ROLE OF VARIOUS NEUROTRANSMITTER SYSTEMS IN THE PHENOMENON OF AMPHETAMINE-INDUCED REVERSE TOLERANCE

by

Imtiaz A. Chaudhry

A dissertation submitted to the faculty of The University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Pharmacology

The University of Utah

August 1990
This dissertation has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

Chair: Ralp Karler

Stuart A. Turkanis
To the Graduate Council of the University of Utah:

I have read the dissertation of Imtiaz A. Chaudhry in its final form and have found that (1) its format, citations and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the supervisory committee and is ready for submission to The Graduate School.

[Signature]

[Date]

Approved for the Major Department

[Signature]

Chair/Dean

Approved for the Graduate Council

B. Gale Dick
Dean of The Graduate School
ABSTRACT

The potential role of various neurotransmitter systems was investigated in the phenomenon of amphetamine-induced reverse tolerance. CF-1 mice were treated daily with amphetamine to produce reverse tolerance to the stereotypic effects of the drug. After amphetamine withdrawal, the enhanced responsiveness characteristic of reverse tolerance persisted for at least 28 days. Associated with reverse tolerance were changes in the glutamate, GABA and serotonin systems, as measured by a decrease in convulsive threshold to N-methyl-DL-aspartate (NMDLA), an increase in convulsive threshold to bicuculline, and an increase responsiveness to 5-hydroxytryptophan in the head-twitch test. In contrast, no changes were observed in the thresholds to arecoline-, strychnine- or aminophylline-induced convulsions. The changes in the NMDLA and bicuculline convulsive thresholds, like reverse tolerance itself, were persistent after amphetamine withdrawal; in contrast, the functional responsiveness of the serotonin system returned to normal within 15 days. Daily pretreatment with the dopaminergic blocker haloperidol or with the N-methyl-D-aspartate antagonists MK-801 or ketamine blocked the development of the reverse tolerance. Pretreatment with haloperidol, MK-801 or ketamine also prevented the changes in convulsive threshold to NMDLA and bicuculline. Pretreatment with the GABAergic drug diazepam or the serotonin antagonist cyproheptadine had no effect on the development of reverse tolerance or changes in the NMDLA or bicuculline convulsive thresholds. The results from these studies illustrate that the development of reverse tolerance to amphetamine involves not only the dopaminergic but also the glutamatergic system.
## CONTENTS

ABSTRACT ........................................................................................................ iv

LIST OF FIGURES ........................................................................................ vi

LIST OF TABLES ........................................................................................... vii

ACKNOWLEDGMENTS ................................................................................ viii

INTRODUCTION .......................................................................................... 1

MATERIALS AND METHODS ....................................................................... 5

  Experimental animals and preparation of drugs ........................................... 5
  Experimental procedures ..................................................................... 5

RESULTS ....................................................................................................... 8

DISCUSSION ................................................................................................. 22

REFERENCES ............................................................................................... 29

VITA ............................................................................................................... 35
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acute dose-response relationship for amphetamine-caused stereotypic effect</td>
<td>9</td>
</tr>
<tr>
<td>2.</td>
<td>Time-course of the development and persistence of reverse tolerance to amphetamine-caused stereotypy</td>
<td>11</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.</td>
<td>Influence of chronic amphetamine treatment on the drug-responsiveness of neurotransmitter-test systems</td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>Influence of withdrawal from chronic amphetamine treatment on various neurotransmitter-test systems</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>Effect of haloperidol pretreatment on the development of reverse tolerance to amphetamine stereotypy</td>
<td>16</td>
</tr>
<tr>
<td>4.</td>
<td>Effect of MK-801 pretreatment on the development of reverse tolerance to amphetamine-caused stereotypy</td>
<td>17</td>
</tr>
<tr>
<td>5.</td>
<td>Influence of haloperidol or MK-801 on the effect of chronic amphetamine pretreatment on NMDLA and bicuculline convulsive thresholds</td>
<td>19</td>
</tr>
<tr>
<td>6.</td>
<td>Influence of ketamine, cyproheptadine or diazepam on the development of reverse tolerance to amphetamine-caused stereotypy</td>
<td>20</td>
</tr>
<tr>
<td>7.</td>
<td>Influence of ketamine, cyproheptadine or diazepam on the effect of amphetamine chronic pretreatment on NMDLA and bicuculline convulsive thresholds</td>
<td>21</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I wish to thank Dr. Ralph Karler for his personal interest and advice during my graduate training, and I am also indebted to all members of the Pharmacology Department for their efforts in my education. Further, the author is grateful to Drs. Ralph Karler, Stuart A. Turkanis, Donald N. Franz, Larry J. Stensaas and James W. Gibb for serving as members of his thesis committee. An expression of thanks is extended to Dr. Ewart A. Swinyard for his encouragement and confidence in me. Sincere thanks are expressed to Dr. Larry D. Calder for his valuable assistance in the laboratory.

Special thanks are extended to my undergraduate professors Edward B. Walker, Gerald R. Grove, Darrell J. Graff and Garth L. Welch of Weber State University for their continued interests in my education and to Ardell and Ray Gottfredson for their much needed help and prayers. Many thanks to Pamela Marshall for her help in the preparation of this manuscript.

A note of gratitude is extended to all my friends whose company I have enjoyed and whose meaningful friendship has resulted in my personal development, especially to Masood Hasan for his unselfish love and support. Finally, to my parents Rashida and Bashir A. Chaudhry, whose prayers and encouragement sustained me, and to my brothers Liaqat Ali, Zulfiqar Ahmad, Fiaz Ahmad and my sister Dr. Samina Bashir Shama who made my days bright, I dedicate this thesis.
INTRODUCTION

Chronic treatment with drugs often results in the appearance of decreased responsiveness to a given effect, or a shift to the right in the dose-response curve, which is commonly called tolerance. Contrary to tolerance, as a consequence of repeated treatment, is the phenomenon of enhanced responsiveness or reverse tolerance, which is a shift to the left in the dose-response curve. The phenomenon of enhanced responsiveness was first described by Tatum and Seevers (1929) in monkeys and by Downs and Eddy (1932) in rats. They reported that chronic administration of cocaine resulted in increased excitability and susceptibility of these species to cocaine-induced convulsions. Almost 50 years later, the phenomenon was rediscovered in rats by Segal and Mandell (1974) who demonstrated reverse tolerance to the stereotypic and motor stimulant effects of amphetamine. Subsequently, reverse tolerance has also been documented for other motor stimulants, such as, methylphenidate (Shuster, Hudson, Anton and Righi, 1982; Kolta, Shreve and Uretskey, 1985b), morphine (Shuster, Webster and Yu, 1975; Babbini, Gaiardi and Bartoletti, 1979, Kalivas and Duffy, 1987), and phencyclidine (Schlemmer, Jackson, Preston, Bederka, Garver and Davis, 1978).

Although reverse tolerance has been studied most extensively with motor stimulant drugs, the phenomenon also occurs for depressant drugs, such as phenytoin and cannabinoids (Karler, Borys and Turkanis, 1982). In addition, reverse tolerance takes place to different types of effects; for example convulsive (Tatum and Seevers, 1929; Karler, Calder, Chaudhry and Turkanis, 1989) and anticonvulsive (Karler et al., 1982) effects, as well as stereotypic (Segal and Mandell, 1974; Leith and Kuczenski, 1982; Karler et al., 1989) and locomotor effects (Downs and Eddy, 1932; Segal and Mandell,
1974; Rebec and Segal, 1979; Chaudhry, Turkanis and Karler, 1988). These observations suggest that reverse tolerance is not merely restricted to stimulants or to drugs of abuse but may represent a property of drugs in general. In addition, the phenomenon has been described in many species, including rats, mice, guinea pigs, cats, dogs, monkeys and humans (Robinson, 1988), which illustrates the generality of the phenomenon.

Some characteristics of reverse tolerance are similar to those described for electrical kindling: Both are examples of neuronal plasticity; both are dose or stimulus-intensity dependent (Sangdee, Turkanis and Karler, 1982; Stripling, 1983); and both are extremely long-lasting after the treatment withdrawal (Racine, 1978; Leith and Kuczenski, 1982; Shuster et al., 1982; Robinson, 1984). The reverse tolerance state (Chaudhry et al., 1988), as with the kindled state (Sangdee et al., 1982), appears to be the result of a selective rather than a generalized change in CNS function. For example, although mice made reverse tolerant to amphetamine are also reverse tolerant to cocaine, they are not reverse tolerant to the motor stimulant effects of morphine (Chaudhry et al., 1988). The enhanced responsiveness appears, therefore, to be the result of a selective change in dopaminergic responses rather than a generalized change in the sensitivity of the CNS.

Clinically, reverse tolerance may be involved in the phenomena of drug-induced paranoid schizophrenia and drug self-administration, and in the actions of antidepressants. The observations of Conell (1958) in humans suggested that the drug-induced symptoms of paranoid schizophrenia increase with the duration of exposure to amphetamine. The repetitive behavior in animals associated with either high or chronic doses of amphetamine or cocaine resembles the behavior seen in humans intoxicated with these drugs; therefore, stereotypy in animals is considered to be a model for paranoid schizophrenia. There is also recent evidence from animal studies linking reverse tolerance to self-administration (Piazza, Deminiere, Moal and Simon, 1989); for example animals made reverse tolerant to motor stimulant effects of amphetamine learned to self-administer amphetamine more
readily. In addition, the slow onset of the therapeutic efficacy of antidepressant drugs suggests that reverse tolerance may contribute to the action of these agents (Willner, 1989). An understanding of the mechanism by which amphetamine produces reverse tolerance in animals may provide insight into an understanding of paranoid psychosis, the actions of antidepressants, and into a fundamental property of all drugs of abuse, that is, their ability to effect self-administration.

Little is understood about the mechanism of reverse tolerance, or even whether reverse tolerance to different behavioral effects shares a common mechanism. The effect however, can not be attributed to changes in biodisposition, or to any pretreatment factors. For example, Segal and Mandell (1974) did not detect any change in rat blood levels following chronic exposure to amphetamine; the possibility of amphetamine accumulation in the brain has also been excluded (Brien, Peachey, Rogers and Kitney, 1977). In addition, Rebec and Segal (1979) administered daily doses of amphetamine by the intraventricular infusion method and still observed increased locomotor response. These studies have ruled out the involvement of any dispositional changes in the development of reverse tolerance and suggest that a central mechanism is responsible for the enhanced behavioral responses after chronic drug pretreatment. The role of drug or environmental conditioning has also been ruled out as the cause of reverse tolerance. For example, Segal and Mandell (1974) and Segal (1975) found that whether they tested rats (previously chronically treated with amphetamine) in the same environment or in a unique test environment, they still observed reverse tolerance. Similarly, Segal (1975) demonstrated that low doses of amphetamine, which are initially motor stimulant ultimately result in the emergence of stereotyped behavior in rats; such a qualitative change in drug response can not be attributed to a conditioning phenomenon.

The dopaminergic system has been implicated in reverse tolerance to amphetamine. For example chronic pretreatment with the dopaminergic antagonist haloperidol prevents the development of reverse tolerance in both rats and mice (Kuczenski and Leith, 1981;
Karler et al., 1989). Despite these data, changes in the dopaminergic system do not appear to account for the phenomenon; and presently there is no satisfactory explanation of the mechanism of reverse tolerance. The following work was designed to investigate the functional status of the glutamatergic, GABAergic, cholinergic, adenosinergic and serotonergic systems in mice made reverse tolerant to amphetamine. These systems were selected because of the availability of relatively selective agonist and antagonist drugs; in addition behavioral responsiveness to these drugs can be determined quantitatively. Preliminary data suggested that the functional responsiveness of the glutamate, GABA and serotonin systems is altered in mice that are reverse tolerant to the stereotypic effects of amphetamine. Subsequent experiments were designed to investigate the potential involvement of these systems in the development, as well as in the persistence of reverse tolerance after drug withdrawal.
MATERIALS AND METHODS

Experimental animals and preparation of drugs

Male CF-1 mice, initially weighing 20-25 g, were used as experimental animals. They were housed in groups of 12-18; fed ad libitum; and maintained on a 12-hr light/dark cycle in which the light cycle corresponded to daylight. DL-Amphetamine sulfate, N-methyl-DL-aspartate (NMDLA), bicuculline, arecoline hydrobromide, 5-hydroxy-L-tryptophan (5-HTP) and diazepam were obtained from Sigma Chemical Co. (St. Louis, MO); aminophylline from LyphoMed, Inc. (Rosemont, IL); ketamine from Parke-Davis (Morris Plains, NJ). Haloperidol was a gift from McNeil Pharmaceutical (Spring House, PA); dizocilpine (MK-801) and cyproheptadine from Merck Sharp & Dohme (Rahway, NJ).

All drugs were prepared in isotonic saline solution; 5-HTP and diazepam required suspension with 2% Tween 80; haloperidol and bicuculline required acidification. All drugs were administered intraperitoneally, except haloperidol and ketamine which were given subcutaneously. For chronic studies, unless otherwise indicated, mice were treated each day with a single dose of drug or vehicle.

Experimental procedures

All behavioral measurements were made between 1000 and 1500 hr. Stereotypy and head-twitch were determined by visual observation for a period of 45 min after drug administration. Both stereotypy and head-twitch were assessed as quantal measures. For stereotypy, the end-point consisted of fast, repetitive head and fore-limb movements with the animal stationary with spread hindlimbs. This end-point is equivalent to a score of 8 on the scale published by Ellinwood and Balster (1974). For head-twitch the end-point
was a characteristic head shaking as previously described (Corne, Pickering and Warner, 1963). To produce reverse tolerance to stereotypy, amphetamine was administered each day for a period of about 15 days. To determine persistence of the effects, mice were chronically treated with amphetamine or saline and their responsiveness to the challenge drugs was assessed after various withdrawal times.

In the animals made reverse tolerant to the stereotypic effects of amphetamine, convulsive thresholds to NMDLA, bicuculline, strychnine, aminophylline and arecoline were assessed. All drugs were infused intravenously through a tail vein with the use of a Harvard Apparatus infusion pump (Boston, MA). A clear plexiglass cylindrical restrainer (5 cm diameter and 8.5 cm deep) was used to hold the animal. This restrainer had a slit in the top portion which provided access to the tail, and the size of the restrainer provided enough room for the animal to exhibit a convulsion. The infusion rate for NMDLA (20 mg/ml) was 0.54 ml/min; bicuculline (0.10 mg/ml), 0.20 ml/min; strychnine (0.05 mg/ml), 0.28 ml/min; aminophylline (19.7 mg/ml), 0.39 ml/min; and arecoline (3 mg/ml), 0.10 ml/min. These infusion rates and drug concentrations were selected to produce convulsions in the normal mouse within 50-100 sec because these times were convenient in determining decreases or increases in thresholds. Heparin was added to all drug solutions (final activity, 10 units/ml) to prevent clotting. Maximal convulsive thresholds were recorded for all intravenously infused drugs, except arecoline, which produces only minimal convulsions. The end-point for minimal convulsions was jaw and/or front-paw clonus; for maximal convulsions, tonic hind-limb extension. The mean dose of each of the drugs needed to produce convulsions after amphetamine withdrawal was determined.

Similarly, reverse tolerant mice were challenged with 5-HTP (50 mg/kg) and the percentage of animals that exhibited the head-twitch response was observed. The challenge dose of 5-HTP was selected from an acute dose-effect curve for head-twitch.

In the experiments designed to block reverse tolerance, mice were pretreated daily with MK-801, ketamine, cyproheptadine, diazepam or haloperidol 15-30 min before the
once daily amphetamine treatment. Twenty-four hr after the last treatment, all groups were challenged with amphetamine and the presence or absence of reverse tolerance was noted. The doses of MK-801 and ketamine were selected on the basis of dose-effect curves for anticonvulsant activity against NMDLA. The dose of diazepam was obtained from the dose-effect response of its elevation of bicuculline-induced convulsions. The haloperidol dose was obtained from a dose-effect curve of its ability to antagonize acute amphetamine-caused stereotypy. The dose of cyproheptadine was selected from a dose-dependent antagonism study of the 5-HTP-caused head-twitch response.
RESULTS

Figure 1 displays the acute dose-response relationship for amphetamine-caused stereotypy in mice. The data illustrate that the effect is linear in the dose range tested. The dose-response curve provided the rationale for the dose selection in the subsequent experiments.

The results in Figure 2 illustrate the time-course of the development and persistence of reverse tolerance to amphetamine-caused stereotypy. Mice were treated with a single, daily, fixed dose of amphetamine for 18 days, at which time most of the mice exhibited stereotypy. These mice were divided into two groups; one group was challenged with the same dose of amphetamine 14 days after treatment withdrawal; the other 28 days after withdrawal. Reverse tolerance was still evident after 28 days of withdrawal from the chronic amphetamine treatment. These data suggest that reverse tolerance is a relatively long-lasting phenomenon.

The data summarized in Table 1 are derived from the results of six separate experiments designed to determine the influence of the reverse tolerant state on the drug-responsiveness of various neurotransmitter-test systems. Of the five convulsant-threshold tests, only the responsiveness to NMDLA and bicuculline was affected. In the head-twitch experiment, reverse tolerance was also associated with an enhanced responsiveness to the challenge dose of 5-HTP. The results from Table 1 indicate that reverse tolerance to amphetamine causes changes in the glutamate, GABA and serotonin systems. The negative results in the other three systems suggest that the enhanced responsiveness does not generalize to all measures of CNS function.
Figure 1. Acute dose-response relationship for amphetamine-caused effect. Each stereotopic response was obtained from 25 mice/group, and is presented as the percentage of the group that displayed stereotypy. Amphetamine was administered i.p. and observations were made for 45 min after the treatment.
Figure 2. Time-course of the development and persistence of reverse tolerance to amphetamine-caused stereotypy. Forty mice were given a single daily dose of 10 mg/kg amphetamine (i.p.) for 18 days; 20 mice were rechallenged with drug on withdrawal day 14 and another 20 mice on withdrawal day 28. All values on days 5-18 are significantly different from that on day 1; values on withdrawal days 14 and 28 are significantly different from their comparable controls, as determined by chi-square test ($p < 0.05$; Spiegel, 1961).
Mice were treated chronically either with 10 mg/kg DL-amphetamine or saline for 15 days. Twenty-four hr after the last treatment their response to the challenge test drugs was assessed. Convulsive threshold data represent the mean values and their standard deviations obtained from 10 mice/group. All convulsant drugs were administered i.v., and their maximal convulsive thresholds were obtained, except for arecoline which produces only minimal convulsions. The percentage of animals displaying the head-twitch response to 50 mg/kg 5-HTP, i.p. was determined in groups of 28-31 mice.

* Mean values are significantly different from their respective controls, as determined by a t-test (p < 0.05; Snedecor and Cochran, 1967).

+ Value significantly different from the control, as determined by a chi-square test (p < 0.01; Spiegel, 1961).
The data presented in Table 2 illustrate the persistence of the withdrawal effects described in Table 1. For this experiment mice were made reverse tolerant to stereotypy and withdrawn from the chronic amphetamine treatment for various time intervals and then tested for the persistence of the observed changes in NMDLA- and bicuculline-caused convulsions, as well as in 5-HTP-caused head-twitch response. The results indicate that the changes in NMDLA- and bicuculline-convulsive thresholds are relatively long-lasting because they had not returned to the control values even after 30 days of drug withdrawal. On the other hand, the response of mice in the 5-HTP head-twitch test returned to the control response within 15 days of withdrawal. The persistence of the NMDLA- and bicuculline-threshold data agrees with that of the reverse tolerance shown in Figure 2. The 5-HTP data, however, suggest that the changes in the serotonergic system are not as persistent and do not parallel the persistence of the reverse tolerance.

The data in Table 3 depict the influence of haloperidol pretreatment on the development of reverse tolerance to the stereotypic effect of amphetamine. Mice were treated daily as indicated, and 24 hr after the withdrawal from the treatment regimen they were given a challenge dose of amphetamine. In these experiments the incidence of stereotypy in mice pretreated chronically with amphetamine is about 3-fold greater than that in the saline controls, illustrating the enhanced responsiveness to amphetamine. In contrast, mice pretreated with haloperidol (0.25 mg/kg) and amphetamine were not reverse tolerant to the amphetamine effect, demonstrating that haloperidol can block the development of the phenomenon. The chronic exposure to haloperidol alone did not alter the test response to amphetamine.

The results in Table 4 indicate that MK-801 pretreatment can also block the development of reverse tolerance. The results presented in Table 4 were obtained 24 hr after the indicated pretreatment withdrawal, at which time all mice were challenged with an equal dose of amphetamine. The data illustrate that mice pretreated with MK-801 and
Table 2

Influence of withdrawal from chronic amphetamine treatment on various neurotransmitter-test systems.

<table>
<thead>
<tr>
<th>Test System</th>
<th>Withdrawal Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Convulsive threshold (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>NMDLA</td>
<td>528 ± 127</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Head-twitch (%)</td>
<td>28</td>
</tr>
<tr>
<td>5-HTP</td>
<td></td>
</tr>
</tbody>
</table>

Mice were treated chronically either with 10 mg/kg DL-amphetamine or saline for 15 days and were withdrawn for the indicated period of time before the test.

Convulsive threshold data represent the mean values and their standard deviations obtained from 10 mice/group. Head-twitch response to 50 mg/kg 5-HTP (i.p.) was determined in groups of 25-28 mice.

*As indicated by analysis of variance and the Newman-Keuls multiple means test, the values are significantly different from their control values (p <0.05; Snedecor and Cochran, 1967).

+Values significantly different from the control value as determined by a chi-square test (p < 0.05; Spiegel, 1961).
Table 3

Effect of haloperidol pretreatment on the development of reverse tolerance to amphetamine-caused stereotypy.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Stereotypy</th>
<th>No Stereotypy</th>
<th>Percent Stereotypy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Chronic amphetamine (10 mg/kg)</td>
<td>19*</td>
<td>3</td>
<td>86*</td>
</tr>
<tr>
<td>Chronic haloperidol (0.25 mg/kg) + Chronic amphetamine (10 mg/kg)</td>
<td>4</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Chronic haloperidol (0.25 mg/kg)</td>
<td>5</td>
<td>18</td>
<td>22</td>
</tr>
</tbody>
</table>

Four groups of 22-24 mice were chronically treated once daily for 15 days with the indicated regimen. On day 16, the number of mice showing signs of stereotypy following a 10 mg/kg (i.p.) amphetamine challenge dose was noted.

*Value significantly different than control as determined by a chi-square test (p < 0.01; Spiegel, 1961).
Table 4

Effect of MK-801 pretreatment on the development of reverse tolerance to amphetamine-caused stereotypy.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Stereotypy</th>
<th>No stereotypy</th>
<th>Percent stereotypy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>5</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Chronic amphetamine (10 mg/kg)</td>
<td>20*</td>
<td>4</td>
<td>83*</td>
</tr>
<tr>
<td>Chronic MK-801 (0.25 mg/kg) + Chronic amphetamine (10 mg/kg)</td>
<td>4</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Chronic MK-801 (0.25 mg/kg)</td>
<td>3</td>
<td>21</td>
<td>14</td>
</tr>
</tbody>
</table>

Four groups of 24-26 mice were chronically treated once daily for 15 days with the indicated regimen. On day 16, the number of mice showing signs of stereotypy following 10 mg/kg amphetamine challenge dose were noted.

*Value significantly different than control as determined by a chi-square test (p < 0.05; Spiegel, 1961).
amphetamine did not develop reverse tolerance, illustrating that the glutamate antagonist can block the development of the phenomenon.

The data shown in Table 5 demonstrate the influence of haloperidol and MK-801 pretreatment on the effect of chronic amphetamine treatment on the NMDLA and bicuculline convulsive thresholds. Twenty-four hr after the last treatment, the thresholds to NMDLA- or bicuculline-caused convulsions were assessed. The results show that haloperidol or MK-801 pretreatment can prevent not only the development of reverse tolerance, but also the concomitant changes in the convulsive thresholds to NMDLA and bicuculline.

The data displayed in Table 6 describe the effect of ketamine, cyproheptadine or diazepam pretreatment on the development of reverse tolerance to amphetamine. Mice were tested 24 hr after the last treatment, and their stereotypic response was recorded. While the pretreatment with ketamine prevented the development of reverse tolerance, the pretreatment with cyproheptadine or diazepam had no effect. The data indicate that the NMDA antagonist ketamine, like MK-801, can also block the development of reverse tolerance.

The data presented in Table 7 illustrate the effect of ketamine, cyproheptadine or diazepam pretreatment on the amphetamine-caused changes in the NMDLA- and bicuculline-convulsive thresholds. Mice were chronically treated with the indicated regimen for 15 days and their threshold to both, the NMDLA- and bicuculline-caused convulsions was obtained 24 hr after the last treatment. Pretreatment with ketamine prevented the decrease in convulsive threshold to NMDLA and the increase in convulsive threshold to bicuculline. On the other hand, pretreatment with cyproheptadine or diazepam had no effect on these changes in convulsive thresholds.
### Table 5

**Influence of haloperidol or MK-801 on the effect of chronic amphetamine pretreatment on NMDLA and bicuculline convulsive thresholds.**

<table>
<thead>
<tr>
<th>Chronic Pretreatment</th>
<th>Convulsive Threshold (mg/kg)</th>
<th>Convulsive Threshold (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NMDLA</td>
<td>Bicuculline</td>
</tr>
<tr>
<td>Saline</td>
<td>507 ± 126</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Amphetamine (10 mg/kg)</td>
<td>384 ± 42*</td>
<td>1.2 ± 0.1*</td>
</tr>
<tr>
<td>Haloperidol (0.25 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>490 ± 118</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>MK-801 (0.25 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>497 ± 115</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Haloperidol (0.25 mg/kg)</td>
<td>506 ± 93</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>MK-801 (0.25 mg/kg)</td>
<td>491 ± 86</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Mice were chronically treated with the indicated drugs or saline once daily for 15 days. Twenty-four hr after the last treatment, their response to NMDLA- and bicuculline-caused convulsions was assessed. Convulsive threshold data represent the mean values and their standard deviations obtained from 10 mice/group.

*As indicated by analysis of variance and the Newman-Keuls multiple means test, the values are significantly different from their control values (p < 0.05; Snedecor and Cochran, 1967).
Table 6

Influence of ketamine, cyproheptadine or diazepam on the development of reverse tolerance to amphetamine-caused stereotypy.

<table>
<thead>
<tr>
<th>Chronic Pretreatment</th>
<th>Stereotypy</th>
<th>No stereotypy</th>
<th>% Stereotypy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Amphetamine (10 mg/kg)</td>
<td>18*</td>
<td>5</td>
<td>78*</td>
</tr>
<tr>
<td>Ketamine (15 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>3</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Cyproheptadine (6 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>20*</td>
<td>4</td>
<td>83*</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>20*</td>
<td>3</td>
<td>87*</td>
</tr>
<tr>
<td>Ketamine (15 mg/kg)</td>
<td>4</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Cyproheptadine (6 mg/kg)</td>
<td>5</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>5</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Groups of 23-26 mice were chronically treated once daily for 15 days with the indicated regimen. On day 16, the numbers of mice showing signs of stereotypy following 10 mg/kg (i.p.) amphetamine challenge dose were recorded.

*Values significantly different than control as determined by a chi-square test (p < 0.01; Spiegel, 1961).
Table 7

Influence of ketamine, cyproheptadine or diazepam on the effect of amphetamine chronic pretreatment on NMDLA and bicuculline convulsive thresholds.

<table>
<thead>
<tr>
<th>Chronic Pretreatment</th>
<th>NMDLA</th>
<th>Bicuculline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>509 ± 64</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Amphetamine (10 mg/kg)</td>
<td>377 ± 43*</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>Ketamine (15 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>491 ± 90</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Cyproheptadine (6 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>390 ± 73*</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg) + Amphetamine (10 mg/kg)</td>
<td>397 ± 59*</td>
<td>1.4 ± 0.1*</td>
</tr>
<tr>
<td>Ketamine (15 mg/kg)</td>
<td>493 ± 96</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Cyproheptadine (6 mg/kg)</td>
<td>482 ± 127</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg)</td>
<td>521 ± 115</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Mice were chronically treated with the indicated drugs or saline for 15 days. Twenty-four hr after the last treatment their response to NMDLA- and bicuculline-caused convulsions was assessed. Convulsive threshold data represent the mean values and their standard deviations obtained from 10 mice/group.

*As indicated by analysis of variance and the Newman-Keuls multiple means test, the values are significantly different from their control values (p < 0.05; Snedecor and Cochran, 1967).
DISCUSSION

The results presented demonstrate that the repeated daily administration of amphetamine results in an enhanced stereotypic behavioral response in mice. This observation is consistent with previous studies in which reverse tolerance was described in mice (Hitzemann, Loh, Craves and Domino, 1973; Short and Shuster, 1976; Chaudhry et al., 1988) and in many other species, including humans (see, for review, Robinson and Becker, 1986). The response remained augmented 28 days after amphetamine withdrawal, suggesting that reverse tolerance is a relatively long-lasting phenomenon. The persistence of reverse tolerance in mice resembles that seen in rats withdrawn from drug treatment for as long as one month (Hitzemann, Tseng, Hitzemann, Sampath-Khanna and Loh, 1977; Koltz, Shreve, De Souza and Uretsky, 1985). Similarly, Ellinwood (1974) reported that cats displayed reverse tolerance to amphetamine-induced stereotypy and dyskinesia for at least two months after drug withdrawal. Thus, chronic amphetamine treatment causes a persistent change in the CNS.

The underlying mechanism of amphetamine-induced stereotypic behavior may be related to dopaminergic function in the striatum (Randrup and Munkvad, 1966; Scheel-Kruger, 1970; Cole, 1978). Amphetamine releases dopamine (Ungerstedt, 1971; Arnold, Molinoff and Rutledge, 1977), which interacts with the postsynaptic dopamine receptors. Support of this notion comes from studies in which the selective destruction of dopamine neurons or dopaminergic nerve terminals by surgical or chemical lesions (Ungerstedt, 1971; Creese and Iversen, 1972; Kelly, Seviour and Iversen, 1975), eliminates amphetamine-induced behavior. Furthermore, pretreatment with the dopaminergic blocker haloperidol prevents the acute effects of amphetamine, presumably because the released
dopamine is unable to react with its postsynaptic receptor sites (Munkvad, Pakkenberg and Randrup, 1968).

The mechanism of the enhanced dopaminergic response after chronic amphetamine treatment is not understood, but there are several ways the system could change. First, it could involve autoreceptor sensitivity. Since the release of dopamine appears to be controlled in part by a negative feedback mechanism (Groves, Wilson, Young and Rebec, 1975), the possibility exists that reverse tolerance is the result of a presynaptic phenomenon. Based on observations that chronic amphetamine decreases [3H]apomorphine binding, Martres, Costentin, Baudry, Marcais, Protais and Schwartz, (1977) and Muller and Seeman (1979) suggested that subsensitivity of autoreceptors may be the mechanism of reverse tolerance. Muller and Seeman (1979) argued that low concentrations of [3H]apomorphine used in their study reflected binding to presynaptic dopamine receptors; however, Conway and Uretsky (1982) demonstrated that subsensitivity of dopamine autoreceptors itself can not account for the enhanced behavioral effects to amphetamine. Their conclusion was based on findings that chronic amphetamine treatment did not alter the inhibitory effect of low doses of apomorphine on the motor activity of rats. Apomorphine was used since it has been demonstrated that relatively low doses decrease motor activity by the stimulation of dopamine autoreceptors which inhibit dopamine release from dopaminergic terminals (Skirboll, Grace and Bunney, 1979).

A second explanation of reverse tolerance is that it involves a change in dopamine postsynaptic receptors. Klawans and Margolin (1975) and Kilby and Ellinwood (1977) suggested that up-regulation of postsynaptic dopaminergic receptors may cause reverse tolerance. Their interpretation was based upon observations that chronic pretreatment of guinea pigs and rats with amphetamine results in an augmented response to apomorphine-caused stereotypy. They assumed that apomorphine acts predominantly at postsynaptic dopamine receptors and concluded that reverse tolerance results from an alteration in
postsynaptic dopamine receptors. These observations, however, were not confirmed by Conway and Uretsky (1982), who demonstrated that apomorphine-caused stereotypy in rats is not enhanced following chronic amphetamine treatment. Paradoxically, studies involving the direct measurement of dopamine receptors report a reduction in radioligand binding to brain dopamine receptors (Howlett and Nahorski, 1979; Nielsen, Nielsen, Ellison and Braestrup, 1980; Robertson, 1983), suggesting that down-regulation rather than up-regulation results from chronic amphetamine treatment.

Finally, reverse tolerance may be due to enhanced release of dopamine by amphetamine. Chronic amphetamine results in enhanced dopamine release from rat striatal slices upon re-exposure to amphetamine (Castaneda, Becker, Wilcox and Robinson, 1985; Kolta et al, 1985a), and the enhanced dopamine release by amphetamine persists for a long period of time after amphetamine withdrawal (Kolta et al., 1985a); this persistence corresponds with the long-lasting reverse tolerant state of animals. Additional evidence for an amphetamine-induced enhanced dopamine release has also been presented by a microdialysis study in freely moving rats (Robinson, Jurson, Bennett and Bentgen, 1988). Contradicting the enhanced dopamine release hypothesis are observations that behavioral differences in acutely and chronically treated animals are not accompanied by comparable differences in dopamine synthesis or metabolism (Kuczenski and Segal, 1988). Furthermore, reverse tolerance occurs to methylphenidate (Shuster et al., 1982), even though its effects are mediated through the inhibition of dopamine reuptake instead of the release of dopamine (Ross, 1978). Nevertheless, striatal tissues taken from animals chronically treated with methylphenidate also show enhanced release of dopamine when perfused with amphetamine (Kolta et al., 1985b). The enhanced release hypothesis, however, does not fit the observations that reverse tolerance can also develop to the direct dopaminergic receptor agonists, such as apomorphine (Baily and Jackson, 1978) and lisuride (Carruba, Ricciardi, Chiesara, Spano and Mantegazza, 1985) which do not involve the release of dopamine. In addition, reverse tolerance can develop to the...
stimulant effects of morphine which do not require the release of dopamine (Villarreal, Guzman and Smith, 1973).

To date, none of the proposed dopaminergic mechanisms satisfactorily accounts for the phenomenon of reverse tolerance. The present study of the mechanism of reverse tolerance was predicated on the assumption that the phenomenon may be the result of a change in the functional properties of other neurotransmitter systems that interact with the dopamine system (Robinson and Becker, 1986); hence, some other neural correlates of reverse tolerance were sought. The rationale was that because reverse tolerance is a long-lasting phenomenon, there may be concomitant long-lasting alterations in the functional status of participating neurotransmitters. Systems were evaluated for which there are relatively selective agonist and antagonist drugs and for which some tests of behavioral responsiveness are available.

The results obtained demonstrate that chronic amphetamine treatment resulted in a change in the functional responsiveness of glutamatergic, GABAergic and serotonergic systems, as measured by NMDLA- and bicuculline-caused convulsions and 5-HTP-caused head-twitch. No changes were observed in the glycinergic, adenosinergic or cholinergic systems, as assessed by strychnine-, aminophylline- or arecoline-caused convulsions. The data in Table 2 reveal that changes in the glutamate system and the GABA system were long-lasting and may coincide with the reverse tolerant state. In contrast, the change in the serotonin system was relatively short-lived because the responsiveness returned to normal within 15 days of amphetamine withdrawal.

The haloperidol studies were conducted to determine if reverse tolerance to amphetamine involves dopaminergic mechanisms. Previously, haloperidol was shown to block the development of reverse tolerance to amphetamine-caused stereotypy in rats (Kuczenski and Leith, 1981); and the present results confirm that such pretreatment can also block the development of reverse tolerance in mice. Furthermore, these results indicate that pretreatment with haloperidol not only blocked reverse tolerance to behavioral
effects of chronic amphetamine, it also prevented the associated changes in the glutamate, GABA and serotonin systems. Such data suggest that the amphetamine-induced alterations in dopamine function during the chronic pretreatment phase are necessary for the subsequent expression of the reverse tolerance phenomenon, as well as for the observed changes in the glutamate, GABA and serotonin systems.

The functional modifications in the glutamate, GABA and serotonin systems posed the question of the involvement of these neurotransmitters in the development of reverse tolerance. In order to test for their involvement, individual systems were evaluated with the use of drugs known to affect them. With the assumption that pretreatment with a GABAergic drug might affect reverse tolerance and the concomitant changes in threshold to NMDLA- and bicuculline-caused convulsions, mice were pretreated with a well-recognized GABAergic drug, diazepam, during the course of chronic amphetamine treatment. The pretreatment, however, did not modify the development of reverse tolerance or the change in responsiveness of the glutamate and GABA systems. A GABAergic drug was selected for use rather than an antagonist like bicuculline because the chronic exposure to the antagonists results in kindling and death (unpublished observations). Previously, Lynch and Leonard (1978) reported alterations in the GABA content of various brain regions after chronic amphetamine treatment; however, they were unable to relate any specific change to reverse tolerance and suggested that changes in the GABA system may be secondary to the reverse tolerance phenomenon.

Serotonin can modulate the acute behavioral effects of amphetamine by virtue of an inhibitory effect on dopaminergic neurotransmission (Mabry and Campbell, 1973). Evidence in support of serotonin's inhibitory role includes the potentiation of amphetamine-induced activity after lesions of the raphe nuclei (Neill, Grant and Grossman, 1972), after pretreatment with p-chlorophenylalanine (Mabry and Campbell, 1973) and after treatment with serotonin receptor blocking agents (Hollister, Breese, Kuhn, Cooper and Schanberg, 1976). There is also evidence that acute amphetamine
causes release of serotonin from the presynaptic serotonergic terminals in the striatum (Geyer, Dawsey and Mandell, 1975), in addition to the release of dopamine. More recent evidence for amphetamine-caused release of serotonin comes from in-vivo intracerebral microdialysis studies (Kuczenski and Segal, 1989). Because serotonin exerts an inhibitory effect on the presynaptic dopamine release acutely, it is possible that with chronic amphetamine treatment, serotonin's ability to inhibit dopamine release is diminished, resulting in enhanced dopamine release. The results from the head-twitch studies suggest an enhanced serotonergic responsiveness following chronic amphetamine treatment. The short-lived enhanced responsiveness of this system, however, indicates that the change is not required for the persistence of reverse tolerance. Such data, however, do not rule out the possibility of the involvement of serotonin system in the development of reverse tolerance. Therefore, the potential role of this system in the development of reverse tolerance was investigated by the use of a serotonergic antagonist, cyproheptadine. Pretreatment with this drug blocked the occurrence of cross-reverse tolerance to the 5-HTP-caused head-twitch, but not the development of reverse tolerance to the stereotypic effects of amphetamine (Karler, Calder and Turkanis, 1990). Pretreatment with cyproheptadine also failed to prevent changes in the glutamate or GABA system observed following the chronic amphetamine. Such data suggest that the serotonin system is not required for the development of reverse tolerance to stereotypy and that the change in the functional status of this system observed following chronic amphetamine is independent of amphetamine reverse tolerance. Furthermore, the relatively short-lived change in the serotonin system appears to be secondary to the changes responsible for persistence of reverse tolerance. It was also found that pretreatment with haloperidol during chronic amphetamine treatment blocked the cross-reverse tolerance to the 5-HTP-caused head-twitch (Karler et al., 1990), suggesting that dopaminergic mechanisms are required for the enhanced serotonergic responsiveness.
The decrease in threshold to NMDLA-induced convulsions implicates the glutamate system in the phenomenon of reverse tolerance. In order to see if such changes are independent of reverse tolerance, the involvement of this system was investigated with the use of the noncompetitive glutamate antagonists MK-801 and ketamine (Anis, Berry, Benton and Lodge, 1983; Wong, Kemp, Priestley, Knight, Woodruff and Iversen, 1986; Kemp, Foster and Wong, 1987). Both of these drugs raise the threshold to NMDLA-induced convulsions, and chronic pretreatment with either MK-801 or ketamine blocked the development of reverse tolerance, as well as the associated changes in the convulsive thresholds to NMDLA and bicuculline.

The data presented demonstrate that the glutamate system, as well as the dopamine system, appear to be involved in the phenomenon of reverse tolerance to the stereotypic effects of amphetamine. Several sets of data support this notion: First, the drug responsiveness of the glutamate system is altered when mice are made reverse tolerant to stereotypy; second the change in the glutamate system associates with the persistence of the enhanced behavioral effect of amphetamine; and third, glutamatergic antagonist block both the appearance of reverse tolerance and the lowering of threshold to NMDLA-caused convulsions. Because haloperidol, a dopaminergic antagonist, also blocks the development of reverse tolerance and the subsequent change in the glutamate system, the data suggest that there exists a functional link between the glutamatergic and the dopaminergic systems. How these two systems interact in the development of reverse tolerance can not be explained by the available data. However, several studies have demonstrated the existence of interactions between glutamatergic and the dopaminergic pathways (Roberts and Anderson, 1979; Rowlands and Roberts, 1980; Kerkorian, Dusticier and Nieoullon, 1987). Further studies are required to define the role of the glutamatergic and the dopaminergic systems in the phenomenon of reverse tolerance.
REFERENCES


