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Responses of the Rat Basal Ganglia Neurotensin Systems to Low Doses of Methamphetamine

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Abstract

Rationale—Administration of high doses of methamphetamine (METH) in a manner mimicking the bingeing patterns associated with abuse, reduces NT release and causes its accumulation and elevated NT levels in extrapyramidal structures by a D1 mechanism. The relevance of these findings to the therapeutic use of METH needs to be studied.

Objectives—The effect of low doses (comparable to that used for therapy) of METH on basal ganglia NT systems was examined and compared to high-dose and self-administration effects previously reported.

Methods—Rats were injected four times (2-h intervals) with either saline or low doses of METH (0.25, 0.50 or 1.00 mg/kg/s.c.). For the DA antagonist studies, animals were pretreated with a D1 (SCH23390) or D2 (eticlopride) antagonist 15 min prior to METH or saline treatments. Rats were sacrificed 5–48 h after last injection.

Results—METH at doses of 0.25 and 0.50, but not 1.00 mg/kg rapidly and briefly decreased NTLI concentration in all basal ganglia structures studied. In the posterior dorsal striatum, the

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reduction in NT level after low-dose METH appeared to be caused principally by D2 stimulation, but both D2 and D1 stimulation were required for the NT responses in the other basal ganglia regions.

Conclusions—A novel finding from the present study was that opposite to abuse-mimicking high doses of METH, the therapeutically relevant low-dose METH treatment reduced NT tissue levels likely reflecting an increase in NT release and a short-term depletion of the levels of this neuropeptide in basal ganglia structures. The possible significance is discussed.

Keywords

dopamine antagonists; methamphetamine; neurotensin

Introduction

Even though methamphetamine (METH, Desoxyn) can be legally prescribed to treat attention deficit disorder and obesity (RxList 2013), nonmedical high-dose use of METH has been associated with major problems due to its addictive, emotional, and social effects (Meredith et al. 2005; Baldessarini et al. 1996). In order to understand the mechanistic difference between the therapeutic versus abuse use of this stimulant, it is important that the dose-response central nervous system (CNS) effects of this potent psychoactive stimulant be understood. To this end, we have studied the role of DA-regulating neuropeptides in mediating the effects of psychostimulants such as METH. For example, feedback neuropeptide systems, such as neurotensin (NT) pathways, associated with the nigrostriatal DA pathways are altered by noncontingent high doses of METH treatment that mimic bingeing abuse patterns (Letter et al. 1987a, b; Merchant et al. 1988; Hanson et al. 1989; Gygi et al. 1994; Castel et al. 1994a, b). This is important because NT is a tridecapeptide that has been shown to antagonize the behavioral effects of commonly abused psychostimulants, such as METH by interfering with the release of DA caused by these stimulants (Nemeroff et al. 1983; Wagstaff et al. 1994).

There is also evidence that altered activity of DA pathways caused by high doses of the potent psychostimulants substantially impact NT systems by increasing NT levels in basal ganglia structures (Letter et al. 1987a; Hanson et al. 1989; Gygi et al. 1994; Castel et al. 1994a, b). Thus, its apparent interaction with dopaminergic systems suggests that NT influences the addictive properties of these psychostimulants under high-dose conditions. Of particular relevance to the current study, our understanding of how NT systems contribute to METH effects has for the most part been based on animal models that employed noncontingent drug administration and analysis of the CNS NT tissue contents (Letter et al. 1987a, b; Merchant et al. 1989a, b), synthesis (Adams et al. 2001), and release (Wagstaff et al. 1996a, b; Frankel et al. 2005). These studies suggested that high doses (5–15 mg/kg) or heavy abuse of METH: (i) reduce release of NT in basal ganglia (Wagstaff et al. 1996a; Hanson et al. 1992); and (ii) increase NT tissue levels by increasing NT synthesis and accumulation (Adams et al. 2001; Letter et al. 1987a; Castel et al. 1994a, b). Recently, the response of these same NT systems to self-administration of METH (contingent on lever pressing and a model for METH dependence) has also been reported (Frankel et al. 2011; Hanson et al. 2012). It was observed that like the response to high doses of noncontingent

METH, NT levels in the basal ganglia structures also increased in rats trained to lever press for METH for 7–15 days. The NT responses were blocked by a pretreatment of a D1, but not a D2, antagonist after both the high-dose noncontingent and self-administration METH treatment paradigms. Consistent with the conclusion that these METH effects are mediated by D1 mechanisms were the findings that like the high-dose of METH, selective D1 agonists also increased the basal ganglia NT levels (Merchant et al. 1989b).

For comparison, we are now studying how the basal ganglia NT systems respond to low doses of METH which are more likely to reflect what would be administered when using METH therapeutically. Thus, the current study examines in detail the changes in NT systems throughout the basal ganglia 5–48 h after a METH low-dose range of 0.25–1.0 mg/kg and analyzes the DA-related mechanisms of the NT responses. These studies determined for the first time that noncontingent low-dose METH treatments have effects opposite of that caused by high-dose METH in that they reduced NT levels throughout the basal ganglia in a rapid, but brief manner through a predominantly D2 mechanism.

Materials and methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) weighing 250–330 g were maintained in a temperature-controlled environment and cared for according to NIH guidelines. Animals were kept on a 12-h light/dark cycle with food and water available *ad libitum*. Animals were allowed to acclimate for at least 2 weeks before their use. All the experiments were performed according to the guidelines of the University of Utah Institutional Animal Care and Use Committee.

Drug treatment and tissue dissection

Following the acclimatization period, two experimental protocols were used in this study. First, animals were injected four times (2-h intervals) with either saline (1.0 mL/kg, s.c.), or METH (0.25, 0.50 or 1.00 mg/kg, dose, s.c.). The selection of low doses of METH was based on previous studies that indicated that a single low-dose administration of METH (0.5 mg/kg, s.c.), increased the release of NT, while a high dose of METH (5–10 mg/kg) either did not alter, or decreased, the extracellular NT levels in the dorsal striatum (Wagstaff et al. 1996a). All rats were sacrificed 5–48 h after the last injection as this has been shown to be an appropriate period of time to cause changes in release or accumulation of NT after METH treatment (Castel et al. 1994a, b; Merchant et al. 1994; Hanson and Keefe 1999; Letter et al. 1987a, b; Wagstaff et al. 1996a). For one experiment, animals were pretreated with a D1 (SCH 23390; 0.5 mg/kg/injection, i.p.) or a D2 (eticlopride; 0.5 mg/kg/injection, i.p.) antagonist 15 min prior to a 0.25 mg/kg dose of METH or saline and killed by decapitation 5 h after treatment.

After drug treatments, brains were removed rapidly, frozen immediately on dry ice and stored at –80 °C until dissected and analyzed. For regional studies, brain areas were dissected from consecutive 1-mm thick coronal slices as previously described (Alburges et al. 2001a, b). Based on the atlas of Paxinos and Watson (1986), the anterior and posterior

dorsal striata, globus pallidus and substantia nigra were removed and subsequently stored at -80°C until assayed for NT levels.

Drugs

Eticlopride hydrochloride (S(-)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride) and SCH 23390 hydrochloride (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine hydrochloride) were acquired from Sigma-Aldrich (St. Louis, MO). Methamphetamine hydrochloride was generously supplied by the National Institute on Drug Abuse, National Institute of Health (Bethesda, MD). All doses were calculated as freebase of the drug and were freshly prepared in physiological saline solution [0.9 % (w/v) NaCl, pH 7.4].

Radioimmunoassay

The solid-phase radioimmunoassay used to analyze the neuropeptide concentrations in this study was adapted from the methods previously described (Maidment et al. 1991; Alburges et al. 1999). Tissue samples were homogenized in 300 μL 0.01 N HCl. The resulting homogenate was then placed in boiling water for 10 min in order to inactivate peptidases (these are standard analytical procedures for measuring protein in solution or after precipitation with acid or other agents see Bradford 1976). Homogenates were centrifuged ($17,000 \times g$) for 30 min. Supernatant was then collected and an aliquot was used to determine the total protein for each tissue sample by the method of Bradford (1976). The remaining sample was lyophilized overnight and stored at -80°C until the radioimmunoassay was performed. The tissue concentrations of neuropeptides were determined with a modified solid-phase radioimmunoassay technique previously described (Maidment et al. 1991; Alburges et al. 1999). Lyophilized samples were reconstituted in 300 μL phosphate-buffered saline (pH 7.4) containing 0.1% (w/v) gelatin and 0.1% (v/v) Triton X-100. Nunc-Immunoplates (ISC BioExpress, Kaysville, UT) were incubated overnight at 4°C with 50 μL of protein G solution (50 ng/100 μL in 0.1 M sodium bicarbonate; pH 9.0). After washing the wells three times with wash buffer [0.15 M K_2HPO_4 , 0.02 M NaH_2PO_4 , 0.2 mM ascorbic acid, 0.2% (v/v) Tween-20 and 0.1% (w/v) sodium azide; pH 7.5], 25 μL of a highly selective antiserum for neurotensin (diluted to 1:20,000) was diluted in assay buffer [same as wash buffer containing 0.1% (w/v) gelatin].

Wells for NT assays were incubated with the antisera for 4 h at room temperature in order to allow the attachment of antibody to the protein G-coated surface. After incubation, wells were washed three times and 25 μL of sample or standard(s) were added to each well and incubated overnight at 4°C . After incubation, 25 μL of the labeled NT (^{125}I NT) diluted with assay buffer to approximately 6500 dpm per 25 μL , were added to the wells and incubated for 2 h at room temperature. After incubation, wells were washed, separated and placed in 12 $\times$ 75-mm polypropylene tubes and counted in a five-channel Packard Cobra II Auto-Gamma counter (Packard Instrument Co., Meriden, CT). The total and nonspecific binding were defined by adding 25 μL of the labeled peptide to protein G-untreated and -treated wells, respectively. Determinations of the quantities of NT were made by comparing bound to free ^{125}I NT in each sample to a standard curve (from 1 to 125 pg/assay tube). The reproducibility of the assay was evaluated using cerebellum tissue spiked with 62 and

250 pg of the peptide. This technique has been demonstrated to be very reproducible, resulting in less than 10% variability between assays and less than 5% between sample and standard duplicates with a uniform NT recovery from 77% to 93%.

Antisera

The NT antiserum was raised in New Zealand White rabbits as previously described (Letter et al. 1987b). The highly selective antiserum recognizes the NT carboxy terminus (~ 80% cross-reactivity with the 8–13 carboxyl-terminal fragment of NT) and does not cross-react with 1000-fold excess concentration of other endogenous neuropeptides such as dynorphin A, met-enkephalin, cholecystokinin or substance P. Consequently, our measurements are referred to as picograms per milligram of protein of neurotensin-like immunoreactivity (NTLI).

Data analysis

Results from these experiments are expressed as percentages of their respective controls in order to facilitate comparisons between groups (mean values $\pm$ SEM). The average control values of NTLI (pg/mg of protein) for regions examined were 291 ± 43 (anterior dorsal striatum), 285 ± 61 (posterior dorsal striatum), 468 ± 47 (globus pallidus), and 598 ± 44 (substantia nigra). Differences between means were analyzed using one-way analysis of variance followed by Newman-Keuls post hoc test. Differences were considered significant when the probability that they were zero was less than 5%.

Results

Dose-response of basal ganglia NT systems to METH low-dose treatments

The responses of NT systems in basal ganglia structures were determined by measuring NTLI tissue levels 5 h after multiple administrations of 0.25, 0.50 or 1.00 mg/kg/injection, s.c. The NTLI content was significantly decreased in anterior ($F[3,33]=7.97$) and posterior ($F[3,33]=6.05$) dorsal striatum, substantia nigra ($F[3,33]=8.50$) and globus pallidus ($F[3,35]=8.12$) after 0.25 and 0.5 ($P<0.05$), but not 1.0 mg/kg METH (Figs. 1a, b, c, d, respectively).

Recovery of basal ganglia NT systems to low-dose METH

Because the most consistent and robust response to the low-dose METH treatment tended to occur after the 0.25 mg/kg/injection (s.c.), this dose was used to assess the temporal NT basal ganglia effects under low-dose METH conditions: i.e., rats were killed 5 and 24 h after METH treatment. As reported in Fig. 1, the NTLI concentrations in anterior dorsal striatum ($F[3,31]=7.37$), posterior dorsal striatum ($F[3,24]=6.90$), globus pallidus ($F[3,32]=5.58$), and substantia nigra ($F[3,30]=12.94$) were significantly decreased 5 h following METH administration ($P<0.05$); however, the NTLI concentrations in these brain regions no longer differed from their respective control, 24 h after drug treatment (Fig. 2a–d). We also checked the NT response 48 h after METH exposure and found no significant difference from their saline controls (data not shown).

Effects of D1 or D2 receptor blockade on NT response to low-dose METH treatment

Pretreatments with D1 or D2 antagonists were used to elucidate the role of DA receptors in the response by basal ganglia NT systems to low-dose METH treatments (0.25 mg/kg METH). As observed before, this low-dose METH treatment significantly reduced NTLI levels ($P<0.05$) in anterior ($F[5,51]=7.76$) and posterior ($F[5,54]=6.06$) dorsal striatum, globus pallidus ($F[5,54]=4.51$) and substantia nigra ($F[5,52]=4.63$) (Figs. 3 and 4). Alone, SCH 23390 had no significant effect on NTLI concentrations in any of the basal ganglia structures examined, whereas eticlopride increased the NTLI content in the anterior dorsal striatal region ($P<0.05$), but not in any other structure examined (Figs. 3 and 4) as previously reported with other D2 antagonists (Letter et al. 1987a; Merchant et al. 1988; Hanson et al. 1989; Alburges and Hanson 1999; Alburges et al. 2011). In general, blockade of the D2 receptor antagonized METH-induced reductions in all of basal ganglia structures (compare eticlopride-saline [E-S] vs. eticlopride-METH [E-M] groups). In contrast, blockade of the D1 receptor prevented the METH-induced effects in the anterior dorsal striatum, substantia nigra and globus pallidus, but not the posterior dorsal striatum (compare SCH23390-saline [SCH-S] vs. SCH23390-METH [SCH-M]).

Discussion

It has been demonstrated that basal ganglia NT systems are substantially altered by high-dose METH administrations that mimic binge taking associated with abuse of this stimulant. Consequently, there are several reported studies that demonstrate increases in tissue levels of NT in basal ganglia regions 18–24 h after 5–15 mg/kg of METH (Hanson et al. 1992; Merchant et al. 1988; Castel et al. 1994b). This METH-dependent increase in NT tissue levels has been associated with a reduction in NT release (Hanson et al. 1992) and an elevated synthesis of NT precursor as measured by *in situ* hybridization (Adams et al. 2001). Of clinical relevance, a similar increase in NT levels throughout the basal ganglia also occurs in rats that have self-administered METH through lever pressing during a daily 4-h session for ~5–15 days (Frankel et al. 2011; Hanson et al. 2012, 2013) suggesting that similar changes in basal ganglia NT systems and related reduced activity of associated NT pathways are linked with METH abuse and dependence. Both of these METH-related contingent and noncontingent increases in basal ganglia NT tissue levels appear to be mediated by increased D1 receptor activity (respectively Letter et al. 1987a and Frankel et al. 2011), a conclusion confirmed by the fact that treatment with a D1 agonist also elevates NTLI content in the dorsal striatum, globus pallidus and substantia nigra (Merchant et al. 1989b).

In contrast, although little research has been done to examine if low doses more relevant to therapeutic use of METH also have a significant impact on NT tissue levels, there are reports that a single administration of 0.5 mg/kg of METH increases NT release (elevated extracellular NTLI content) from regions of NT-related striatal efferent cell bodies (dorsal striatum) and terminals (globus pallidus and substantia nigra) (Wagstaff et al. 1994, 1996a; Frankel et al. 2005). The mechanism of this low-dose METH-induced NT release was reported to be linked with increased activity of D2 receptors in all tissues studied, in combination with increased activity of D1 receptors in some (Wagstaff et al. 1996b; Frankel

et al. 2005). These conclusions are consistent with observations that a D2 agonist generally elevates NT release throughout the basal ganglia with a corresponding reduction in striatal, pallidal and nigral NT tissue levels (Wagstaff et al. 1996b; Merchant et al. 1989b). Thus, it has been suggested that the consequence of the low-dose METH-induced extrapyramidal NT release is increased turnover of this peptide perhaps with a corresponding reduction in NT throughout the basal ganglia structures thereby reducing associated NT tissue levels (Wagstaff et al. 1996a). To assess this possibility, the current study has examined in detail the impact of low doses of METH on NT tissue levels in major basal ganglia structures.

The functional importance of understanding the dose-dependent response of NT systems to METH treatments relates to the feedback role of this neuropeptide in regulating the activity of basal ganglia DA pathways critical to drug dependence and psychiatric disorders and the potential therapeutic benefits of NT-targeted drugs (Tyler-McMahon et al. 2000; Cáceda et al. 2006; Norman et al. 2008; Liang et al. 2010; Briody et al. 2010; Nemeroff et al. 1977; Richelson et al. 2003). For example: (i) pretreatment with NT receptor agonists blocks stimulant-induced neurochemical and behavioral consequences (Norman et al. 2008; Briody et al. 2010); (ii) NT receptor knockout mice manifest DA-related hyperactivity (Liang et al. 2010); and (iii) blockade of NT activity by either a NT-selective antibody or receptor antagonist, increases effects of a low, therapeutically relevant, dose of METH suggesting that activated NT function mitigates DA responses to METH under these low-dose conditions (Wagstaff et al. 1994). Although the precise neurophysiological mechanism whereby NT pathways antagonize dopaminergic pathways is not currently well understood, there are some studies suggesting that NT indirectly regulates presynaptic dopaminergic transmission by increasing glutamate release followed by elevated GABA activity and a subsequent inhibitory action on DA transmission (Ferraro et al. 1998, 2007). Because of its apparent antagonistic suppression of DA activity, NT has been referred to as a natural neuroleptic (Hadden et al. 2005; Feifel et al. 2008) and activation of its receptors has been suggested to be useful in the management of the excessive DA activity associated with schizophrenia (Chartoff et al. 2004; Feifel et al. 2008).

In order to elucidate further how low doses of METH impact NT activity in the present study, we determined NT tissue content in basal ganglia structures after multiple administrations of METH. In a dose-dependent manner, METH reduced tissue NTLI in basal ganglia regions associated with both the cell bodies (anterior and posterior dorsal striatum) and terminals (globus pallidus and substantia nigra) of the NT-related striatal efferent pathways (Fig. 1a–d) 5 h after injections of 0.25 and 0.50, but not 1.0 mg/kg/ administration.

Studies designed to identify the time course of 0.25mg/kg METH-induced NT changes demonstrated that the changes in all basal ganglia structures examined after 5 h had returned to control levels by 24 h (Figs 2 a–d). This pattern of response suggests that reduction in NT levels is relatively rapid and short-term, consistent with the interpretation that these changes are caused by NT release previously reported to be caused by a low-dose of METH and subsequent depletion of the NT stores (Wagstaff et al. 1996a). The fact that NT tissue levels throughout the basal ganglia were also unaltered 48 h after the METH treatment suggests

that there was no long-term consequence (e.g., rebound) of this low dose of METH on these associated NT systems.

We employed pretreatment by selective D1 or D2 antagonists in order to identify the role of specific DA receptors in the reduced basal ganglia NT levels caused by low-dose METH treatment. We observed that eticlopride blocked the METH-induced NT changes in all basal ganglia structures examined; however, the D1 antagonist (SCH23390) blocked the METH effect in the anterior dorsal striatum, globus pallidus and substantia nigra, but not the posterior dorsal striatum (Figs 3 a, b and 4 a, b). These findings confirmed our conclusion that the reduced NT levels reflect the ability of low doses of METH to release NT and cause its depletion because as with the patterns of low-dose METH-induced release the effects are mediated by activation of D2 receptors in all structures, while the D1 receptor appears to play a role in most, but not all areas (Wagstaff et al. 1996a; Frankel et al. 2005).

The findings revealed that multiple administrations of low doses of METH cause rapid dose- and time-dependent decreases in NT tissue contents in basal ganglia structures and these effects likely reflect increased release and turnover of this neuropeptide: it is noteworthy that this is opposite the increase in NT tissue levels following high-dose METH treatments that are used to model drug abuse patterns. The clinical relevance is related to the fact that METH self-administration affects NT systems in a manner similar to the noncontingent high-dose treatments. Also of clinical relevance is the observation that the low-dose METH-mediated changes in the basal ganglia NT systems resemble the reduction in NT levels recently reported in METH self-administering rats during the early stages of extinction (i.e., lever-pressing transitions from administering METH to saline) (Hanson et al. 2013). This similarity suggests that during the early stages of extinction from METH self-administration, the effect on DA systems is similar to that caused by the therapeutically relevant low doses of this drug and is associated with elevated release of NT in the basal ganglia and its inhibitory feedback influence.

In summary, the potential clinical relevance of the current findings is based on how traditional therapeutic doses of METH (e.g., for ADHD) relate to our novel mechanistic low-dose findings. Because a normal therapeutic oral dose of METH ranges approximately from 5 to 15 mg for an adult (Drugs.com 2013), it is likely that resultant concentrations of this drug in the brain during such therapy approach those that occur after a s.c. injection of 0.25–0.5 mg/kg, rather than that after 5–15 mg/kg. Based on this assumption, it is possible that the responses of NT systems observed in this low-dose METH study (i.e., an increase in NT release and related feedback inhibition in the basal ganglia resulting in reduced NT tissue levels) are relevant to the therapeutic benefits of this drug, while in contrast the high-dose METH effects (reduced release of NT and related feedback inhibition resulting in increases in NT tissue levels) are more relevant to use patterns associated with the development of METH dependence and addiction.

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Fig. 1.

Dose-dependent effects of low doses of METH on NTLI content in anterior (a) and posterior (b) dorsal striatum, globus pallidus (c) and substantia nigra (d). Rats were administered 4 injections (2-h intervals) of either saline (SAL; s.c.) or METH (M; 0.25, 0.50 or 1.00 mg/kg/injection, s.c.) and killed 5 h after treatment. Results are expressed as percentages of respective SAL controls and represent mean values $\pm$ SEM. (N= 8–10). * $p < 0.05$ versus respective SAL or M-1.00 groups.

Fig. 2.

Temporal effects of a low-dose (0.25 mg/kg/administration) METH treatment on NTLI content in anterior (a) and posterior (b) dorsal striatum, globus pallidus (c) and substantia nigra (d). Rats were treated as described in Fig. 1 and killed 5 or 24 h following treatment. Results are expressed as percentages of respective saline controls (SAL-5h) and represent mean values $\pm$ SEM (N= 8–10). *p < 0.05 versus corresponding saline (SAL-5h).

Fig. 3.

Role of DA receptors in mediating changes in neurotensin levels of anterior (a) and posterior (b) dorsal striatum after treatment of 0.25 mg/kg METH. The rats received s.c. injections of 0.25 mg/kg/administration of METH (M) or saline (S) as described for Fig. 2, except 15 min prior to these treatments they received i.p. injections of saline (S-S, S-M), SCH23390 (SCH-S, SCH-M) or eticlopride (E-S, E-METH). Values represent the means $\pm$ SEM expressed as percentages of corresponding S-S controls (N= 8). * $P < 0.05$ versus corresponding S-S and E-M groups. ** $P < 0.05$ vs. corresponding S-M group. *** $P < 0.05$ versus corresponding S-S group.

Fig. 4.

Role of DA receptors in mediating changes in neurotensin levels of globus pallidus (a) and substantia nigra (b) after treatment of 0.25 mg/kg METH. The rats were treated with METH (M) or saline (S) as described for Fig.3: also as for Fig. 3, 15 min prior to these treatments they received i.p. injections of saline (S-S, S-M), SCH23390 (SCH-S, SCH-M) or eticlopride (E-S, E-METH). Values represent the means $\pm$ SEM expressed as percentages of corresponding S-S controls (N= 8). *P<0.05 versus corresponding S-S groups. **P<0.05 vs. corresponding S-M group.