Interaction Between Hyperthermia and Oxygen Radical Formation in the 5-Hydroxytryptaminergic Response to a Single Methamphetamine Administration

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ABSTRACT
Administration of a single high dose of methamphetamine (METH) causes a rapid and reversible decrease in the activity of the tryptophan hydroxylase (TPH), the rate-limiting enzyme in the synthesis of 5-hydroxytryptamine. This effect can be reversed completely by exposing the METH-impaired enzyme to a reducing environment, which suggests that the decrease in TPH activity is a reversible oxidative consequence of free radical formation. Consistent with this hypothesis, a single METH administration to male rats increased oxygen radical formation, as demonstrated by increased striatal dihydroxybenzoic acid formation after co-administration of salicylate with METH. Prevention of METH-induced hyperthermia attenuated both the increase in dihydroxybenzoic acid formation and the decrease in TPH activity observed 1 h after METH administration. These data suggest that both reactive oxygen species and hyperthermia contribute to the acute decrease in TPH activity which results from a single METH administration.

Administration of the frequently abused amphetamine analog, METH, has profound effects on central nervous system function. For example, a single high-dose administration of METH to rats causes a rapid and reversible decrease in the content of 5HT and its metabolite, 5HIAA, which follows this acute reduction in enzyme activity (Peat et al., 1985). Because TPH per se is rapidly inactivated by oxidizing factors (Kuhn et al., 1980) and reactive oxygen species are known to be formed after METH administration (Kondo et al., 1994; Giovanni et al., 1995; Hirata et al., 1995), it has been suggested that METH-induced reactive oxygen species may play a role in decreasing core body temperatures. Whereas this drug-induced hyperthermia likely contributes to the neurotoxic effects of multiple administrations of this stimulant (Bowyer et al., 1994; Bronstein and Hong, 1995; Farfel and Seiden, 1995; Albers and Sonsalla, 1995), the role of body temperature in mediating acute effects of METH, including the rapid decrease in TPH activity, is less established. However, the recent finding by Che et al. (1995) that the acute decrease in TPH activity caused by the METH-related drug, MDMA, is attenuated when MDMA-induced hyperthermia is prevented suggests that increased body temperatures contribute to the immediate effects of a single METH administration as well.

Because both oxygen radicals and hyperthermia may play a role in the acute effects of METH, we examined the possibility that the interaction between these factors may effect the acute decrease in TPH activity resulting from a single injection of the stimulant. The results reveal that hyperthermia contributes to both the oxygen radical generation and the decreased TPH activity which result from METH administration. These findings suggest a correlation between METH-induced formation of free radicals and the immediate reduction in TPH activity caused by this stimulant.

ABBREVIATIONS: DHBA, dihydroxybenzoic acid; METH, methamphetamine; 5HT, 5-hydroxytryptamine; 5HIAA, 5-hydroxyindoleacetic acid; 5HTP, 5-hydroxytryptophan; HPLC, high-performance liquid chromatography; MDMA, methylenedioxymethamphetamine; TPH, tryptophan hydroxylase; HEPES, N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid].
Methods

Animals. Male Sprague Dawley rats (200–300 g; Simonsen Laboratories, Gilroy, CA) were maintained under conditions of controlled temperature and lighting, with food and water provided ad libitum. On the day of the experiment, rats (mean rectal temperature of approximately 37.3°C) were injected with drug or saline vehicle, and then housed in groups in plastic cages that were either placed on ice (environmental temperature, 6°C), at room temperature (26°C) or on a heating pad (environmental temperature, 30°C). Where indicated, core body temperatures were measured 10 min before decapitation with use of a digital thermometer (Physiobtemp Instruments, Inc., Clifton, NJ). All procedures were conducted in accordance with approved National Institutes of Health guidelines.

Drugs and chemicals. (+)-METH hydrochloride was supplied generously by the National Institute on Drug Abuse. Analytical reference materials for METH determination (+METH and deuterated METH [METH-d8]) were obtained from Radian Corporation (Austin, TX). Sodium salicylate, 2,3-DHBA and 2,5-DHBA were purchased from Aldrich Chemical Co. (Milwaukee, WI). Drugs were administered as indicated in the legends of appropriate figures; with the exception of sodium salicylate, doses were calculated as the respective free bases.

TPH activity. TPH activity was determined in tissue homogenates using HPLC by measuring 5HTP formation resulting from the hydroxylation in vitro of tryptophan according to a modification of the method described by Johnson et al. (1992). Frozen tissue samples were sonicated in 150 to 300 μl of ice-cold 50 mM HEPES buffer (pH 7.4) containing 0.2% Triton X-100 and 6 mM dithiothreitol; where specified (see legend to fig. 1), 50 μM FeCl2 was included in this buffer. The resulting suspension was centrifuged (16,000 × g for 15 min; 4°C). Unless otherwise specified (see legend to fig. 1), duplicate aliquots (15 μl) of supernatant were then incubated for 10 min (37°C) with 10 μl reaction mixture (52.8 mM HEPES, 50 mM β-mercaptoethanol, 8 mM tryptophan, 1.25 mM m-hydroxybenzylhydrazine (NSD 1015) and 3.38 mM DL-6-methyl-5,6,7,8-tetrahydrobiopterin dihydrochloride). Boiled supernatant was used for blanks. The reaction was terminated by placing the tubes on ice and adding HPLC mobile phase (see below). The resulting mixture was centrifuged (2500 × g for 10 min; 4°C), and the supernatant retained for 5HTP quantification with HPLC coupled with electrochemical detection (C-18 Microsorb column (Rainin, Woburn MA); glassy carbon electrode set at +0.73 V relative to a Ag/AgCl reference electrode). The HPLC mobile phase (pH 2.7) consisted of 0.05 M sodium phosphate dibasic, 0.03 M citric acid, 0.1 mM ethylenediaminetetraacetic acid, 0.6 mM sodium octyl sulfate and 15% methanol.

METH determination. Brain tissues (whole brains minus the cerebellum, hippocampi, striatum and some frontal cortex) were each homogenized in 2 ml distilled water and frozen at −70°C. On the day of the assay, these homogenates were equilibrated to room temperature. Three hundred nanograms of deuterated METH (METH-d8) were added as internal standards to each sample. Samples were vortexed for 5 s and then made alkaline (pH 8) by adding sodium octyl sulfate and 15% methanol.

Concentrations of METH were determined with a Finnigan 4500 MAT mass spectrometer operating in positive chemical ionization mode (methane/ammonia reagent gas) coupled to a DB5 MS-30 M-0.25 μm capillary column. Ions monitored were 263 m/z and 271 m/z (METH and METH-d8, respectively). Accuracy was within 6 to 17% of spiked METH target values for brain homogenate quality control samples.

DHBA and salicylate determination. Dihydroxybenzoic acids were quantified by HPLC with electrochemical detection according to a modification of the method of Althaus et al. (1995). This method was selected because, as reported by Chieuh et al. (1994), other HPLC procedures do not readily separate DHBA from dopamine and its metabolites. Because salicylate concentrations can differ among experiments, salicylate concentrations were determined by HPLC with ultraviolet detection (Althaus et al., 1995) in all experiments that used the DHBA technique. Frozen striata were sonicated in modified HPLC mobile phase buffer (14.15 g/ml monochloroacetic acid, 10% acetonitrile, 0.05% mannitol, 0.02% deferoxamine; pH 2.4) and the resulting tissue suspension frozen immediately on dry ice. This suspension was then centrifuged (22,000 × g 20 min) and the resulting supernatant split for electrochemical and ultraviolet analysis (mobile phase consisting of 10% acetonitrile, 10% tetrahydrofuran, 14.15 g/ml monochloroacetic acid in double-distilled H2O; pH 2.4; Hewlett Packard 25-cm C-18 column; 0.65 V for EC and 295 nm for UV detection). The lower limits of sensitivity for salicylate and DHBA detection in tissue were approximately 2 nmol/g tissue and 1 to 2 pmol/g tissue, respectively. DHBA was detected in salicylate-treated control rats (i.e., rats that received salicylate, but not METH: this appearance is inherent in the technique and may reflect scavenging of hydroxyl radicals formed under normal physiological conditions. However, much of the base-line DHBA detected in control samples likely arises from atmospheric exposure of salicylate-containing samples. Hence, samples were kept shielded from light and frozen until immediately before analysis to prevent spontaneous oxidation of salicylate.

Data analysis. Statistical analyses between two groups were conducted by a two-tailed Student’s t test. Analyses among three or more groups were conducted with analysis of variance followed by Fisher’s test. Differences among groups were considered significant if the probability of error was less than 5%.

Results

Results presented in figure 1 (left panel) demonstrate that a single high-dose (15 mg/kg s.c.) METH injection decreased TPH activity in rat striatum 1 h after administration. METH-inactivated TPH was completely reactivated in vitro by3ho f anaerobic preincubation (37°C) and each organic phase (containing analytes of interest) was transferred to a clean tube. This step was repeated twice.

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Results presented in figure 1 (left panel) demonstrate that a single high-dose (15 mg/kg s.c.) METH injection decreased TPH activity in rat striatum 1 h after administration. METH-inactivated TPH was completely reactivated in vitro by 3-h aerobic preincubation (37°C) and each organic phase (containing analytes of interest) was transferred to a clean tube. This step was repeated twice.

The finding that the METH-induced loss in TPH activity presented in figure 1 is reversed after exposure of METH-induced tissue to a reducing environment suggests that the decrease in enzyme activity was an oxidative consequence of administering the stimulant. To directly assess whether a single administration of METH causes oxygen radical formation, production of the hydroxylated salicylate metabolites, 2,3-DHBA and 2,5-DHBA, was measured by a modification of the method of Althaus et al. (1995). Before use of this technique to assess effects of METH, optimal dosing and timing conditions for salicylate administration were determined (fig. 2). Similar to that described previously (Stone et al., 1989a). The finding that the METH-induced loss in TPH activity presented in figure 1 is reversed after exposure of METH-induced tissue to a reducing environment suggests that the decrease in enzyme activity was an oxidative consequence of administering the stimulant. To directly assess whether a single administration of METH causes oxygen radical formation, production of the hydroxylated salicylate metabolites, 2,3-DHBA and 2,5-DHBA, was measured by a modification of the method of Althaus et al. (1995). Before use of this technique to assess effects of METH, optimal dosing and timing conditions for salicylate administration were determined (fig. 2). Similar to that described previously (Stone et al., 1989a), intraperitoneal salicylate administration effected a dose- and time-related increase in both salicylate and DHBA detection in tissue were approximately 2 nmol/g tissue and 1 to 2 pmol/g tissue, respectively. DHBA was detected in salicylate-treated control rats (i.e., rats that received salicylate, but not METH: this appearance is inherent in the technique and may reflect scavenging of hydroxyl radicals formed under normal physiological conditions. However, much of the base-line DHBA detected in control samples likely arises from atmospheric exposure of salicylate-containing samples. Hence, samples were kept shielded from light and frozen until immediately before analysis to prevent spontaneous oxidation of salicylate.

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Fig. 1. Effects of preincubation in a reducing environment on METH-induced decreases in TPH activity. Rats received METH (15 mg/kg s.c.; solid columns) or saline vehicle (1 ml/kg s.c.; open columns) 1 h before decapitation. Striatal tissue from these rats was sonicated in HEPES-Triton X-100 dithiothreitol buffer containing 50 μM FeCl2 and centrifuged (16,000 × g 15 min) as described under “Methods.” Quadruplicate aliquots were taken from the resulting supernatants; two aliquots were immediately frozen on dry ice (i.e., “no preincubation”) and two were incubated in an atmosphere of 100% helium for 3 h before placement on dry ice. Frozen samples were then thawed and incubated (37°C; 10 min) with reaction mixture as described under “Methods.” Columns represent means and vertical lines represent 1 S.E.M. of determinations in five rats. *Value for METH-treated rats that is significantly different from saline-treated controls (P < .05).

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affected significantly by either METH treatment or variation in rectal temperatures (see legend to fig. 4). Rats with a final rectal temperature of 37.9°C (i.e., which had been placed in a 6°C environment) had whole brain METH concentrations 1.5-fold greater than that of rats with a final temperature of 42.1°C (i.e., which had been placed in a 30°C environment; 12.8 ± 0.7 and 18.7 ± 2.0 ng/mg tissue for rats exposed to 30°C and 6°C environments, respectively; n = 7–8).

Discussion

Because of the increased frequency of METH abuse, elucidation of its consequent neurochemical impact is important. Of particular interest are reports that monoaminergic systems are especially sensitive to administration of this potent stimulant. For instance, a single METH administration (10–15 mg/kg, as used in the present study) causes a rapid and reversible decrease in the activity of TPH (Bakhit and Gibb, 1981), and a corresponding reduction in 5HT content and reversible decrease in the activity of TPH (Bakhit and Gibb, 1981), and a corresponding reduction in 5HT content.

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To assess more directly whether a single administration of METH causes reactive oxygen species formation, we measured production of the hydroxylated salicylate metabolites, 2,3-DHBA and 2,5-DHBA. Both are used as indices of reactive oxygen species formation, although 2,5-DHBA is less reliable as it can be formed both by interacting with hydroxyl radicals and by nonreactive oxygen species-mediated processes (Halliwell et al., 1991). The results reveal that coadministration of METH with salicylate increased DHBA formation 1 h after drug treatment. These findings are consistent with those of Kondo et al. (1994) and Giovanni et al. (1995) who demonstrated increased DHBA and presumably hydroxyl radical formation, albeit after multiple high-dose administrations of METH. This acute increase in free radical formation observed 1 h after METH administration correlates temporally with the rapid METH-induced decrease in TPH activity described above.

In 1995, Che et al. found that decreases in TPH activity caused by the methylenedioxy analog of METH, MDMA, are attenuated when the drug-induced hyperthermia is prevented. Consistent with this finding, prevention of METH-induced hyperthermia by placement of rats in a 6°C environment attenuated the decrease in TPH activity resulting from a single METH injection. Because prevention of hyperthermia attenuated, but did not abolish, the METH-induced decrease in TPH activity, factors other than body temperature must be important for effecting METH-induced decreases in TPH activity.

Because prevention of METH-induced hyperthermia attenuates the drug-induced decrease in TPH activity, and because the effect of METH on TPH is associated with oxygen radicals, the relationship between body temperature and oxygen radicals was assessed. Results reveal that prevention of METH-induced hyperthermia blocked increases in 2,3-DHBA formation. These data reveal that hyperthermia contributes to the hydroxyl radical generation resulting from a single METH administration.

The finding of Stone et al. (1989b) that METH-induced decreases in TPH activity are completely reversed by exposing METH-inactivated enzyme to a reducing environment suggests that oxidative processes are solely responsible for the METH effect. Nevertheless, results presented in figure 3 demonstrate that TPH activity is still somewhat decreased in the absence of METH-enhanced 2,3-DHBA formation, which suggests that other reactive species, in addition to hydroxyl radicals, likely contribute to the decrease in TPH activity. Consistent with this possibility, superoxide radicals have been demonstrated to mediate METH-induced effects on 5HT neurons, as evidenced by findings that increased superoxide dismutase activity protects against METH-induced neurotoxicity (Hirata et al., 1995).

One possible explanation for our finding that there is an association among METH-related elevated rectal temperature, increased DHBA content and decreased TPH activity is that drug-induced hyperthermia may alter the pharmacokinetics of either METH or salicylates. For example, if elevated body temperature increased the distribution of either or both of these drugs into the striatum, this could increase DHBA content in a temperature-sensitive manner. However, in this experiment, brain levels of salicylate were not elevated significantly in hyperthermic animals. Furthermore, METH content was actually greater in the brains of nonhyperthermic (final core temperature, 37.9°C) versus hyperthermic (final core temperature, 42.1°C) rats. These results, although limited in that METH and salicylate levels were assessed at only one time point (i.e., immediately before decapitation), indicate that variations in pharmacokinetic factors caused by changes in body temperature were not likely responsible for our changes in DHBA formation after METH treatment.

It is interesting to speculate about the nature of reactive oxygen species resulting in the acute impairment of TPH that results from a single METH administration. Dopamine, released after METH administration, is a plausible candidate because: 1) depletion of dopamine prevents the acute METH-induced decrease in TPH activity (Schmidt et al., 1985); and 2) dopamine may have oxidative consequences by causing formation of hydrogen peroxide, superoxide, dopamine quinones (Graham, 1978; Baez et al., 1995) or the neurotoxin 6-hydroxydopamine (Seiden and Vosmer, 1984). Hydrogen peroxide and superoxide, in turn, can cause hydroxyl radical formation. 5HT, also released after METH administration, can undergo nonenzymatic oxidation to produce the toxin 5,6-dihydroxytryptamine (Commins et al., 1987) and other toxic species (Wrona et al., 1995) which may contribute to the oxidative consequences of METH treatment. Carrier- or diffusion-mediated uptake of any of these reactive products could conceivably effect the oxidative decrease in TPH activity observed after METH administration. Further experiments identifying the precise reactive species involved in METH-induced effects on monoaminergic neurons are required.
In summary, we have confirmed and extended the observation by Stone et al. (1989b) that a single high dose of METH causes a reactive oxygen species-mediated decrease in striatal TPH activity which is reversed by 3 h exposure to reducing conditions. Our findings support the hypothesis that this immediate change in enzyme activity is caused by free radical formation by demonstrating that there is a relatively rapid concomitant increase in the production of DHBA metabolites from salicylate after a single METH administration. In addition, we observed that the METH-induced formation of reactive oxygen species is temperature sensitive and is attenuated in nonhyperthermic, METH-treated animals: this finding suggests that hyperthermia facilitates formation of oxidative species resulting from the administration of high-dose METH treatment.

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References


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