The effects of methamphetamine (METH) on pro-oxidant processes and on the production of reactive oxygen species were examined in vivo in the rat brain. The presence of oxidative damage in striatum, as revealed by the oxidation of lipid, also was assessed via the measurement of the lipid peroxidation product malondialdehyde. To elucidate further the mechanisms mediating METH-induced oxidative stress, we studied the possible reversal of the long-term METH-induced decrease in striatal dopamine content by antioxidants through iron chelation and trapping of free radicals. The uric acid concentration in the striata of rats killed 1 hr, but not 24 hr, after a four-injection regimen of METH was increased significantly compared with saline-injected control rats. METH increased the in vivo formation of the hydroxylated products of salicylate and d-phenylalanine, as evidenced by the elevated extracellular concentrations of 2,3 dihydroxybenzoic acid and p-tyrosine, respectively. The local perfusion of the striatum with the iron chelator deferoxamine attenuated the long-term depletions of striatal dopamine content produced by METH. In other experiments, malondialdehyde concentrations in incubated striatal homogenates were elevated significantly in METH-treated rats. Finally, pretreatment with the spin trapping agent phenylbutyl- nitronitrone before the METH injections attenuated the subsequent long-term depletions in striatal dopamine content. Overall, the results illustrate that METH increases pro-oxidant processes and offer supportive evidence that METH produces oxidative damage. These studies also demonstrate that iron may be involved in mediating the long-term damage to dopamine neurons after repeated administrations of METH.

High doses of METH produce losses in several markers of brain dopamine and serotonin neurons. Striatal dopamine and 5HT concentrations, dopamine and 5HT uptake sites, and tyrosine and tryptophan hydroxylase activities are reduced after the administration of METH (for review, see Seiden and Ricaurte, 1987). The exact mechanism(s) mediating these changes, however, is (are) unknown. The decreases in dopamine parameters appear to be mediated by the excessive acute increases in dopamine release produced by METH. Inhibition of dopamine synthesis before METH attenuates the decrease in tryptophan hydroxylase activity (Gibb and Kogan, 1979), and this attenuation is reversed by l-dopa administration (Schmidt et al., 1985). In addition, inhibition of dopamine release with dopamine uptake blockers attenuates METH-induced striatal dopamine depletions (Schmidt et al., 1985; Stephens and Yamamoto, 1994). Thus it appears that the magnitude of dopamine release is related to the long-term toxic effects of METH on dopamine neurons.

Glutamate also plays a role in METH-induced neurotoxicity to dopamine neurons. Glutamate antagonists block the METH-induced decreases in dopamine content and tyrosine hydroxylase activity (Sonsalla et al., 1989; 1991). METH also increases the extracellular concentrations of glutamate (Abekawa et al., 1994; Nash and Yamamoto, 1992; Stephens and Yamamoto, 1994; 1996). The increase in glutamate is blocked by dopamine antagonists, which also block the decreases in tyrosine hydroxylase activity and dopamine content produced by METH (Sonsalla et al., 1989; Stephens and Yamamoto, 1994).

A common underlying mechanism involving both dopamine and glutamate that may mediate the damage to dopamine neurons is through the production of ROS and oxidative stress. Dopamine itself can produce neurotoxicity (Filloux and Townsend, 1993) and generate hydroxyl radicals (Michel et al., 1988; Rosenblatt and Hefti, 1990; Rosenberg, 1988; Tanaka et al., 1991). The enzymatic degradation or auto-oxidation of dopamine results in the formation of hydrogen peroxide and superoxide radical. Hydrogen peroxide is susceptible to iron-catalyzed formation of hydroxyl free radicals via the Fenton reaction (Olanow, 1992; Kopin, 1992). Similarly, increases in glutamatergic transmission also can produce ROS (Bondy and Lee, 1993; Dugan et al., 1995; Lafood-Cazal et al., 1993) through the release of arachidonic acid (Dumuis et al., 1988) or through the activation of nitric oxide synthase and the.

ABBREVIATIONS: METH, methamphetamine; ROS, reactive oxygen species; 2,3-DHBA, 2,3 dihydroxybenzoic acid; PBN, phenylbutynitrone.
generation of nitric oxide (Dawson et al., 1992). Nitric oxide can react with superoxide to form peroxynitrite ion (Huie and Padmaja, 1993), with the eventual formation of hydroxyl radical.

The possibility that oxidative stress and ROS mediate METH-induced damage to dopamine neurons is supported by several findings. METH increases intracellular oxidation in vitro, as indicated by dichlorodihydrofluorescein fluorescence in cultures of ventral midbrain dopamine neurons. Consistent with this finding is that Giovanni et al. (1995) have reported that the oxidation products of intraventricularly administered salicylate, which are indicative of ROS formation, were increased in vivo after METH. Conversely, antioxidants and free radical spin trapping agents acting as free radical scavengers attenuate the decrease in striatal dopamine content (Wagner et al., 1980; DeVito and Wagner, 1989; Wagner et al., 1985; Cappon et al., 1996). In addition, decreases in dopamine transporter function and tryptophan hydroxylase activity induced by METH appear to be mediated by ROS and oxidative stress (Stone et al., 1989a, 1989b; Fleckenstein et al., 1997a, b, c, d). Furthermore, the decreases in dopamine content and uptake sites produced by METH are attenuated in mice that overexpress the gene coding for the antioxidant defense enzyme copper-zinc superoxide dismutase (Cadet et al., 1994; Hirata et al., 1996).

In the present study, we used several approaches to examine whether METH increases pro-oxidant processes and the production of ROS in vivo in the striatum. To assess the presence of oxidative damage produced by METH, we evaluated the oxidation of lipid by measuring the lipid peroxidation product malonyldialdehyde. To elucidate further the oxidation of lipid by measuring the lipid peroxidation product malonyldialdehyde. To elucidate further the generation of nitric oxide (Dawson et al., 1992). Nitric oxide can react with superoxide to form peroxynitrite ion (Huie and Padmaja, 1993), with the eventual formation of hydroxyl radical.

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Materials and Methods

Animals. Male Sprague-Dawley (Zivic-Miller, Allison Park, PA) rats were used for all experiments.

Drugs and reagents. The following drugs and chemicals were used in their study: all reagents (Fluka Chemical Co., Ronkonkoma, NY), d-methamphetamine, Dulbecco's powdered medium, uric acid, o- m-, and p-tyrosine, d-phenylalanine, 1,1,3,3-tetraethoxypropane, deferoxamine and 2,3 and 2,5 dihydroxybenzoic acid (Sigma Chemical Co., St. Louis, MO), ethyl hexadecyl dimethyl ammonium bromide (Kodak Chemical Co., Rochester, NY), phenylbutylnitrone (Alcal Chemical Co., Milwaukee, WI), salicylate (Fluka Chemical Co.) and acetonitrile (Optima) and methanol (Optima) (Fisher Scientific, Pittsburgh, PA).

Drug administration. A dose of 10 mg/kg (+)METH or saline was administered intraperitoneally every 2 hr over an 8-hr period.

In vivo measurement of hydroxyl radicals. Surgery—Rats were anesthetized with xylazine (6 mg/kg i.m.) and ketamine (70 mg/kg i.m.). A guide cannula with a stylet obturator was cemented to the skull above the striatum (1.2 mm anterior to bregma and 3.2 mm lateral to the midline suture; Paxinos and Watson, 1986). Rats were allowed 3 days to recover from surgery. On the day of the dialysis experiment, the stylet obturator was removed from the guide cannula, and the dialysis probe was inserted into and through the guide cannula. The vertical placement of the dialysis probe was predetermined by gluing a ring of PE20 tubing at a measured distance along the length of the probe. The PE tubing served as a mechanical “stop” when the probe was inserted through the dialysis membrane and into the stratum such that the exposed part of the dialysis membrane (4.0 mm) extended into the stratum. The ventral tip of the striatal probe was located 7 mm from the cortical surface. Microdialysis probes were constructed as previously described (Yamamoto and Pehek, 1990). The probes were perfused at a rate of 2 μl/min with a modified Dulbecco’s phosphate-buffered saline containing 138 mM NaCl, 2.7 mM KCl, 0.5 mM MgCl2, 1.5 mM KH2PO4, 1.2 mM CaCl2 and 5 mM d-glucose; pH 7.4.

In vivo trapping—The methods for in vivo trapping of hydroxyl radicals were based on those previously reported (Giovanni et al., 1995; Kaur and Halliwell, 1994; Sun et al., 1993) that the trapping agents were perfused directly through a microdialysis probe implanted in the brain. The hydroxyl radical trapping agents salicylate (2.5 mM free acid) or d-phenylalanine (2.5 mM) was dissolved in the perfusion medium and perfused through the microdialysis probe at a rate of 2.0 μl/min. The perfusion of salicylate or d-phenylalanine was initiated immediately after insertion of the probe and was continued throughout the entire time course of the experiment. After a 3-hr equilibration period, base-line samples were collected every 30 min for a 1.5-hr period. Saline or METH was administered as described above. The formation of 2,3-DHBA or p-tyrosine was measured in the perfusates collected during salicylate or d-phenylalanine perfusion, respectively.

2,3 DHB and 2,5-DHBA were separated on a reverse-phase Inertisil C18 column (100 × 2.0 mm I.D., 5-μm particle size; Metachem Technologies Co., Torrance, CA) with a mobile phase (pH 4.5) consisting of 50 mM sodium phosphate dihydrate (monobasic), 1 mM disodium EDTA, 10 mM NaCl, 10% (v/v) acetonitrile and 6% (v/v) methanol. The flow rate was 0.27 ml/min. The column was at ambient temperature. We measured 2,3-DHBA and 2,5 DHBA with electrochemical detection (LC-4C Amperometric Detector, Bioanalytical Systems Inc, Lafayette, Indiana), using a glassy carbon working electrode vs. a Ag/AgCl reference electrode maintained at a potential of 530 mV.

p-Tyrosine was separated from o- and m-tyrosine and quantitated by HPLC with UV detection (Perkin-Elmer Corp., Norwalk, CT). The separations were performed on a reverse-phase C18 column (250 × 3.0 mm I.D., 5-μm particle size; Nucleosil, Phenomenex Co., Torrance, CA) with a mobile phase (pH 3.0) of potassium dihydrogen phosphate (30 mM) and 6% methanol (v/v). The mobile phase was pumped at a flow rate of 0.44 ml/min at 28°C. Absorbance was monitored at 216 nm at a sensitivity of 0.001 AUFS.

Measurement of malonyldialdehyde and uric acid. Striata were dissected from the brains of saline-treated and METH-treated animals 1 day after drug administration. Tissue was sonicated, the homogenate centrifuged and the supernatant assayed for uric acid or malonyldialdehyde by HPLC with electrochemical detection or UV, respectively. For the malonyldialdehyde assay, the homogenate was incubated at 37°C before centrifugation.

A widely used assay for malonyldialdehyde is based on its reaction with thiobarbituric acid (TBA). This assay requires acid heating at 95°C for 15 to 60 min, which gives a pink complex with an absorption at 532 nm. However, this method, which measures total malonyldialdehyde, itself can generate malonyldialdehyde, lacks specificity and has other limitations (Draper and Hadley, 1990; Valenzuela, 1991; Cini et al., 1994). TBA reacts with a number of other compounds, such as oxidized amino acids and carbohydrates that form products that absorb at near 532 nm. Thus the data obtained are often expressed as “thiobarbituric acid-reactive substances,” or “TBARS.” This could be important, particularly when malonyldialdehyde is determined in tissue homogenates or after oxidative stress. Therefore, the method chosen for this series of studies was to measure malonyldialdehyde with UV detection that does not involve a reaction with a marker compound. Furthermore, because free iron can generate free radicals to induce lipid peroxidation as well as break down malonyldialdehyde, we examined the effect of the iron chelator deferoxamine. The striatum was sonicated and the homogenate was incubated at 37°C for 1 hr in 250 μl of 40 mM Tris buffer .
was eluted on a reverse-phase C18 column (250 μm I.D., 5-μm particle size; Prodigy Phenomenex Co.) with a mobile phase (pH 7.25) consisting of 50 mM sodium phosphate (dibasic), 25 mM NaCl, 3.0 mM ethylhexadecyl dimethyl ammonium bromide, 5% acetonitrile and 3% methanol (v/v). The mobile phase was pumped at a flow rate of 0.2 ml/min at ambient temperature. Absorbance was monitored at 267 nm with a gain sensitivity of 0.001 AUFs. A standard stock solution of malondialdehyde was prepared by dissolving 101.05 mg of 1,1,3,3-tetraethoxypropane in 100 ml of deionized Millipore filtered water. HCl (50 μl of 1M) was added and the contents heated for 60 min in a water bath maintained at 50°C. The solution was then cooled to room temperature and adjusted with distilled water to a final volume of 500 ml. Aliquots of this solution were diluted with water to different concentrations for determination of a standard curve. The limit of detection for this assay was 200 pg/injection.

Glutamate has been implicated in mediating the neurotoxicity observed after METH, so the activation of calcium-dependent proteases and xanthine oxidase by glutamate could increase uric acid as well as hydroxyl radicals. Therefore, because uric acid and its oxidation products may be markers of oxidant generation as well as hydroxyl radicals. Therefore, because uric acid and its oxidation products may be markers of oxidant generation, uric acid metabolites were separated on a C18 reverse-phase column (Prodigy 3 μm particle size, Phenomenex Co.) using a mobile phase of sodium dihydrogen phosphate dihydrate and disodium EDTA (1 mM). The pH of the mobile phase was adjusted to 2.5 with phosphoric acid. The mobile phase was pumped at a flow rate of 0.5 ml/min at room temperature. Uric acid was quantitated with an electrochemical detection system (BAS LC-4B Amperometric Detector, Bioanalytical Systems, Inc., W. Lafayette, IN) consisting of a glassy carbon electrode 6 mm in diameter maintained at a potential of 750 mV vs. a Ag/AgCl reference electrode. The protein content of each sample was measured by the Bradford protein assay.

**Pretreatment with PBN and dopamine content.** The oxygen radical spin trapping agent phenylbutylhydrazine (150 mg/kg i.p.) was simultaneously administered with the first and third injections of METH. Seven days after the METH and PBN drug administration regimen, striata were dissected from frozen coronal sections 400 μm thick, sonicated in 300 μl of cold 0.1 N HClO4 and centrifuged at 14,000 × g for 10 min at 4°C. The supernatant was injected directly onto an HPLC system. Uric acid was eluted on a reverse-phase C18 column (250 × 2.0 mm I.D., 5-μm particle size; Prodigy, Phenomenex Co.) with a mobile phase of sodium dihydrogen phosphate dihydrate and disodium EDTA (1 mM). The pH of the mobile phase was adjusted to 2.5 with phosphoric acid. The mobile phase was pumped at a flow rate of 0.5 ml/min at room temperature. Uric acid content was determined by reductive chemiluminescence using a Fluoromax 2 fluorimeter. The protein content of each sample was measured by the Bradford protein assay.

**Deferoxamine perfusion.** Rats were implanted bilaterally with guide cannulas positioned on the cortex dorsal to the striatum as described above. Rats were allowed 3 days to recover from surgery. On the day of the dialysis experiment, dialysis probes were inserted into each striatum, and the rats were placed into a round plastic testing chamber (30 cm diameter). The probes were perfused with Dulbecco’s phosphate-buffered saline as described above except that one probe was perfused with medium containing 50 μM deferroxamine. The probe on the contralateral side was perfused with normal Dulbecco’s medium without deferroxamine. After a 3-hr equilibration period, either saline or METH (10 mg/kg i.p.) was injected at 2-hr intervals over an 8-hr period. Rats were then returned to their home cage for 7 days. After 7 days, rats were killed by decapitation, and the brains were removed and quickly frozen on powdered dry ice. The brains were sliced into coronal sections 200 μm thick, the probe tracks were visualized, and the frozen tissue immediately adjacent to the probe tracks in both striata was dissected with a micro knife from the frozen sections visualized under a microscope (40× magnification). The dissected tissues were assayed by HPLC with electrochemical detection for dopamine content as described.

**Statistical analysis.** Data from the experiments involving the perfusion of salicylate or d-phenylalanine and the subsequent trapping of hydroxyl radicals were analyzed by two-way ANOVA with repeated measures over time (main effects of time and drug and time × drug interaction effect). Differences in dopamine content after perfusion with deferroxamine or after the administration of PBN were determined by two-way ANOVA for independent measures (main effect of pretreatment and METH vs. saline). Significant differences in malondialdehyde content were measured by a paired t test. Significance was determined at P < .05.

**Results.** The effects of METH on uric acid content in striatum are illustrated in figure 1. METH significantly increased striatal uric acid content by 29% from saline control concentrations of 9.4 mg/mg protein. METH-treated rats had concentrations of 12.1 ng/mg of protein when killed 1 hr after the fourth injection. No significant differences were observed between saline controls and METH-treated rats when rats were killed 24 hr after the fourth injection.

Compared with saline-injected controls, METH-treated rats exhibited significantly increased formation of 2,3 DHB during salicylate perfusion (fig. 2) as well as an increase in the extracellular concentration of p-tyrosine during d-phe-
nalalanine perfusion (fig. 3). This was determined by a significant overall time × METH vs. saline interaction effect (P < .05) and an overall significant drug treatment effect (saline vs. METH) (P < .05). Although there was a trend toward an increase in 2,5 DHBA, concentrations of 2,5 DHBA did not change significantly after METH because of variability (data not shown). METH had no effect on the extracellular concentrations of o- and m-tyrosine during d-phenylalanine administration (data not shown). No changes were observed in p-tyrosine after METH or saline treatment of rats that were perfused with Dulbecco’s in the absence of d-phenylalanine.

The perfusion with deferroxamine did not affect the METH-induced increase in extracellular dopamine. Extracellular dopamine increased and peaked at an average of 28-32-, 29- and 20-fold over baseline on both the deferroxamine- and the dulbecco’s-perfused sides 60 min after each of the four injections of METH (fig. 4a). The effects of the perfusion of deferroxamine on striatal dopamine content 7 days after...
multiple injections of saline or METH are shown in figure 4b. Each rat had probes implanted bilaterally into the striatum. In saline-injected controls, deferroxamine perfusion did not have an effect on striatal dopamine content (side perfused with deferroxamine vs. contralateral side perfused with normal Dulbecco’s vehicle). METH significantly reduced dopamine content (significant overall METH effect; \( P < .01 \)). A significant interaction effect was observed between the side perfused with deferroxamine or normal Dulbecco’s and the systemic administration of saline or METH (\( P < .05 \)). A paired t test revealed a significant difference between the side perfused with deferroxamine and the contralateral side perfused with normal Dulbecco’s medium (90.6 vs. 59.4 pg/ \( \mu \)g of protein; \( P < .025 \)).

Figure 5 illustrates malonyldialdehyde concentrations after incubation of striatal homogenates prepared from saline-treated or METH-treated rats. One day after the beginning of the METH or saline injection regimen, malonyldialdehyde concentrations in the striatum were higher in METH-treated rats than in saline-treated controls (\( P < .02 \)). It should be noted that these results were obtained from striatal homogenates incubated in the presence of deferroxamine. Experiments also were performed with incubations in Tris buffer that did not contain deferroxamine. These latter results revealed that striata from METH-treated rats had significantly lower malonyldialdehyde concentrations than those from controls (3.97 ± 0.48 vs. 2.93 ± 0.33 ng/mg of protein; \( P < .05 \)).

The effect of phenylbutylnitrone pretreatment and METH on dopamine content in the striatum 7 days after the drug injection regimen is illustrated in figure 6. Dopamine content in rats pretreated with PBN immediately before the first and third injections of saline (PBN/Sal) did not differ from control rats pretreated with saline (Sal/Sal). In rats pretreated with saline but administered METH (Sal/Meth), dopamine content was significantly reduced (71.4 pg/ \( \mu \)g; \( P < .01 \)) compared with saline controls (Sal/Sal) (150.8 pg/ \( \mu \)g). However, striatal dopamine content in rats pretreated with PBN and administered METH (PBN/Meth) (121.6 pg/ \( \mu \)g) did not differ from either of the saline control groups (Sal/Sal, PBN/Sal) (156.0 pg/ \( \mu \)g) or the METH-treated group (Sal/Meth).

**Discussion**

The present study examined the effect of METH on several markers of oxidative stress in the striatum. METH increased uric acid concentrations as well as the extracellular concentrations of the hydroxylated metabolites of salicylate and d-phenylalanine. In addition, a separate series of experiments addressed the possibility of a pharmacological reversal or attenuation by antioxidants of the long-term METH-induced depletions of striatal dopamine. The results indicated that local perfusion with the iron chelator deferroxamine or systemic administration of the spin trapping agent phenylbutylnitrone attenuated the long-term dopamine depletions produced by METH. Moreover, the results offer supportive evidence that METH produces oxidative damage in vivo as measured by an increase in the lipid peroxidation product malonyldialdehyde.

The increase in the concentration of uric acid in the striatum after METH suggests that xanthine oxidase, which converts xanthine to uric acid, is activated after METH. This could occur as a consequence of glutamate and the activation of calcium-dependent proteases (such as calpain) (Mccord, 1985; Dumuis et al., 1988) and would be consistent with previous studies indicating that glutamate mediates the neurotoxic effects of METH (Sonsalla et al., 1989; Nash and Yamamoto, 1992; Abekawa et al., 1994; Stephens and Yamamoto, 1994). It also has been shown that uric acid and its oxidation products may be markers of oxidant generation in vivo (Grootveld and Halliwell, 1987; Halliwell et al., 1988). In contrast, uric acid has been shown to possess antioxidant properties as well (Ames et al., 1981). Therefore, an increase in uric acid concentration may indicate a reactive increase in endogenous antioxidant protective mechanisms as a consequence of increased oxidative stress. In contrast, increased uric acid in the presence of \( O_2 \) could allow for the formation of urate peroxy radicals and contribute to the production of oxidative stress (Maples and Mason, 1988). We recognize
that it is difficult to interpret tissue concentrations of uric acid alone as evidence of oxidative stress. However, in conjunction with the other reported measures of oxidative and antioxidant processes in the present study, the increase in uric acid provides additional supportive evidence that uric acid is involved in oxidative responses after the administration of METH.

The perfusion of salicylate and α-phenylalanine through a microdialysis probe and the simultaneous formation of the hydroxylated metabolites 2,3 DHBA and p-tyrosine, respectively, were employed to measure localized changes in the extracellular concentrations of reactive oxygen species in the striatum (figs. 2 and 3). Both methods have been shown to be effective and sensitive indicators of hydroxyl radical formation (Giovanni et al., 1995; Colado et al., 1997; Stone et al., 1989a, b; Kondo et al., 1994; Fleckenstein et al., 1997a, b, c, d).

There were slight differences in the time courses of 2,3 DHBA and p-tyrosine formation. The increase in 2,3 DHBA was significantly different from saline controls between the second and third injections of METH, whereas a significant increase in p-tyrosine over the saline group was not evident until the fourth injection of METH. Moreover, the pattern of changes in 2,3 DHBA appear to be similar to what would be expected for the extracellular changes in dopamine (Stephans and Yamamoto, 1994). The rate constant for reaction of phenylalanine with OH is about five times less than that of salicylate (Kaur and Halliwell, 1994), so the greater capacity and reactivity of salicylate to trap hydroxyl radicals at the 2 and 3 positions to form 2,3 DHBA, compared with the single hydroxylation of α-phenylalanine at the p position, may account for the apparent greater sensitivity to changes in hydroxyl radicals with the salicylate. α-Phenylalanine was chosen over l-phenylalanine because the d-isomer is not a substrate for phenylalanine hydroxylase and therefore minimizes any possible disruption in catecholamine biosynthesis. Although the extracellular concentrations of p-tyrosine increased during α-phenylalanine infusions, no changes were observed in m- and o-tyrosine. The preferred hydroxylation of tyrosine at the p position may be due to the steric hindrance by the amine group against the hydroxylation at the m and o positions on the phenyl ring. Because no changes after METH were observed in p-tyrosine in the absence of d-phenylalanine infusions, METH does not appear to increase endogenously p-tyrosine. Therefore, increases in p-tyrosine during d-phenylalanine infusion could be attributed to the trapping of hydroxyl radicals by d-phenylalanine to form p-tyrosine. Clearly, trapping hydroxyl radicals with either d-phenylalanine or salicylate has relative disadvantages and advantages. Regardless, both approaches consistently revealed localized increases in hydroxylated metabolites after METH, and together, they are effective for revealing METH-induced increases in ROS formation.

The local perfusion of the striatum with the iron chelator deferoxamine attenuated the long-term depletion of striatal dopamine content produced by repeated injections of METH. This is the first evidence that free iron may be involved in mediating the neurotoxicity observed after METH. Iron is known to catalyze the generation of OH− from hydrogen peroxide via the Fenton reaction and may be involved in the generation of ROS after METH. Chelation of free iron with deferoxamine may have blocked the METH-induced formation of OH− and consequently attenuated the depletion of dopamine content. It remains to be determined whether the concentrations of free iron, existing as Fe2+ or Fe3+, are increased by METH. One speculation is that METH, which increases extracellular glutamate (Nash and Yamamoto, 1992; Stephans and Yamamoto, 1994; Abebacha et al., 1994) also increases ROS generated through the glutamate-mediated increases in nitric oxide and superoxide radical (see Olano, 1993). The OH− formed may damage proteins such as transferrin, ferritin and hemoglobin that store iron and may thus subsequently increase the concentrations of free iron. The iron, in turn, can generate more OH− in the presence of hydrogen peroxide produced by the enzymatic and auto-oxidation of dopamine (Olanow, 1990) that has been released by METH. Therefore, the increase in both glutamate and dopamine release produced by METH may have additive or synergistic effects on the production of ROS. It is possible that the concentrations of deferoxamine used in this study could have altered dopamine transmission through nonspecific effects on either the synthesis or the uptake of dopamine and consequently attenuated the long-term depletion of dopamine content produced by METH; however, deferoxamine did not alter METH-induced dopamine release. Therefore, it appears that the neuroprotective effect of deferoxamine is independent of any acute effects on the increases in extracellular dopamine. Thus the present results suggest that iron could play a role in mediating METH-induced damage to dopamine neurons. These data are consistent with the hypothesized role of iron in the degeneration of nigrostriatal dopamine neurons in Parkinson's disease (Sofic et al., 1991; Hirsch et al., 1991; Dexter et al., 1989; Ben-Shachar and Youdim, 1991).

A more definitive characterization of oxidative stress produced by METH should include evidence of oxidative damage. The finding that METH increases the lipid peroxidation product malondialdehyde (fig. 5) is supportive of the findings that METH produces oxidative stress (Wagner et al., 1985; Stone et al., 1989a, b; Cadet et al., 1994; Fleckenstein et al., 1997a, b, c, d). As mentioned in "Materials and Methods," rather than use the thiobarbituric acid reaction method, we chose to measure malondialdehyde directly because it is specific for malondialdehyde. Although it was not determined in the present study, future studies are needed to demonstrate conclusively that the increase in malondialdehyde is dependent on ROS formed by the action of METH. An interesting note to these findings is that a METH-induced increase in malondialdehyde is seen only when deferoxamine is added to the homogenization/incubation Tris medium. When deferoxamine was omitted, malondialdehyde concentrations were lower in striatal tissue exposed to METH. The fact that iron can decompose lipid hydroperox-
ides at 37°C (Gutteridge and Halliwell, 1990) would explain the decrease in malondialdehyde after METH when the striatum is incubated in the absence of deferroxamine. This assumes, however, that more free iron is available to decompose lipid hydroperoxides in the tissue exposed to METH. Because iron also can catalyze the conversion of H₂O₂ to OH⁻, chelation of free iron may also decrease the formation of ROS and oxidative damage after METH. Although the iron content of the striatum was not measured, the present study did show that deferroxamine attenuated the METH-induced long-term depletions of striatal dopamine (fig. 4).

Pretreatment with PBN attenuated the METH-induced depletions of striatal dopamine content. This finding is similar to that previously reported (Cappon et al., 1996). The most likely explanation for this effect is that PBN reacts with free radicals to form nitroxyl products (Floyd and Carney, 1992). Strategies that reduce amphetamine-induced dopamine or 5HT depletion also prevent hyperthermia (Bowyer et al., 1992; Farfel and Seiden, 1995 a, b; Miller and O’Callaghan, 1994; Che et al., 1995). Therefore, an alternative but less likely explanation for the attenuation of the dopamine depletion produced by pretreatment with PBN is that PBN could have produced hypothermia or prevented the METH-induced hyperthermic response. Although the present study did not examine the effect of PBN on body temperature, Cappon et al. (1996) reported that PBN did not prevent the METH-induced hyperthermia and suggested that the neuroprotective effect of PBN is not related to its interaction with METH on body temperature. In contrast, Che et al. (1995) have shown that PBN itself produces hypothermia and reverses 3,4-methylenedioxyamphetamine (MDMA)-induced decreases in tryptophan hydroxylase activity. This neuroprotection was dependent on the ability of PBN to prevent the hyperthermic response to MDMA. Therefore, it is possible that PBN can attenuate the METH-induced deficits in striatal dopamine content by preventing the hyperthermia after METH. Further study certainly is warranted to characterize precisely the neuroprotective role of PBN in METH toxicity.

Overall, the present study supports the conclusion that METH increases pro-oxidant processes and provides additional evidence of oxidative damage after METH. Moreover, these studies illustrate that iron may be involved in mediating the long-term damage to dopamine neurons that follows the repeated administration of METH.

References


Send reprint requests to: Bryan K. Yamamoto, Ph.D., Department of Psychiatry, University Hospitals of Cleveland, Hanna Pavilion, 11100 Euclid Avenue, Cleveland, Ohio 44106.