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Causes and Consequences of Methamphetamine and MDMA Toxicity

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ABSTRACT

Methamphetamine (METH) and its derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) are 2 substituted amphetamines with very high abuse liability in the United States. These amphetamine-like stimulants have been associated with loss of multiple markers for dopaminergic and serotonergic terminals in the brain. Among other causes, oxidative stress, excitotoxicity and mitochondrial dysfunction appear to play a major role in the neurotoxicity produced by the substituted amphetamines. The present review will focus on these events and how they interact and converge to produce the monoaminergic depletions that are typically observed after METH or MDMA administration. In addition, more recently identified consequences of METH or MDMA-induced oxidative stress, excitotoxicity, and mitochondrial dysfunction are described in relation to the classical markers of METH-induced damage to dopamine terminals.

KEYWORDS: substituted amphetamines, excitotoxicity, oxidative stress, mitochondrial dysfunction, glutamate, blood-brain barrier

INTRODUCTION

Methamphetamine (METH) and its derivative 3,4-methylenedioxymethamphetamine (MDMA) are substituted amphetamines with potent central nervous stimulant effects. Acutely, METH and MDMA have numerous peripheral and central effects, leading to psychomotor activation, euphoria, decreased appetite, and hyperthermia. Because of their strong euphoric properties, METH and MDMA have a high abuse liability, and chronic use of either one can lead to psychotic and violent behaviors. Abuse of METH and MDMA is a growing problem in the United States: According to the 2003 national surveys on drug use and health (NSDUH), 1.4 and 2.1 million Americans aged 12 and older had tried METH and MDMA, respectively, within the month prior to the survey. The substance abuse and mental health services and administration (SAMSHA) reported that emergency room visits involving amphetamines increased by 54% between 1995 and 2002.

Preclinical studies have revealed that both METH and MDMA produce long-term damage to dopaminergic and serotonergic nerve terminals in multiple brain areas. Specifically, METH administration has been shown to produce long-term decreases in numerous measures of both dopaminergic and serotonergic function such as enzyme activity, monoamine content, and monoamine transporters, while the MDMA-induced depletions have typically been more specific to serotonergic terminal markers.[1-3] Despite the extensive clinical evidence that the substituted amphetamines are neurotoxic, the mechanisms involved in producing those effects are still not well understood. However, increasing evidence suggests that METH and MDMA-induced neurotoxicity involves the production of reactive oxygen and reactive nitrogen species,[4] and a subsequent induction of oxidative stress. The excessive extracellular dopamine (DA) concentrations resulting from the ability of the substituted amphetamines to reverse the carrier-mediated dopamine uptake system[5] can be oxidized enzymatically and nonenzymatically to form highly reactive dopamine quinones and reactive oxygen species, leading to increase in oxidative stress.[6]

Glutamate has also been implicated in the toxicity produced by the substituted amphetamines. METH, but not MDMA, has been shown to increase the extracellular concentrations of glutamate in the striatum and the hippocampus.[4,7-10] Increases in extracellular glutamate levels have been associated with increases in reactive nitrogen species as well as activation of calcium-dependent proteases and cytoskeletal damage, supporting a role for excitotoxicity in mediating the long-term neurotoxicity produced by METH. Finally, alterations in energy metabolism have been hypothesized to mediate METH and MDMA-induced neurotoxicity, as substituted amphetamines have been shown to inhibit mitochondrial function.[11,12] The purpose of this review is to

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present the causes of substituted amphetamine-induced toxicity, namely, oxidative stress, excitotoxicity, and mitochondrial inhibition, and to discuss how they interact and synergize to produce long-lasting neurotoxicity.

Amphetamine Neurotoxicity

It is now well documented that high doses of both METH and MDMA produce long-term neurotoxicity in rodents and nonhuman primates. Preclinical studies have revealed long-term decreases in tyrosine and tryptophan hydroxylase activities after either METH[13] and/or MDMA administration.[14] METH has been shown to produce long-lasting depletions in dopamine and its metabolites, as well as dopamine reuptake sites in the rat and primate striatum, but not in other dopamine rich areas such as the nucleus accumbens and the prefrontal cortex.[2,15-17] In contrast to the neurotoxic effects to striatal DA terminals, METH administration affects serotonergic terminals in multiple brain areas, including the prefrontal cortex, hippocampus, and striatum.[3,18] Methamphetamine toxicity to DA and 5-HT terminals has been shown to persist for up to 4 years after drug administration in nonhuman primates,[19] and reduced levels of striatal dopamine transporter were found in human abstinent methamphetamine users, 3 years or more after the last use of drug.[20] MDMA, unlike METH, has been shown to be selectively neurotoxic to serotonergic terminals. For instance, in rats, MDMA administration produced a reduction of 5-HT reuptake sites[21] and decreased striatal 5-HT and its metabolites.[22,23] Ricaurte et al also demonstrated the neurotoxic effects of MDMA in nonhuman primates,[24] and evidence of MDMA-induced damage to serotonergic terminals in humans have been reported.[25,26] Examples of the effects of METH and MDMA on 5-HT and DA tissue content or reuptake sites in the rat striatum are shown in Figures 1 and 2. Figure 1 depicts serotonin and dopamine striatal tissue content in rats 7 days after administration of METH or MDMA (10 mg/kg, intraperitoneally [ip], every 2 hours for a total of 4 injections). METH administration produced significant decreases in both 5-HT and DA tissue content, while MDMA administration produced a selective 50% decrease in 5-HT, but not DA content. Figure 2 shows the corresponding effects of METH and MDMA treatment on the dopamine transporter (DAT) and the 5-HT transporter (SERT), respectively. In addition, MDMA and METH both produce a notable hyperthermic response, which has been shown to play an essential role in the development of long-term toxicity to the 5-HT and DA terminals. Conversely, drugs or cool ambient temperatures that prevent hyperthermia have been shown to attenuate the toxic effects of METH and MDMA.[27,28]

In addition to the loss of various markers of 5-HT and/or DA terminals, evidence for axonal degeneration has been

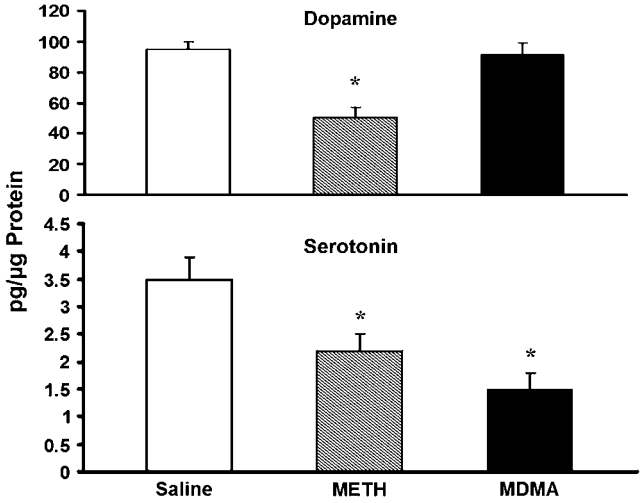

Figure 1. Effects of METH and MDMA on 5-HT and DA tissue content in the striatum. METH (10 mg/kg), MDMA (10 mg/kg), or saline (1 mL/kg) were administered intraperitoneally every 2 hours for a total of 4 injections. Rats were killed 7 days later. The data represent mean ± SEM of dopamine or serotonin content (in pg/μg of proteins) in the striatum. **P* < .05, when compared with the saline controls, as measured by *t* test.

reported after METH administration. For instance, in rats, METH has been shown to increase silver- and fluoro-Jade-staining.[29-31] Similarly, glial fibrillary fluorescent protein (GFAP), a marker for central nervous system (CNS) trauma or injury[32] has been reliably induced by METH treatment in rats[33] and mice.[34] Unlike the multiple histochemical studies supporting that METH could produce axonal degeneration, little evidence for degenerating neurons has been shown after MDMA administration.[35]

Causes of Toxicity

Oxidative Stress

The free radical hypothesis of substituted amphetamine-induced toxicity was proposed as early as 1989 by Gibb et al, after it was found that that MDMA-induced inactivation of tryptophan hydroxylase was reversed by sulfhydryl-reducing compounds.[36,37] The role of oxidative stress is further supported by the findings that the neurotoxic effects of METH and MDMA can be attenuated by free radical scavengers and antioxidants.[38-40] Similarly, administration of N-acetyl-cysteine was shown to decrease METH-induced damage to DA nerve terminals in the rat striatum.[41] It has been hypothesized that the excessive increases in intracellular dopamine that follow METH and MDMA administration could lead to the formation of dopamine quinones and reactive oxygen species (ROS).[6] Accordingly, there is considerable evidence that the increase in dopaminergic activity evoked by METH and MDMA plays an important role in mediating the toxicity to 5-HT and/or DA terminals.[42,43] For

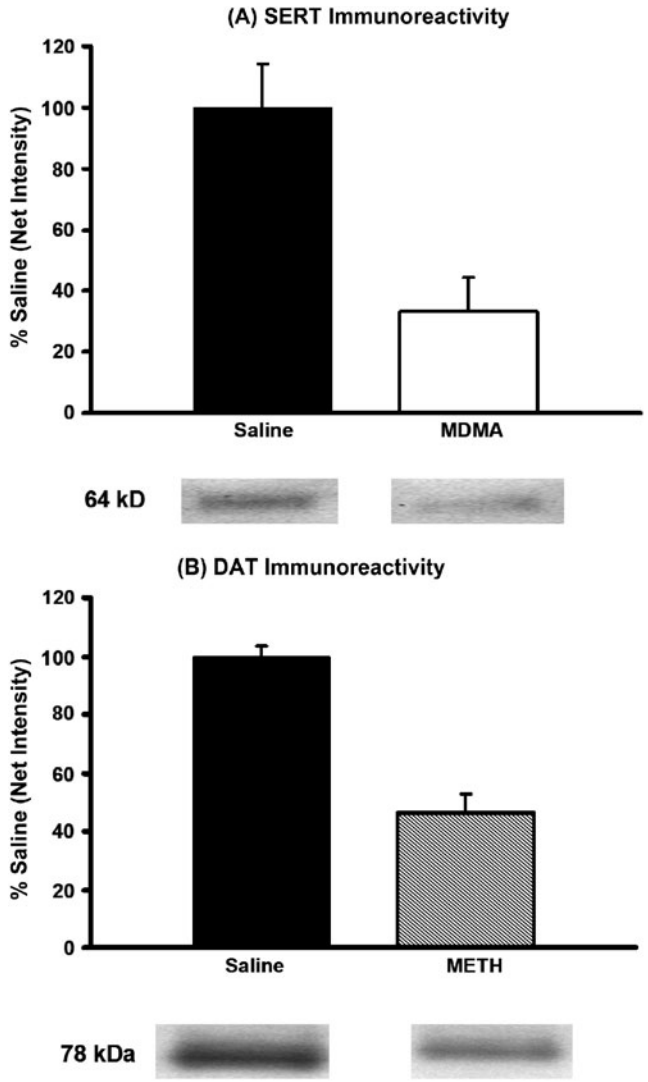

Figure 2. Effects of METH and MDMA on DAT and SERT-immunoreactivity in the striatum. METH (10 mg/kg), MDMA (10 mg/kg), or saline (1 mL/kg) were administered intraperitoneally every 2 hours for a total of 4 injections. Rats were killed 24 hours later and striatal DAT or SERT immunoreactivity was measured by Western blotting. Data are expressed as relative optical density (% saline controls).

instance, attenuation of DA release by lesioning DA neurons or blocking the DA transporter have been shown to protect against the long-term toxicity produced by METH and MDMA.[43-46] In the case of MDMA, which is only neurotoxic to 5-HT terminals, it is believed that the excessive DA released in the synapse can be either taken up by the activated 5-HT transporter into the terminals or synthesized nonenzymatically inside the terminals from tyrosine, where it subsequently generates free radicals and produces oxidative damage.[47,48] In addition, oxidation of 5-HT, MDMA itself, and thioether metabolites of MDMA have also been implicated in MDMA-induced neurotoxicity.[49-51] MDMA has now been shown to increase hydroxyl radical formation in rats.[52-54] Similarly, METH-induced hydroxyl radical generation has been demonstrated in rats[28,55] as well as in mice[56]; consequently these highly reactive free radicals can lead to the generation of lipid peroxides and oxidize proteins in the nerve terminals.

In fact, MDMA was shown to produce lipid peroxidation and to reduce antioxidant content in 5-HT nerve terminals,[52,57,58] events that usually accompany free radical formation. Our laboratory showed that METH administration led to a rise in the formation of 2,3-dihydroxybenzoic acid (2,3DHBA) after local infusion of salicylate into the striatum, demonstrating that METH could locally increase the formation of free radicals in the brain.[59] These results were in accordance with previous studies that showed that 2,3DHBA formation could be augmented by METH using the peripheral administration of salicylate to trap hydroxyl radicals.[28,55] We also showed that malonyldialdehyde, a lipid peroxidation product, was increased in the rat striatum 24 hours after the injection of METH, suggesting that oxidation of lipid also occurs in response to METH. Lipid peroxidation following METH administration was also demonstrated by other groups in both rats[60] and mice.[61]

The role of iron also appears to play an important role in the pro-oxidant effects of METH. The long-term depletion of dopamine content was attenuated by deferroxamine, an iron chelator.[59] This was the first evidence that iron may be involved in the neurotoxic effects of METH. Because iron is known to catalyze the production of free radicals from hydrogen peroxide through the Fenton reaction, this finding further supports the hypothesis that amphetamine toxicity is mediated in part by oxidative stress.

In addition to reactive oxygen species, reactive nitrogen species (RNS) now appear to play a major role in mediating METH and MDMA-induced neurotoxicity. METH-induced toxicity in the mouse striatum can be attenuated by nitric oxide synthase (NOS) inhibition.[62] Similarly, Colado et al reported in 2001 that neuronal NOS inhibitors provided significant neuroprotection against MDMA-induced long-term dopamine depletion in mice.[63] However, owing to the substantial differences between the effects of MDMA in rats and mice, such as MDMA's ability to produce DA depletions in mice, caution must be taken when making conclusions regarding the mechanisms of MDMA-induced toxicity in mice. More recently, it has been demonstrated that systemic administration of 2 NOS inhibitors, *N*ω-nitro-L-arginine methyl ester hydrochloride and *S*-methyl-L-thiocitrulline (*S*-MTC), as well as the peroxynitrite decomposition catalyst Fe(III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (FeTPPS) attenuated the MDMA-induced depletion of striatal 5-HT in the rat brain.[64] Of importance, this attenuation was obtained without altering the hyperthermic effects of MDMA. Therefore, the finding that NOS inhibitors could attenuate MDMA-induced

toxicity without attenuating its hyperthermic effects clearly demonstrated the involvement of nitric oxide in the MDMA-induced 5-HT depletions.[64] In addition, MDMA-administration significantly increased the formation of nitric oxide as well as nitrotyrosine concentrations in the striatum. Figure 3 depicts the effects of METH and MDMA administration on nitrotyrosine concentration in the rat striatum. Both METH and MDMA produced more than a 2-fold increase in nitrotyrosine concentrations, suggesting that the substituted amphetamines can potentiate the formation of peroxynitrites, which in turn induce the nitration of tyrosine residues on various proteins. It is conceivable that these nitrated proteins include some that are essential to the functioning of 5-HT or DA terminals such as the tyrosine or tryptophan hydroxylase or vesicular monoamine transporters. In fact, nitration of tyrosine hydroxylase by peroxynitrites has been shown by Kuhn et al.[65] Lipid peroxidation and protein oxidation or nitration induced by METH and MDMA could, therefore, play an important role in mediating their toxic effects on 5-HT and DA terminals.

Excitotoxicity

Excessive glutamate release has been associated with neuronal cell death. This process, termed excitotoxicity, is mediated by the activation of ionotropic and group 1 metabotropic glutamate receptors (iGluR and mGluR, respectively), leading to a rise in intracellular Ca^{2+} levels. This rise in Ca^{2+} leads to activation of multiple kinases, lipases, and proteases, eventually causing the breakdown of cytoskeletal proteins, generation of free radicals, and DNA damage.[66] In fact, glutamate and other excitatory amino acids have been linked to several neurodegenerative disorders,[67] suggesting a possible role of glutamate in METH or MDMA-induced terminal degeneration. Sonsalla et al showed in 1989 that METH-induced toxicity to DA terminals in mice can be attenuated by N-methyl-D-aspartate (NMDA) glutamate receptor antagonists.[68] Similarly, the 2 glutamate receptor antagonists MK-801 and CGS19755 attenuated the toxic effects of both METH and MDMA in the rat striatum.[69] However, the results of these studies were confounded by the fact that NMDA-receptor antagonists typically block METH-induced hyperthermia.[70] A more recent study by Battaglia et al[71] showed that blockade of metabotropic glutamate receptors could attenuate METH-induced DA depletions in mice, independent of the hyperthermic effects of METH, providing more evidence of the role of glutamate in amphetamine-induced toxicity.

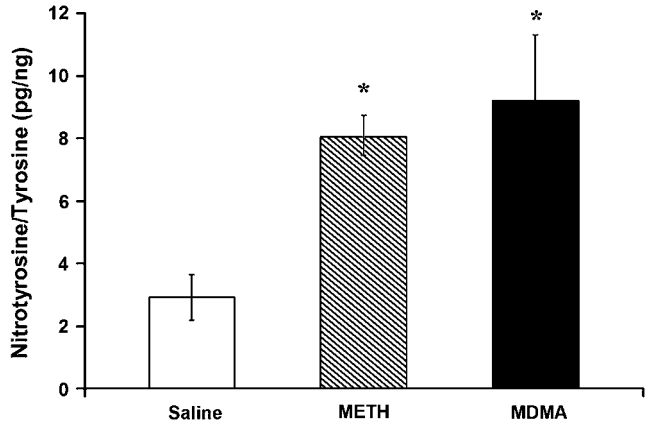

Figure 3. Effects of METH and MDMA on nitrotyrosine concentration in the rat striatum. METH (10 mg/kg), MDMA (10 mg/kg), or saline (1 mL/kg) were administered intraperitoneally every 2 hours for a total of 4 injections. Rats were killed 7 days later. Data are expressed as pg of nitrotyrosine per ng of tyrosine. *$P < .05$, when compared with the saline controls, as measured by *t* test.

Our laboratory reported that repeated injections of METH, but not MDMA, could produce an increase in glutamate release in the rat striatum.[4,7] In these studies, we showed that extracellular concentrations of glutamate began to rise ~4 hours after the first METH injection. Striatal glutamate levels remained elevated for up to 24 hours after drug administration (unpublished observations, June 2005). In support of these data, Burrows et al[72] showed that glutamate immunoreactivity was decreased in nerve terminals in the rat striatum and motor cortex 12 hours after METH administration. To elucidate how METH may increase the release of glutamate, we have shown that the increase in extracellular glutamate after METH administration involves the activation of striatonigral GABAergic transmission, leading to an inhibition of the nigrothalamic GABA outflow and a subsequent disinhibition of glutamatergic thalamocortical afferents. The increase in glutamate release in the cortex then activates the corticostriatal pathways, leading to the increase in extracellular striatal glutamate concentrations.[8] In these studies, we also showed that inhibition of METH-induced glutamate release by intranigral perfusion of the $GABA_A$ receptor antagonist bicuculline attenuated the METH-induced striatal DA depletions observed 7 days after drug administration, suggesting an important role of glutamate in mediating METH-induced toxicity. In preliminary studies, it was shown that the increases in extracellular glutamate concentrations were paralleled by increases in vesicular glutamate transporter-1 (VGLUT-1) mRNA and protein levels 12 and 24 hours after METH administration, respectively.[73] These increases in VGLUT-1 protein and mRNA levels were accompanied by increases in glyceraldehyde phosphate dehydrogenase (GADPH), and enzyme necessary to facilitate glutamate uptake into VGLUT-1 containing vesicles.[73] Taken together, these observations suggest that the rise in glutamate that follows METH administration may, in part, occur through METH-induced increases in VGLUT-1 function.[73]

Among the enzymes activated by the glutamate-induced increases in intracellular calcium, calpain I activation has been specifically linked to excitotoxicity. Calpain is a protease that, upon activation, leads to the proteolysis of several cytoskeletal proteins such as spectrin,[74] tau, and microtubule-associated protein2 (MAP 2).[75] We recently demonstrated that METH treatment was associated with calpain-mediated spectrin proteolysis in the rat striatum 5 and 7 days after drug administration.[76] This effect was specific to calpain-mediated proteolysis, as no changes in the immunoreactivity for the caspase-specific breakdown products were measured. The increases in METH-induced spectrin proteolysis were completely blocked by the AMPA receptor antagonist GYKI 52466, while the NMDA receptor antagonist MK801 had no effect. This blockade could not be attributed to changes in body temperature as the METH-induced hyperthermia was maintained in all animals receiving the antagonists. Surprisingly, despite the attenuation of METH-induced spectrin proteolysis observed after treatment with GYKI 52466, no attenuation of the METH-induced DA depletions was observed after pretreatment with either of the antagonists.

The evidence that substituted amphetamines can increase glutamatergic function and markers of excitotoxicity also strengthens the findings that reactive nitrogen species are involved in substituted amphetamine-induced toxicity. Indeed, the increased calcium influx produced by activation of NMDA receptors is associated with an increase in NOS activation, which can in turn lead to the formation of reactive nitrogen species.[77] In addition, reactive oxygen species can also be generated in response to increases in intracellular calcium,[78] suggesting that the excitotoxic effects of substituted amphetamine could synergize with their ability to induce oxidative stress to produce their toxic effects.

Mitochondrial Dysfunction

In addition to the increased oxidative stress and excitotoxicity produced by METH and MDMA administration, more recent evidence suggests an important role of the mitochondrial electron transport chain (ETC) in mediating the toxic effects of substituted amphetamines. In 1994, METH was reported to deplete striatal ATP content in mice, in regions typically affected by METH-induced toxicity.[79] The theory that METH and MDMA could disrupt mitochondrial function received more attention after the finding that inhibitors of the ETC could enhance MDMA[80] and METH-induced damage in both mice[81] and rats,[82] and that substrates of energy metabolism could attenuate METH and MDMA toxicity.[83,84] Because DA terminals have been shown to be more sensitive than other striatal terminals to the toxic effects of inhibitors of mitochondrial function,[85] METH-induced mitochondrial dysfunctions could explain the susceptibility of striatal dopaminergic terminals to METH-induced toxicity.

The studies by Stephans et al[83] were the first to assess the involvement of the ETC in the toxic effects of METH. In these studies, we showed that the striatal dopamine depletions observed 7 days after 4 injections of METH (10 mg/kg, every 2 hours) were significantly attenuated by intrastriatal infusions of decylubiquinone or nicotinamide for 6 hours beginning immediately after the last drug injection. Decylubiquinone is a component of the electron transport chain, while nicotinamide is a precursor for the electron carrier molecule NAD, and administration of either one presumably enhances oxidative phosphorylation on the mitochondria. Of interest, infusion of either energy substrate during drug administration did not protect against the toxicity to dopaminergic terminals, suggesting that the effects of METH on the ETC are delayed. In related studies, we showed that extracellular lactate concentrations increased in the striatum and, to a lesser extent the prefrontal cortex, shortly after the first METH injection.[83] These observations were in accordance with the idea that METH could possibly increase energy use through an increase in glycolysis. Similarly, Darvesh et al[86] showed that MDMA administration in rats led to increased extracellular glucose concentrations in the striatum. This effect was paralleled by a decrease in glycogen content in the brain 30 minutes to 1 hour after drug administration, demonstrating that, like METH, MDMA administration also leads to an increase in energy use.

The first direct evidence of METH and MDMA-induced inhibition of the ETC was provided in studies by Burrows and Meshul,[87] where we measured cytochrome oxidase activity (Complex IV of the ETC) in the rat brain after 4 injections of either substituted amphetamine. We observed a significant inhibition of cytochrome oxidase activity in the substantia nigra (SN), the nucleus accumbens, and the striatum 2 hours after the last injection of either METH (10 mg/kg) or MDMA (15 mg/kg). Complex IV activity returned to normal within 24 hours of the drug treatment. The involvement of the ETC in the toxic effects of MDMA was also supported by Nixdrof et al.[80] In these studies, MDMA (100 μM) was reverse-dialyzed into the striatum since this route of administration of MDMA eliminated any peripheral effects of the drug that were related to hyperthermia. While local administration of MDMA did not affect DA or 5-HT tissue content 7 days later, co-administration of MDMA with malonate, an inhibitor of complex II of the ETC, produced toxicity to both DA and 5-HT terminals.

We recently showed that the activity of succinate dehydrogenase (complex II of the ETC) was inhibited by 20% to 30% in the striatum, but not the hippocampus, of rats 1 to 24 hours after METH administration (10 mg/kg, every 2 hours, for a total of 4 injections).[12] In contrast, the activity of

complex I to III was not affected by that treatment regimen. Of interest, METH-induced mitochondrial inhibition was still observed when the rats were maintained normothermic, suggesting that this phenomena occurs independently of the hyperthermic effects of METH. In addition, we also showed that co-administration of the glutamate NMDA receptor antagonist MK-801 or the peroxynitrite scavenger Fe-TPPS prevented the METH-induced inhibition of complex II of the ETC, providing the first evidence for a convergence of the excitotoxic effects of METH and the inhibition of mitochondrial function. In contrast to the selective effects of METH for complex II inhibition, MDMA decreased the activities of both complexes I and II in striatal mitochondria 12 hours after administration (Figure 4).

It is likely that the METH- and MDMA-induced increases in reactive oxygen and nitrogen species (ROS and RNS) contribute to their disruptive effects on the mitochondrial function. In addition, the increases in intracellular calcium concentrations that follow the METH-induced rise in glutamate can also lead to inhibition of the ETC.[88] Taken together, these studies suggest that the mitochondria are common targets for the oxidative species and glutamate-mediated excitotoxicity produced by METH and MDMA, and that mitochondrial dysfunction and increased energy use play an important role in mediating the pro-oxidant and neurotoxic effects of the substituted amphetamines.

Consequences of Exposure to the Substituted Amphetamines

Vesicular Monoamine Transporter 2 Protein Loss and Oxidation

Oxidative stress, excitotoxicity, and mitochondrial dysfunction produced by administration of either METH or MDMA can converge to mediate their toxic effects. In accordance with previous reports demonstrating decreases in the activity of the vesicular monoamine transporter 2 (VMAT-2) after METH,[89,90] we recently showed that the protein concentrations of VMAT-2 were decreased in vesicular preparations from the striatum of rats treated with METH, 1 hour after drug treatment.[91] Since VMAT-2 is responsible for the uptake of dopamine from the cytosol into the vesicles, thereby preventing it from being enzymatically oxidized or auto-oxidized to produce free radicals or quinones in the nerve terminals, an inhibition of its function could lead to oxidative stress. In fact, Larsen et al[92] showed cytosolic DA concentrations and markers of oxidative damage after METH-administration were increased in VMAT-2 knockout mice. More important, we showed that VMAT-2 levels were decreased by 50% in vesicular, synaptosomal, and membrane-associated fractions as early as 24 hours after METH-administration, a time at which the classical markers of METH-induced toxicity are not affected or evident.[91] The finding that VMAT-2 depletions precede changes in other markers of toxicity suggests that these changes may contribute to nerve terminal degeneration through an increase in intracellular dopamine concentration and a subsequent increase in ROS generation. In addition to changes in protein levels, preliminary studies by Eyerman and Yamamoto[93] reported that VMAT-2 protein was nitrosylated within 1 hour after METH administration, as measured by an increase in nitrocysteine immunoreactivity in immunoprecipitated VMAT-2. These results suggest that METH-induced excitotoxicity and the subsequent generation of reactive nitrogen species may damage and decrease the activity of VMAT-2. Consequently, the decrease in DA reuptake into the vesicles could contribute to the dopaminergic terminal degeneration observed after METH administration.

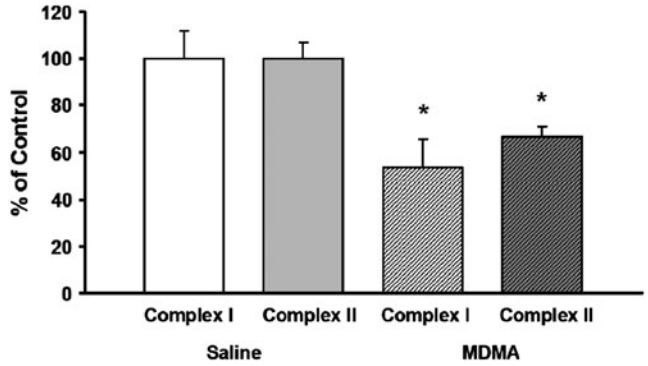

Figure 4. Effects of MDMA on complex I and II activity in the striatum. MDMA (10 mg/kg) or saline (1 mL/kg) were administered intraperitoneally every 2 hours for a total of 4 injections. Rats were killed 12 hours after the last injection and complexes I and II activities were measured in the striatum. Data are expressed as pg of nitrotyrosine per ng of tyrosine. *$P < .05$, when compared with the saline controls, as measured by *t* test.

Cell Loss Upon Therapeutic Intervention

We have previously reported that METH administration produced an increase in GABA release in the SN.[8] In these experiments, it was demonstrated that intranigral infusion of the D1 receptor antagonist SCH23390 prevented the METH-induced increase in extracellular GABA concentrations in the SN that was dependent upon D1 dopamine receptor activation. However, little is known about the effects of METH on the indirect pathway from the caudate putamen to the SN involving the globus pallidus and the subthalamic nucleus (STN), and using glutamate as a neurotransmitter. In this regard, we recently reported that glutamate release in the SN could be evoked by activating muscarinic receptors in the STN with carbachol.[94] Of interest, the carbachol-induced glutamate release in the SN was enhanced and prolonged by administration of the dopamine D2 receptor antagonist raclopride (100 μM) in the SN.

Accordingly, these studies also demonstrated that activation of nigral D2 receptors by local administration of quinpirole blocked the carbachol-induced increases in extracellular glutamate concentrations. In contrast, local infusion of the D1 receptor agonist SCH23390 in the SN had no effect on carbachol-induced nigral glutamate release. These data demonstrate that glutamate release in the SN is under the inhibitory influence of dopamine, through activation of D2 receptors. It was therefore reasonable to assume that under normal conditions, METH would not affect glutamate release in the SN, owing to its ability to increase extracellular dopamine release in that area,[95] which in turn activates D2 receptors to inhibit the subthalamonigral glutamatergic terminals. Indeed, METH administration (10 mg/kg, ip, every 2 hours for a total of 4 injections) produced no effect on extracellular concentrations of glutamate in the SN.[96] However, when METH was administered in the presence of the D2 receptor antagonist raclopride, a 500% increase in extracellular glutamate concentration was observed, suggesting a potentially excitotoxic consequence of the combined effects of METH and D2 receptor antagonists. In fact, a subchronic regimen of another D2 antagonist, haloperidol (0.5 mg/kg/d for 5 days), after METH administration produced dendritic spectrin proteolysis in the SN and, of more importance, resulted in a 32% decrease in NeuN positive staining in substantia nigra pars reticulata (SNr).[97] These data suggest that administration of haloperidol after METH leads to cell body damage in the SNr, a brain area mainly populated with GABAergic output neurons from the basal ganglia. Damage to this area responsible for the main outflow from the basal ganglia could potentially lead to extrapyramidal motor dysfunction. These observations have marked significance because administration of D2 receptor antagonists such as haloperidol represents the typical therapeutic intervention for METH overdose.

Disruptions of Blood-Brain-Barrier Permeability

Oxidative stress and reactive oxygen species have been associated with disruptions of the integrity of the blood-brain barrier (BBB).[98] In addition, MDMA and METH produce other acute physiological changes such as changes in body temperature, hyponatremia, and hypertension, events also associated with changes in BBB permeability.[99] We recently showed that MDMA administration (10 mg/kg, every 2 hours for a total of 4 injections) produced long lasting disruptions of BBB permeability.[100] More specifically, we showed that transcardial perfusion of trypan blue dye 10 weeks after drug administration led to an increase in staining at the level of the striatum and the dorsal hippocampus of the MDMA treated rats, when compared with the saline controls. Administration of the antioxidant vitamin C during the MDMA regimen reduced staining in the striatum, but not the hippocampus of the MDMA-treated rats, demonstrating the involvement of oxidative stress in the MDMA-induced disruptions of the BBB in proximity to the striatum. This MDMA-induced long-term damage to the BBB suggests that MDMA abuse could enhance the vulnerability of the brain to infection and environmental toxins.

CONCLUSIONS

Oxidative stress, excitotoxicity, and mitochondrial dysfunction appear to be causal events that converge to mediate METH and MDMA-induced neurotoxicity, as measured by loss of various markers of dopaminergic and serotonergic terminals (see Table 1 and diagram on Figure 5). In particular, the mitochondria appear to be a common target for the oxidative and glutamate-derived species produced by the substituted amphetamines. Among the various consequences of these phenomena, is the nitrosylation, and loss of VMAT2

Table 1. Causes of METH and MDMA-induced Neurotoxicity: A Summary of Our Previous Studies

Evidence for Substituted Amphetamine-induced Oxidative Stress

- METH increases in free-radical generation[59]
- METH induces the production of malonyldehaldehyde[59]
- METH and MDMA increase nitrotyrosine in the striatum[64]
- nNOS inhibition attenuates MDMA-induced serotonergic depletions[64]
- Peroxynitrite scavengers inhibit METH and MDMA-induced toxicity[12,64]

Evidence for Substituted Amphetamine-induced Excitotoxicity

- METH increases striatal glutamate release[4,7]
- METH increases vGLUT-1 protein and mRNA levels[8,73]
- METH induces spectrin proteolysis in the striatum[76]

Evidence for Substituted Amphetamine-induced Mitochondrial Dysfunction

- MDMA increases extracellular glucose and decreases glycogen use[86]
- METH increases extracellular lactate concentration in the striatum[83]
- METH and MDMA decrease cytochrome oxidase activity[87]
- The mitochondrial inhibitor malonate enhances MDMA-induced toxicity[80]
- Complex I and II activities are decreased after METH and MDMA[12]
- Ubiquinone infusions protect against METH-induced toxicity[83]

*METH indicates methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine (ecstasy); and NOS, nitric oxide synthase.

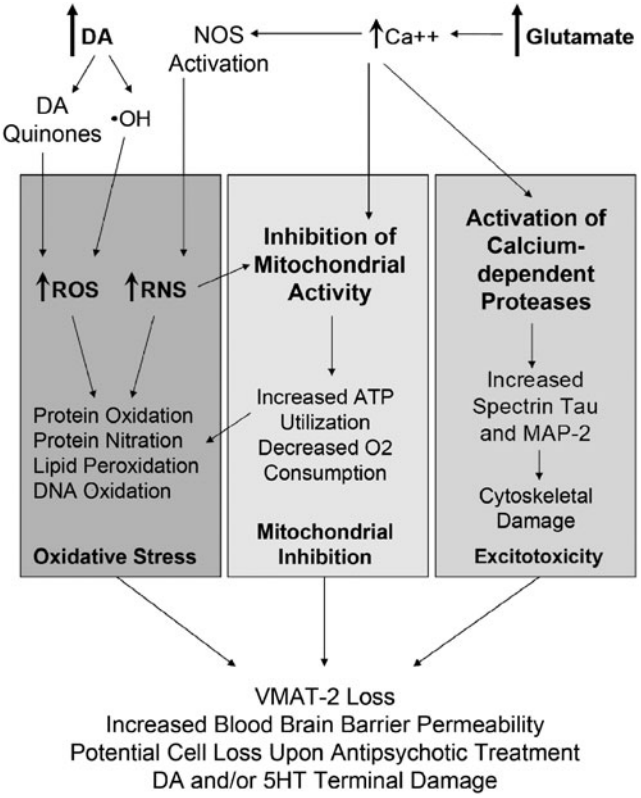

Figure 5. Schematic diagram of the causes and consequences of METH and MDMA toxicity. The increase in extracellular dopamine that follows METH or MDMA (DA) leads to the formation of DA quinones and hydroxyl radicals (•OH) and the production of reactive oxygen species (ROS). In addition, the rise in extracellular glutamate produced by the substituted amphetamines leads to an increase in intracellular calcium concentrations that leads to the activation of nitric oxide synthase (NOS) and the consequent generation of reactive nitrogen species (RNS). The ROS and RNS can alter proteins, lipids, and DNA, as well as inhibit mitochondrial function to produce energy deficits in the nerve terminals. In addition, the increases in intracellular calcium concentrations can directly inhibit mitochondrial function, as well as activate caspase that produce the breakdown of cytoskeletal proteins such as spectrin, tau, and microtubule-associated protein 2 (MAP-2). Taken together, these events lead to damage to the nerve terminals and produce loss of essential proteins such as the vesicular monoamine transporter 2 (VMAT-2), increase BBB permeability, and produce unsafe interactions with drugs such as the antipsychotics.

protein. This could possibly lead to inactivation of the sequestration of dopamine into vesicles, produce an excessive increase in intracellular dopamine concentrations, and result in consequent damage to the dopaminergic nerve terminals through accumulation of dopamine-derived ROS. In addition, we showed that the lack of D2 receptor activation after METH administration could lead to a potentially toxic interaction with typical antipsychotic drugs in the substantia nigra. This finding raises concerns regarding the safety of using these drugs for the treatment of METH overdose, as the resulting cell loss could potentially lead to disruptions in extrapyramidal motor function. Finally, we showed that the oxidative stress produced by the substituted amphetamines may mediate disruptions in BBB permeability, possibly increasing the vulnerability of the brains of drug users to further damage by viruses or environmental toxins.

REFERENCES

1. Hotchkiss AJ, Morgan ME, Gibb JW. The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life Sci*. 1979;25:1373-1378.

2. Wagner GC, Ricaurte GA, Seiden LS, Schuster CR, Miller RJ, Westley J. Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Res*. 1980;181:151-160.

3. Ricaurte GA, Schuster CR, Seiden LS. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. *Brain Res*. 1980;193:153-163.

4. Stephans SE, Yamamoto BK. Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. *Synapse*. 1994;17:203-209.

5. Raiteri M, Cerrito F, Cervoni AM, Levi G. Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine. *J Pharmacol Exp Ther*. 1979;208:195-202.

6. Michel PP, Hefti F. Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. *J Neurosci Res*. 1990;26:428-435.

7. Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res*. 1992;581:237-243.

8. Mark KA, Soghomonian JJ, Yamamoto BK. High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. *J Neurosci*. 2004;24:11449-11456.

9. Yamamoto BK, Gudelsky G, Stephans SE. Amphetamine neurotoxicity: Roles for dopamine, glutamate and oxidative stress. In: *Progress in HPLC-HPCE.* Utrecht, The Netherlands: VSP; 1998:223-244.

10. Rocher C, Gardier AM. Effects of repeated systemic administration of d-fenfluramine on serotonin and glutamate release in rat ventral hippocampus: comparison with methamphetamine using in vivo microdialysis. *Naunyn Schmiedebergs Arch Pharmacol*. 2001;363:422-428.

11. Brown JM, Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. *Pharmacol Ther*. 2003;99:45-53.

12. Brown JM, Quinton MS, Yamamoto BK. Methamphetamine-induced inhibition of mitochondrial complex II: roles of glutamate and peroxynitrite. *J Neurochem*. 2005;95:429-436.

13. Hotchkiss AJ, Gibb JW. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. *J Pharmacol Exp Ther*. 1980;214:257-262.

14. Schmidt CJ, Taylor VL. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. *Biochem Pharmacol*. 1987;36:4095-4102.

15. Eisch AJ, Marshall JF. Methamphetamine neurotoxicity: dissociation of striatal dopamine terminal damage from parietal cortical cell body injury. *Synapse*. 1998;30:433-445.

16. Seiden LS, Fischman MW, Schuster CR. Long-term methamphetamine induced changes in brain catecholamines in tolerant rhesus monkeys. *Drug Alcohol Depend*. 1976;1:215-219.

17. Finnegan KT, Ricaurte G, Seiden LS, Schuster CR. Altered sensitivity to d-methylamphetamine, apomorphine, and haloperidol in rhesus monkeys depleted of caudate dopamine by repeated administration of d-methylamphetamine. *Psychopharmacology (Berl)*. 1982;77:43-52.

18. Green AR, De Souza RJ, Williams JL, Murray TK, Cross AJ. The neurotoxic effects of methamphetamine on 5-hydroxytryptamine and dopamine in brain: evidence for the protective effect of chlormethiazole. *Neuropharmacology*. 1992;31:315-321.

19. Woolverton WL, Ricaurte GA, Forno LS, Seiden LS. Long-term effects of chronic methamphetamine administration in rhesus monkeys. *Brain Res*. 1989;486:73-78.

20. McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. *J Neurosci*. 1998;18:8417-8422.

21. Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, de Souza EB. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. *J Pharmacol Exp Ther*. 1987;242:911-916.

22. O'Hearn E, Battaglia G, de Souza EB, Kuhar MJ, Molliver ME. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J Neurosci*. 1988;8:2788-2803.

23. Finnegan KT, Ricaurte GA, Ritchie LD, Irwin I, Peroutka SJ, Langston JW. Orally administered MDMA causes a long-term depletion of serotonin in rat brain. *Brain Res*. 1988;447:141-144.

24. Ricaurte GA, Forno LS, Wilson MA. (+/−)3,4-Methylene-dioxymethamphetamine selectively damages central serotonergic neurons in nonhuman primates. *JAMA*. 1988;260:51-55.

25. Steele TD, McCann UD, Ricaurte GA. 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): pharmacology and toxicology in animals and humans. *Addiction*. 1994;89:539-551.

26. McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after (+/−)3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. *Neuropsychopharmacology*. 1994;10:129-138.

27. Malberg JE, Sabol KE, Seiden LS. Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. *J Pharmacol Exp Ther*. 1996;278:258-267.

28. Fleckenstein AE, Wilkins DG, Gibb JW, Hanson GR. Interaction between hyperthermia and oxygen radical formation in the 5-hydroxytryptaminergic response to a single methamphetamine administration. *J Pharmacol Exp Ther*. 1997;283:281-285.

29. Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY. Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Res*. 1982;235:93-103.

30. Eisch AJ, Schmued LC, Marshall JF. Characterizing cortical neuron injury with fluoro-jade labeling after a neurotoxic regimen of methamphetamine. *Synapse*. 1998;30:329-333.

31. O'Dell SJ, Marshall JF. Neurotoxic regimens of methamphetamine induce persistent expression of phospho-c-Jun in somatosensory cortex and substantia nigra. *Synapse*. 2005;55:137-147.

32. O'Callaghan JP, Sriram K. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. *Expert Opin Drug Saf*. 2005;4:433-442.

33. Pu C, Fisher JE, Cappon GD, Vorhees CV. The effects of amfonelic acid, a dopamine uptake inhibitor, on methamphetamine-induced dopaminergic terminal degeneration and astrocytic response in rat striatum. *Brain Res*. 1994;649:217-224.

34. Miller DB, O'Callaghan JP. Environment-, drug- and stress-induced alterations in body temperature affect the neurotoxicity of substituted amphetamines in the C57BL/6J mouse. *J Pharmacol Exp Ther*. 1994;270:752-760.

35. Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS. Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther*. 1987;241:338-345.

36. Stone DM, Johnson M, Hanson GR, Gibb JW. Acute inactivation of tryptophan hydroxylase by amphetamine analogs involves the oxidation of sulfhydryl sites. *Eur J Pharmacol*. 1989;172:93-97.

37. Gibb JW, Johnson M, Hanson GR. Neurochemical basis of neurotoxicity. *Neurotoxicology*. 1990;11:317-321.

38. Wagner GC, Carelli RM, Jarvis MF. Ascorbic acid reduces the dopamine depletion induced by methamphetamine and the 1-methyl-4-phenyl pyridinium ion. *Neuropharmacology*. 1986;25:559-561.

39. Gudelsky GA. Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin. *J Neural Transm*. 1996;103:1397-1404.

40. Colado MI, Green AR. The spin trap reagent alpha-phenyl-N-tert-butyl nitrone prevents "ecstasy"-induced neurodegeneration of 5-hydroxytryptamine neurones. *Eur J Pharmacol*. 1995;280:343-346.

41. Fukami G, Hashimoto K, Koike K, Okamura N, Shimizu E, Iyo M. Effect of antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. *Brain Res*. 2004;1016:90-95.

42. Stone DM, Johnson M, Hanson GR, Gibb JW. Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *J Pharmacol Exp Ther*. 1988;247:79-87.

43. Schmidt CJ, Ritter JK, Sonsalla PK, Hanson GR, Gibb JW. Role of dopamine in the neurotoxic effects of methamphetamine. *J Pharmacol Exp Ther*. 1985;233:539-544.

44. Gudelsky GA, Nash JF. Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. *J Neurochem*. 1996;66:243-249.

45. Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol Rev*. 2003;55:463-508.

46. Marek GJ, Vosmer G, Seiden LS. Dopamine uptake inhibitors block long-term neurotoxic effects of methamphetamine upon dopaminergic neurons. *Brain Res*. 1990;513:274-279.

47. Sprague JE, Everman SL, Nichols DE. An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology*. 1998;19:427-441.

48. Breier JM, Bankson MG, Yamamoto BK. L-tyrosine contributes to (+)-3,4-methylenedioxymethamphetamine-induced serotonin depletions. *J Neurosci*. 2006;26:290-299.

49. Bai F, Jones DC, Lau SS, Monks TJ. Serotonergic neurotoxicity of 3,4-(+/−)-methylenedioxyamphetamine and 3,4-(+/−)-methylendioxymethamphetamine (ecstasy) is potentiated by inhibition of gamma-glutamyl transpeptidase. *Chem Res Toxicol*. 2001;14:863-870.

50. Jones DC, Lau SS, Monks TJ. Thioether metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine inhibit human serotonin transporter (hSERT) function and simultaneously stimulate dopamine uptake into hSERT-expressing SK-N-MC cells. *J Pharmacol Exp Ther*. 2004;311:298-306.

51. Jiang XR, Wrona MZ, Alguindigue SS, Dryhurst G. Reactions of the putative neurotoxin tryptamine-4,5-dione with L-cysteine and other thiols. *Chem Res Toxicol*. 2004;17:357-369.

52. Colado MI, O'Shea E, Granados R, Murray TK, Green AR. In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. *Br J Pharmacol*. 1997;121:889-900.

53. Shankaran M, Yamamoto BK, Gudelsky GA. Involvement of the serotonin transporter in the formation of hydroxyl radicals induced by 3,4-methylenedioxymethamphetamine. *Eur J Pharmacol*. 1999;385:103-110.

54. Shankaran M, Yamamoto BK, Gudelsky GA. Mazindol attenuates the 3,4-methylenedioxymethamphetamine-induced formation of hydroxyl radicals and long-term depletion of serotonin in the striatum. *J Neurochem*. 1999;72:2516-2522.

55. Giovanni A, Liang LP, Hastings TG, Zigmond MJ. Estimating hydroxyl radical content in rat brain using systemic and intraventricular salicylate: impact of methamphetamine. *J Neurochem*. 1995;64:1819-1825.

56. Imam SZ, el Yazal J, Newport GD, et al. Methamphetamine-induced dopaminergic neurotoxicity: role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite decomposition catalysts. *Ann N Y Acad Sci*. 2001;939:366-380.

57. Shankaran M, Yamamoto BK, Gudelsky GA. Ascorbic acid prevents 3,4-methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT. *Synapse*. 2001;40:55-64.

58. Sprague JE, Nichols DE. The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. *J Pharmacol Exp Ther*. 1995;273:667-673.

59. Yamamoto BK, Zhu W. The effects of methamphetamine on the production of free radicals and oxidative stress. *J Pharmacol Exp Ther*. 1998;287:107-114.

60. Acikgoz O, Gonenc S, Kayatekin BM, et al. Methamphetamine causes lipid peroxidation and an increase in superoxide dismutase activity in the rat striatum. *Brain Res*. 1998;813:200-202.

61. Gluck MR, Moy LY, Jayatilleke E, Hogan KA, Manzino L, Sonsalla PK. Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment. *J Neurochem*. 2001;79:152-160.

62. Itzhak Y, Ali SF. The neuronal nitric oxide synthase inhibitor, 7-nitroindazole, protects against methamphetamine-induced neurotoxicity in vivo. *J Neurochem*. 1996;67:1770-1773.

63. Colado MI, Camarero J, Mechan AO, et al. A study of the mechanisms involved in the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') on dopamine neurones in mouse brain. *Br J Pharmacol*. 2001;134:1711-1723.

64. Darvesh AS, Yamamoto BK, Gudelsky GA. Evidence for the involvement of nitric oxide in 3,4-methylenedioxymethamphetamine-induced serotonin depletion in the rat brain. *J Pharmacol Exp Ther*. 2005;312:694-701.

65. Kuhn DM, Sadidi M, Liu X. Peroxynitrite-induced nitration of tyrosine hydroxylase: identification of tyrosines 423, 428, and 432 as sites of modification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and tyrosine-scanning mutagenesis. *J Biol Chem*. 2002;277:14336-14342.

66. Sattler R, Tymianski M. Molecular mechanisms of calcium-dependent excitotoxicity. *J Mol Med*. 2000;78:3-13.

67. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med*. 1994;330:613-622.

68. Sonsalla PK, Nicklas WJ, Heikkila RE. Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. *Science*. 1989;243:398-400.

69. Finnegan KT, Taraska T. Effects of glutamate antagonists on methamphetamine and 3,4-methylenedioxymethamphetamine-induced striatal dopamine release in vivo. *J Neurochem*. 1996;66:1949-1958.

70. Albers DS, Sonsalla PK. Methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice: pharmacological profile of protective and nonprotective agents. *J Pharmacol Exp Ther*. 1995;275:1104-1114.

71. Battaglia G, Fornai F, Busceti CL, et al. Selective blockade of mGlu5 metabotropic glutamate receptors is protective against methamphetamine neurotoxicity. *J Neurosci*. 2002;22:2135-2141.

72. Burrows KB, Meshul CK. Methamphetamine alters presynaptic glutamate immunoreactivity in the caudate nucleus and motor cortex. *Synapse*. 1997;27:133-144.

73. Mark KA, Eyerman DJ, Yamamoto BK. The Effects of Methamphetamine on the Vesicular Glutamate Transporter 1. Program No. 681.18.2005 Abstract Viewer/Itinerary Planner. 2005; Available at: http://sfn.scholarone.com/itin2005/main.html?new_page_id=126&abstract_id=16249&p_num=681.18&is_tech=0. Accessed November 15, 2005.

74. Siman R, Noszek JC, Kegerise C. Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. *J Neurosci*. 1989;9:1579-1590.

75. Minger SL, Geddes JW, Holtz ML, et al. Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia. *Brain Res*. 1998;810:181-199.

76. Staszewski RD, Yamamoto BK. Methamphetamine-induced spectrin proteolysis in the rat striatum. *J Neurochem*. 2006;96:1267-1276.

77. Schmidt HH, Hofmann H, Schindler U, Shutenko ZS, Cunningham DD, Feelisch M. No $^{\cdot}$NO from NO synthase. *Proc Natl Acad Sci USA*. 1996;93:14492-14497.

78. Kahlert S, Zundorf G, Reiser G. Glutamate-mediated influx of extracellular Ca2+ is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. *J Neurosci Res*. 2005;79:262-271.

79. Chan P, Di Monte DA, Luo JJ, DeLanney LE, Irwin I, Langston JW. Rapid ATP loss caused by methamphetamine in the mouse striatum: relationship between energy impairment and dopaminergic neurotoxicity. *J Neurochem*. 1994;62:2484-2487.

80. Nixdorf WL, Burrows KB, Gudelsky GA, Yamamoto BK. Enhancement of 3,4-methylenedioxymethamphetamine neurotoxicity by the energy inhibitor malonate. *J Neurochem*. 2001;77:647-654.

81. Albers DS, Zeevalk GD, Sonsalla PK. Damage to dopaminergic nerve terminals in mice by combined treatment of intrastriatal malonate with systemic methamphetamine or MPTP. *Brain Res*. 1996;718:217-220.

82. Burrows KB, Nixdorf WL, Yamamoto BK. Central administration of methamphetamine synergizes with metabolic inhibition to deplete striatal monoamines. *J Pharmacol Exp Ther*. 2000;292:853-860.

83. Stephans SE, Whittingham TS, Douglas AJ, Lust WD, Yamamoto BK. Substrates of energy metabolism attenuate methamphetamine-induced neurotoxicity in striatum. *J Neurochem*. 1998;71:613-621.

84. Wan FJ, Lin HC, Kang BH, Tseng CJ, Tung CS. D-amphetamine-induced depletion of energy and dopamine in the rat striatum is attenuated by nicotinamide pretreatment. *Brain Res Bull*. 1999;50:167-171.

85. Zeevalk GD, Derr-Yellin E, Nicklas WJ. Relative vulnerability of dopamine and GABA neurons in mesencephalic culture to inhibition of succinate dehydrogenase by malonate and 3-nitropropionic acid and protection by NMDA receptor blockade. *J Pharmacol Exp Ther*. 1995;275:1124-1130.

86. Darvesh AS, Shankaran M, Gudelsky GA. 3,4-Methylenedioxymethamphetamine produces glycogenolysis and increases the extracellular concentration of glucose in the rat brain. *J Pharmacol Exp Ther*. 2002;301:138-144.

87. Burrows KB, Gudelsky G, Yamamoto BK. Rapid and transient inhibition of mitochondrial function following methamphetamine or 3,4-methylenedioxymethamphetamine administration. *Eur J Pharmacol*. 2000;398:11-18.

88. Kushnareva YE, Wiley SE, Ward MW, Andreyev AY, Murphy AN. Excitotoxic injury to mitochondria isolated from cultured neurons. *J Biol Chem*. 2005;280:28894-28902.

89. Brown JM, Hanson GR, Fleckenstein AE. Methamphetamine rapidly decreases vesicular dopamine uptake. *J Neurochem*. 2000;74:2221-2223.

90. Riddle EL, Topham MK, Haycock JW, Hanson GR, Fleckenstein AE. Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine. *Eur J Pharmacol*. 2002; 449:71-74.

91. Eyerman DJ, Yamamoto BK. Lobeline attenuates methamphetamine-induced changes in vesicular monoamine transporter 2 immunoreactivity and monoamine depletions in the striatum. *J Pharmacol Exp Ther*. 2005;312:160-169.

92. Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D. Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *J Neurosci*. 2002;22:8951-8960.

93. Eyerman DJ, Yamamoto BK. A Rapid and Long-lasting Decrease in Vesicular Monoamine Transporter 2 Immunoreactivity in Striatal Synaptosomes After Methamphetamine. Program No. 981.17.2005. Abstract Viewer/Itinerary Planner 2005; Available at: http://sfn.scholarone.com/itin2005/main.html?new_page_id=126&abstract_id=17188&p_num=918.17&is_tech=0. Accessed April 27, 2006.

94. Hatzipetros T, Yamamoto BK. Dopaminergic and GABAergic modulation of glutamate release from subthalamic nucleus efferents to the rat substantia nigra. *Brain Res*. 2006;1076:60-67.

95. Bustamante D, You ZB, Castel MN, et al. Effect of single and repeated methamphetamine treatment on neurotransmitter release in substantia nigra and neostriatum of the rat. *J Neurochem*. 2002;83:645-654.

96. Hatzipetros T, Yamamoto BK. Methamphetamine-induced Spectrin Proteolysis and Dopamine Depletions in the Substantia Nigra. Program No. 563.15.2004. Abstract Viewer/Itinerary Planner. 2004; Available at: http://sfn.scholarone.com/itin2004/main.html?new_page_id=126&abstract_id=10146&p_num=563.15&is_tech=0. Accessed April 27, 2006.

97. Hatzipetros T, Yamamoto BK. The Effects of Haloperidol Treatment After Methamphetamine On the Neuronal Population of the Rat Substantia Nigra. Program No. 918.5.2005. Abstract Viewer/Itinerary Planner. 2005; Available at: http://sfn.scholarone.com/itin2005/main.html?new_page_id=126&abstract_id=3411&p_num=918.5&is_tech=0. Accessed April 27, 2006.

98. Stanimirovic DB, Wong J, Ball R, Durkin JP. Free radical-induced endothelial membrane dysfunction at the site of blood-brain barrier: relationship between lipid peroxidation, Na,K-ATPase activity, and 51Cr release. *Neurochem Res*. 1995;20:1417-1427.

99. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev*. 2005;57:173-185.

100. Bankson MG, Yamamoto BK. MDMA Causes Long-term Increases in Blood Brain Barrier Permeability. Program No. 190.4.2005 Abstract Viewer/Itinerary Planner 2005; Available at: http://sfn.scholarone.com/itin2005/main.html?new_page_id=126&abstract_id=15187&p_num=190.4&is_tech=0. Accessed April 27, 2006.