Effects of Amphetamine Analogs on Central Nervous System Neuropeptide Systems

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INTRODUCTION

Substantial efforts have been devoted to elucidating the effects of amphetamine analogs on central nervous system (CNS) monoaminergic pathways. These agents enhance the activity of such neuronal systems by causing release of their transmitter substances as well as by interfering with transmitter metabolism and reuptake. However, little is known about the consequences of the monoaminergic changes resulting from the administration of these agents, i.e., the eventual effect of these drugs on transmitter systems directly influenced by the monoaminergic pathways. Such effects are important in transmitting the monoamine-initiated messages to those brain regions that eventually mediate the drug-related behavioral changes. In addition, these systems likely have important feedback functions on the amphetamine-sensitive monoaminergic pathways. Consequently, drug-induced changes in these feedback pathways might contribute to phenomena such as tolerance and sensitization.

Of interest to the present work are the neuropeptide neuronal projections associated with extrapyramidal structures and the responses of peptidergic pathways to treatments with amphetamine analogs. These peptide systems were selected for study because of their close association with the mesostriatal dopaminergic neuronal circuitry, a system thought to contribute to the locomotor and mood-altering effects of the amphetamine compounds. For example, neurons containing substance P (SP), which originate within the shiatum and terminate in the substantia nigra, are thought to serve an excitatory feedback function on the mesostriatal dopamine (DA) pathway. Thus, intranigral injections of SP cause striatal release of DA (Reid et al. 1988) and stimulate locomotion (Herrera-Marschitz et al. 1986). Nigral administration of SP has no effect on locomotor activity in animals that have received 6-hydroxydopamine lesions to their mesostriatal DA pathway (Herrera-Marschitz et al. 1986).

The interactions between neurotensin (NT) pathways and the extrapyramidal dopaminergic system are somewhat more complex. As the vast majority of striatal and nigral NT receptors are associated with DA neurons (Quirion et al. 1985). NT pathways certainly contribute to the regulation of extrapyramidal DA activity. The overall CNS pharmacology of NT has been compared to that of neuroleptic drugs (Nemeroff 1986), while intraventricular administration of this peptide is reported to antagonize some of the behavioral activity of amphetamine and cocaine (Skoog et al. 1986). Finally, dynorphin (Dyn) A1-17 is associated with striatal-nigral neurons that, like the SP pathway, have been postulated to be part of a feedback system to the nigral-striatal DA neurons (Herrera-Marschitz et al. 1983). However, such a feedback role for Dyn has been questioned recently, as the locomotor activity induced by nigral Dyn injections is not impaired by elimination of the nigral-striatal DA pathway (HerreraMarschitx et al. 1986).

EVALUATION OF NEUROPEPTIDE RESPONSE TO AMPHETAMINE ANALOGS

This chapter discusses the responses of these extrapyramidal neuropeptide systems to the amphetamine analogs methamphetamine (METH), methylene dioxyamphetamine (MDA), and methylenedioxymethamphetarnine (MDMA). These drugs were selected for this study because they represent somewhat diverse mechanisms of action. While all three agents are able to enhance extrapyramidal serotonergic activity (Schmidt et al. 1987). only METH has been characterized as a substantial stimulant of the DA system. The effects of MDA and MDMA on extrapyramidal DA systems have not been well elucidated. Thus, evaluating and comparing the responses of the SP, NT, and Dyn extrapyramidal systems to these drugs will help to determine the nature of the DA responses to METH, MDA, and MDMA administrations.

Methods

Sprague Dawley rats (180 to 220 g) were treated with METH, MDA, and MDMA generously donated by the National Institute on Drug Abuse. Following drug treatments, rats were sacrificed, brains removed, and the striatal and nigral areas dissected out. Tissue samples were rapidly frozen and stored until analyzed. The responses of these neuropeptide systems to treatments by the amphetamine analogs were assessed with radioimmunoassay techniques by measuring drug-induced changes in the tissue content of neuropeptidelike immunoreactivity. Highly selective and sensitive antibodies were used in the detection of SP (Hanson and Loveberg 1980), NT (Letter et al. 1987). and Dyn (Hanson et al. 1987). The mean nigral contents of SP, NT, and Dyn for the control groups were 12 nanograms (ng), 595 picograms (pg), and 766 pg per mg protein, respectively. The mean striatal contents for SP, NT, and Dyn for the control groups were 1,250,127, and 380 pg/mg protein, respectively. To characterize the

METH-induced changes in neuropeptide levels, selective D_1 (SCH 23390) and D_2 (sulpiride) dopaminergic receptor antagonists were coadministered. The results are expressed as percent of control to facilitate comparisons; each value represents the mean ± SEM of five to seven animals. Data were subjected to either a Student's r-test (figures 4 and 5) or ANOVA analysis followed by a multiple comparisons test (figures 1, 2, and 3). Significance was set at the .05 level.

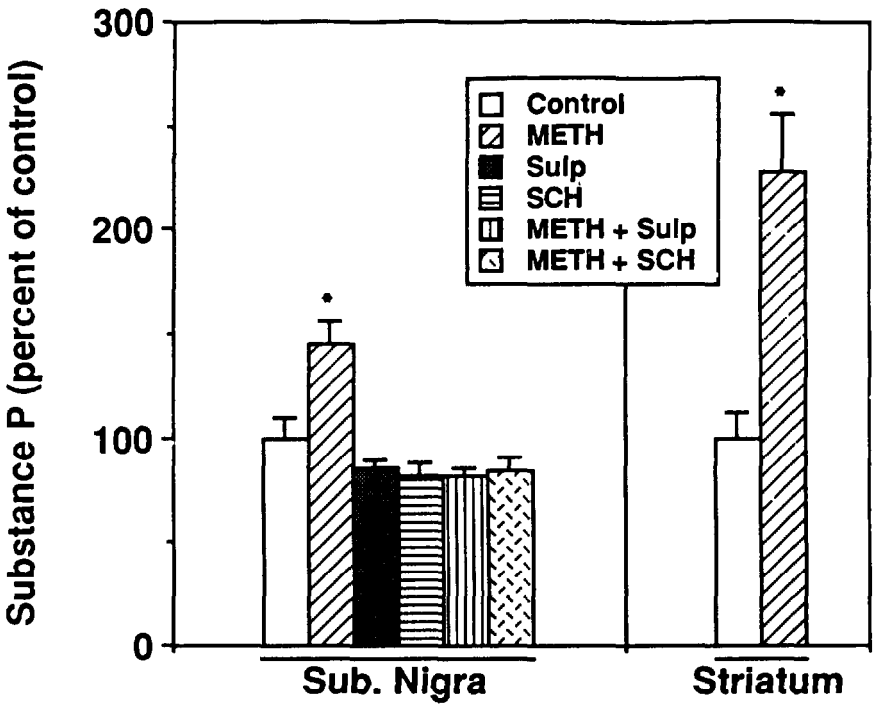

FIGURE 1. *Effects of METH on extrapyramidal SP content*

*p<0.02 compared to corresponding groups.

NOTE: METH was injected alone or concurrently with either SCH 23390 (SCH) (0.5 mg/kg/injection) or sulpiride (sulp) (80 mg/kg/injection).

Results

Administrations of five injections of METH (15 mg/kg/injection; 6-hour intervals between injections) caused substantial increases in the striatal and nigral levels of all three neuropeptides examined in rats sacrificed 18 hours following treatment. Figures 1 to 3 present the effects of blocking the D_1 and D_2 dopaminergic receptors on the responses by these peptide systems

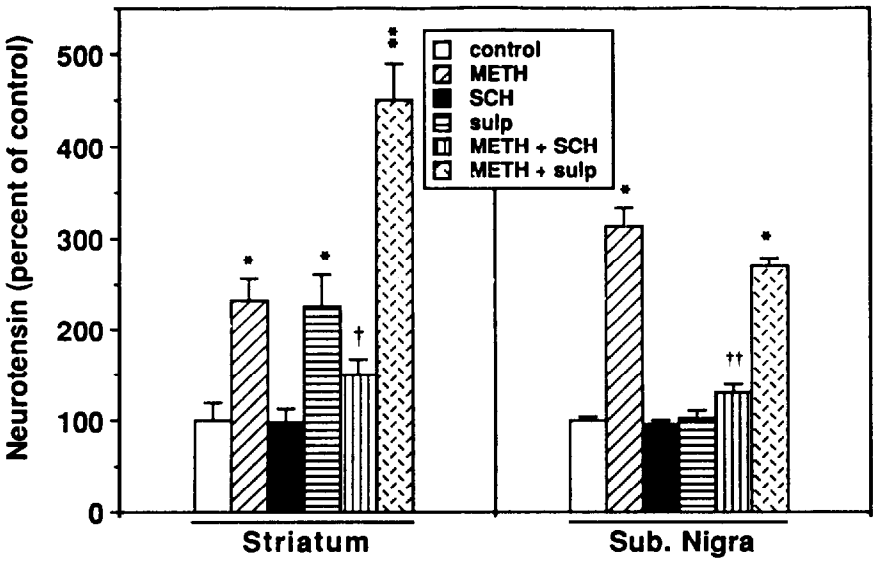

FIGURE 2. *Effects of METH on extrapyramidal NT content*

*p<0.02 compared to corresponding control.

**p<0.01 compared to the corresponding METH- and sulpride (sulp)-treated groups, and p<0.001 compared to the corresponding control.

†p<0.05 compared to the corresponding METH-treated groups.

††p<0.01 compared to the corresponding METH-treated group.

NOTE: Animals were treated as described in figure 1.

to METH treatment. Figures 4 and 5 present the responses of SP, NT, and Dyn extrapyramidal pathways to MDMA and MDA treatments, respectively.

Following METH administration, levels of SP were elevated to 150 percent of control in the substantia nigra and 227 percent of control in the striatum (figure 1). Blockade of either D_1 or D_2 receptors totally prevented the METH-induced rise in nigral SP content.

In rats sacrificed 18 hours following METH treatment, nigral and striatal levels of both NT (figure 2) and Dyn (figure 3) increased dramatically, to 200 to 400 percent of respective controls. However, the effects of D_1 and D_2 receptor antagonism on the METH-induced changes in these peptide systems were somewhat different. Administration of sulpiride alone caused an increase in striatal NT levels. METH administration in the presence of

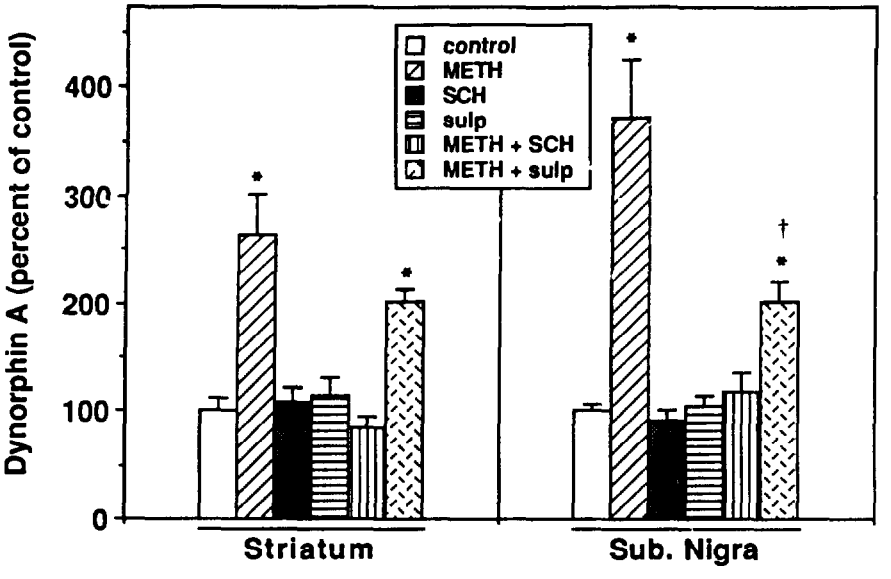

FIGURE 3. *Effects of METH on extrapyramidal Dyn A content*

*p<0.02 compared to corresponding controls.

†p<0.05 compared to the corresponding METH-treated group.

NOTE: Animals were treated as described in figure 1.

this D_2 blocker resulted in increases of striatal NT content approximately equal to the summation of the effects of the two drugs when given individually. In contrast, the D_1 antagonist, SCH 23390, had no effect alone and substantially attenuated the METH-induced striatal changes in NT content. The SCH 23390 compound also completely blocked the METH-mediated elevation of nigral NT levels, while sulpiride had no effect of its own, nor did its presence significantly influence the response of the nigral NT system to METH treatment.

Administration of sulpiride or SCH 23390 alone did not alter the striatal or nigral content of Dyn. Blockade of D_1 receptors substantially interfered with the METH-induced changes in both striatal and nigral Dyn levels. Blockade of D_2 receptors by sulpiride appeared to attenuate the METH-related changes in the Dyn levels, especially in the substantia nigra, but its interference with the METH effects was less than that of the SCH 23390 compound.

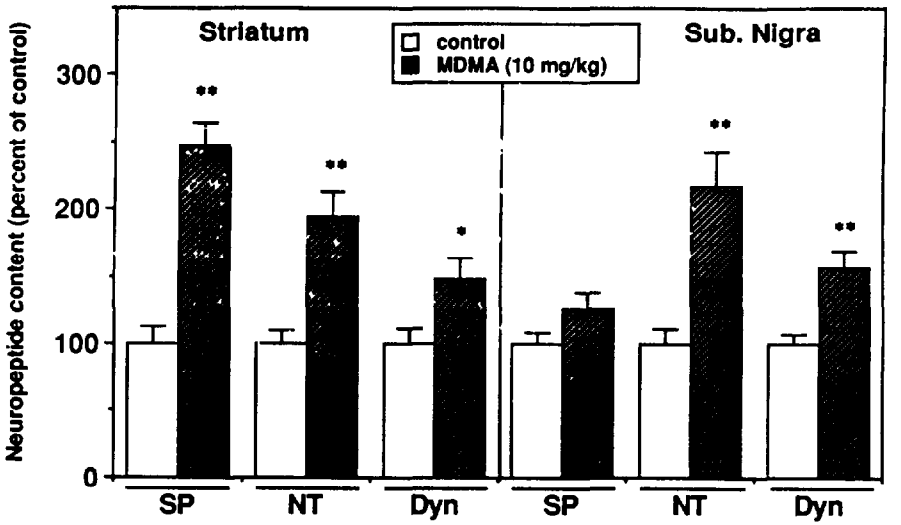

FIGURE 4. *Effects of MDMA on extrapyramidal neuropeptide contents*

*p<0.05 compared to corresponding controls.

**p<0.001 compared to corresponding controls.

NOTE: Animals were given multiple injections of MDMA (10 mg/kg/injection) and sacrificed 18 hours after treatment. Striatal and nigral content of SP, NT, and Dyn were examined

The effects of five injections of MDMA (10 mg/kg/injection) on striatal and nigral neuropeptide content are presented in figure 4. Animals were treated in a manner similar to that used for the METH experiments. Following multiple MDMA administrations, the striatal levels of SP, NT, and Dyn were elevated to 248 percent., 195 percent, and 148 percent, respectively, of corresponding controls. Nigral content of these same peptides were increased to 127 percent, 217 percent, and 157 percent, respectively, compared to their controls. These effects resembled those observed with METH treatment, Similar SP and NT responses were observed following MDA treatment (figure 5).

CONCLUSION

These findings demonstrate that some neuropeptide systems associated with mesostriatal dopaminergic projections are profoundly altered by treatment with each amphetamine analog examined.Although the significance of these drug-induced increases in striatal and nigral contents of SP, NT, and

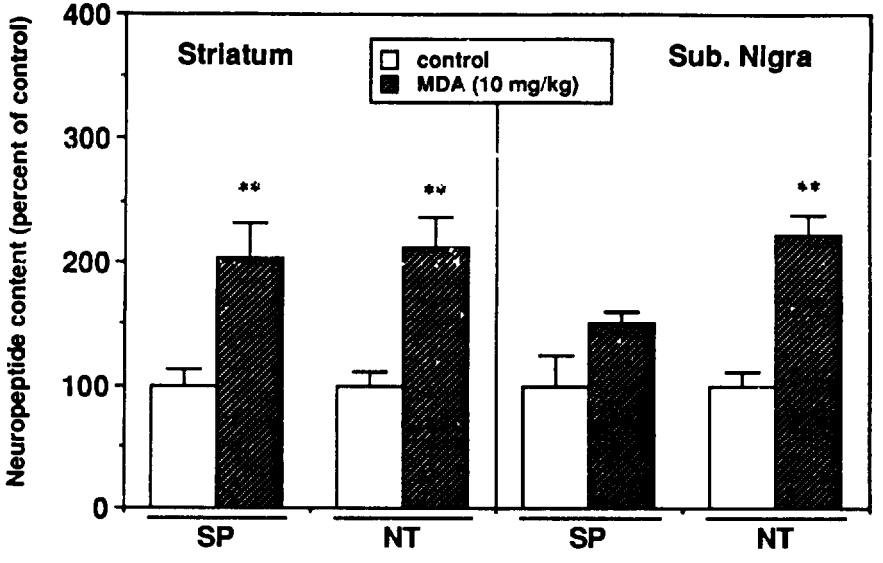

FIGURE 5. *Effects of MDA on extrapyramidal neuropeptide contents*

**p<0.01 compared to corresponding controls.

NOTE: Animals were given multiple injections of MDA (10 mg/kg/injections) and sacrificed 18 hours after treatment. Striatal and nigral levels of SP and NT were determined.

Dyn is not yet known, it is likely that such changes reflect variations in the activity of the associated pathways. One possible explanation is that increases in neuropeptide tissue levels are due to decreased release of the transmitter, which diminishes the extracellular peptide metabolism and results in accumulation of these peptide substances. Another possible contributing factor is a drug-related alteration in neuropeptide synthesis. For example, Bannon et al. (1987) reported that METH administration increased the quantity of striatal messenger RNA for the SP precursor preprotachykinin. Thus, increases in peptide synthesis might contribute to increases in peptide content caused by treatment with METH or the other amphetamine analogs.

The dramatic responses to METH reported herein were most certainly a consequence of drug-mediated changes in postsynaptic dopaminergic activity. It is interesting that each neuropeptide response to METH treatment was subtly unique. The increases in SP content cased by METH appeared to occur primarily by activation of D_2 receptors (figure 1). This conclusion is based on previously reported findings that D_2 agonists also increase nigral SP levels, while D_1 agonists actually cause a decrease in the nigral SP

concentration (Sonsalla et al. 1984). Even so. D_1 receptors appeared to play a facilitatory role in this drug effect, as blockade of this receptor completely prevented the METH effects. The effects of METH on the NT systems appeared to be mediated completely by D_1 receptors, as the presence of SCH 23390 almost entirely blocked the METH-mediated changes in NT levels, while sulpiride did not appear to interfere with the METH effects (figure 2). Finally, these data suggest that the actions of METH on the Dyn systems were mediated primarily by D_1 receptors; even so, D_2 receptors also contributed to these effects as their blockade attenuated, although to a lesser degree than D_1 blockade, the METH-related increases in Dyn levels (figure 3).

The present data demonstrate that the amphetamine analogs MDA and MDMA influence the extrapyramidal neuropeptide systems in a METH-line manner (figures 4 and 5). As already discussed, the METH effects on these peptide systems are dopaminergically mediated, thus, it is likely that the amphetamine designer drugs also influence SP, NT, and Dyn extrapyramidal pathways by enhancing extrapyramidal dopaminergic activity. In support of this conclusion, we have observed that blockade of D_1 receptors with SCH 23390 completely blocks the increases in striatal NT and Dyn induced by MDMA treatment (unpublished observation). This finding is consistent with observations that MDMA and MDA stimulate the release of striatal DA from tissue slices (Schmidt et al. 1987) and intact animals (Yamamoto and Spanos 1988). In addition, Stone et al. (1986) reported that treatments with MDA and MDMA resulted in increases in striatal concentrations of homovanillic acid, a DA metabolite, which reflects the extent of DA release.

While perhaps quantitatively different, each of the amphetamine analogs examined had substantial effects on the extrapyramidal SP, NT, and Dyn pathways. Thus. these peptide pathways likely contribute to the behavioral effect of this group of agents in general; specifically, they might participate in mediating the changes in locomotion or mood or the development of psychotic disorders associated with administration of high doses of the amphetamine analogs. More studies are necessary to identify specific contributions ma& by each of these peptide systems to the pharmacological profiles of these agents. In addition, these neuropeptide changes are of interest as nemochemical markers for the effects of the amphetamine drugs on postsynaptic dopaminergic activity and could be useful in the study of such consequences of these drugs as tolerance and sensitization.

DISCUSSION

QUESTION: The last slide referred to postsynaptic actions of the drugs. Do you mean postsynaptic consequences of their presynaptic actions?

ANSWER: Yes. We all know that the dopamine comes out The question is: What happens after the dopamine comes out? We know that if nothing occurred following the dopamine release as far as other transmitter systems being influenced, them would be no behavioral effect. So downstream systems like these peptides are probably involved in mediating those monoaminergic messages to some other parts of the brain or playing feedback roles and altering the way that the monoamine systems respond So they may play roles in sensitization or tolerance by impacting on the activity of those projections.

QUESTION: Did you mention that 6-hydroxydopamine blocks or elevates neurotensin levels?

ANSWER: Yes, 6-hydroxydopamine by itself elevates neurotensin levels. When you combine it with methamphetamine, you do not get any additivity. It is just a 6-hydroxydopamine action. It is a bit complicated to interpret, but it appears that it is still the nigral striatal dopamine pathway that is mediating the methamphetamine effect.

QUESTlON: Have you had the opportunity to look at substance P, possibly in the spinal cord? I am thinking about some of the work that Dr. Seiden presented and potentially a role in analgesia.

ANSWER: We have not looked in the spinal cord at all for substance P. Everything has been in the extrapyramidal and limbic systems.

COMMENT: Another reason why you should be looking at substance P in the spinal cord is that, in spinal cord, substance P is cocontained in neurons together with tyrosine hydroxylase.

RESPONSE: Right. And we have asked ourselves the question because of the issue of coexistence, not only with substance P but with neurotensin and probably dynorphin. Is this the reason these things are changing? Because if they are coexisting with dopamine projections and there is some alteration in dopamine, then maybe there is an intraneuronal action that results in the peptide changes.

COMMENT: I was thinking about this, but I couldn't remember if substance P had been shown to be colocalized in the striatum.

RESPONSE: No, if there is any, it is very, very small coexistence of substance P and tyrosine hydroxylase in the striatum. That is why we don't feel that that is the explanation for these changes.

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