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Effect of low doses of methamphetamine on rat limbic-related neurotensin systems

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Abstract

Administration of methamphetamine (METH) alters limbic-related (LR) neurotensin (NT) systems. Thus, through a D1-receptor mechanism, noncontingent high doses (5–15 mg/kg), and likely self-administration, of METH appears to reduce NT release causing its accumulation and an elevation of NT-like immunoreactivity (NTLI) in limbic-related NT pathways. For comparison, we tested the effect of low doses of METH, that are more like those used in therapy, on NTLI in the core and shell of the nucleus accumbens (NAc and NAs), prefrontal cortex (PFC), ventral tegmental area (VTA), the lateral habenula (Hb) and basolateral amygdala (Amyg). METH at the dose of 0.25 mg/kg in particular, but not 1.00 mg/kg, decreased NTLI concentration in all of the LR structures studied, except for the prefrontal cortex; however, these effects were rapid and brief being observed at 5 h but not at 24 h after treatment. In all of the LR areas where NTLI levels were reduced after the low dose of METH, the effect was blocked by pretreatment with either a D1 or a D2 antagonist. Thus, opposite to high doses like those associated with abuse, the therapeutic-like low-dose METH treatment induced reduction in NT tissue levels likely reflected an increase in NT release and a short-term depletion of the levels of this neuropeptide in LR

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structures, manifesting features comparable to the response of basal ganglia NT systems to similar low doses of METH.

Graphical Abstract

Keywords

dopamine antagonists; methamphetamine; neurotensin; stimulant; basal ganglia

INTRODUCTION

Abuse of methamphetamine (METH) causes major problems due to its addictive, emotional, and social effects (Meredith et al., 2005; Baldessarini et al., 1996), thus, it is important that the central nervous system (CNS) effects of this potent psychoactive stimulant be understood. Unfortunately, there are no FDA-approved medications (including DA direct agonist or antagonists) to treat METH abuse/addiction at the present time (EurekAlert.org 2010; Elkashef et al., 2008; NIDA Research 2006); consequently, the National Institute on Drug Abuse has solicited research to help elucidate pharmacological mechanisms of this potent stimulant in order to develop novel strategies for treating METH dependence (Cáceda et al., 2006). Thus, others and 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 LR DA pathways are altered by noncontingent high doses (5–15 mg/kg) of METH, like those used in abuse (Letter et al., 1987; Merchant et al., 1988; Hanson et al., 1989; Gygi et al., 1994; Castet et al., 1994a, b). This is important because NT is a tridecapeptide that has been shown to antagonize the behavioral effects of commonly abused CNS psychostimulants, such as cocaine and 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 LR structures (Letter et al., 1987; Hanson et al., 1989; Gygi et al., 1994; Castet et al., 1994a, b). Thus, its apparent interaction with mesolimbic dopaminergic systems suggests that NT influences the addictive properties of these psychostimulants. 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., 1987; 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 of METH: (i) reduce release of NT in the nucleus

accumbens (Wagstaff et al., 1996a; Hanson et al., 1992); and (ii) increase NT tissue levels by increasing LR NT synthesis and accumulation (Adams et al., 2001; Letter et al., 1987; Castel et al., 1994a, b). Recently, the response of these same NT systems to self-administration of METH (contingent on lever pressing) was also reported (Hanson et al., 2012). It was observed that like the high doses of noncontingent METH, NT levels in the LR structures 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 are the findings that like the high-dose of METH, selective D1 agonists also increase limbic NT levels (Merchant et al., 1989b).

In order to thoroughly appreciate the role of LR NT systems in mediating METH effects, it is important to conduct a complete dose-response analysis of the NT responses to METH treatments. While reports have suggested that the D1-mediated changes in basal ganglia NT systems described above for METH high-doses occur for drug doses of 5 to 15 mg/kg, little is known as to how these NT LR pathways respond to low doses (e.g., <1 mg/kg) of METH that are used therapeutically to treat conditions such as ADHD (Drugs.com, 2013). One study examining the effects of low and high doses of METH on NT release in dorsal striatum found that 0.5 mg/kg of METH tended to reduce striatal NT levels 3 h after treatment. In this study no other structures, doses or times were evaluated (Wagstaff et al., 1996a). Consequently, the current study examines in detail the changes in NT systems throughout LR structures 5–24 h after a METH low-dose range of 0.25–1.0 mg/kg and analyzes the mechanisms of the NT responses.

These studies determined that a noncontingent METH dose of 0.25 mg/kg reduced NT levels throughout many LR system in a rapid, but brief manner through both D1 and D2 mechanisms.

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. The treatment of four low-dose administrations was used in order to compare the results directly with those in the literature associated with four high-dose administrations, a treatment that represents a METH binge often associated with METH abuse

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 administration of the METH dose of 0.5 mg/kg, s.c., increases the release of NT while decreasing its tissue levels in some regions of the dorsal striatum (Wagstaff et al., 1996a). All rats were sacrificed 5–24 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., 1987; 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 shell and core of the nucleus accumbens, the prefrontal cortex, the ventral tegmental area, the lateral habenula and the basolateral amygdala were removed by punch dissection 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 % wt/vol 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 mol/L 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% (wt/vol) gelatin and 0.1% (vol/vol) 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 mL in 0.1 mol/L sodium bicarbonate; pH 9.0). After washing the wells three times with wash buffer (0.15 mol/L

K_2HPO_4 , 0.02 mol/L NaH_2PO_4 , 0.2 mmol/L ascorbic acid, 0.2% [vol/vol] Tween-20 and 0.1% [wt/vol] sodium azide; pH 7.5), 25 μ L of a highly selective antiserum for neurotensin (diluted to 1:20,000) was diluted in assay buffer (same as wash buffer containing 0.1% [wt/vol] 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 two 25 μ L aliquots of each tissue sample or standard(s) were added to two separate wells and incubated overnight at 4 °C. After incubation, 25 μ L of the labeled NT ($[^{125}I]$ NT) diluted with assay buffer to approximately 6500 dpm per 25 μ L, 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 μ L 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) and the average of the duplicates for each tissue sample was determined and used for the calculation. 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., 1987). 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 573 ± 46 (nucleus accumbens shell), 456 ± 49 (nucleus accumbens core), 65 ± 6 (prefrontal cortex), 823 ± 81 (ventral tegmental area), 979 ± 76 (lateral habenula), and 510 ± 41 (basolateral amygdala). Differences between one variable were analyzed using one-way analysis of variance followed by Newman-Keuls post hoc test. Differences among two variables were analyzed by two-way analysis of variance followed by a Bonferroni post hoc test. Differences were considered significant when the probability was less than 5%.

RESULTS

Dose-response of LR NT systems to METH low-dose treatments

The responses of NT systems in LR 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. (Fig. 1). The NTLI content was significantly ($P<0.05$) decreased in shell (NAs, $F[3,29]=4.31$), and core (NAc, $F[3,29]=3.09$) of the nucleus accumbens, ventral tegmental area (VTA, $F[3,34]=3.37$) lateral habenula (Hb, $F[3,33]=17.46$), and basolateral amygdala (Amyg, $F[3,35]=4.72$), but not the prefrontal cortex (PFC), after 0.25 mg/kg METH (Fig. 1). The 0.5 mg/kg METH dose significantly reduced NTLI levels in Hb and Amyg, but not in any of the other LR structures, whereas the 1.0 mg/kg dose had no significant effects on any of the LR areas examined.

Recovery of LR NT systems to low-dose METH

Because the most consistent and robust response to the low-dose METH treatment occurred after the 0.25 mg/kg/injection (s.c.), this dose was used to assess the temporal NT limbic effects under low-dose METH conditions: i.e., rats were killed 5 and 24 h after METH treatment. There was a significant time $\times$ METH treatment interaction, with NTLI concentrations in the NAs ($F[1,30]=9.19$), NAc ($F[1,32]=10.41$), VTA ($F[1,32]=14.67$), Hb ($F[1,30]=19.66$), and Amyg ($F[1,25]=5.97$) 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. 2). 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 LR NT systems to low-dose METH treatments (0.25 mg/kg METH). There was a significant pretreatment by METH treatment interaction in the NAs ($F[2,54]=7.37$), and NAc ($F[2,53]=5.69$) with the pretreatment of SCH23390 or eticlopride blocking the effect of the low-dose METH exposure. In general, selective blockade of either the D1 or D2 receptor antagonized low-dose METH-induced reductions in all of LR structures [compare eticlopride-Sal (E-S) vs. eticlopride-METH (E-M) or SCH-Sal (SCH-S) vs. SCH-METH (SCH-M) groups]. In the habenula (Hb), there was also a significant pretreatment by METH treatment interaction ($F[2,54]=11.08$), but only with the pretreatment SCH 23390 blocked the effect of the low-dose METH exposure whereas pretreatment with eticlopride actually increased NTLI in METH exposed rats. In contrast, in the VTA and Amyg there was a significant effect of low-dose METH exposure (VTA: $F[1,54]=11.94$; Amyg: $F[1,50]=7.94$), but no significant effect of pretreatment (VTA: $F[2,54]=0.30$; Amyg: $F[2,50]=2.16$) or pretreatment by low-dose METH exposure interaction (VTA: $F[2,54]=1.14$; Amyg: $F[2,50]=1.47$). In both the VTA and Amyg low-dose METH exposure decreased NTLI.

Discussion

It has been demonstrated that LR NT systems such as those associated with the NAs, NAc and VTA are substantially altered by high-dose METH administrations. Consequently, there are several reported studies that demonstrate increases in tissue levels of NT in these LR structures 18–24 h after 5–15 mg/kg doses 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, increases in NT tissue levels throughout the LR system also occur 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; Hanson et al., 2013) suggesting that similar changes in LR NT systems and related reduced activity of associated NT pathways are linked with METH dependence. Both the METH-related contingent and non-contingent increases in limbic NT tissue levels appear to be mediated principally by increased D1 receptor activity (Merchant et al., 1988; Hanson et al., 2012), a conclusion confirmed by the fact that treatment with a D1 agonist also elevates NTLI content in the nucleus accumbens (Singh et al., 1992). It has been speculated that high-dose METH treatment causes a D1 receptor-dominant effect on NT systems because D1 receptors are most sensitive to high levels of DA release, thus activating the low-affinity D1 receptor. In contrast, the predominant NT responses caused by the high affinity D2 receptors are particularly receptive to a low dose of METH resulting in low levels of DA release (Marcillino et al. 2012).

While the NT responses in some of the LR structures to METH high doses have been previously published, there are no reports that suggest the effects of METH on Hb or Amyg NT systems have been previously examined. Despite this lack of information concerning METH's effects on the NT systems associated with these two structures, there are reports that: (i) Hb and Amyg may play critical roles in regulating the DA pathways associated with VTA and nucleus accumbens (Jhou et al., 2009) and high concentrations of NT have been found to be associated in both of these LR regions with possible links to DA systems (Moyse et al. 1987; Day et al. 2002); (ii) efferents from the Hb may contribute to the effect of METH on mesencephalic dopamine systems (Sasaki et al., 1990); and (iii) NT and VTA-linked DA systems appear to interact in the amygdala to cause avoidance behavior (Day et al., 2002; László et al., 2012; Pontieri et al., 2000). Consequently, we evaluated the response of NT in these structures following a low dose of METH. This is the first report that low doses of METH influence the NT systems associated with these LR structures.

Although little research has been done to examine if low doses of METH 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 LR regions such as the nucleus accumbens (Wagstaff et al., 1996a). The mechanism of this low-dose METH-induced NT release from the nucleus accumbens was reported to be linked with increased activity of D2 receptors, (Wagstaff et al., 1996b). This conclusion is consistent with observations that a D2 agonist generally increases NT release in the nucleus accumbens and reduces accumbens 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 depleting NT in several LR 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 LR structures.

The 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 LR 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., 2008; 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; Hanson et al., 2012); (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 dose of METH suggesting activated NT function mitigates DA responses to METH under these 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 LR structures after four injections of METH. In a dose-dependent manner, METH reduced tissue NTLI in most LR regions associated with both the cell bodies (nucleus accumbens and VTA) and terminals (nucleus accumbens) of the NT-related LR pathways (Fig. 1) 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 LR structures identified after 5 h had returned to control levels by 24 h (Fig. 2). This pattern of response suggests that reduction in limbic 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 LR structures 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 LR NT levels caused by low-dose METH treatment.

We observed that both eticlopride and SCH23390 appeared to block the low-dose METH-induced NT changes in all LR structures where METH-induced NT changes occurred (Fig. 3). 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 both D1 and D2 receptors (Wagstaff et al., 1996a; Frankel et al., 2005).

The findings revealed that four administrations of low doses of METH cause rapid dose- and time-dependent decreases in NT tissue contents in LR structures and these effects likely reflect increased release and turnover of this neuropeptide. It is noteworthy that this is opposite of the increase in NT tissue levels observed following high-dose METH treatments (Hanson et al. 1992). The clinical relevance perhaps relates to the fact that METH self-administration affects NT systems in a manner similar to the noncontingent high-dose treatments. In contrast, the low-dose METH-mediated changes in the LR 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 very low doses of this drug and is associated with elevated release of NT in the LR system and its inhibitory feedback influence.

Finally, our low-dose METH findings have therapeutic relevance (e.g. for ADHD; Drugs.com 2013). 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 body during such therapy are more likely to approach those that occur after a s.c. injection of 0.25–0.5 mg/kg, than that after 5–15 mg/kg. Based on this assumption, it is possible that the responses of NT LR systems observed in this low-dose METH study (i.e., an increase in NT release and related feedback inhibition in the LR systems resulting in reduced NT tissue levels) are more 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. This possibility deserves further consideration.

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Fig. 1.

Dose-dependent effects of low doses of METH on NTLI content in the shell (NAs) and core (NAc) of the nucleus accumbens, ventral tegmental area (VTA), lateral habenula (Hb), basolateral amygdala (Amyg) and prefrontal cortex (PFC). 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 group; †P < 0.05 versus respective M-1.00 group; ††P < 0.05 versus respective M-0.5 group.

Fig. 2.

Temporal effects of a low-dose (0.25 mg/kg/administration) METH treatment on NTLI content in the shell (NAc) and core (NAc) of the nucleus accubens, ventral tegmental area (VTA), lateral habenula (Hb) and basolateral amygdala (Amyg). 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 the shell (NAs) and core (NAc) of the nucleus accumbens, ventral tegmental area (VTA), lateral habenula (Hb) and basolateral amygdala (Amyg). The rats were given 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-M), SCH23390 (SCH-S, SCH-M; 0.5 mg/kg) or eticlopride (E-S, E-M; 0.5 mg/kg). Values represent the means $\pm$ SEM expressed as percentages of corresponding S-S controls (N= 8). * $P < 0.05$ versus corresponding S-S, ^a $P < 0.05$ E-S vs. E-M. ^b $P < 0.05$ main effect of METH treatment.