Mechanisms Mediating Biogenic Amine Deficits Induced by Amphetamine and Its Congeners

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INTRODUCTION

In other chapters of this monograph, evidence is presented that the neurotoxicity of drugs of abuse can be assessed by monitoring behavioral, neurophysiologic, and neuroanatomic parameters. In this chapter, evidence is cited that neurotoxicity also can be assessed by determining the effects of drugs of abuse on neurochemical elements. Because Gibb and colleagues at the University of Utah have investigated primarily methamphetamine (METH) and its congeners over the past two decades, this group of abused agents is used as an example. In monitoring neurochemical parameters, one can measure neurotransmitter concentrations ex vivo or by in vivo dialysis. Enzyme activity, either V_{max} and/or K_m, can be determined. Loss of uptake sites (Battaglia et al. 1987) or alteration of binding sites also can be assessed.

In 1971, Koda and Gibb (1971, 1973) attempted to simulate in rats the conditions that exist in METH abusers who characteristically inject the drug intravenously at frequent intervals over a period of 2 to 3 days. METH (10 to 15 mg/kg, subcutaneously [SC]) was administered every 6 hours for 30 hours, and the effect on tyrosine hydroxylase (TH) activity and on the concentrations of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid and homovanillic acid, in the neostriatum was determined. Following drug treatment, these parameters were decreased to approximately 60 percent of normal. Because it was known that amphetamines release catecholamines, it was not surprising that the concentrations of DA and its metabolites were depressed, but an explanation for the decrease in TH activity was not immediately apparent. Induction of TH in the adrenal gland, superior cervical ganglion, and selected areas of the brain had been reported in stressed animals (Zigmond et al. 1980; Masserano et al. 1981), but a decline in the activity of this enzyme was rather perplexing; it was acknowledged that 6-hydroxydopamine (6-OHDA), a potent neurotoxin, also decreased TH activity.

After a comparable dosing schedule, METH had similar effects on the serotonergic system (Hotchkiss et al. 1979; Hotchkiss and Gibb 1980). Tryptophan hydroxylase (TPH) activity was markedly decreased after a single dose of METH; a decrease in concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) was also observed. A major difference in the response of the 5-HT and DA systems was the more rapid onset and more profound response observed in the 5-HT system compared with the DA system. The question then arose as to whether these large, repeated doses of METH were neurotoxic to all neurotransmitter systems or to selected neurons. The effect of METH on the cholinergic system was assessed by measuring choline acetyltransferase activity in the neostriatum, nucleus accumbens, and olfactory tubercle after METH; because this enzyme, which is responsible for acetylcholine synthesis, was not altered, it was concluded that cholinergic systems remained intact after METH (Hotchkiss et al. 1979). Using a similar strategy, glutamic acid dehydrogenase was measured to determine whether the γ-aminobutyric acid (GABA) system was affected; the activity of this enzyme was unaffected. It appears, then, that the neurotoxic effect of large doses of METH is limited to the dopaminergic and serotonergic systems.

Ricaurte and colleagues (1985) reported that an analog of amphetamine, 3,4-methylenedioxyamphetamine (MDA), decreased concentrations of neostriatal 5-HT and 5-HIAA but had no effect on DA or norepinephrine content. Morphological changes, as demonstrated by the Fink-Heimer technique, also were observed; moreover, 5-HT uptake sites were compromised. Stone and coworkers (1986) examined the effects of MDA and 3,4-methylenedioxymethamphetamine (MDMA) on the activity of both TPH and TH. Whereas METH characteristically decreases both TH and TPH activity, MDA or MDMA depressed only TPH activity. The more selective effect of MDMA or MDA on the serotonergic system is reminiscent of the response to p-chloroamphetamine.

A compound cannot be assumed to be neurotoxic if the neurochemical changes are transient; however, when neurochemical alterations persist, neurotoxicity is suspected. Bakhit and colleagues (1981) and Stone and colleagues (1987) administered multiple doses of METH or MDMA to rats, stopped the drug, and waited for varying periods of time after the dosing was discontinued to measure the neurochemical response. When five administrations of MDMA or METH (10 mg/kg) were given, TPH activity and concentrations of 5-HT and 5-HIAA were still depressed at 110 days. Because MDMA affects the serotonergic system selectively, comparable results were obtained for the dopaminergic system only when METH was administered. These data, coupled with the morphological evidence from other laboratories (Ricaurte et al. 1984; Commins et al. 1987; O'Hearn et al. 1988), are highly suggestive that large doses of METH are neurotoxic to dopaminergic neurons, whereas MDMA is selectively neurotoxic to the serotonergic system.

DOPAMINE IS ESSENTIAL FOR THE NEUROTOXIC RESPONSE

The mechanism by which the neurotoxic effect of the amphetamine analogs occurs has been under consideration from the very beginning of Gibb and colleagues' studies. Buening and Gibb (1974) reported that the DA antagonists, chlorpromazine and haloperidol, prevent the usual response to METH. In subsequent studies, Sonsalla and coworkers (1986*a*) observed that either D_1 or D_2 antagonists block the effects on the DA system; the effects of METH on the 5-HT system were blocked only by D_1 antagonists.

Further evidence that DA is essential for the METH-induced neurotoxicity was then obtained (Gibb and Kogan 1979; Hotchkiss and Gibb 1980; Schmidt et al. 1985). DA synthesis was blocked at the rate-limiting step by inhibiting TH by administering α-methyl-p-tyrosine (MT). The METH-induced alterations in both the dopaminergic and the serotonergic systems were prevented by concurrent administration of MT. When synthesis of DA was reinstated by administering L-dopa concomitantly with the METH and MT, the neurochemical deficits returned.

Recently, it was found that when the nigrostriatal dopaminergic projections are destroyed by prior injection of 6-OHDA into the substantia nigra, METH (Johnson et al. 1987; Sonsalla et al. 1986*b*) or MDMA (Stone et al. 1988) does not cause the characteristic decrease in striatal TPH activity. However, METH or MDMA still decreases TPH activity in the cerebral cortex or hippocampus of 6-OHDA-pretreated animals. Thus, dopaminergic innervation is critical for the METH or MDMA to cause the neurotoxic response.

Catecholamines were then depleted by prior administration of reserpine. In animals so depleted, MDMA was ineffective in causing its usual deficit in TPH activity (Stone et al. 1988). This observation provides further evidence that DA is essential for the neurotoxicity observed after MDMA or METH. Additional evidence for the necessity of DA in the neurotoxic process was recently provided by Chris Schmidt (personal communication, May 1991), who demonstrated that L-dopa enhanced the deficits in rat brain 5-HT content 1 week after administering an otherwise ineffective single dose of MDMA or METH.

IS GLUTAMATE INVOLVED IN THE NEUROTOXICITY?

Sonsalla and colleagues (1989) recently reported that MK-801, an N-methyl-D-aspartate (NMDA) antagonist, protects against the METH-induced deficits in the dopaminergic and serotonergic systems, which suggests that glutamate is associated with the METH-induced neurochemical events that lead to toxicity. Johnson and coworkers (1989) confirmed these observations but found that MK-801 does not protect against the MDMA-induced neurotoxicity. Glutamate appears to play a role in the METH-induced neurotoxicity but not in the MDMA-induced deficits.

ROLE OF AN OXIDATIVE PROCESS IN NEUROTOXICITY

The above data provide significant evidence that DA and/or its reactive metabolites are essential for neurotoxicity. The question remains as to the mechanisms by which DA causes the response. Stone and colleagues (1989) have evidence that an oxidative process is responsible for the serotonergic alterations associated with the neurotoxicity. It was reported earlier that TPH activity is rapidly decreased within 15 minutes after a single dose of METH (Bakhit and Gibb 1981). Schmidt and Taylor (1987) reported no change in the K_m for substrate or pterin cofactor, but a decrease in V_{max} was observed after MDMA. TPH is activated by a variety of conditions, including exposure of the enzyme to certain phospholipids, mild proteolysis, and phosphorylating conditions (Hamon et al. 1981; Vitto and Mandell 1981; Garber and Makman 1987); under these conditions, the affinity of the enzyme is increased, and the apparent K_m for tryptophan and/or the pterin cofactor is decreased.

In contrast, a strong reducing environment (5 mM dithiothreitol [DTT] and 50 mM Fe^{2+} under a nitrogen atmosphere) increases the apparent V_{max} of TPH but does not alter the apparent K_m (Hamon et al. 1978). It has been previously reported that in vitro exposure of TPH to oxygen inactivates the enzyme (Kuhn et al. 1980). The oxygen-inactivated TPH can be reactivated by incubating the enzyme in an anaerobic milieu under reducing conditions. The similarity between the inactivation by molecular oxygen and by MDMA prompted Stone and colleagues (1989) to investigate the role of enzyme oxidation in the acute decline of TPH by MDMA.

Rats were administered a single dose (10 mg/kg) of MDMA and killed 3 hours later. Crude enzyme from the cerebral cortex (27,000 x g supernatant) from saline- or MDMA-treated rats was placed in test tubes, and DTT and Fe^{2+} were added. The preparation was incubated under nitrogen at 25 °C for varying periods of time, and TPH activity was assayed by a modified $^{14}CO_2$-trapping procedure (Ichiyama et al. 1970). The time course of in vitro reactivation is depicted in figure 1. Addition of DTT and Fe^{++} to the enzyme from saline-treated rats prevented deterioration of the enzyme activity observed with incubation in water. When the reducing mixture was incubated with TPH from MDMA-treated rats, enzyme activity was restored from 40 percent of control to normal over a 24-hour incubation period. Several other sulfhydryl-reducing agents were examined for their ability to reactivate MDMA-inactivated TPH. Although all compounds slightly elevated the inactivated enzyme, only DTT and 2-mercaptothanol caused significant reactivation of MDMA-depressed enzyme during the 24-hour preincubation period.

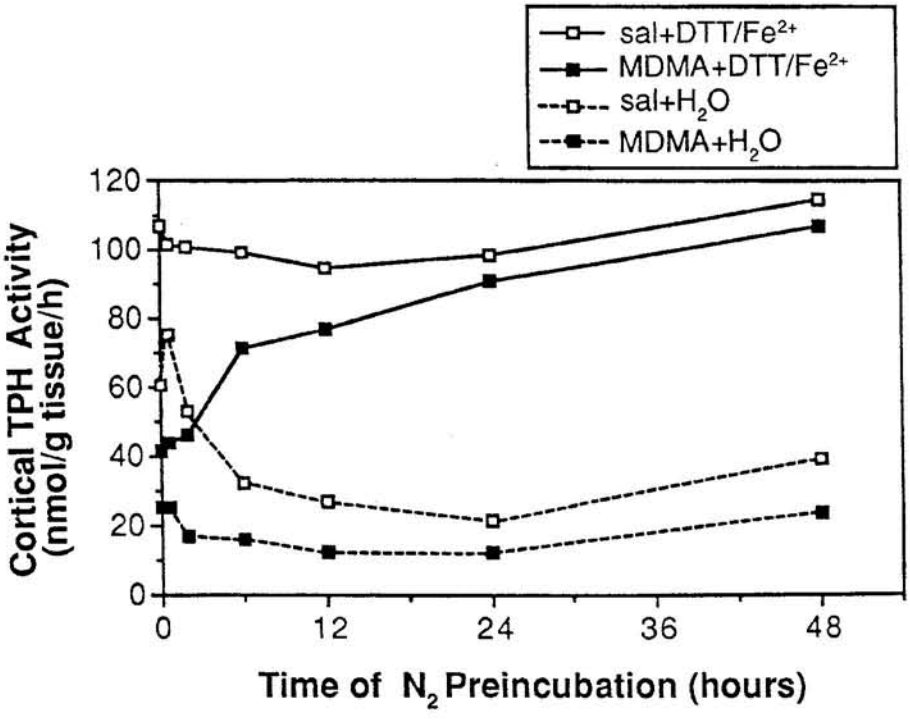

FIGURE 1. *Time course of in vitro reactivation of MDMA-inactivated cortical TPH. Enzyme solutions were prepared from rats killed 3 hours after a single SC injection of MDMA (10 mg/kg) or saline (sal). Reactivation mixture (DTT/Fe^{2+}) or water was added to enzyme preparations, which were then assayed immediately (zero time point); the remainder was subsequently preincubated under N_2 for up to 48 hours. Each point represents the mean of four determinations.*

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Because MDMA decreases TPH activity by decreasing V_{max} rather than the affinity for either the substrate or cofactor (Schmidt and Taylor 1987), the effect of reducing conditions on the kinetics of the enzyme was determined (figure 2). When cortical TPH from MDMA-treated rats was incubated under reducing conditions, enzyme reactivation essentially doubled the V_{max} of TPH, whereas the K_m for either tryptophan or the cofactor, 6-methyl-5,6,7,8-tetrahydropterin (6-MPH_4), was not significantly altered.

Insight into the time course of the toxicity induced by MDMA in vivo could likely be obtained by assessing the ability of reducing conditions to reactivate the

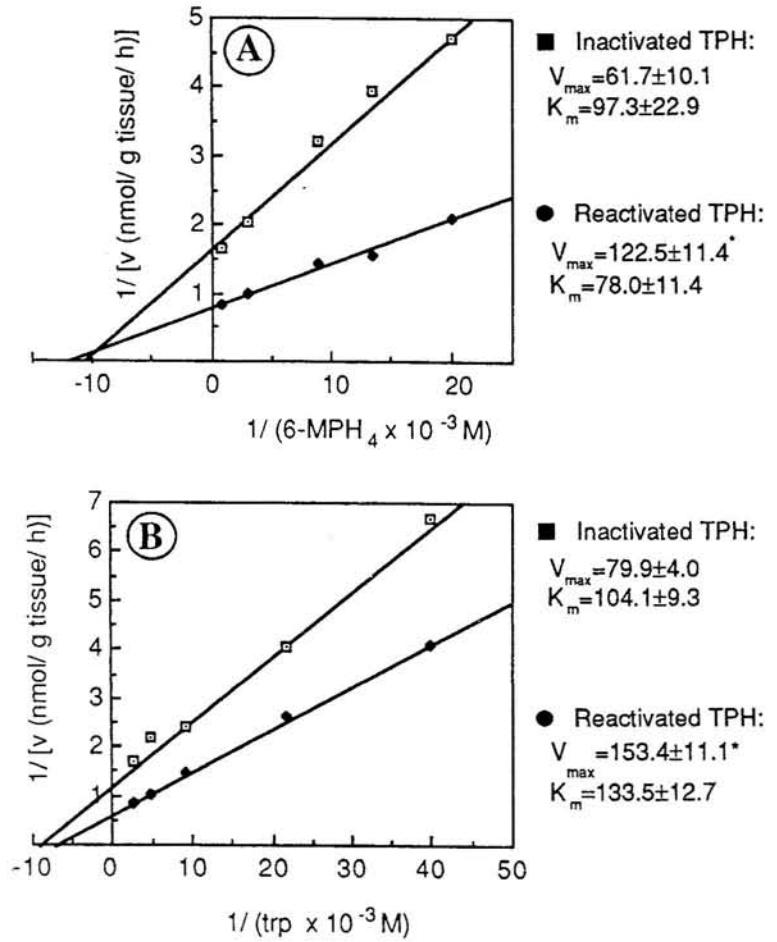

FIGURE 2. *Double reciprocal plots of inactivated and reactivated TPH activity from MDMA-treated rats, as a function of 6-MPH$_4$ (A) or tryptophan (trp) (B) concentrations. TPH solutions were prepared from rats killed 3 hours after a single dose of MDMA (10 mg/kg, SC). Following addition of reactivation mixture, inactivated and reactivated enzyme solutions were preincubated for 24 hours in room air at 0 °C and under N_2 at 25 °C, respectively. The K_m and V_{max} values are the means±SEM derived from double reciprocal plots of each of three enzyme samples (each sample was prepared from the pooled cortices of five rats). The lines shown are based on the mean K_m and V_{max} values; each point represents the mean value for 1/velocity at that substrate concentration.*

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enzyme at increasing times after administering the drug. If less and less enzyme is reactivated as the time of in vivo exposure to MDMA increases, this would suggest the development of irreversible drug-induced damage. Rats were killed at varying times after MDMA administration, and the enzyme was incubated under reactivating conditions (table 1). Neurotoxicity occurred rapidly following MDMA (10 mg/kg).

Within 6 hours after MDMA was administered, <50 percent of inactivated TPH could be reactivated; by 3 days, essentially all the MDMA-inactivated enzyme (>80 percent) had been irreversibly altered.

TABLE 1. *Cortical TPH activity from rats killed at various times after saline or MDMA treatment, before and after preincubation under reactivating conditions*

	TPH Before Preincubation		TPH After Preincubation		
Time	Saline	MDMA	Saline	MDMA	Percent Recovery
3 hours	98.0±5.9	54.4±10.3 (55.6±10.5)	91.5±6.8	93.1±9.0 (101.7±9.8)†	103.8
6 hours	103.0±8.2	38.0±5.1 (36.9±5.0)	100.4±6.8	69.8±6.6 (69.5±6.5)†	51.7
3 days	99.6±3.1	48.6±4.0 (48.8±4.0)	91.1±4.9	51.8±4.9 (56.9±5)	15.8
1 week	80.6±2.9	50.2±2.9 (62.4±3.6)	82.9±6.0	52.6±5.0 (63.5±6.0)	2.9
30 days*	53.7±4.5	29.9±2.7 (55.7±5.0)	65.4±3.9	30.3±3.2 (46.3±4.9)	-21.2

NOTE: Rats were killed at indicated times following a single saline or MDMA (10 mg/kg) injection. Cortical TPH activity (nmol/g of tissue/hour) from each rat was assessed following the addition of reactivation mixture, both before and after a 20- to 24-hour preincubation under N_2. Results are presented as the means±SEM (n=5 to 7). Numbers in parentheses indicate percentage of saline control. Percent recovery was calculated as: ([percent after preincubation – percent before preincubation]/[100 – percent before preincubation]) X 100.

*30 days after multiple MDMA doses (10 mg/kg per dose; five doses, one every 6 hours)
†$p<0.05$ vs. corresponding percentage before preincubation

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Because congeners of MDMA also decrease TPH activity, it was determined whether enzyme from animals injected with a single dose of METH (10 mg/kg), p-chloroamphetamine (5 mg/kg), or MDMA (10 mg/kg) could be restored by incubating under reducing conditions (figure 3). Incubation with DTT/Fe^{2+} for 24 hours reactivated the TPH from animals treated with each of the congeners. It appears that oxidative inactivation is common to the neurotoxicity of all three amphetamine congeners.

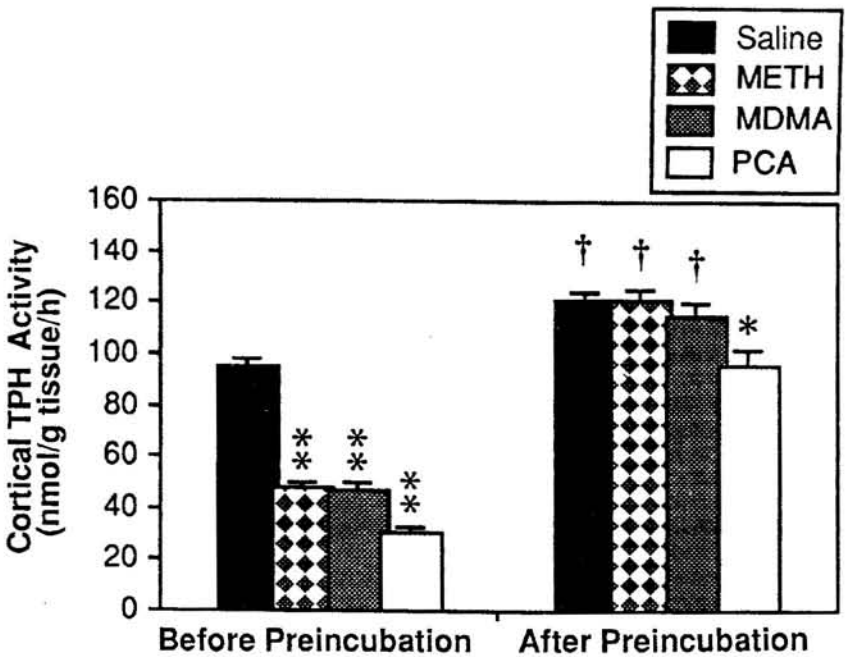

FIGURE 3. *Comparison of cortical TPH activity, before and after reactivation, from rats treated with MDMA, METH, or p-chloroamphetamine (PCA). Rats were administered a single SC injection of MDMA (10 mg/kg), METH (10 mg/kg), PCA (5 mg/kg), or saline and killed 3 hours later. Cortical TPH activity (nmol/g of tissue/hour) was assessed following the addition of reactivation mixture, both before and after a 24-hour preincubation under N_2. Results are the means±SEM (n=6).*

*p<0.05

**p<0.001 vs. time-matched saline

† Denotes a significant difference (p<0.01) between percentage after preincubation and percentage before preincubation (percent=[TPH activity from drug-treated rats/TPH activity from time-matched saline-treated rats] X 100)

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CONCLUSIONS

Amphetamine and its analogs cause neurochemical deficits in brain dopaminergic and serotonergic systems. Because these deficits persist for long periods after discontinuing the drug, neurotoxicity is inferred. Endogenous DA is essential for neurotoxicity to occur. TPH, inactivated by administering amphetamine congeners to rats, can be reactivated by incubating the enzyme under reducing conditions; this suggests that the serotonergic deficits are associated with oxidative reactions.

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DISCUSSION

Q: Methamphetamine, do you have a single dose?

A: Do I have a single dose? I don't have one here, but we do have it. You get more effect with amphetamine on the dopamine system than on the serotonergic system. And so, obviously with one dose, you are not going to see much effect on the dopamine system.

Q: You give high doses?

A: Yes.

Q (Seiden): I wonder if you could state whether you think that the dopamine is exerting its effects by activating other neurons or is it exerting some effects on the chemistry of the system independent of neuroactivity?

A: I don't know the answer to your question, but my speculation would be that the dopamine is auto-oxidized and that auto-oxidation results in the formation of hydroquinone or in free radicals. I think those reactive metabolites might be

responsible for causing the neurotoxicity. At this point that's my guess, and only time will tell, after the appropriate experiments have been performed.

Q (Seiden): Just before I left for this meeting, I read a paper in which they showed that 5,7-DHT could catalyze the reduction of glutathione.

A (Gibb): That should be explored.

Q (Ricaurte): Could you comment on the difference between an amphetamine and methamphetamine? The basis for the question is that if dopamine release is the key, and both amphetamine and methamphetamine are very good dopamine releasers, both would be expected to have similar neurotoxic properties. Yet there is no question that methamphetamine is much more toxic than amphetamine to serotonin systems.

A: Yes

Q (Ricaurte): Why do you think that there is such a difference?

A: I don't know. There are obviously some factors that still need some sorting out. In our hands at least, we see toxicity with both. But there is more; I agree with you. In our hands, compared to amphetamine, methamphetamine is more toxic to the serotonergic than to the dopaminergic system.

COMMENT (Cho): Let me comment on that. We have done some studies comparing methamphetamine- and amphetamine-induced release of dopamine after an intravenous dose, and it is very clear that the rate at which dopamine comes out is much greater with methamphetamine. I think that this is just a question of the rapidity with which methamphetamine gets into the brain. The volume of distribution of methamphetamine is much larger than amphetamine. I think that is because the stuff gets in the brain more quickly and it is causing release faster. So it's like comparing morphine and heroin.

Q (Cho): I want to go back to your thiol protein recovery experiments. When you did the thiol experiments, was this a whole cell preparation or was it cell-free?

A (Gibb): We homogenized and spun it down at 27,000 g. So this was 27,000 g supernatant.

Q (Cho): I guess what I am really asking is where is the enzyme that you are activating? Is it the bottom of the cell particle?

A (Gibb): Yes, I understand your question. I don't know the answer, but the 27,000 g supernatant still has cell particles.

COMMENT (Cho): Sometimes glutathione has trouble getting access to sites, and that was why I was asking that question.

COMMENT (Gibb): In other words, you are saying that glutathione can't get in.

Q (Cho): If you could get a soluble enzyme, you could go after it. The other question is why you used iron 2 with the DTT?

A (Gibb): Thank you for bringing that up. I should have mentioned it. The iron is not essential in the cortical preparation, but in the striatal preparation, it enhances the response. I don't know why. Perhaps there is enough iron bound to the enzyme that you don't need the iron. But if you get a more purified enzyme, then iron is essential.

Q (Cho): What do you think iron is doing?

A: I don't know.

COMMENT (Cho): If you have iron 2 and you continually generate it (presumably you would with the thiol reagent present), you would have ideal conditions for generating hydroxyl radical, because it is the iron 2 and oxygen and peroxide that generate this stuff. So, in a way, within that system, it sounds to me as if you were fighting the cell. That was the thing that should have bothered me.

COMMENT (Gibb): I suppose there are enough protective capabilities within the preparation that that is not occurring.

COMMENT (Cho): But then I looked at your cortical preparation, and you didn't get any better results in the absence of iron.

COMMENT (Gibb): And the only reason we included it, Art, was that in the neostriatum it does seem to improve the situation to some extent. That has been observed not only with MDMA but with oxygen as well.

Q: What do you think we should make of what looks like an increase in TPH activity as you use agents that modify the dopamine release?

A (Gibb): Yes. That is an inconsistent finding, but I think that it is fascinating; and I think that it demonstrates that there is possibly some interaction that is going on between the dopamine and the serotonergic system.

Q: Maybe it is a basal interaction?

A (Gibb): Yes. I think that has been demonstrated neurophysiologically as well as neurochemically. But what controls that interaction, I am not sure. But it is very pronounced. We can get up to 130 percent of control when we give reserpine or somehow interfere with the dopamine input to the serotonergic system.

Q: It could be determined by your basal dopamine?

A (Gibb): Yes, but I think there are those projections, those interactions that are very real.

Q (Seiden): Did you have any EDTA [ethylenediaminetetraacetic acid] present?

A: No, we didn't.

Q (Olney): Why does MK801 protect?

A (Gibb): That is a good question. Based on some other experiments that we have done, we think that there is a link between the dopamine and the glutamate systems. Glen Hanson has done a lot of experiments with the neuropeptides, which is another story and is very fascinating, and I think that it offers another way that we can get at some of these problems. But it seems that when we give NMDA, we have been aggravating the situation. It appears that way, that maybe it is a sequence.

COMMENT (Olney): So there is an NMDA receptor involved?

COMMENT (Gibb): Yes. I am sure that you are happy about that.

COMMENT (Olney): Well, I am puzzled.

COMMENT (Gibb): So are we at this point.

COMMENT (Olney): I am also happy. Makes things nice and challenging and complicated. Ordinarily, you would think of an NMDA receptor being on dendrites or cell bodies; and if it is mediating any toxicity, it would be toxic to the cell bodies and dendrites. That is the conventional arrangement. But you are dealing here with an apparent toxic effect impinging on axons and axon terminals.

COMMENT (Gibb): We are.

COMMENT (Olney): We don't know of any way that NMDA receptors would be involved in that kind of toxicity, but it is a fascinating possibility that you're putting your finger on a new type of excitotoxicity that hasn't been invented before.

COMMENT (Gibb): Right. We have found some very encouraging results, John; but as I said about the neuropeptide story, we can simulate or at least cause similar effects with NMDA as with methamphetamine on the increase in neuropeptides that Hanson has observed. We thought that with MK801 we can sort out those interrelationships better with the neuropeptides than we can neurotoxicity. It may be a little more physiologic than the neurotoxicity.

COMMENT (Ricaurte): John, just in relation to this whole question, we have envisioned that NMDA receptors are on DA terminals and hypothesized that if NMDA receptor activation is involved in the toxicity of amphetamines, we should be able to reproduce the toxicity by giving NMDA directly into the striatum. When you inject NMDA into the striatum, you see damage, but it appears to be nonspecific since DA levels per se are unaffected by high doses of NMDA. There is no question that the competitive and noncompetitive NMDA receptor blockers protect against methamphetamine toxicity. Just a few weeks ago at a New York Academy of Sciences meeting, John Marshall showed some very nice data obtained with in vivo microdialysis demonstrating that a compound like MK801 was very effective in interfering with methamphetamine-induced dopamine release; so indeed the basis of protection may involve NMDA receptors, perhaps by modulating DA release.

COMMENT (Gibb): Somehow I don't know which one comes first in the sequence, but obviously, it appears to be a component.

COMMENT (Seiden): There is no question that this result is reproducible.

Q (Landfield): What calcium channel blockers did you try?

A (Gibb): Nimodipine, which makes it worse. I have forgotten the others, but we tried quite a few.

COMMENT (Cho): I would just like to comment, because flunarizine is quite different chemically from some of the other calcium channel blockers. You are right; it is a dirty drug. But this goes back to what clearly represents my bias, but it seems that we ought to know the effects of these multiple drugs on the disposition of some of these compounds because part of the selectivity or part of the discrepancies that one might see could be due to the disposition or changes in the brain.

A (Gibb): We've certainly entertained that thought. It doesn't seem to fit with anything else. It could very well be that it is a disposition problem or it also has other effects on other systems. I think the thing that we have found fascinating about it is that it works selectively on one system and not the other. And that came as a bit of a surprise. It just so happens that it is the opposite of the MK801, and so it makes it kind of an interesting story.

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