

Methamphetamine and Methylenedioxymethamphetamine Neurotoxicity: Possible Mechanisms of Cell Destruction

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BACKGROUND

Methamphetamine and Related Drugs Have High Abuse Liability

Methamphetamine and amphetamine, potent indirectly acting sympathomimetic amines and related compounds, are self-administered by experimental animals and abused by humans. Methylenedioxymethamphetamine (MDMA) shares some discriminative properties with amphetamine and has been reported to be heavily used for recreational purposes among certain groups. Although the abuse liability of methamphetamine and its congeners was recognized shortly after the recognition of their pharmacological properties, a concerted effort to assess long-term effects in the central nervous system (CNS) was only made in the last 15 years. The effort to determine possible neurotoxic effects was in part prompted by epidemics of methamphetamine abuse between 1950 and 1970 in Japan, Sweden, Great Britain, and the United States (Brill and Hirose 1969; Jonsson and Gunne 1970; Kramer et al. 1967). Since the middle 1970s, cocaine abuse has increased to epidemic proportions. The acute psychoactive effects of methamphetamine are similar to those of cocaine, but the effects of methamphetamine last longer (Seiden et al. 1993).

Data are available on the neurotoxic effects of long-term administration of high doses of methamphetamine to experimental animals, but there are no similar data for cocaine. Although some of the potentially dangerous effects of methamphetamine on the human brain are known, the duration of these effects and/or their physiological and behavioral consequences are not well understood. Such information would provide valuable insight and guidance for treatment and prevention programs, and it would further the understanding of neurobiological principles of drug-induced CNS injury. Understanding how these drugs' biochemical and pharmacological interactions lead to cell death may enhance understanding of cell death in the

CNS caused by disease, environmental toxins, and aging, and lead to preventive or ameliorative therapies.

The social problems caused by abuse of these drugs may result from or be compounded by their neurotoxic effects. While the neurotoxic doses of amphetamine and methamphetamine are between 10 and 20 times the dose required to affect behavior (Koda and Gibb 1973; Seiden and Ricaurte 1987), the toxic dose of MDMA is only 2 to 4 times that required to affect behavior. Methamphetamine (100 milligrams per kilogram (mg/kg)) and MDMA (40 mg/kg) can cause the same toxic response with only one injection of the drug at a somewhat higher unit dose than the eight injections over 4 days used in the authors' original paradigm (Seiden and Ricaurte 1987).

Methamphetamine and MDMA Have Toxic Effects on Monoamine-Containing Nerve Cells

Methamphetamine is selectively toxic to dopamine (DA) and 5-hydroxytryptamine (5-HT) nerve terminals in the CNS, while MDMA is selectively toxic to 5-HT terminals. The neurotoxicity is evidenced by: 1) long-lasting depletions of the specific neurotransmitter in the CNS (Seiden and Ricaurte 1987); 2) reduction of V_{max} for the rate-limiting enzymes (in the case of destruction of DA and 5-HT terminals the enzymes are tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), respectively); 3) reduction in the number of DA and 5-HT uptake sites (Commins et al. 1987b; Wagner et al. 1980b); 4) morphological evidence of neurotoxicity showing that cells in DA and 5-HT regions are argyrophilic after methamphetamine or MDMA treatment (Steranka and Sanders-Bush 1980; Wagner et al. 1980a, 1980b); and 5) immunohistochemical evidence showing swelling and fragmentation of axons in the short term, and decreased immunoreactivity in the long term, with morphology that is consistent with cell death being the result of necrosis (Axt and Molliver 1991; O'Hearn et al. 1988) as opposed to programmed cell death or apoptosis.

An important issue with respect to neurotoxicity involves the long-term effects of methamphetamine and MDMA as indicated by the length of time these effects are observed after drug treatment, which engenders depletion of the transmitter. In the rhesus monkey, there are data showing that changes persist for over 3 years (Woolverton et al. 1989). Several reports exist in which the long-term effect of MDMA on the 5-HT system in the rat was investigated. 5-HT tissue concentrations show a pattern of partial recovery, but continue to be significantly

reduced at 52 weeks posttreatment (De Souza et al. 1990). De Souza and colleagues (1990) used a treatment regimen of 20 mg/kg administered eight times at 12-hour intervals. Using a lower dose (10 mg/kg four times at 1-hour intervals), Scanzello and colleagues (1993) found significant reductions of 5-HT tissue concentrations at 2 to 32 weeks (depending on the region), but complete recovery at 52 weeks post-treatment. The number of cortical 5-HT uptake sites (as measured by specific binding to the transporter) was completely recovered (Battaglia et al. 1988; Scanzello et al. 1993) at 52 weeks posttreatment, while hippocampal 5-HT uptake sites were still significantly decreased after 52 weeks (Scanzello et al. 1993). Functional uptake (as measured by the transport of 5-HT across the cell membrane), while showing a pattern of recovery, was found to be significantly reduced 1 year posttreatment (20 mg/kg eight times at 12-hour intervals) (Lew et al. 1993). Although these three reports are not in complete agreement on the extent of recovery of the 5-HT system at 52 weeks post-MDMA treatment, they do agree that each demonstrates a pattern of serotonergic recovery after high-dose MDMA treatment. Whether this recovery persists or reverses (see Zaczek et al. 1990) remains to be determined.

In addition to the measures discussed above, the long-term effects of methamphetamine and related compounds on DA receptors have been investigated. The results obtained are equivocal; increases, decreases, and lack of effects have been reported (Robinson and Becker 1986). The absence of consistent results may be attributable to the use of slightly different binding techniques (e.g., use of different displacing agents) as well as varying dosing regimens. Since most previous studies also used low repeated doses of methamphetamine, it is difficult to determine whether the changes observed were related to neurotoxicity. Several studies using high doses of methamphetamine have demonstrated decreases in DA receptor binding (McCabe et al. 1987; Schmidt et al. 1985a, 1985b). Interestingly, McCabe and colleagues (1987) reported that DA type 1 (D_1) receptors remained decreased in the substantia nigra as long as 21 days after a neurotoxic methamphetamine regimen.

PROPOSED MECHANISMS OF METHAMPHETAMINE AND MDMA NEUROTOXICITY

In an overview of selective neurotoxicity, Baumgarten and Zimmerman (1992) proposed general mechanisms that can cause cell death. These are conceptually useful as a framework for understanding the mechanisms

underlying nerve cell death engendered by drugs. As noted by Baumgarten and Zimmerman (1992), specific types of pathology were observed in different neuroanatomical regions of the CNS resulting from hypoxia and ischemia. Baumgarten and Zimmerman (1992) discussed three types of trauma that induce neurotoxicity that are not mutually exclusive. First, an inadequate supply of glucose and/or oxygen to the CNS depletes energy stores and results in cell death. Second, synaptic transmission mediated by excitatory transmitters such as glutamate may lead to high Ca^{++} influx into neurons which, if high enough, can cause cell death. Third, specific neurotoxicity is engendered by a toxin that has high and specific affinity for the membrane transporter, which is responsible for uptake of the transmitter. Toxins transported into neurons may be formed by auto-oxidation of endogenous neurotransmitters (e.g., DA and 5-HT) to form hydroxy derivatives. Although the mechanism by which these (6-hydroxydopamine and 5,7-dihydroxytryptamine) compounds cause neurotoxicity is uncertain, these transporter-specific toxins are highly reactive and may themselves generate destructive free radicals or cross-link proteins that contain reactive sulfhydryl groups.

A Toxic Metabolite of the Amphetamine Analog Is Formed

An approach used in the search for a toxic metabolite of amphetamine-like compounds was to directly inject the parent drug into the brain. If the parent drug is effective, then one can rule out metabolites that are formed in the periphery (Sherman et al. 1975); however, a toxic metabolite may be formed in the brain. Direct injections of MDMA into the brain did not mimic peripheral injections in its acute (Schmidt and Taylor 1988) or long-term effects (Paris and Cunningham 1991). However, when MDMA was infused into the brain over a 1-hour period, the behavioral and neurochemical acute effects were observed (Schmidt and Taylor 1988).

Intracerebral injections of two metabolites of parachloroamphetamine (PCA), 3-chloro-4-hydroxy amphetamine and 4-chloro-3-hydroxy amphetamine, were minimally effective in changing serotonin levels. Only the 4-Cl-3-OH compound was active, and only at 24 hours postinjection, not at 2 weeks. McCann and Ricaurte (1991) showed that intracerebral injections of two metabolites of methylenedioxymphetamine (MDA) (which itself is a metabolite of MDMA), alpha-methyldopamine and 3-O-methyl-a-methyldopamine, did not cause MDA-induced serotonergic neurotoxicity. In addition, systemic injection of the two MDA metabolites

did not cause long-term effects on the serotonin system (McCann and Ricaurte 1991).

Steele and colleagues (1991) found that alpha-methylepinephrine, a metabolite of MDMA formed by demethylenation, failed to damage the 5-HT system in rats. In addition, Lewander (1971) reported that guinea pigs, a species that does not metabolize amphetamine by para-hydroxylation, still showed neurotoxic damage from amphetamine. Finally, when ipindol treatment (which inhibits para-hydroxylation of the parent drug) (Freeman and Sulser 1972) precedes PCA (Sherman et al. 1975), the short-term and long-term effects on the 5-HT system are not blocked or attenuated. Ricaurte and colleagues (1984a) showed that at a dose of amphetamine which was ineffective in producing long-term DA depletions, the combination of amphetamine plus ipindol resulted in long-lasting DA depletions, suggesting that the prolongation of the half-life of amphetamine caused the toxicity of amphetamine (Ricaurte et al. 1984a). Based on the above discussion, the toxic drug metabolite theory of amphetamine (and related compounds) neurotoxicity has little support. It should be noted, however, that an exhaustive study of all possible metabolites of the amphetamine class of drugs has not been done.

DA Is Important for Neurotoxicity Induced by Amphetamine-Like Drugs

An intact DA system appears to be necessary for methamphetamine- and MDMA-induced neurotoxicity to the DA and 5-HT systems of the brain (Nash et al. 1990; Schmidt et al. 1985a, 1992b). Inhibition of DA synthesis with alpha methyltyrosine (AMT) blocks MDMA- and methamphetamine-induced damage to both the DA and 5-HT systems (Axt et al. 1990; Schmidt et al. 1985b). Administration of l-dihydroxyphenylalanine (L-dopa), thus replacing the AMT-depleted DA, blocks the protective effects of AMT (Schmidt et al. 1985b). The induction of DA depletion with 2,3,5-trihydroxyphenethylamine (6-OHDA) also blocks MDMA toxicity to the 5-HT system (Schmidt et al. 1990b; Stone et al. 1988). These results led to the theory that DA mediates methamphetamine- or MDMA-induced 5-HT neurotoxicity (Schmidt et al. 1985b). One difficulty with this hypothesis is that much of the 5-HT terminal damage occurs in brain regions which have essentially no dopaminergic innervation (e.g., hippocampus) (Verhage et al. 1992). The anatomical location for a putative DA and 5-HT interaction is not presently understood, but may occur in the brainstem.

An Excitatory Feed-Forward Loop Enhanced by Methamphetamine May Produce Metabolic Conditions That Cause Neurotoxicity

Carlsson (1992, 1995; Carlsson et al. 1995) has elaborated on the feed-forward neural circuit, which coincides with the extrapyramidal motor system. Carlsson proposes that when the system is stimulated by methamphetamine, the excessive neural activity may mediate methamphetamine-induced neurotoxicity to the DA system. Theoretically, the pathway involved (cortex-striatum-pallidus-thalamus-subthalamus-cortex) is excited by methamphetamine or related compounds, causing a continued excitation of 5-HT and DA neurons. This maintained activity of the DA and 5-HT systems demands excess energy. During repeated activity, the cell is depolarized and repolarized; Na^+ and Ca^{++} move into the cell and must be removed. The cells cannot maintain homeostasis and therefore die. This theory is discussed in detail elsewhere (Carlsson 1992, 1995; Carlsson et al. 1995), and is consistent with the some of the data (see below) concerning pharmacological treatments that prevent methamphetamine-induced neurotoxicity.

NMDA Receptor Mediation of Neurotoxicity Induced by Amphetamine-Like Compounds: A Role for Glutamate

Sonsalla and colleagues (1989) first reported that MK-801 (a noncompetitive antagonist at the N-methyl-D-aspartate (NMDA) glutamatergic site) could antagonize the methamphetamine-induced neurotoxicity to DA neurons. The protective effects of MK-801 are consistent with a Ca^{++} theory of methamphetamine neurotoxicity. MK-801 blocks Ca^{++} entry into the cell; this blockade may be important for two reasons. Keeping extracellular Ca^{++} from entering the neuron would diminish the probability of Ca^{++} -induced cell death (Nicotera et al. 1990). In addition, by blocking Ca^{++} entry into the cell, subsequent Ca^{++} -induced Ca^{++} release from intracellular stores could also be blocked (Frandsen and Schousboe 1992; Lei et al. 1992).

MK-801's protective effect may also be related to temperature regulation. Schmidt and colleagues (1990a) and Bowyer and colleagues (1992) have shown that lowering ambient temperature can protect against MDMA and methamphetamine neurotoxicity. In a series of studies, Bowyer and colleagues (Bowyer et al. 1992, 1993, 1994) have shown that rats injected with methamphetamine at an ambient temperature of 23 °C had significant depletions of striatal DA, whereas rats that were injected in an

ambient temperature of 4 °C did not show any depletion in striatal DA. In addition, they have shown that rats which became very hyperthermic in response to methamphetamine treatment, but were cooled to prevent death, had larger DA depletions than rats that did not show the same degree of methamphetamine-induced hyperthermia. Bowyer and colleagues concluded that the hyperthermia induced by methamphetamine is related to the DA depletions, but hyperthermia alone does not cause the DA depletions produced by methamphetamine.

The noncompetitive NMDA receptor antagonist MK-801 attenuates depletions of 5-HT induced by MDMA. MK-801 has been shown to induce hypothermia in rat models of ischemia. The question arose as to whether MK-801 and two other glutamate antagonists, CGS 19755 (CGS) and NBQX, protect against MDMA-induced 5-HT depletions by induction of hypothermia. Male Sprague-Dawley rats were injected with either saline (SAL), MK-801 (2.5 mg/kg), CGS (25.0 or 50.0 mg/kg x 2), or NBQX (30.0 mg/kg x 2 or 55.0 mg/kg x 3) followed by either MDMA (40.0 mg/kg) or SAL. Core body temperature was monitored for 4 hours or longer using radiotelemetry. Baseline temperature was between 37.0 ° and 37.6 °C. Administration of MK-801 with MDMA significantly decreased temperature to 34.0 ± 0.39 °C within 2 hours of the MDMA injection, and it also protected against serotonergic toxicity. Neither MDMA alone nor MK-801 alone had a significant effect on temperature over the same time period. When rats were treated with MK-801 plus MDMA and temperature was maintained between 38.4 °C and 40.4 °C for 4 hours, protection against 5-HT depletion was abolished. Coadministration of the competitive NMDA antagonist CGS with MDMA resulted in a decrease in temperature to 34.5 ± 0.27 °C and provided partial protection against 5-HT depletions. When the AMPA receptor antagonist NBQX was administered with MDMA, temperature did not differ from rats treated with saline plus MDMA, and NBQX did not protect against 5-HT depletions.

The data from this study (Farfel 1993) show that coadministration of NMDA antagonists with MDMA induces hypothermia in dose combinations which protect against serotonergic toxicity, and neuroprotection is abolished when temperature is maintained above 38.4 °C (Farfel 1993). These data indicate that hypothermia induced by NMDA receptor antagonism plays a role in protection against serotonergic toxicity. MK-801, when given in combination with MDMA, decreases body temperature by 3 to 5 °C (Farfel 1993). In addition, if rats are kept at normal body temperature through artificial heating, the protective

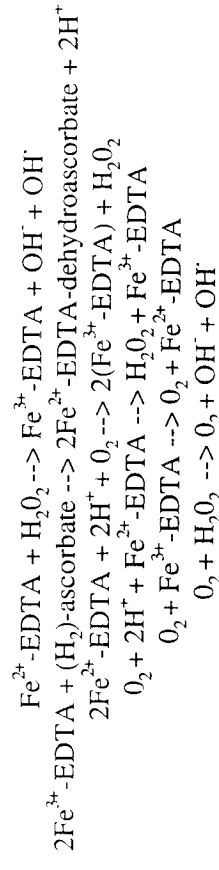
effect of MK-801 is reversed. Therefore, MK-801 may be protecting against methamphetamine- and MDMA-induced neurotoxicity by slowing down cellular processes, including the toxic process.

Holson and colleagues (1993) have reported that haloperidol and diazepam, which protect against amphetamine neurotoxicity, also lower core body temperature. As suggested by these authors, any compound that is shown to protect against toxicity may have an effect on temperature regulation mechanisms. This is a developing issue in the field of amphetamine-analog neurotoxicity. By determining which compounds protect by cooling alone, researchers may be able to narrow the field of possible mechanisms of neurotoxicity.

Hydroxy Radical Formation and Methamphetamine Neurotoxicity

Senoh and Wiktop (1959) observed the presence of trihydroxyphenethylamines in the urine of some schizophrenic patients, which suggested the formation of an unusual metabolite of DA. Substitution of a hydroxy group in the fifth position on the phenyl ring of DA leads to the formation of 6-OHDA. Cohen and Heikkilä (1974) showed that DA could be converted to one of three trihydroxyphenethylamines via the Fenton-Huber-Weiss reactions in a system where there was Fe^{++} , hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA), and DA.

Fenton-Huber-Wise reactions:



Based on the reports of Senoh and colleagues (1959) and Cohen and Heikkilä (1974), the authors hypothesized that injections of large doses of methamphetamine could result in the formation of a toxic metabolite of DA. The *in vivo* condition seemed close to the *in vitro* conditions described by Cohen and Heikkilä. Riederer and colleagues (1989) and Halliwell (1989) showed that there is Fe^{++} stored in many regions of the brain. Hydrogen peroxide is a product of monoamine oxidase metabolism, and its concentration is normally kept small by catalase. If there is excess

hydrogen peroxide, however, it could undergo Fe^{++} catalysis and result in hydroxy radical formation. Hydroxy radicals are characterized by single unpaired electrons in their outer orbit and are, therefore, highly reactive (Cohen and Heikkilä 1974; Halliwell and Gutteridge 1984). The hydroxy radical, once formed, could react with DA to form 6-OHDA. It is possible that with large amounts of DA in the synaptic cleft after high-dose methamphetamine treatment, a small proportion of DA could be metabolized to 6-OHDA and be transported back into the DA neuron through the DA transporter. Once inside the neuron, it can be converted to a semiquinone. The reactive semiquinone seeks an electron donor such as the sulphydryl groups on cysteine or methionine (components of long-chain proteins). When the semiquinone and long-chain proteins are cross-linked through sulphydryl bonds, the proteins are denatured and no longer functional (Fornstedt and Carlsson 1989; Fornstedt et al. 1986).

Seiden and Vosmer (1984) have detected 6-OHDA in the striatum of rats and 5,6-dihydroxytryptamine (5,6-DHT) in the hippocampus (Commins et al. 1987a) after a single large dose of methamphetamine. The authors assumed that both conversions proceeded according to a Fenton-type reaction. Attempts to replicate this work have proved difficult; there were instances when neither 6-OHDA nor 5,6-DHT could be detected in any of the rats treated with methamphetamine. Rollema and colleagues (1986) failed to detect extracellular 6-OHDA in rats treated with methamphetamine using the *in vivo* dialysis technique. In addition, other investigators have tried to measure tissue concentrations of 6-OHDA after methamphetamine treatment, but then either found the results inconsistent from rat to rat or could not detect any of the hydroxylated derivatives of DA (Cohen and Gibb, personal communication, 1989). Recently, Wagner and colleagues (1993) reported the formation of 6-OHDA in the microgram range after the rats were treated with methamphetamine; in this experiment a monoamine oxidase (MAO) inhibitor and a catechol-O-methyltransferase inhibitor were administered before treatment with methamphetamine. Similar results have been obtained with the use of an MAO inhibitor (Marek et al. 1990c). Although the data are inconclusive at present, the *in vivo* formation of the neurotoxins 6-OHDA and 5,6-DHT would account for the specificity of methamphetamine effects on DA and 5-HT neurons.

Zigmond and colleagues (Hastings and Zigmond 1992; Zigmond and Hastings 1992) investigated the role of endogenous DA in DA neurotoxicity induced by methamphetamine. They reported the oxidation of DA and the formation of cysteinyl-DA adducts using both *in vitro* and *in*

METHAMPHETAMINE- AND MDMA-INDUCED NEUROTOXICITY CAN BE ANTAGONIZED PHARMACOLOGICALLY

AMT Attenuates Methamphetamine and MDMA Neurotoxicity

AMT prevents methamphetamine-induced depletion of DA and 5-HT (Axt et al. 1990; Ricaurte et al. 1984b; Schmidt et al. 1985b; Wagner et al. 1983). AMT also prevents the MDMA-induced depletion of 5-HT (Stone et al. 1988) and partially attenuates PCA depletion of 5-HT (Axt and Seiden 1990). An interpretation of these findings is that DA release is necessary for methamphetamine- or MDMA-induced neurotoxicity to DA and 5-HT neurons (Schmidt et al. 1985b). The data obtained with AMT are also consistent with the idea that DA is important in driving a potentially toxic, feed-forward, striatal-thalamic-cortical loop (Carlsson 1992, 1995; Carlsson et al. 1995). The AMT results are also consistent with the proposal that the release of DA engenders the formation of neurotoxic metabolites of DA (Commins et al. 1987a; Giovanni et al. 1992; Hastings and Zigmond 1992; Liang et al. 1992; Seiden and Vosmer 1984; Zigmond and Hastings 1992). AMT pretreatment has been shown to decrease amphetamine-induced DA release (Butcher et al. 1988); AMT, therefore, decreases the availability of DA for hydroxy radical reactions. The AMT results do not provide direct support for the drug metabolite or NMDA receptor hypotheses. However, there are preliminary results from the authors' laboratory (unpublished observations) that the combination of methamphetamine plus AMT causes a decrease in core temperature in rats; therefore, the mechanism of action of AMT may be similar to that of MK-801.

DA Receptor Antagonists Block Methamphetamine and MDMA Neurotoxicity

DA antagonists (haloperidol, chlorpromazine) prevent methamphetamine- and MDMA-engendered neurotoxicity (Hotchkiss and Gibb 1980; Schmidt et al. 1990a; Sonsalla et al. 1986). The most parsimonious explanation for DA antagonism by haloperidol in the context of current theories is that the antagonist alters output of the striatal-thalamic-cortical circuit as described above (Carlsson 1992, 1995; Carlsson et al. 1995). By blocking striatal DA receptors, one could theoretically interrupt the dopaminergic influence on the striatal-thalamic-cortical loop. The protection afforded by DA antagonists is difficult to integrate with other theories of neurotoxicity. Haloperidol does not block amphetamine-

vivo systems. Although DA oxidation can proceed nonenzymatically (see table 1), they examined the formation of the hydroxy radical as an enzymatic reaction. Peroxidase enzymes are capable of catalyzing the conversion of DA to reactive DA quinones. Since peroxidase enzymes are not present in brain, they tested a similar enzyme, prostaglandin (PG) synthase, which is present in brain. When purified PG synthase was combined with DA and bovine serum albumin, they identified a DA quinone and a cysteinyl-DA adduct. It was inferred from this reaction that hydroxy radicals could be formed (see enzymatic reaction in table 1). They concluded that DA oxidation could be catalyzed by PG synthase and, importantly, that the oxidized quinone was a potential mechanism for cytotoxicity.

TABLE 1. Toxic metabolite formation.

Nonenzymatic reaction	Enzymatic reaction
$H_2O_2 + Fe^{3+} \rightarrow OH + OH^{\cdot}$	$H_2O_2 + DA \xrightarrow{PG\text{-}SMT} \text{Quinone} + OH + OH^{\cdot}$
$OH + DA \rightarrow 6\text{-OHDA}$	$\text{Quinone} + \text{Cysteine} \rightarrow \text{Cysteinyl-DA adduct}$
$6\text{-OHDA} \rightarrow \text{Semiquinone}$	
$\text{Semiquinone} + \text{Cysteine} \rightarrow \text{Cysteinyl-DA adduct}$	

Hydroxy radicals in rat brain have recently been detected by allowing them to react with injected salicylates to form 2,5-dihydroxybenzoic acid (Liang et al. 1992). This proves to be a useful technique for measurement of hydroxy radical formation in vivo (Giovanni et al. 1992). Methamphetamine (12.5 mg/kg 4 x 2 hour) caused an increase in free hydroxy radicals as measured by the salicylate techniques, and the increase in free radicals was blocked by AMT. These results again suggest that high neurotoxic doses of methamphetamine promote the formation of free radicals and that DA plays a role in the formation of free radicals when methamphetamine is given in neurotoxic doses.

induced DA release (Nash and Yamamoto 1992), and in fact it increases DA synthesis (Carlsson and Lindqvist 1963). The haloperidol result, therefore, does not fit well with the hydroxy radical theory because the synthesis of DA as well as its release are increased. Nor does the neuroprotection of haloperidol fit well with the idea that an intact DA system is needed for neurotoxicity: With haloperidol, the DA neuron itself and its ability to release DA remain intact. Finally, the haloperidol results provide no direct support for the toxic drug metabolite and the NMDA receptor theories of amphetamine-analog toxicity.

5-HT₂ Antagonists Block Methamphetamine- and MDMA-Induced Neurotoxicity

The 5-HT₂ antagonist ketanserin protects against MDMA-induced damage to the serotonin system (Azmitia et al. 1990; Nash et al. 1990). Nash and colleagues (1990) also found that ketanserin inhibits DA synthesis after MDMA treatment, and they suggested that MDMA-induced neurotoxicity involves the activation of DA neurons via 5-HT₂ receptors on DA cell bodies. In addition, Nash (1990) demonstrated that ketanserin attenuated MDMA-induced DA release *in vivo*. The neuroprotective effects of 5-HT₂ antagonists were reproduced with other 5-HT₂ antagonists (Schmidt et al. 1991, 1992a, 1992b). In addition to blocking MDMA-induced neurotoxicity, MDMA-induced DA release, and MDMA-induced increases in DA synthesis, 5-HT₂ antagonists also block the MDMA-induced decreases in DA cell firing (Schmidt et al. 1992a). This series of experiments support the view that DA mediates the MDMA-induced damage to the 5-HT terminal, and the 5-HT₂ blocking agents prevent this neurotoxicity by interacting with DAergic activity.

The neuroprotective effects of 5-HT₂ antagonists are also consistent with a Ca⁺⁺ theory of methamphetamine and MDMA neurotoxicity. 5-HT₂ receptors are linked to the second messenger inositol-1-4-5-trisphosphate (IP₃) (Minchin 1985). IP₃ in turn stimulates the release of intracellular Ca⁺⁺ from sequestration compartments (Berridge and Irvine 1989; Gandhi and Ross 1987). Blockade of the 5-HT₂ receptor should, therefore, diminish the amount of intracellular free Ca⁺⁺ and decrease the likelihood of Ca⁺⁺-induced cell death (Azmitia et al. 1990). 5-HT₂ antagonists administered with MDMA (e.g., MK-801, AMT) also cause a substantial decrease in core temperature that may be responsible for its protective effects (Malberg et al. 1994; Schmidt et al. 1992a). The 5-HT₂ antagonist result is consistent with the excitatory feed-forward loop hypothesis in that the 5-HT₂ receptors are probably involved in the circuitry (e.g., on

the DA cell body). The 5-HT₂ antagonist result is also consistent with the hydroxy radical theory since it has been shown that the 5-HT₂ antagonist ketanserin attenuates the MDMA-induced release of DA (Nash 1990). The toxic drug metabolite theory and the NMDA receptor theory do not receive direct support from the 5-HT₂ antagonist result.

MK-801 and Other NMDA Antagonists Block Methamphetamine- and MDMA-Induced Neurotoxicity

Sonsalla and colleagues (1989) reported that MK-801 protects against methamphetamine-induced damage to DA terminals, and other noncompetitive as well as competitive NMDA antagonists protected against methamphetamine-induced neurotoxicity (Sonsalla et al. 1991). MK-801 also protects against methamphetamine- and MDMA-induced damage to the serotonin system (Farfel et al. 1992; Johnson et al. 1989a). These results are consistent with an NMDA receptor-mediated calcium mechanism of neurotoxicity. Alternatively, MK-801 may protect against methamphetamine- and MDMA-induced neurotoxicity by interacting with temperature regulation mechanisms (Bowyer et al. 1994; Farfel and Seiden 1992); that is, the protection afforded by MK-801 may be due to lowering of body temperature rather than blockade of an NMDA receptor-mediated toxic process (see above).

The protective effects of MK-801 could be consistent with the DA mediation and the hydroxy radical theory of methamphetamine and MDMA neurotoxicity. MK-801 has been shown to decrease methamphetamine-induced DA release *in vivo* (Weihmuller et al. 1991), diminishing the availability of DA for conversion into a neurotoxic DA metabolite. However, Kashiwara and colleagues (1991) failed to replicate this finding *in vivo*, and Bowyer and colleagues (1991) failed to block methamphetamine-induced DA release *in vitro*. These issues will remain controversial until the relationship between glutamate release and DA release as mediated by the glutamate NMDA receptor is clarified.

The protection afforded by MK-801 is consistent with the idea of interrupting an excitatory feed-forward loop. The MK-801 results do not provide direct support for the toxic drug metabolite theory.

Antioxidants Can Block Methamphetamine-Induced Neurotoxicity

Ascorbic acid (Wagner et al. 1986) protects against the DA damage induced by methamphetamine, and cysteine (Schmidt and Kehne 1990; Stranka and Rhind 1987) protects against PCA- and MDMA-induced serotonergic toxicity. The protective effects of these antioxidants are consistent with the hydroxy radical theory of amphetamine-analog neurotoxicity, insofar as the antioxidant would neutralize the hydroxy radical before it can oxidize DA. Whether auto-oxidation occurs enzymatically or nonenzymatically, the antioxidants could function in a similar manner by forming a nonreactive complex with the hydroxy radical or protecting DA from quinone formation. The antioxidant results could support the toxic drug metabolite theory in that antioxidants may block the conversion of the parent drug to a toxic metabolite; they could also support the DA mediation theory. The antioxidant results provide no direct support for the excitatory feed-forward loop and NMDA receptor theories.

DA and 5-HT Transporter Inhibitors Block Methamphetamine and MDMA Neurotoxicity

DA uptake inhibitors protect against methamphetamine-induced damage to the DA system, but not against serotonergic damage (Marek et al. 1990b; Schmidt and Gibb 1985b). Similarly, 5-HT uptake inhibitors protect against methamphetamine- or MDMA-induced damage to the 5-HT system, but not the DA system (Ricaurte et al. 1983; Schmidt 1987; Schmidt and Gibb 1985b). Mazindol, which blocks both DA and 5-HT uptake, protects against both DA and 5-HT depletions (Marek et al. 1990b). Amfonelic acid blocks methamphetamine-induced DA toxicity when administered up to 8 hours after methamphetamine (Fuller and Hemrick-Luecke 1982; Marek et al. 1990b). Fluoxetine blocks MDMA-induced 5-HT damage when administered 3 to 6 hours post-MDMA (Schmidt 1987).

Since uptake inhibitors have been shown to block or attenuate the transmitter release induced by amphetamine-like compounds (Butcher et al. 1988), these results suggest that DA release is important for DA toxicity, and 5-HT release is important for 5-HT toxicity. This interpretation is consistent with the hydroxy radical theory of amphetamine toxicity: Uptake inhibitors result in less extracellular DA or 5-HT available for conversion to the toxin 6-OHDA or 5,6-DHT. The inhibition of methamphetamine-induced neurotoxicity with uptake inhibitors is also consistent

with the idea that methamphetamine-induced neurotoxicity is dependent on a striatal-thalamic-cortical loop. Decreasing methamphetamine-induced DA release diminishes DA's influence on this circuit, resulting in protection against methamphetamine- or MDMA-induced neurotoxicity.

The pattern of protection afforded by uptake inhibitors does not completely generalize, however. The DA uptake inhibitor benztropine does not protect against either methamphetamine-induced DA or 5-HT depletion (Marek et al. 1990b); the DA uptake inhibitor GBR 12909 partially protects against MDMA-induced decreases in the serotonin synthetic enzyme TPH (Stone et al. 1988); and finally, the DA uptake inhibitor amfonelic acid has been shown to protect against methamphetamine-induced damage to the serotonin system (Schmidt and Gibb 1985a). These inconsistencies may reflect some limitations in pharmacological understanding of the drugs being used as specific tools, or they may suggest that the DA and 5-HT systems are somewhat interactive in the mechanism of amphetamine toxicity. For example, the protection against serotonergic damage by the DA uptake inhibitor GBR 12909 (Stone et al. 1988) supports the view that DA release is important for 5-HT toxicity. In addition, the failure of the selective DA uptake inhibitor benztropine (Marek et al. 1990a) to protect against methamphetamine-induced DA depletion brings into question the parallel between release and toxicity within a given transmitter system. The uptake inhibitor results would support the toxic drug metabolite hypothesis if it could be demonstrated that the uptake inhibitor blocked uptake of the toxic drug metabolite into the neuron. The uptake inhibitor results do not support the NMDA receptor theory in any direct manner.

6-OHDA Lesions Protect Against MDMA-Induced Damage to the 5-HT System

Bilateral 6-OHDA lesions of the substantia nigra partially block MDMA-induced deficits to the 5-HT system (Schmidt et al. 1990b; Stone et al. 1988). These results are consistent with the DA mediation theory of serotonergic toxicity and the excitatory feed-forward loop theory. They provide no support for the NMDA receptor and hydroxy radical theories, but more work is needed for clarification. These results are not consistent with a toxic drug metabolite theory of amphetamine-analog neurotoxicity.

GABA Transaminase Inhibitors and GABA Agonists Protect Against Methamphetamine-Induced Neurotoxicity

Amino-oxyacetic acid inhibits gamma-aminobutyric acid (GABA) transaminase, an enzyme responsible for GABA degradation. Chlormethiazole, an agonist at the GABA-A receptor, also protects against methamphetamine-induced DA and 5-HT damage (Green et al. 1992). GABA is an important inhibitory transmitter in the striatal-thalamic-cortical circuit. It can be postulated that as the levels of GABA increase, the toxic overexcitation of this circuit is diminished, allowing for protection against methamphetamine or MDMA treatment. Since GABA is an ubiquitous inhibitory transmitter, any agent that increases GABA activity will possibly decrease or counteract glutamate activity. In this way the GABA transaminase inhibitor results would be consistent with a glutaminergic mechanism (NMDA receptor-mediated) theory of amphetamine toxicity. The GABA transaminase inhibitor results provide no obvious support for the hydroxy radical theory, the DA theory, or the toxic drug metabolite theory of amphetamine-analog neurotoxicity.

The list of agents discussed above that can affect amphetamine neurotoxicity is not exhaustive. For example, adrenalectomies (Johnson et al. 1989b) and protein synthesis inhibitors (Finnegan and Karler 1992) both protect against amphetamine analog toxicity, while acetone, which activates several cytochrome P450 enzymes, enhances MDA toxicity (Michel and George 1993). This list of protective agents is likely to grow with future research.

SUMMARY

Methamphetamine and MDMA as well as similar substituted phenethylamines are toxic to DA and/or 5-HT neurons. The duration and magnitude of these effects are dose dependent and are accompanied by different degrees of recovery. MDMA-induced 5-HT damage persists for up to 52 weeks in the rat, and methamphetamine-induced DA damage persists for up to 3 years in the rhesus monkey.

Several possible mechanisms of amphetamine-analog toxicity have been reviewed. The excitatory feed-forward loop theory is best supported by the literature. This theory, however, is very wide ranging and difficult to prove or disprove. The hydroxy radical and DA mediation theories are both well supported by the data reviewed. It should be noted that these

two hypotheses are closely related to each other. The DA mediation theory is based on the requirement of an intact DA system for methamphetamine and MDMA neurotoxicity to occur. The hydroxy radical theory is also based on the presence of DA and 5-HT; in addition, it suggests the formation of toxic hydroxy radicals from DA or 5-HT as the specific mechanism for the amphetamine-analog neurotoxicity. The hydroxy radical theory also accounts for the fact that amphetamine-analog neurotoxicity is selectively toxic to the DA and/or 5-HT systems of the brain; that is, the toxin is formed either in the synapse or within the neurons that release DA and/or 5-HT as a result of amphetamine analog treatment.

The toxic drug metabolite theory, while not exhaustively studied, has little support from the literature at present. Similarly, the NMDA receptor mediation theory, in its most straightforward form, also has little support from the literature. The protective effects of the NMDA receptor antagonist MK-801 may be a modulatory effect resulting from changes in temperature regulation, rather than a direct effect of antagonizing a link in the toxic mechanism itself. It should be noted that the effects of the protective agent plus amphetamine-analog combinations on body temperature, when thoroughly investigated, may serve to separate agents which protect through a cooling mechanism from agents that protect by interfering with the toxic process itself.

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Stress, Glucocorticoids, and Mesencephalic Dopaminergic Neurons: A Pathophysiological Chain Determining Vulnerability to Psychostimulant Abuse

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INTRODUCTION: VULNERABILITY TO DRUGS AND DRUG ABUSE

It is common knowledge that enormous individual differences exist in drug intake by humans (De Wit et al. 1986). A large number of people have tried drugs at least once, but for most of them drug use experiences are restricted to a single or a few incidents. Among those who persist in taking drugs, drug use can remain an occasional behavior limited, for example, to weekends or parties. Only some drug users develop drug abuse (i.e., a compulsive drug use that becomes the principal goal-directed behavior of the subject) (O'Brien et al. 1986). The origin of the peculiar vulnerability to develop drug abuse observed in some individuals is one of the principal questions to be answered about addiction.

Individual differences in the vulnerability to drug abuse may be explained from two very different points of view. The first is a drug-centered vision of addiction that sees drug abuse as the consequence of the modifications induced in the brain by repeated drug intake. Through the development of tolerance, sensitization, and conditioning, repeated exposure to the drug induces drug dependence—the real cause of abuse. In this viewpoint, vulnerable individuals are those who have greater chances to be, and actually are, the most exposed to the drug because of the environment that surrounds them (peer and/or social pressure are the most often cited causes). The second view may be considered an individual-centered theory of addiction that regards drug abuse as the consequence of a peculiar, pathological reaction to the drug. From this perspective, vulnerable individuals are those who, because of a specific