of these findings is confounded by conflicting reports. On the one hand, direct administration of neurotensin into the striatum stimulates the release of striatal dopamine and enhances locomotor activity (Chapman et al., 1992). On the other hand, some studies suggest that neurotensin

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has an overall inhibitory influence on the stimulatory behavioral effects of psychostimulants (Ervin et al., 1981; Skoog et al., 1986; Kalivas et al., 1984), possibly through an indirect mechanism involving increased striatal gamma-aminobutyric acid activity (Ferraro et al., 1998, 2001).

Whereas the role of neurotensin in regulating striatal dopamine function requires further elucidation, more is known about how changes in dopamine activity influence the striatal efferent neurotensin systems. These studies have identified variations in striatal and nigral tissue levels of neurotensin in response to activation of dopamine receptors either directly by selective agonists (Merchant et al., 1989a,b) or indirectly by psychostimulants such as methamphetamine (Letter et al., 1987). This research revealed that stimulation of dopamine D1 receptors or administration of high doses of methamphetamine or cocaine dramatically elevates tissue levels of neurotensin in the striatum, the substantia nigra and the globus pallidus. In contrast, activation of the dopamine D2 receptor with a selective agonist or administration of a low dose of methamphetamine actually decreases striatal, nigral and pallidal neurotensin tissue content (Wagstaff et al., 1996a,b) suggesting opposite dose-dependent effects of methamphetamine on these neurotensin responses.

Dopamine-related changes in the neurotensin systems of the basal ganglia have also been monitored by assessing the synthesis of this neuropeptide through measurements of the mRNA for its neurotensin/neuromedin N precursor. Such studies demonstrated that changes in dopamine receptor activity caused by either selective direct dopamine D1 receptor agonists or indirect agonists such as methamphetamine significantly increase the expression of striatal neurotensin mRNA (Hanson and Keefe, 1999; Castel et al., 1994a; Merchant et al., 1994). The effects of dopamine D2 agonists or low doses of methamphetamine on neurotensin mRNA expression have not been reported. Taken together, these studies suggest that activation of dopamine D1 receptors may alter neurotensin synthesis, resulting in increased tissue accumulation of neurotensin. In contrast, dopamine D2 receptor activation may have an opposite effect. To better appreciate the interaction between basal ganglia dopamine and neurotensin systems, it is necessary to identify how the actual release of neurotensin is affected by these pharmacologically induced changes in dopamine function. To this end, Wagstaff et al. (1996a) reported that neurotensin release is increased in the striatum after a low, but not a high, dose of methamphetamine. The present study extends these findings by examining the effects of low and high doses of methamphetamine on extracellular neurotensin levels in the substantia nigra and globus pallidus, terminal regions of the two principal efferent projection pathways from the striatum that contain neurotensin (Castel et al., 1994b).

In summary, the present study demonstrated that a single low dose of methamphetamine (0.5 mg/kg) significantly increased the extracellular levels of neurotensin in the substantia nigra, much like the effect previously reported in the striatum (Wagstaff et al., 1996a). In the globus pallidus, the low dose of methamphetamine elicited increases in extracellular neurotensin in 50% of the animals. In the other 50%, there were no significant changes in the pallidal extracellular neurotensin content. The high dose of methamphetamine (10 mg/kg) did not significantly alter extracellular neurotensin levels in either the substantia nigra or globus pallidus. Antagonism of either the dopamine D1 or D2 receptor blocked the effect of the low dose of methamphetamine on nigral extracellular neurotensin levels. The potential significance of these findings is discussed, especially in light of dose-dependent overall effects of methamphetamine on striatal efferent neurotensin systems.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Simonson Laboratories, Gilroy, CA or Charles River Laboratories, Raleigh, NC) were allowed to acclimate for at least 2 weeks and were used when they weighed 280 to 320 g. Animals were maintained in a temperature-controlled room with a 12-h light/dark cycle and given free access to food and water. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee and adhered to the National Academy of Sciences *Guide for the Care and Use of Laboratory Animals*.

2.2. Microdialysis

Concentric microdialysis probes similar to those previously described (Wagstaff et al., 1996a,b) were constructed. Briefly, one end of a 20-mm length of 24-gauge, thin-walled stainless-steel hypodermic tubing was inserted into approximately 3 cm of polyethylene tubing (PE 20). Fused silica tubing served as the outlet. A dialysis membrane (Hospal Industrie, Meyziu, France) with a 40,000-Da molecular weight cut-off (ID 0.22 mm, OD 0.31 mm) was slipped over the fused silica and extended 0.25 mm into the stainless steel tubing and then was cemented into place. The active dialyzing length of the probe was 2 mm.

Probe recovery was tested by placing probes in a test tube with known concentrations of synthetic neurotensin. Several 60-μl samples of this solution were collected and recovered neurotensin was quantified by radioimmunoassay as described below. Probes with recovery of synthetic neurotensin of approximately 7–8% were used for the experiments. Control values reported below were not corrected for probe recovery. Approximately 20 to 24 h before the experiment, probes were stereotaxically implanted into the left globus pallidus (from bregma: A/P −1.3, L 3.2, V −8.0) and/or the right substantia nigra (from bregma: A/P −5.5, L 6.1, V −8.5 at an angle of 32°) in rats anesthetized with 500 mg/kg chloral hydrate. The coordinates were determined using the atlas of Paxinos and Watson (1998) and confirmed by visual inspection of coronal sections after the experiment. The probes were cemented in place with dental acrylic and skull screws and then connected to an infusion pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and perfused at a flow rate of 2 μl/min with artificial cerebrospinal fluid (aCSF; pH 7.4) containing: NaCl, 145 mM; KCl, 2.4 mM; $CaCl_2$, 1.1 mM; $MgCl_2$, 0.82 mM; KH_2PO_4, 0.5 mM; $NaHPO_4$, 1.7 mM; glucose, 5.9 mM

and bovine serum albumin, 0.08% (w/v). Animals were perfused overnight before collecting samples for the experiments. Animals tolerated the procedure well and had no appearance of stress on the day of the experiment. Dialysis samples were collected into microcentrifuge tubes to which 6.6 µl of 10× assay buffer (see below) had been added. Samples were collected every 30 min for a total volume of 66.6 µl/sample, immediately frozen and stored at −80 °C until assayed for neurotensin immunoreactivity.

After overnight perfusion, three consecutive samples were collected to determine basal extracellular levels of neurotensin-like immunoreactivity before treatments. The extracellular neurotensin levels in these initial control samples were found to be stable and the mean was used for comparison of drug effects within the individual animals (represented as the 0 min time point in all figures); i.e., all samples were expressed as a percentage of the mean basal value in the same animal, as previously described (Wagstaff et al., 1996a). With these procedures, mean basal levels (±S.E.M.) of extracellular neurotensin in dialysate samples from the substantia nigra ranged from 30.2±4.4 to 89.9±15.7 pg/25 µl. Globus pallidus mean basal levels (±S.E.M.) of extracellular neurotensin ranged from 29.7±4.2 to 41.6±8.3 pg/25 µl. After collection of the baseline samples, a single dose of saline or methamphetamine (0.5 or 10.0 mg/kg, s.c.) was administered. Samples (30 min) were collected for another 180 min (Figs. 1A and 3A). In experiments examining the roles of dopamine D1 and D2 receptors in methamphetamine-induced changes in extracellular levels of neurotensin (Fig. 2), two doses of either SCH 23390 (0.5 mg/kg, i.p.) or eticlopride (0.5 mg/kg, i.p.; Fig. 2B) were administered after baseline samples were collected in order to minimize the number of animals used while also assessing the effects of blocking dopamine D1 or D2 receptors alone and prior to methamphetamine (Hanson et al., 2002). That is, a dose of an antagonist was given 2 h prior to the methamphetamine in order to determine if either antagonist altered baseline neurotensin levels, and then an additional dose of the antagonist was administered 15 min prior to methamphetamine in order to ensure blockade of each respective dopamine receptor. After methamphetamine was injected, samples were collected for another 180 min. In order to insure proper controls for this experiment, dialysate samples from the substantia nigra were collected in separate sets of animals following three injections of saline or two injections of saline followed by methamphetamine (Fig. 2A).

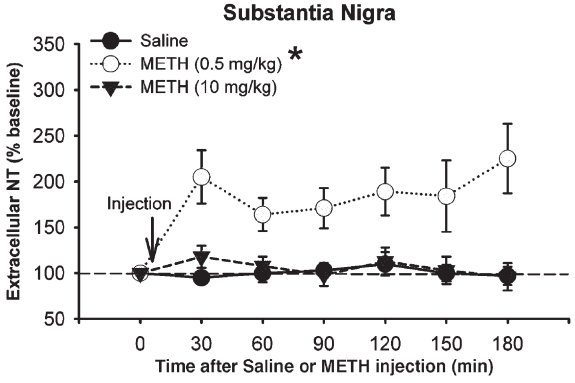

Fig. 1. Extracellular levels of neurotensin (NT) in the substantia nigra following a single injection (s.c.) of saline or methamphetamine (METH; 0.5 or 10 mg/kg, s.c.). Data (means±S.E.M. of 9–16 animals) are expressed as percentages of the baseline (0 min on time axis; average of preceding three time points, individual samples not shown). *$p<0.05$ vs. saline-treated animals.

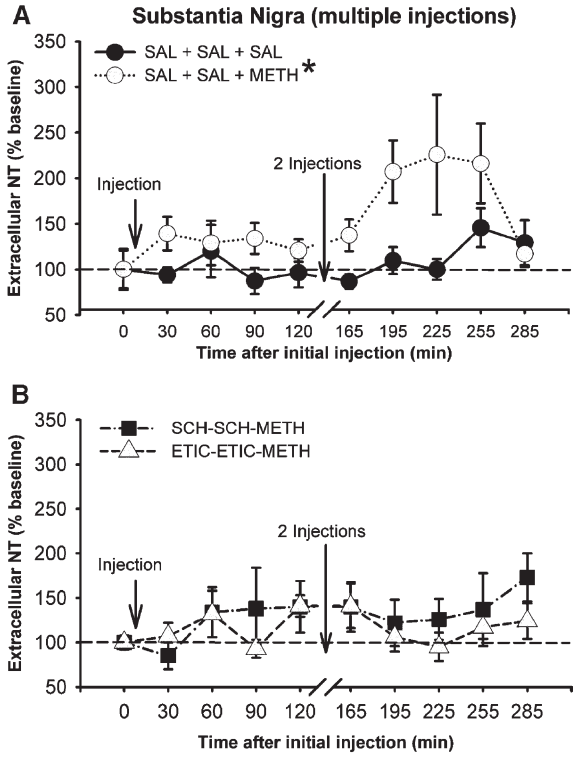

Fig. 2. Extracellular levels of neurotensin (NT) in the substantia nigra following multiple injections of saline (SAL) and methamphetamine (METH; 0.5 mg/kg) (A) or SCH 23390 (SCH; 0.5 mg/kg) and METH or eticlopride (ETIC; 0.5 mg/kg) and METH (B). The initial injection (i.p.) occurred just after baseline samples were collected. The second injection (i.p.) was administered 2 h after the first. The third injection (s.c.) was administered 15 min after the second. There was no sample collected between the second and third injections. A statistical analysis employing a two-way ANOVA (see Materials and methods) included all four groups; however, for ease of comparison, SAL–SAL–SAL and SAL–SAL–METH are plotted separately (A) from SCH–SCH–METH and ETIC–ETIC–METH (B). Data (means±S.E.M. of 8–14 animals) are expressed as percentages of the baseline (0 min on time axis; average of the preceding three time points, individual samples not shown). *$p<0.05$ vs. SAL–SAL–SAL, SCH–SCH–METH and ETIC–ETIC–METH.

After each experiment, animals were euthanized by an overdose of anesthetic (100 mg/kg sodium pentobarbital). The brains were then removed and frozen and the dialysis probe placement assessed by visual inspection of coronal sections. If inspection revealed that the probe was not located in the region intended, the dialysate samples from these animals were excluded from the study.

2.3. Radioimmunoassay

The solid-phase radioimmunoassay used to analyze the extracellular levels of neurotensin immunoreactivity in this study was adapted from the methods described by Maidment et

al. (1991) and was similar to that used previously by this lab (Wagstaff et al., 1996a,b). Removable 96-well immunoplates (MaxiSorb, Nunc) were incubated for at least 2 h at room temperature with a protein G solution (50 ng/100 ml in 0.1 M sodium bicarbonate; pH 9.0). This solution was aspirated, and the wells washed three times with wash buffer (0.15 M K_2HPO_4, 0.02 M NaH_2PO_4, 0.2 mM ascorbic acid, 0.2% Tween 20, 0.1% sodium azide, pH 7.5). A highly selective antiserum for neurotensin (see below) was diluted 1:20,000 in assay buffer (same as wash buffer with 0.1% gelatin), and 25 μl was added to each well. The antiserum solution was incubated for at least 4 h, at room temperature, in order to allow antibody attachment to the protein G-coated surface. The wash procedure was repeated, and samples and standards were added to the wells in a volume of 25 μl. Samples and standards were incubated at room temperature overnight. The next day, I^{125}-labeled neurotensin (NEN Du Pont, Wilmington, DE) was diluted to approximately 5000 dpm/25 μl in assay buffer, and 25 μl was added to each well. Labeled neurotensin was also added to four wells treated with only protein G solution to assess nonspecific binding and to four untreated wells to determine the total radioactivity. Labeled neurotensin was incubated in the wells for 2.5 to 4 h at room temperature. After this period, wells were washed, broken apart, placed in 75×12 mm polypropylene tubes and counted on a five-channel gamma counter. This procedure allowed for reliable detection of 250 fg of neurotensin per 50 μl sample, well below the sensitivity required to reliably determine the amounts of neurotensin in the 30-min fractions collected by microdialysis in this study (see above).

2.4. Antiserum

The neurotensin antiserum was raised in New Zealand White rabbits as previously described (Letter et al., 1987; Ritter et al., 1984; Wagstaff et al., 1996a). This antiserum recognizes the neurotensin carboxy terminus and is highly selective, expressing no cross-reactivity with 1000-fold excess concentrations of other endogenous neuropeptides such as dynorphin A, [met^5] enkephalin, cholecystokinin, substance P or substance K. There was also no appreciable cross-reactivity to any of the components of the aCSF used in the study.

2.5. Drugs

SCH 23390 hydrochloride (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1-*H*-3-benzazepine hydrochloride) and eticlopride hydrochloride (*S*(−)-3-chloro-5-ethyl-*N*-[(l-ethyl-2-pryolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride) were purchased from Sigma. (±)-Methamphetamine hydrochloride was a generous gift from the National Institute on Drug Abuse (NIDA). Methamphetamine (0.5 mg/kg or 10 mg/kg) and SCH 23390 (0.5 mg/kg) were dissolved in saline. Eticlopride (0.5 mg/kg) was dissolved in water. All drug doses are calculated as the free base. Saline and methamphetamine (0.5 mg/kg or 10 mg/kg) were injected s.c., whereas SCH 23390, eticlopride or their vehicle were administered i.p.

2.6. Statistics

Due to variability between animals and to facilitate statistical and graphic comparison of mean values±S.E.M., extracellular levels of neurotensin-like immunoreactivity were calculated as a percentage of each animal's baseline (the average of the first three samples collected before treatment). The basal extracellular levels of neurotensin expressed as pg/50 μl sample are given above. Data in all figures were analyzed with a two-way analysis of variance (ANOVA) with repeated measure across time followed by post hoc analysis with the Fisher PLSD ($p<0.05$; SAS V8). The between factor was treatment.

3. Results

The effect of a single, low (0.5 mg/kg) or high (10 mg/kg) dose of methamphetamine on the activity of striatal efferent neurons containing neurotensin was determined by employing microdialysis to assess the extracellular content of neurotensin immunoreactivity in the terminal regions of the striatal efferent projections. A two-way ANOVA revealed a significant main effect of treatment [$F_{(17,269)}=4.82$, $p<0.01$] in the substantia nigra, with no significant main effect of time (interval) and no significant interaction ($p>0.05$). Post hoc analysis with Fisher's PLSD revealed that, within the substantia nigra, the low (0.5 mg/kg), but not the high (10 mg/kg), dose of methamphetamine significantly increased neurotensin levels relative to saline-treated controls (Fig. 1). Because the high-dose methamphetamine treatment did not significantly alter extracellular neurotensin levels (Fig. 1), no further experiments were conducted with this dosing paradigm in the substantia nigra.

In order to determine the mechanism of action for the effects of the low-dose methamphetamine treatment on nigral extracellular neurotensin, animals were pretreated with either saline or selective dopamine D1 and D2 receptor antagonists prior to an injection of 0.5 mg/kg methamphetamine. The pretreatment with either saline or the dopamine receptor antagonists consisted of two injections: the first administration was given to assess whether SCH 23390 (dopamine D1 receptor antagonist) or eticlopride (dopamine D2 receptor antagonist) had effects of their own on extracellular levels of neurotensin. A second injection of each antagonist was administered 120 min later to assure a blockade of the respective receptor when the methamphetamine treatment occurred after an additional 15 min. In order to verify that the injection paradigm itself did not alter the nigral neurotensin response, additional animals were tested with saline. Data from both Fig. 2A and B were analyzed together; however, in order to better illustrate the comparisons between groups, the data were separated into saline and methamphetamine treatments and dopamine antagonist treatments. A two-way ANOVA revealed

a significant main effect of treatment [$F_{(35,337)}=1.65$, $p<0.05$] with no significant main effect of time (interval) and no significant interaction ($p>0.05$). Post hoc comparison indicated that pretreatment with the initial two injections of saline did not prevent the stimulatory effect of a low dose of methamphetamine (0.5 mg/kg) as these animals produced significantly increased extracellular neurotensin levels ($p<0.01$) relative to saline (Fig. 2A). Additionally, post hoc comparisons indicated that neither the SCH 23390 nor the eticlopride-treated rats (Fig. 2B) were significantly different from saline-treated rats controls (Fig. 2A). However, the SCH 23390-treated and eticlopride-treated groups were significantly different (Fig. 2A) from the animals receiving methamphetamine subsequent to saline injections (Fig. 2A).

In contrast to our findings in the substantia nigra, a two-way ANOVA revealed that there were no significant main effects of treatment or time (interval) and no significant interaction with respect to extracellular neurotensin levels in the globus pallidus ($p<0.08$; Fig. 3A). However, further inspection of the data from rats treated with the low dose of methamphetamine (0.5 mg/kg) suggested distinct populations within this treatment group. Half of the rats ($n=16$) treated with the low dose of methamphetamine (0.5 mg/kg; "responders") showed an increase in extracellular neurotensin levels beyond the means (plus two standard deviations) of the levels observed in control animals receiving a saline injection. The other half of the rats treated with drug ($n=16$) had extracellular levels of neurotensin that were not distinct from saline-treated animals ("non-responders"; Fig. 3B). Of note, evaluation of these groups by a two-way ANOVA revealed a significant main effect of treatment [$F_{(17,305)}=5.27$, $p<0.05$] with no significant main effect for time (interval) and no interaction ($p>0.05$). Post hoc analysis with Fisher's PLSD revealed that the "responder" group displayed extracellular neurotensin levels that were significantly increased relative to both the "non-responder" and saline-treated groups ($p<0.05$; Fig. 3B).

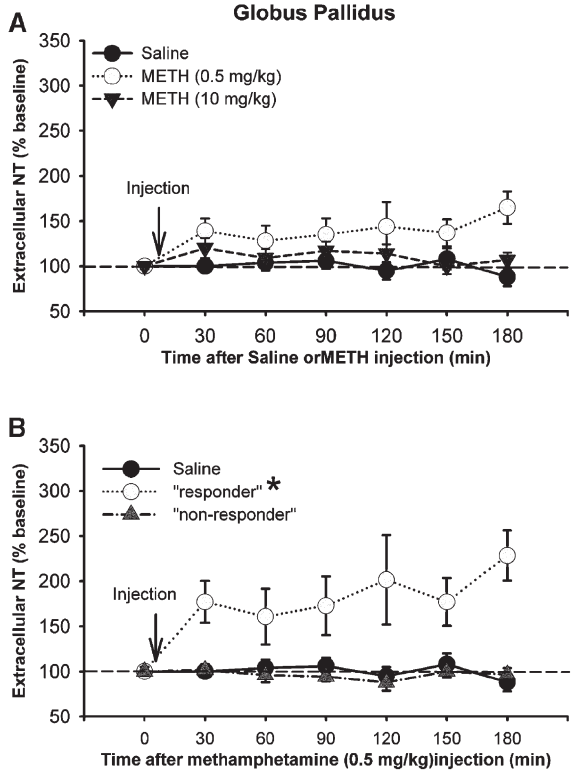

Fig. 3. Extracellular levels of neurotensin (NT) in the globus pallidus in all animals tested following a single injection (s.c.) of saline or methamphetamine (METH; 0.5 or 10 mg/kg, s.c. (A)). Data are means (±S.E.M. of 13–32 animals) and are expressed as percentages of the baseline (0 min on time axis; average of the preceding three time points, individual samples not shown). (B) Animals receiving a single injection of methamphetamine (0.5 mg/kg) were divided into two populations ("responders" or "non-responders"), as described in Results, to illustrate the response patterns of animals administered methamphetamine (0.5 mg/kg (B)). *$p<0.05$ vs. saline and "non-responders".

4. Discussion

This study examined the dose-dependent effects of the psychostimulant methamphetamine on the activity of neurotensin systems in the terminal regions of striatal efferent neuron projections. This was accomplished by analysis of extracellular neurotensin levels via in vivo microdialysis analysis coupled to a sensitive solid-phase radioimmunoassay with the assumption that changes in extracellular neurotensin immunoreactivity levels are caused by changes in the release of this neuropeptide. However, while there is no previous evidence that methamphetamine alters the degradation of neuropeptides, this approach does not eliminate the possibility that changes in neurotensin metabolism may also contribute to variations in extracellular levels of neurotensin-like immunoreactivity. Despite this caveat, the possibility that the release of neurotensin in extrapyramidal pathways might be differentially influenced by low and high doses of methamphetamine was suggested by an earlier report using in vivo microdialysis to assess the extracellular levels of neurotensin in the striatum. Those findings showed that a low dose of methamphetamine (0.5 mg/kg) increases the release of striatal neurotensin, whereas a higher dose of methamphetamine (10 mg/kg) does not (Wagstaff et al., 1996a). Because striatal efferent projections containing neurotensin terminate in both the substantia nigra and globus pallidus (Castel et al., 1994b), we employed in vivo microdialysis to evaluate the extracellular levels of this peptide in these structures as an index of altered neurotensin release from striatal efferent terminals. The pattern of methamphetamine-induced changes in neurotensin levels following an acute injection was similar in the substantia nigra to that previously reported for neurotensin in the striatum and nucleus accumbens. That is, the low dose of methamphetamine (0.5 mg/kg) significantly elevated the nigral extracellular content of neurotensin (Fig. 1), whereas the 10 mg/kg dose had little effect on this parameter (Fig. 1; Wagstaff et al., 1996a). These findings suggest that a low, but not a high, dose of methamphetamine stimulates the release of neurotensin from the terminals of striatal efferent neurons.

The role of dopamine D1 and D2 receptors in mediating the increase in extracellular levels of neurotensin in the substantia nigra induced by the low dose of methamphetamine was also determined. In order to establish the effects of selective dopamine receptor antagonists on extracellular levels of neurotensin in the same animals that were to be treated with methamphetamine, the rats were first pretreated with either SCH 233390 or eticlopride 2 h prior to methamphetamine administration. To assure continued blockade of the dopamine receptors, a second injection of either dopamine receptor antagonist was subsequently given just prior to the injection of methamphetamine. As stated in Materials and methods, an additional set of rats underwent the same injection protocol with the exception that they were treated with saline. These experiments were conducted to assure that repeated injections per se did not influence extracellular neurotensin levels. There was no significant change observed in the extracellular levels of neurotensin in the substantia nigra when rats were given three injections of saline (Fig. 2A), or following a single injection of either SCH 23390 or eticlopride (Fig. 2B), suggesting that the basal extracellular levels of neurotensin in the substantia nigra are not under the influence of tonic stimulation of either dopamine receptor subtype.

These findings in the substantia nigra are in contrast to our previous findings in the striatum, in which the dopamine D2 receptor antagonists haloperidol or eticlopride (Huang and Hanson, 1997; Wagstaff et al., 1996a) alone decreased the extracellular levels of neurotensin. A possible explanation is that the extracellular levels of neurotensin in the striatum are associated with the dopamine D2 receptor-dominated striatopallidal neurons whereas the extracellular levels in the substantia nigra are associated with the striatonigral projection. The fact that both the dopamine D1 and D2 receptor antagonists completely blocked the methamphetamine-induced changes in extracellular neurotensin levels in the substantia nigra (Fig. 2A and B) suggests that co-activation of dopamine D1 and D2 receptors is necessary for low doses of methamphetamine to increase the nigral release of neurotensin. Other groups have reported that concomitant activation of dopamine D1 and D2 receptors is often required for dopamine-mediated effects in the basal ganglia (c.f., Barone et al., 1986; Ruskin and Marshall, 1994; Weick and Walters, 1987), including activation of the striatonigral efferent pathway (Wang and McGinty, 1997; Ruskin and Marshall, 1994; but see Castel et al., 1994a).

Another means of assessing neuropeptide responses is to measure changes in tissue peptide levels. It is assumed that the total tissue content of neuropeptides in some way reflects a balance between release and subsequent peptide degradation and replacement by synthesis. For neurotensin, it has been previously reported that high doses of methamphetamine increase tissue levels in the striatum, substantia nigra and globus pallidus (Letter et al., 1987; Gygi et al., 1994). Although not as thoroughly investigated, low doses of methamphetamine appear to decrease the tissue content of neurotensin in some striatal regions (Wagstaff et al., 1996a). Assuming the extracellular responses to methamphetamine treatment reported herein reflect release patterns, a better perspective on the overall response of basal ganglia neurotensin systems to methamphetamine exposure is possible by considering the combination of these parameters. For example, a high dose of methamphetamine does not appear to alter release of neurotensin from the terminals of striatal efferent neurons. At the same time, there is a significant increase in striatal neurotensin mRNA expression within 3 h (Adams et al., 2001; Hanson and Keefe, 1999; Merchant et al., 1994; Castel et al., 1994a), suggestive of increased neurotensin precursor synthesis. This combination of no increase in release and turnover of neurotensin coupled with increased synthesis would likely result in accumulation of neurotensin (probably inside of striatonigral neurons) and elevation of striatal and nigral neurotensin tissue levels (Letter et al., 1987; Castel et al., 1994b). The finding that the methamphetamine-induced changes in tissue levels of neurotensin in striatum and substantia nigra are blocked by dopamine D1, but not D2, receptor antagonism (Letter et al., 1987) and that administration of a selective dopamine D1 receptor agonist causes an increase in striatal neurotensin mRNA (Hanson and Keefe, 1999), suggest that this high-dose effect is a dopamine D1 receptor-dominated phenomenon.

The low-dose methamphetamine effects on the neurotensin striatonigral pathway are not as well studied and, consequently, less conclusive. From the microdialysis findings described herein (Figs. 1 and 2) and previously (Wagstaff et al., 1996a), it is apparent that 0.5 mg/kg of methamphetamine increases the release of neurotensin via activation of both dopamine D1 and D2 receptors. Previous studies suggested that low doses of methamphetamine reduce neurotensin tissue levels in some basal ganglia regions (Wagstaff et al., 1996a). Preliminary data from our lab suggests that low doses of methamphetamine do not alter neurotensin mRNA expression at either 30 min or 3 h post injection (data not published). A possible interpretation of these findings is that 0.5 mg/kg of methamphetamine causes an immediate increase in neurotensin release in both striatum and substantia nigra, leading to an increase in neurotensin turnover and a reduction in peptide tissue levels. Based on previous reports (Wagstaff et al., 1996a,b), the current study supports the hypothesis that activation of both the dopamine D1 and D2 receptors are required for the effects of a low dose of methamphetamine on the striatonigral neurotensin system.

As was the case in the substantia nigra, the acute injection of a low, but not a high, dose of methamphetamine resulted in a trend towards an increase in neurotensin release in globus pallidus (Fig. 3B). It is interesting to note, upon further inspection, individual rats seemed to fall into two distinct groups with respect to the effects of a low dose of methamphetamine on pallidal extracellular levels of neurotensin. Fifty percent of the rats ($n=16$) appeared to be “responders”, exhibiting an increase in the extracellular levels of neurotensin. The other half of the animals, classified as “non-responders”, showed no response to the methamphetamine, resembling saline-treated animals with respect to extracellular levels of neurotensin. While the increase in extracellular levels of pallidal neurotensin in the “responders” resembled that found in the substantia nigra, the nigral effects in

individual rats did not predict the response by the pallidal neurotensin systems (data not shown).

In summary, this study used in vivo microdialysis to examine the differential effects of a low- and high-dose treatment of methamphetamine on neurotensin systems in the terminal regions of striatal efferent neurons. The results showed that an acute low dose of methamphetamine increases the release of this peptide from striatonigral neurons through co-activation of D1 and D2 dopamine receptors, whereas a high dose of methamphetamine does not. These findings are consistent with our previous work examining the effects of these doses of methamphetamine on the extracellular levels of substance P in the substantia nigra (Hanson et al., 2002), and therefore further suggest that a low, but not a high, dose of methamphetamine activates striatonigral neurons. The present data, along with our previous work examining the effects of low and high doses of methamphetamine on tissue levels of [met^5]enkephalin in globus pallidus (Alburges et al., 2001), also suggest that a similar differential regulation of striatopallidal efferent neuron function by low and high doses of methamphetamine may occur, although further studies examining the release of neurotensin, as well as [met^5]enkephalin responses are required to determine the magnitude and the pharmacology underlying this effect. Only by fully elucidating these mechanisms will it be possible to understand the regulatory nature of the neuropeptides and what roles they play in basal ganglia-dependent behaviors especially in response to psychostimulants, such as methamphetamine.

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