Recommendations for Future Research on Amphetamines and Related Designer Drugs

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INTRODUCTION

This volume has focused on several amphetamine analogs in addition to amphetamine and methamphetamine, especially 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA). Among the pharmacologic actions of these drugs, their behavioral effects in humans and in laboratory animals have been discussed, with some attention to electrophysiologic and electroencephalographic effects. Other functional effects, such as effects on neuroendocrine regulation, sleep, thermoregulation. and appetite and body weight have not been discussed. Consideration of toxic effects mainly focused on neurotoxic actions that the drugs can have on specific brain monoaminergic neurons. In relation to this action, two other amphetamine analogs, *p*-chloroamphetamine and fenfluramine, have been compared because of their similar neurotoxic actions in rats.

SOCIAL IMPLICATIONS

General concerns about abuse of amphetaminerelated drugs are similar to concerns about other illicit or addictive drugs. Dr. G. Nahas wrote an editorial for the *Wall Street Journal* arguing that "a strongly expressed sentiment of societal disapproval" of illicit drugs is necessary for prohibitive measures to be effective *(Wall Street Journal,* July 11, 1988, p. 16). He cited examples from history to support his contention that when illicit addictive drugs are socially accepted and easily available, they have a very damaging effect on individuals and on a society. His examples included the use of cannabis in the Islamic-dominated world several centuries ago, the chewing of coca leaf in Peru, the use of opium in China at the beginning of this century, the epidemic of amphetamine abuse in Japan in the 1950s, and others. In some cases, there was widespread social acceptance. Although there is acceptance of MDMA and amphetamines in only limited segments of our society, Nahas argues there is not forceful enough disapproval of illicit drugs in general in our society today.

The behavioral and dependence-producing effects of some of these drugs can be damaging to individuals, but neurotoxic damage to particular brain neurons can result when these drugs are given to animals, including nonhuman primates. There continue to be inadequate data about whether such damage occurs in humans.

Patterns of Abuse of Amphetamine Analogs

The need for more accurate and precise information about human use of MDMA and MDA was aptly stated by Dr. Gawin (this volume) and others. There is a general perception that these drugs are widely used, especially on college campuses, but there are relatively few hard data on the geographic distribution of use, on the pattern(s) and frequency of use, the doses used, and so on. For many reasons, such information is needed.

Behavioral Effects of Amphetamines: How Useful, What Mechanisms?

The behavioral effects of amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, and fenfluramine are not identical. Except for the last drug, all can cause some degree of behavioral stimulation, but exact behavioral effects differ markedly. More complete definition of their behavioral differences is a prerequisite to a better understanding of the mechanism(s) of these drugs.

Apparently there are psychiatrist and nonpsychiatrist clinicians whose experience convinces them that MDMA can have therapeutic uses, mainly as an adjunct to psychotherapy. Despite these convictions, there appear to be no published data to support these claims. There is an urgent need for objective data from well-controlled, blinded clinical studies, if these claims of therapeutic usefulness are to be taken seriously. If a bona fide use is evident, then it may be possible to produce other drugs with the same desirable action, lacking the toxicity inherent in MDMA.

NEUROCHEMICAL MECHANISMS

Aside from the therapeutic usefulness of MDMA, there is scientific importance to elucidating further the mechanism(s) involved in the seemingly unique behavioral effects of MDMA and MDA. Apparently, a major action of these drugs is the release of serotonin and dopamine from brain neurons, leading to enhanced serotonergic and dopaminergic input to those neuronal systems with which they make synaptic contact. In addition, MDMA has been shown to interact *in vitro* with sites including $5HT_2$, $5HT_{1A}$, and α_2 adrenergic receptors, among others (Battaglia, this volume). Do those interactions occur *in vivo,* and does MDMA interact as an agonist or as an antagonist at these sites? If the interactions occur *in vivo,* how do they contribute to the profile of behavioral effects of MDMA? These questions can and should be approached experimentally. Further unraveling

of the effects of MDMA and MDA on serotonergic and dopaminergic function is also needed. Serotonin neurons and dopamine neurons are known to interact in many brain regions (Bosler et al. 1984; Benkirane et al. 1987; Herve et al. 1987), so the release of dopamine may influence serotonergic function, just as the release of serotonin may influence dopaminergic function.

Neurotoxicity of Amphetamines

The recognition that amphetamines can be neurotoxic in brain can be traced back to *p*-chloroamphetamine studies. In the middle 1960s, *p*-chloroamphetamine and *p*-chlorometbamphetamine were found to cause selective depletion of brain serotonin (Pletscher et al. 1963; Fuller et al. 1965). The long duration of this depletion was not appreciated until later (Sanders-Bush et al. 1972). and it was subsequently established that the loss of serotonin was accompanied by changes in other parameters specifically associated with serotonin neurons, e.g., a loss in tryptophan hydroxylase, a loss in serotonin uptake capacity, and a reduction in serotonin turnover, as well as by histologic evidence of neurotoxicity (Puller and Snoddy 1974; Harvey et al. 1975; Sekerke et al. 1975; Massari et al, 1978). Fenfluramine was recognized to have similar effects on brain serotonin neuron parameters (Harvey and McMaster 1975; Clineschmidt et al. 1978), although there has been controversy about histologic changes (Sotelo and Zamora 1978).

During the 1970s, evidence accumulated that amphetamine and methamphetamine could also be neurotoxic (Ellison et al. 1978; Hotchkiss and Gibb 1980; Wagner et al. 1980). The effects of amphetamine seem mostly limited to dopamine neurons, whereas methamphetamine affects dopamine and serotonin neurons (Warren et al. 1984). Most recently, MDMA and MDA have been shown to produce neurotoxicity toward brain serotonin neurons much like that of the halogenated amphetamines (Ricaurte et al. 1985; Stone et al. 1986).

Role of Uptake Carriers

The neurotoxic effects of all these compounds are antagonized by inhibitors of monoamine uptake (table 1), implicating the membrane uptake carrier on serotonin and dopamine neurons in the mechanism of neurotoxicity. In this regard, these amphetamines are like a drug somewhat related in structure, namely 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a Parkinsonism-causing neurotoxic drug that has been studied intensely since 1983 (Langston and Irwin 1986). In the case of MPTP, the mechanism by which inhibitors of the dopamine uptake carrier block the neurotoxicity toward dopamine neurons (mainly nigrostriatal dopamine neurons) seems clear. A metabolite of MPTP, l-methyl-4-phenylpyridinium (MPP+), has been shown to be a substrate for the dopamine uptake carrier (Javitch et al. 1985). Thus accumulation of MPP+, formed metabolically from

MPTP, into dopamine neurons seems to be essential, and blockade of that accumulation prevents the neurotoxicity. MPP+ also can be transported into norepinephrine neurons (Javitch et al. 1985), leading to neurotoxicity toward cortical norepinephrine neurons, an effect blocked by inhibitors of the norepinephrine uptake carrier (Sundstrom and Jonsson 1985).

TABLE 1. *Characteristics of monoaminergic neurotoxicity induced by amphetamine and related compounds in laboratory animals*

Drug	Membrane Uptake Carrier Involved in Neurotoxicity	Drug Metabolite Involved in Neurotoxicity
Amphetamine	Yes	?
Methamphetamine	Yes	?
MDMA, MDA	Yes	?
p-Chloroamphetamine	Yes	?
Fenfluramine	Yes	?
MPTP	Yes	Yes

The mechanism by which uptake inhibitors block the neurotoxic effects of amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, and fenfluramine is not so clear. A simple explanation (analogous to that with MPTP) might be that these drugs are accumulated into serotonin or dopamine neurons via the membrane uptake carrier, and that uptake inhibitors prevent the neurotoxicity by preventing that accumulation. However, none of these compounds have been shown to be a substrate for dopamine or serotonin uptake carriers. Their lipophilicity leads one to believe they would enter neurons readily without requiring active transport. There may be, however, both entry and accumulation of these drugs. It is conceivable that the amphetamines do enter brain monoaminergic neurons and other cells by passive diffusion. They may in addition be accumulated by brain monoaminergic neurons, if amphetamines are substrates for the membrane uptake carriers. For example, *p*-chloroamphetamine may enter all cells, but may be selectively concentrated in serotonin neurons, due to its accumulation via the membrane uptake carrier. That concentration may be required for the short-term and long-term depletion of brain serotonin, so that inhibition of the uptake carrier blocks the depletion. No direct evidence to support this possibility is available. Uptake of radioactive *p*-chloroamphet-amine by the serotonin uptake carrier has not been shown *in vitro,* although *p*-chloroamphetamine does have high affinity for that carrier (Wong et al. 1973).

There has been some evidence that *p*-chloroamphetamine is preferentially localized in synaptasomal fractions of brain homogenates (Wong et al. 1972), and recently Ask and Ross (1987) have published evidence

consistent with an accumulation of pchloroamphetamine in serotonergic synaptosomes *in vitro*. They evaluated the ability of reversible inhibitors of monoamine oxidase to be accumulated in serotonin nerve endings by the membrane uptake carrier by comparing two conditions of serotonin deamination: first, when the radioactive serotonin was being deaminated, mainly inside serotonergic synaptosomes, after it was accumulated via the uptake carrier; and second, when serotonin was being deaminated by other synaptosomes, because its transport via the serotonin uptake carrier was blocked. In this way, they evaluated the ability of certain reversible inhibitors of monoamine oxidase to be themselves concentrated in serotonergic synaptosomes due to their transport via the membrane uptake carrier. Study of *p*-chloroamphetamine, which is a reversible inhibitor of monoamine oxidase (Fuller 1978), did indicate accumulation within serotonergic synaptosomes.

Further investigation of the possibility that inhibitors of the serotonin uptake carrier protect against serotonin depletion by *p*-chloroamphetamine, fenfluramine, MDMA, MDA, and methamphetamine because they prevent the accumulation of those drugs within serotonin nerve terminals is warranted, but at present compelling evidence for this mechanism does not exist.

Possible Role of Dopamine Release

A second possible mechanism, supported by some existing data on methamphetamine and MDMA, is that these drugs release dopamine, which is then taken up into serotonin neurons via the membrane uptake carrier, leading to neurotoxic effects on the serotonin neurons. Inhibitors of dopamine synthesis or of the dopamine uptake carrier, e.g., α-methyltyrosine and GBR 12909, have been reported to prevent the depletion of serotonin by methamphetamine and by MDMA (Schmidt et al. 1985; Gibb et al., this volume). MDMA does release dopamine both *in vitro* and *in vivo* (Yamamoto and Spanos 1988). Dopamine can be transported into serotonergic synaptosomes (Schmidt and Lovenberg 1985). Further investigation is needed, especially to see if the involvement of dopamine is a general phenomenon in the neurotoxic effects of amphetamines. We have found that potent inhibitors of dopamine uptake, including mazindol and nomifensin, block depletion of striatal dopamine by MPTP in mice, but do not block depletion of brain serotonin by *p*-chloroamphetamine in mice.

Possible Role of an Active Metabolite of the Drug in the Neurotoxicity of Amphetamine Analogs

The possibility that an active metabolite is involved in the neurotoxic effects of amphetamine analogs receives limited discussion in this chapter and has been considered previously, especially with *p*-chloroamphetamine (Miller et al. 1986.) Partly because the chemical structures of these amphetamines do not suggest ways in which they would be toxic to neurons, the

possibility that conversion to a more reactive metabolite accounts for the neurotoxicity has been attractive. The study of numerous analogs of *p*-chloroamphetamine and other neurotoxic amphetamines has not yielded a strong candidate for such a neurotoxic metabolite. Most potential metabolites of p-chloroamphetamine caused less depletion of serotonin (Fuller 1978). Although N-hydroxy-*p*-chloroamphetamine did deplete serotonin, it was metabolized rapidly and almost quantitatively to *p*-chloroamphetamine (Fuller et al. 1974). Inhibitors and inducers of drug metabolism have generally failed to influence neurotoxicity of amphetamines. MDA is metabolixed to a-methyldopamine (Marquardt et al. 1978; Midha et al. 1978), a metabolite that should be considered as a possible mediator of neurotoxicity, especially in view of the properties of dopamine discussed below.

Involvement of a Metabolite of the Neurotransmitter in the Neurotoxicity of Amphetamines

Also a possibility is that a product formed from one of the neurotransmitters affected mediates the neurotoxic effects of amphetamines. This possibility was suggested by Seiden and Vosmer (1984), who reported the presence of 6-hydroxydopamine in the rat caudate nucleus after a single injection of a high, neurotoxic dose of methamphetamine. They suggested that 6-hydroxydopamine was formed from endogenous dopamine released by methamphetamine and that the 6-hydroxydopamine was responsible for the neurotoxicity to dopaminergic terminals. Other investigators have not found 6-hydroxydopamine to be present in rat striatum after amphetamine or methamphetamine administration (Rollema et al. 1986).

Commins et al. (1987) have also reported the formation of 5,6-dihydroxytryptamine in rat hippocampus after a single, high doses of methamphetamine. They suggested that the formation of 5,6-dihydroxytryptamine, a known neurotoxic substance, may mediate the neurotoxic effects of methamphetamine toward serotonergic nerve terminals.

Molliver (this volume) made the provocative suggestion that a metabolite of serotonin released from blood platelets by *p*-chloroamphetamine may mediate the neurotoxic effects of *p*-chloroamphetamine on cortical serotonergic neurons in the rat. Such a possibility would be compatible with the observations of Molliver and his colleagues (this volume) that *p*-chloroamphetamine is not effective when pumped directly into the brain or when added to brain slices *in vitro.* Their demonstration that a combination of *p*-chlorophenylalanine and reserpine prevented the neurotoxic effects of *p*-chloroamphetamine led them to suggest that platelet serotonin stores were involved in the neurotoxic mechanism (Berger et al., submitted for publication). This interesting idea deserves testing in various ways. Since it would not cross the blood-brain barrier, 5,6-dihydroxytryptamine would not

seem to be a candidate for their hypothesized metabolite, unless the integrity of that barrier had been lost due to the drug treatment.

Role of Dopamine Involvement in the Neurotoxicity of Amphetamines

Some data suggest that dopamine itself is involved in certain of the neurotoxic effects. It is worth asking if dopamine might account for the neurotoxicity of all the amphetamine analogs toward both dopaminergic and serotonergic neurons. At least three ways in which dopamine might lead to cytotoxicity have been suggested, First, dopamine might be converted to 6-hydroxydopamine, a known neurotoxin, as discussed above. Second, dopamine metabolism by monoamine oxidase is known to produce hydrogen peroxide, and excess hydrogen peroxide formation from this source might, under some conditions, have deleterious effects on the cell (Cohen and Mytilineou 1985). Third, dopamine itself is known to undergo auto-oxidation analogous to, but slower than, that of 6-hydroxydopamine (Graham et al. 1978; Graham 1984). Persistently increased intraneuronal but extragranular concentrations of dopamine due to amphetamine-induced release of granular stores of dopamine and protection against dopamine oxidation by monoamine oxidase type A have been suggested as possibly mediating the neurotoxic effects of amphetamine (Fuller and Hemrick-Luecke 1982). Uptake of dopamine into serotonergic terminals (Schmidt and Lovenberg 1985) might lead to destruction of serotonergic terminals after treatment with drugs like methamphetamine and MDMA. It is not clear why such effects should be less with amphetamine than with methamphetamine, yet amphetamine seems to affect dopaminergic neurons primarily, whereas methamphetamine is neurotoxic toward serotonin neurons as well as dopamine neurons (Hotchkiss and Gibb 1980; Ricaurte et al. 1984). Investigation of the possible involvement of dopamine in the different neurotoxic process is needed.

IMPLICATIONS OF NEUROTOXICITY

Functional Deficits Resulting from Amphetamine Neurotoxicity

Rats that have lost dopamine and/or serotonin terminals following treatment with amphetamine, methamphetamine, MDMA, MDA, *p*-chloroamphetamine, or fenfluramine show little in the way of overt changes in appearance or behavior. Dr. Ricaurte (this volume) emphasized the need for more studies in primates, since MPTP-treated mice also show little in the way of observable functional changes, whereas MPTP-treated monkeys show marked neurologic deficits. It may be necessary to do more detailed analysis of specific behaviors and other functional outputs that are influenced by dopamine and/or serotonin neurons, to detect functional deficits induced by some neurotoxic drugs. For instance, specific behaviors such as appetite-controlled behavior (Leibowitz and Shor-Posner 1986), muricidal behavior (Katz 1980), and sexual behavior (Tucker and File 1983) elicited by drugs

or environmental conditions ate known to be influenced by serotonergic input. Careful analysis of these behaviors in rats that have received neurotoxic doses of *p*-chloroamphetamine, MDMA, MDA, fenfluramine, or methamphetamine may reveal functional deficits. Electroencephalographic patterns, nociception, sleep, thermoregulation, and endocrine regulation are other brain-controlled functions that are influenced by serotonergic pathways. Careful studies of these functions, especially measuring responses elicited by serotonergic drugs or by environmental stimuli whose actions are mediated by serotonergic systems (insofar as that is known) may reveal functional deficits associated with loss of serotonergic terminals. For example, we have found that the acute increase of serum corticosterone in rats given *p*-chloroamphetamine, an increase that appears to be mediated by release of serotonin from central neurons making input to cells that release corticotropin-releasing factor in the hypothalamus, is blunted in rats pretreated with a neurotoxic dose of *p*-chloroamphetamine. There are few examples of studies of this sort, in which a functional correlate of the loss in serotonin content has been sought in rats that have received neurotoxic doses of any of the amphetamine analogs in question. It seems important for such studies to be done for several masons, including the goal of learning more about physiologic functions of the serotonin and dopamine pathways that are affected, and to suggest ways in which possible neurotoxicity in humans might be investigated.

Does Neurotoxicity Occur in Humans

All the neurotoxic drugs discussed have been taken by human subjects. Amphetamine and methamphetamine have a long history of therapeutic use along with illicit misuse. To a limited extent, *p*-chloroamphetamine has been used in humans as an investigational drug (Van Praag et al. 1971). MDMA and MDA have no approved medical uses, but they appear to be rather widely abused drugs at present. Fenfluramine continues to be marketed as an appetite suppresant A key question, to which there is no current answer, is whether any of these drugs produce, in humans, neurotoxic effects on dopamine and/or serotonin neurons in brain analogous to those produced in rodents and in nonhuman primates (table 2). It is remarkable that no data exist on this issue, given that the neurotoxic effects of some of these drugs in animals have been know for more than a decade.

There are several ways in which possible neurotoxic effects might be studied. First, measurement of cerebrospinal fluid concentrations of dopamine or serotonin metabolites would be a straightforward way of assessing neurotoxicity. There are pitfalls in this approach (as outlined by Dr. Ricaurte (this volume), such as the facts that lumbar cerebrospinal fluid might reflect spinal cord neurochemistry more than it reflected brain neurochemistry, and drugs like *p*-chloroamphetamine affect serotonin neurons in spinal cord less than they do those in brain (Sanders-Bush

et al. 1975). Nonetheless, fenfluramine has been shown to produce marked decreases in 5-hydroxyindoleacetic acid concentration in the cerebrospinal fluid during treatment (Shoulson and Chase 1975), and it would be important to know if those concentrations return to control levels when fenfluramine is discontinued.

TABLE 2. *Nature of neurotoxic damage to brain monoaminergic neurons*

Drug	Brain Monoaminergic Neurons Showing Neurotoxic Damage	Neurotoxicity Occurring in Humans
Amphetamine	Dopamine	?
Methamphetamine	Dopamine, Serotonin	?
MDMA, MDA	Serotonin	?
p-Chloroamphetamine	Serotonin	?
Fenfluramine	Serotonin	?
MPTP	Dopamine, Norepinephrine	Yes

A second approach might be to measure dopamine and serotonin along with their metabolites and other specific neuronal constituents such as tyrosine hydroxylase and tryptophan hydroxylase or uptake carrier sites in brain tissue obtained at autopsy. Accumulating data in this way might be a slow and tedious process, and drug dosing history might be uncertain and variable; nonetheless, the approach deserves consideration.

A third approach would be to measure some indicator of functional output of dopamine and/or serotonin neurons. As mentioned previously, studies in laboratory animals can be invaluable in defining parameters that change in correlation with directly measurable neurotoxic effects in the brain. Changes in serum hormones elicited by a drug whose effects are mediated by dopamine or serotonin neurons are especially attractive possibilities, since these changes are already being used as a means of assessing the functional state of brain serotonergic pathways (Siever et al. 1984). In this regard, it is intriguing that Coccaro et al. (1987) have observed recently a blunted elevation in serum prolactin concentration elicited by fenfluramine in psychiatric patients who had received a previous dose of fenfluramine within a 12-day period. While there may be numerous possible explanations, one could be that the first dose of fenfluramine had damaged or destroyed a traction of the serotonin neurons from which release of serotonin is the mechanism of prolactin elevation by fenfluramine.

A fourth approach to evaluating the intactness of dopamine and/or serotonin neurons in human subjects who have taken one of the amphetamine analogs might be to use a probe for labeling a constituent of those neurons in position emission tomography scanning studies. A label for the serotonin or dopamine uptake carrier, or a label for tryptophan hydroxylase or tyrosine