Effect of Methamphetamine Self-Administration on Neurotensin Systems of the Basal Ganglia

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ABSTRACT

Methamphetamine (METH) dependence causes alarming personal and social damage. Even though many of the problems associated with abuse of METH are related to its profound actions on dopamine (DA) basal ganglia systems, there currently are no approved medications to treat METH addiction. For this reason, we and others have examined the METH-induced responses of neurotensin (NT) systems in the basal ganglia. This neuropeptide is associated with inhibitory feedback pathways to nigrostriatal DA projections, and NT tissue levels are elevated in response to high doses of noncontingent METH because of its increased synthesis in the striatonigral pathway. The present study reports the contingent responses of NT in the basal ganglia to self-administration of METH (SAM). Intravenous infusions of METH linked to appropriate lever-

pressing behavior by rats significantly elevated NT content in both dorsal striatum (210%) and substantia nigra (202%). In these same structures, NT levels were also elevated in yoked METH animals (160 and 146%, respectively) but not as much as in the SAM rats. These effects were blocked by a D1, but not D2, antagonist. A NT agonist administered before the day 5 of operant behavior blocked lever-pressing behavior in responding rats, but a NT antagonist had no significant effect on this behavior. These are the first reports that NT systems associated with striatonigral pathway are significantly altered during METH self-administration, and our findings suggest that activation of NT receptors during maintenance of operant responding reduces the associated lever-pressing behavior.

Introduction

The personal and social damage caused by METH abuse/addiction (Meredith et al., 2005) is probably due, at least in part, to its profound actions on dopamine (DA) basal ganglia systems (Fleckenstein et al., 2007). However, there currently are no approved medications for these METH problems, including drugs with direct DA actions (Elkashaf et al., 2008). As a consequence, it is important to research novel systems to develop effective medications to treat METH addiction. To this end, we and others have investigated the role of the neuropeptide, neurotensin (NT) in METH-abuse models. Neurotensin is synthesized in dorsal striatal cell bodies and linked with the two major striatal efferent projections referred to as the direct (striatonigral) and indirect (striatopallidal) feedback pathways to the nigrostriatal DA neurons (Gerfen et al., 1990; Castel et al., 1994). This dual anatomical arrangement makes NT uniquely positioned to differentially regulate the basal ganglia DA pathway through either D1 (direct feedback pathway) or D2 (indirect feedback pathway) receptor mechanisms. Overall, activation of NT systems or receptors antagonizes DA activity (Chartoff et al., 2004; Feifel et al., 2008), with an apparent physiological role to counteract overactive DA pathways (Wagstaff et al., 1994), leading to the conclusion that NT is a natural neuroleptic and that NT agonists would be an effective treatment for hyperDA psychoses such as schizophrenia (Hadden et al., 2005; Feifel et al., 2008).

Study of the role of DA receptors in regulating NT systems has revealed that stimulation of D1 or D2 receptors has opposite effects, either increasing (selectively in striatonigral neurons; Castel et al., 1994) or decreasing (selectively in striatopallidal neurons; Merchant et al., 1989b; Castel et al., 1994) the NT tissue levels in the dorsal striatum, respectively. In contrast, D1 receptor blockade alone has been re-

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ABBREVIATIONS: METH, methamphetamine; DA, dopamine; SAM, self-administering methamphetamine; YM, yoked-methamphetamine; YS, yoked-saline; NT, neurotensin; DYN, dynorphin; SCH23390, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; FR, fixed ratio; PD149163, Lys(CH₂NH)Lys-Pro,Trp-*tert*-Leu-Leu-Oet; SR48692, 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-imethoxyphenyl)pyrazol-3-yl)carbonylamino]tricyclo(3.3.1.1^{3,7})decan-2-carboxylic acid.

ported to have no effect on dorsal striatal NT levels, but D2 receptor antagonism increases NT levels in striatopallidal neurons (Merchant et al., 1989a,b; Castel et al., 1994).

Much like D1 receptor activation, noncontingent high doses of METH (approximately 10 mg/kg) also increase NT tissue levels and increase related mRNA expression in the dorsal striatum associated with striatonigral neurons (Letter et al., 1987; Castel et al., 1994; Merchant et al., 1994). These effects are blocked by pretreatment with SCH23390 [a D1 antagonist; *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; Research Biochemicals Inc., Natick, MA], but not eticlopride [a D2 antagonist; *S*(-)-3-chloro-5-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-6-hydroxy-2-methoxybenzamide hydrochloride; Research Biochemicals Inc.] (Castel et al., 1994). These elevated NT tissue levels do not seem to be related to changes in NT release per se because 10 mg/kg METH does not change either striatal or nigral extracellular NT levels as measured by microdialysis (Wagstaff et al., 1996b; Frankel et al., 2005). In contrast, stimulation of D2 receptors as mentioned above reduces NT levels in the dorsal striatum, an effect that probably reflects a significant release and turnover of NT (Wagstaff et al., 1994, 1996a), causing a feedback inhibition that can be blocked by either central infusion of a selective NT antibody or antagonist, thereby enhancing both the drug-induced DA and locomotor response (Wagstaff et al., 1994). A D2 antagonist has the opposite effects (Merchant et al., 1989a; Wagstaff et al., 1996a).

Although these types of noncontingent studies implicate NT systems in the immediate pharmacological effects of METH and its neurobiology, their relevance to human abuse is uncertain. For example, it is not clear under contingent conditions (i.e., METH access is linked to operant behavior) how NT systems are affected and whether a response will be dominated by 1) a somewhat quiescent D1-dominated NT striatonigral feedback pathway or 2) a D2-dominated active feedback inhibitory pathway. Such information is necessary to elucidate the nature of the role of NT in METH use and assess the therapeutic potential of this neuropeptide system.

To mimic more accurately the real-life situation of human psychostimulant abuse, the present study determined the response of NT systems to METH self-administration (contingent on appropriate lever-pressing behavior) paradigms. In these studies, the contingent NT responses in rats were determined by comparing NT tissue levels in basal ganglia structures previously shown to be sensitive to investigator-administered METH treatments (Letter et al., 1987) during an abbreviated (5 days) but stable pattern (referred to as maintenance) of self-administration of METH (SAM) to those of yoked-METH (YM) and yoked-saline (YS) animals based on a modification of the operant-training method described by Fuchs et al. (2005) and See et al. (2007).

Materials and Methods

Animals. Male Sprague-Dawley rats (300–350 g; Charles River Laboratories, Raleigh, NC) were allowed to acclimate for at least 1 week before experimentation. For most studies, the rats to be used for self-administration were initially group-housed during food training (Fuchs et al., 2005) and then housed individually after catheter implantation. These rats were killed 6 h after the final fifth self-administration session. In the study represented in Fig. 1, some rats received a single noncontingent administration of 10 mg/kg s.c. METH and were killed 12 h later. To study the effect of lever-

Fig. 1. METH-induced increases in the NT levels of the striatum after both noncontingent and contingent drug treatment. The noncontingent rats received one METH injection (10 mg/kg/injection of METH or saline s.c.) and were sacrificed 12 h after treatment. In contrast, the contingent SAM and YM rats received >3.0 mg i.v. of METH over five sessions in the operant chambers and were killed 6 h after the final session. Values represent the mean percentages of respective controls (Saline or YS) $\pm$ S.E.M. ($n = 7-10$). *, $P < 0.05$ versus respective saline or YS and YM. **, $P < 0.05$ versus YS.

pressing behavior for food, rats were exposed to either active levers that delivered a food pellet when appropriately pressed (food-trained rats) or to inactive levers (control rats). See *Food Training* for more details. These animals were also killed 6 h after the fourth daily session.

Drugs and Chemicals. Methamphetamine hydrochloride was furnished by the National Institutes of Health National Institute on Drug Abuse (Bethesda, MD), and infusion quantities were calculated as the free base. In the experiments to study the DA receptor mechanisms underlying the NT responses to contingent METH treatments, yoked-METH rats were pretreated by intraperitoneal injections of 0.5 mg/kg concentrations of either the D1- (SCH23390) or D2- (eticlopride) receptor antagonists. This pretreatment has been used previously by us (Merchant et al., 1989b; Wagstaff et al., 1996a,b) and others (Castel et al., 1994; Beauvais et al., 2010) in noncontingent studies to determine the selective role of these two DA receptor subtypes in the pharmacological actions of METH. These drugs were administered 15 min before each of the five METH self-administration sessions.

In a separate study, the effect of stimulating and blocking the NT receptors on METH SA was examined by administering a NT agonist [Lys(CH₂NH)Lys-Pro,Trp-*tert*-Leu-Leu-Oet (PD149163); obtained from the National Institutes of Health National Institute of Mental Health], a stable NT fragment (NT[8-13]) that stimulates central nervous system NT1 receptors when delivered systemically (0.5 mg/kg s.c.; Feifel et al., 2008), and an antagonist [0.3 mg/kg 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonylamino]tricyclo(3.3.1.1^{3,7})decan-2-carboxylic acid (SR48692); purchased from Tocris Bioscience, Ellisville, MO] (Antonelli et al., 2007; Wagstaff et al., 1994) before day 5 (PD149163) or days 5 to 7 (SR48692) of METH SA. Follow-up studies suggested that PD149163 alone did not impair motor functions because the performance of rats on the rotor rod, treated with this NT agonist, was identical to saline-treated animals at 6, 9, and 12 rpm (i.e., no falls from the rotating rod regardless of revolutions per minute rate; $n = 6$ rats). We observed that eating behavior of both saline- and PD149163-treated rats were identical after returning to their home cage after the sessions (note: during these sessions, both groups gained an average of 10 to 20 g on this feeding regimen). In addition, the observation that the PD149163 compound alone did not increase motor behavior such as METH administrations suggests that NT agonists are not stimulants and do not have pharmacological prop-

erties such as METH. This is consistent with previous reports that 1) pretreatment with intracerebroventricular NT blocks stimulant (including amphetamine)-induced locomotion (Norman et al., 2008); 2) NT receptor stimulation blocks stimulant-induced DA release (Liang et al., 2008); and 3) NT receptor knockouts have hyperactivity (Liang et al., 2010).

Food Training. Because we have observed that, in our treatment paradigm, food training predicted an animal's ability to acquire drug self-administration, all of the rats used were required to pass food training as described previously (Fuchs et al., 2005; See et al., 2007). In brief, rats were restricted to approximately 85% of their free-feeding food quantity and then placed in operant chambers connected to a PC computer running Graphic State software (Coulbourn Instruments, Whitehall, PA). Each chamber was equipped with two retractable levers, a food pellet dispenser between the levers, and a house light on the wall opposite the levers. One lever was the "active" lever resulting in the delivery of a food pellet, whereas the other lever had no programmed consequences. Training consisted of a schedule of food reinforcement (45 mg of Rodent Grain food pellets; Bio-Serv Delivering Solutions, Frenchtown, NJ) at FR 1, with only the stimulus-appropriate (drug lever) eliciting the reward. If during the overnight food-training session (15 h), a rat received 50 pellets, the FR was increased to 2. If the rat obtained another 50 food pellets, the FR increased to 3 for the balance of the session. Rats remained in the food-training phase for either 4 days or until they achieved two consecutive sessions on the FR 3 schedule, after which they received a jugular vein catheter implant (approximately 90% of the rats successfully completed food training and on average press the active lever from 800 to 1000 times during the final session).

Catheter Implantation. After food training, rats were anesthetized with equithesin (intraperitoneally) and indwelling catheters consisting of a screw-type connector, Silastic tubing (1.19 mm; i.d., 10 cm, o.d., 0.64 mm). Prolite polypropylene monofilament mesh and cranioplastic cement were implanted beneath the skin of the back (at the shoulder blades). The outlet of the catheter ran subcutaneously around the underside of the animal, with the end inserted into the right jugular vein. The catheter was secured to the surrounding tissue with sutures. A 0.1-ml antibiotic solution contained cefazolin (10.0 mg/ml) dissolved in heparinized saline (70 U/ml; Sigma-Aldrich, St Louis, MO) was flushed through the catheter for 3 days after surgery to extend catheter patency. Thereafter, catheters were flushed with 0.1 ml of heparinized saline before and after each self-administration session to prevent clotting (Frankel et al., 2008). Stylets were inserted into the catheters when rats were not connected to infusion pumps. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee and adhered to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

METH Self-Administration Training. Operant training was based on procedures as described previously (Fuchs et al., 2005; See et al., 2007). Self-administration sessions were conducted during the light cycle; however, animals were exposed to a 14-h/10-h light/dark environment while in their home cages. Each SAM rat underwent 4-h sessions in Coulbourn operant chambers for 5 consecutive days and were exposed to the presentation of an identical right and left lever. One of the levers was selected as active, such that appropriate pressing resulted in a primary stimulus of METH infusion (0.06 mg/infusion) followed by lever retraction for a 20-s timeout period until subsequent presentation of the levers. The SAM rats selectively pressed the active lever >90% of the time. Data collection and reinforcer delivery were controlled by a PC computer using Graphic State Notation (Coulbourn Instruments). Before initiation of self-administration training, each SAM rat was randomly paired with two yoked rats. The yoked animals were prepared and treated identically as the SAM animals, with the exception that both levers in the operant chamber had no programmed consequences. Furthermore, these yoked animals received either METH (0.06 mg/infusion; YM) or saline (equal volume; YS) at times determined by the behavior of

the SAM rats. After each self-administration session, all rats were returned to their home cages and given access to 6 g of rat chow (Purina, St. Louis, MO). After 1 day of METH self-administration at an FR 1, the FR was increased within the session to an FR 2 after 25 presses, an FR 3 after 30 presses, and finally an FR 5 after 45 presses (rats typically reached the FR 5 stage toward the conclusion of the second session of acquisition and remained throughout the remainder of the study). For these studies, SAM rats were considered to have reached criterion if they maintained at least 3 consecutive days of relatively steady lever-pressing behavior (with at least 0.6 mg/kg/day METH infusions and self-administered a total of at least 3 mg of intravenous infusion of METH for the experiment; this was typical of 80% of our SAM animals). Six hours after the fifth self-administration session, the rats were killed, and brains were removed and frozen on dry ice.

Analysis of Neuropeptide Levels in Tissue. Levels of NT, dynorphin (DYN), and Met-enkephalin were determined by specific and sensitive RIAs as described previously (Frankel et al., 2007). The striatum and the substantia nigra were dissected (Paxinos and Watson, 1982) and frozen at -80°C until assayed for the appropriate neuropeptide. Typical striatal peptide levels (picograms per milligram of protein) in control animals were 110 (Fig. 1) and 78 (Fig. 5) for NT and DYN, respectively; typical nigral peptide levels (picograms per milligram of protein) in control animals were 331 (Fig. 1) and 231 (Fig. 5), respectively. To facilitate comparisons, data were normalized by dividing with respective control mean values and expressed as the percentage of control.

Statistical Analysis. The data presented are means $\pm$ S.E.M. All data were analyzed using the SAS 9.1 program (SAS Institute, Inc., Cary, NC). For analysis of NT and DYN levels, three or more groups were compared by one-way analysis of variance followed by a Newman-Keuls post hoc test or Student's *t* test. Two groups were assessed using a Student's *t* test. Pressing behaviors were analyzed using a repeated measures analysis of variance. A test of sphericity was performed on repeated measures factors to ensure symmetry of the variance-covariance matrix. The Huynh-Feldt correction was used for any matrices that were significantly nonspherical. All results were considered significant when $p < 0.05$.

Results

Rats that received saline infusions (YS), averaged 19 ± 12 lever presses, on what had been the active lever for food training, during their first session of being yoked to SAM animals. The average lever-pressing behavior dropped off to $4 \pm 5 \pm 3$ presses/session for the fourth and fifth sessions before sacrifice. For the SAM rats, the pattern of lever pressing from days 1 to 4 is shown in Fig. 4 (note: Figs. 2 and 3 show rats that had 5 days of self-administration with the lever-pressing patterns for days 1 to 4, similar to that shown in Fig. 4 and day 5 activity = 38 ± 3 pushes). Notable is the finding that the SAM animals maintained a relatively stable pattern of METH self-administration for sessions three to five.

To assess the METH-induced responses of NT systems associated with the basal ganglia, the tissue levels of this neuropeptide were measured in the dorsal striatum of rats that noncontingently received one subcutaneous injection of METH (10 mg/kg per administration). As reported previously (Letter et al., 1987), investigator-administered METH increased the striatal level of NT ($t_{11} = 3.22$, $p < 0.01$; Fig. 1). To compare this METH effect with that in rats that self-administered this stimulant, NT levels of the striatum were measured in animals that received intravenous METH infusions in response to appropriate lever-pressing behavior (la-

Fig. 2. METH-induced increases in striatal NT content of YM versus YS rats were blocked by pretreatment with the D1 (SCH, SCH23390), but not D2 (Etic, eticlopride), antagonist. Exposure to patterns of intravenous infusions of METH (YM), identical to that determined by a yoked SAM, significantly elevated the NT content but not as much as in the corresponding SAM animals. The eticlopride treatment in YS rats elevated NT levels in this brain region. These two treatments together had an additive effect on the NT response (YM+Etic). $n = 7-17$. Values represent mean percentages of YS $\pm$ S.E.M. *, $P < 0.05$ versus all other groups and each other.

Fig. 3. METH-induced increases in nigral NT of YM versus YS rats were blocked by pretreatment with the D1 (SCH, SCH23390), but not D2 (Etic, eticlopride), antagonist. Exposure to patterns of intravenous infusions of METH (YM), identical to that determined by a yoked SAM, significantly elevated the nigral NT content but not as much as in the corresponding SAM animals. Values represent mean percentages of YS $\pm$ S.E.M. ($n = 7-16$). *, $P < 0.05$ versus all other groups. **, $P < 0.05$ versus all other groups but not each other.

beled contingent SAM in Fig. 1). As with the noncontingent drug treatment, NT tissue content in the SAM animals was significantly elevated versus YS animals ($F_{2,25} = 24.76$, $p < 0.001$). A similar but significantly less increase in striatal NT levels was also found in the corresponding YM group. To confirm that the changes in striatal NT levels were due to exposure to METH and not to food training itself, we measured NT levels in the striatum of rats killed 6 h after the final session of food training. We observed that animals exposed to food training (61 ± 8 pg of striatal NT/mg protein) versus those that were exposed to inactive levers (91 ± 7 pg of striatal NT/mg protein) did not manifest an increase but rather a decrease in striatal NT levels ($t_{14} = 2.26$, $p < 0.05$), confirming that the elevation in NT levels in SAM and YM

Fig. 4. Effect of pretreatment with the NT agonist PD149163 (PD) or antagonist SR48692 (SR) on lever-pressing behavior in SAM rats. The PD compound was administered 15 min before the fifth session ($n = 7$), whereas the SR compound was administered 15 min before each of sessions 5–7 ($n = 8$). Activation of NT receptors significantly, but temporarily, reduced lever-pressing behavior versus that occurred on the day 4 of self-administration (*, $P < 0.05$) ($n = 15$); however, blockade of NT receptors had no significant impact.

rats was related to the METH exposure and not food training per se.

We next investigated the mechanism underlying the effect of METH self-administration on striatal NT systems (Fig. 2). Because it has been reported that the lever-pressing behavior of rats self-administering METH or related drugs is altered by D1 and D2 antagonists (Brennan et al., 2009), we avoided this confound by employing an indirect approach for these studies; this was done by pretreating the YM rats with either a D1 (SCH23390) or D2 (eticlopride) antagonist before each of the five sessions of METH infusions, which were determined by the lever-pressing behavior of linked SAM animals. As demonstrated in Fig. 1, we again observed elevated NT levels in both SAM+Sal and YM+Sal rats ($F_{6,71} = 51.07$, $p < 0.001$), which had been pretreated with saline, with the effect in the SAM group being greater. The YM effect compared with the YS group was blocked by a SCH23390 pretreatment. In contrast, the eticlopride pretreatment itself elevated striatal NT content (YS+etic), an effect that was additive with the METH-induced increase (YM+etic). These findings suggest that D1, but not D2, receptors are involved with the effects caused by METH self-administration on the dorsal striatal NT systems.

Because the findings in Fig. 2 suggested that the NT response to METH self-administration was D1-mediated, it is possible that the D1-dependent striatonigral pathway played an important role in this response. To test this possibility, we also examined NT levels in the substantia nigra (Fig. 3) in a manner similar to that described above for Fig. 2. As with the striatal NT response, we observed that nigral NT levels were also increased in both the SAM and YM rats compared with the YS animals ($F_{6,94} = 15.68$, $p < 0.001$), with the effects being greater in the SAM tissues. In addition to the similarity to the striatal responses, the results revealed that the nigral NT changes in the YM versus YS rats were prevented by D1, but not D2, receptor antagonism. In contrast to the striatal response, eticlopride alone had no significant effect on nigral NT content, thereby supporting the conclusion that

the striatonigral NT system is particularly linked to D1 and not D2 systems.

Although these findings demonstrate that basal ganglia NT pathways are altered by METH self-administration through D1 mechanisms, they do not provide much insight into the functional significance of these elevations in NT tissue levels. To this end, we determined the consequence of either activating or blocking the NT receptor on the level-pressing activity of SAM rats (Fig. 4). This was achieved by administration of either a NT receptor agonist (PD149163) or antagonist (SR48692) 15 min before placement of SAM rats into their respective operant chambers on either day 5 or days 5 to 7 of METH self-administration, respectively. Stimulation of NT receptors on day 5 reduced pressing by the SAM rats on the active lever from 39 ± 5 to 6 ± 3 , whereas blockade of these receptors had no significant effects on lever-pressing behavior in SAM rats on days 5 to 7 ($F_{6,72} = 4.84$, $p < 0.001$).

Finally, to test the selectivity of the NT response to METH self-administration, we measured the effect of this contingent behavior on the basal ganglia tissue levels of DYN and Met-enkephalin. These are neuropeptides that are associated selectively with either the D1-related striatonigral pathway (Jiang et al., 1990) or the D2-related striatopallidal pathway (Durieux et al., 2009), respectively, and we determined their striatal and nigral content in SAM, YM, and YS rats (Fig. 5). The DYN content in both the striatum and substantia nigra was elevated in SAM rats (striatum: $F_{2,26} = 15.16$, $p < 0.001$; substantia nigra: $F_{2,25} = 8.77$, $p < 0.01$). In the substantia nigra the DYN levels were also elevated in the YM animals. In contrast, no significant changes were observed in the striatal Met-enkephalin levels of either SAM or YM rats ($F_{2,24} = 1.00$, ns; data not shown).

Discussion

Neurotensin in the basal ganglia is associated with striatal medium spiny efferent neurons that project to both the substantia nigra and globus pallidus (Gerfen et al., 1990; Castel et al., 1994) and probably contributes to the regulation of DA-related basal ganglia function. Although little is known about its precise role, studies have suggested that NT has an

inhibitory influence on the stimulatory behavioral effects of psychostimulants (Wagstaff et al., 1994; Chartoff et al., 2004; Feifel et al., 2008), possibly through an indirect mechanism involving increased striatal GABA activity and subsequent reduced DA release (Ferraro et al., 1998). This inhibitory action has been speculated to be of potential clinical value, suggesting that activation of NT receptors may have a neuroleptic action and be useful in the management of conditions of excessive DA activity (Chartoff et al., 2004; Feifel et al., 2008).

Although the role of NT in regulating striatal DA function requires further elucidation, more is known about how changes in DA activity influence the striatal efferent NT systems. These studies have identified variations in striatal and nigral NT levels in response to activation of DA receptors, either directly by selective agonists (Merchant et al., 1989a,b) or indirectly by psychostimulants such as METH (Letter et al., 1987). This research has found that stimulation of D1 receptors or administration of high doses of METH elevates tissue levels of NT in the striatum and substantia nigra. In contrast, activation of the D2 receptor with a selective agonist or administration of a low dose of METH decreases striatal neurotensin content (Merchant et al., 1989b; Wagstaff et al., 1996a).

Dopamine-related changes in the NT systems of the basal ganglia have also been monitored by assessing the synthesis of this neuropeptide through measurements of expression for the mRNA of its NT/neuromedin N precursor. Such studies demonstrated that changes in DA receptor activity caused by either selective direct D1-receptor agonists or indirect agonists such as METH significantly increase the levels of striatal NT mRNA and that the elevated synthesis probably contributes to increases in the associated NT tissue content (Castel et al., 1994; Hanson and Keefe, 1999).

Taken together, these studies suggest that activation of D1 receptors, either by a selective D1 agonist or a high dose of METH, influences NT signaling and synthesis, increasing tissue accumulation of this peptide. In contrast, D2 receptor activation seems to have an opposite effect (i.e., it decreases striatal NT content). To better appreciate the interaction between basal ganglia NT and DA systems, especially after METH administration, it is necessary to identify how the actual release of NT is affected by METH treatment. To this end, Wagstaff et al. (1996a,b) and Frankel et al. (2005) reported that NT release is increased in striatum and substantia nigra after a low but not high dose of METH that is required to elevate striatal NT levels. Taken together, these findings suggest that investigator-controlled administration of high doses of METH does not change NT release patterns per se but does increase synthesis and levels of NT in the striatonigral pathway.

As mentioned previously, until the present report, all of the studies of METH-induced NT changes in the basal ganglia have been based on noncontingent models. As described above, we and others have elucidated many of the elements of this interaction; however, whereas these findings have helped us to better understand the pharmacology of this potent stimulant, its link to the critical issues related to human abuse is unclear. Thus, the relevance of these NT responses to important drug-dependent elements, such as drug wanting, anticipation, contingent administration, and extinction, are unknown. The design of the present METH

Fig. 5. Effect of METH self-administration on striatal and nigral dynorphin levels. Increases in striatal and nigral DYN were observed in SAM rats, but in YM animals, a significant elevation only occurred in the substantia nigra. *, $P < 0.05$ versus YS and YM in the striatum and versus YS in the substantia nigra ($n = 9-10$).

self-administration study was intended to begin addressing these important questions. As described under *Results*, the rats behaved as expected in that previously food-trained animals manifested lever-pressing extinction very quickly once placed in the operant chambers when their learned operant behavior no longer was associated with a reward or any consequence. It was also observed that those SAM rats that received METH infusions, contingent to appropriate operant responding, stably pressed the lever to receive a consistent daily dose of METH per session during the final 3 days of self-administration.

The data in Fig. 1 validate previous reports that the immediate pharmacology of high doses of noncontingently administered METH increases NT levels in basal ganglia areas (Letter et al., 1987). In addition, they extend this well established finding to suggest that these NT systems are associated with elements of METH self-administration and might contribute to basal ganglia functions, such as the transferring and processing of information in motor, cognitive, and limbic domains associated with reward-related activity (Tisch et al., 2004). In particular, the finding that changes in striatal and nigral NT systems were greater in SAM rats than the corresponding YM animals suggest that these NT systems are somehow linked to altered DA activities in the respective systems related to learning a response to the positive reinforcer METH.

The fact that METH self-administration elevated dorsal striatal NT levels suggested that the mechanism for this NT response was activation of D1 receptors as a result of METH-induced release of DA associated with the nigrostriatal pathway (Castel et al., 1994). This possibility was tested by pretreating rats before METH exposure, with either a D1 (SCH23390) or D2 (eticlopride) antagonist. Because it has been reported that D1 and D2 antagonists alter the lever-pressing pattern (and consequently the amount of METH administered) in SAM animals (Brennan et al., 2009), we administered the antagonists before the sessions for the YM group to avoid this confound. The results in Fig. 2 supported the hypothesis that the striatal NT effects were mediated by D1 and not D2 receptors. Because previous noncontingent studies demonstrated that D1-mediated elevation in striatal NT tissue content is due to activation of NT synthesis and apparently not associated with release of NT per se (Castel et al., 1994; Wagstaff et al., 1996b), we tested whether this was true in our self-administration model. This was accomplished by determining the lever-pressing pattern of SAM animals that were pretreated with either a NT agonist or antagonist on day 5 or days 5 to 7 of self-administering METH, respectively (Fig. 4). We found that stimulation of NT receptors with the PD149163 almost completely eliminated lever-pressing behavior. Although lever-pressing behavior was still diminished the next day, it returned to normal levels by day 7. This confirmed conclusions by other investigators and us that activation of this NT system has a general inhibitory influence on METH-mediated behaviors that have a DA basis, and we now know that this probably includes some factors that are associated with drug self-administration (Wagstaff et al., 1994; Nicola 2007; Feifel et al., 2008). Pretreatment with the NT antagonist (SR48692) before days 5 to 7 enabled us to assess the state of endogenous NT release during METH self-administration. Because blockade of the NT receptor did not significantly alter the lever-pressing behavior of the SAM rats,

we conclude that NT release did not influence the METH self-administration patterns under these conditions. This finding suggests that, similar to high doses of METH, the D1-mediated elevation in NT tissue levels observed in SAM rats did not reflect changes in NT release and are most probably due to an increase in NT mRNA expression as discussed above.

Another conclusion that is implied by these findings is that the NT system primarily responsible for these effects of METH self-administration is probably associated with the striatonigral pathway because of the dominant D1 linkage with this efferent projection. This was confirmed by the finding that nigral NT levels were elevated in both SAM and YM rats, with the SAM effect being greater, and the finding that this response was also selectively blocked in the YM rats by pretreatment with SCH23390 but not eticlopride (see Fig. 3). In addition, our observation that METH self-administration elevated striatal and nigral DYN (Fig. 5), but not striatal Met-enkephalin, levels may be due to a selective action on the striatonigral but not the striatopallidal pathways.

In summary, the present findings support the hypothesis that the striatonigral NT system is involved in mediating responses to METH self-administration and may contribute to elements that influence METH dependence. In addition, these somewhat selective NT responses seem to be associated specifically with increases in D1 receptor activity related to the striatonigral pathway and confirm the relevance of much of the previous noncontingent METH studies to issues of dependence and addiction. Finally, under these circumstances, the increases in NT tissue levels during METH self-administration do not seem to be related to an increase in NT release, given our observation that a NT antagonist does not significantly alter lever-pressing behavior in the SAM animals, even though stimulation of the NT receptor with an agonist suppresses this operant behavior in these animals.

Authorship Contributions

Participated in research design: Frankel, Hoonakker, Alburges, McFadden, Fleckenstein, and Hanson.

Conducted experiments: Frankel, Hoonakker, Alburges, and McDougall.

Performed data analysis: Frankel, Hoonakker, McFadden, and Hanson.

Wrote or contributed to the writing of the manuscript: McFadden, Fleckenstein, and Hanson.

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