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Body temperature effect on methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity

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Abstract

Brain tryptophan hydroxylase activity decreases within 15 min after a single administration of 3,4-methylenedioxymethamphetamine. In the present study, the effect of body temperature on this acute decrease of tryptophan hydroxylase activity was examined. 2 h after a single dose of 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.), rats exhibited hyperthermia (38.7°C) or hypothermia (35.8°C) when maintained at 25°C or 6°C, respectively. The rectal temperature of control animals maintained at 6°C was not altered. Tryptophan hydroxylase activity measured in the hippocampus, striatum and frontal cortex of hyperthermic rats treated with 3,4-methylenedioxymethamphetamine was decreased to 61%, 65%, and 71% of control levels, respectively, 2 h after drug treatment. However, in hypothermic rats, 3,4-methylenedioxymethamphetamine had no effect on tryptophan hydroxylase activity in the hippocampus, striatum or frontal cortex. Non-drug-induced hyperthermia or hypothermia did not affect tryptophan hydroxylase activity. Since hypothermia may prevent the 3,4-methylenedioxymethamphetamine-induced decrease in tryptophan hydroxylase activity by reducing the formation of free radicals, the effect of a free radical scavenging agent, *N-tert*-butyl-α-phenylnitrone, was examined. *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) alone caused hypothermia but had no direct effect on tryptophan hydroxylase activity. Preadministration of *N-tert*-butyl-α-phenylnitrone prevented 3,4-methylenedioxymethamphetamine from raising the temperature above normal and attenuated the drug-induced decrease in tryptophan hydroxylase activity in hippocampus, striatum and frontal cortex. However, when the rats treated with a combination of *N-tert*-butyl-α-phenylnitrone and 3,4-methylenedioxymethamphetamine were maintained at hyperthermic conditions, *N-tert*-butyl-α-phenylnitrone had no protective effect. These results suggest that body temperature plays a prominent role in the 3,4-methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); Tryptophan hydroxylase; Hyperthermia; Hypothermia

1. Introduction

3,4-Methylenedioxymethamphetamine, a ring-substituted analogue of amphetamine, produces both a long-term and short-term loss in the activity of tryptophan hydroxylase, the rate-limiting enzyme of 5-hydroxytryptamine synthesis (Stone et al., 1986,1987,1989b; Commins et al., 1987; Schmidt et al., 1986; O'Hearn et al., 1988; Battaglia et al., 1987,1988). The long-term loss of enzyme activity is thought to be associated with damage to serotonergic neurons (Stone et al., 1987; Schmidt and Taylor, 1987) although the role of the short-term decrease in tryptophan hydroxylase activity in this neurotoxicity is unclear.

Administration of methamphetamine or 3,4-methylenedioxymethamphetamine causes hyperthermia (Zalis et al., 1967; Nash et al., 1988; Henry et al., 1992). Because of the major role of serotonergic pathways in thermoregulation in rats and other species (Lin, 1994), the relationship between hyperthermia and neurotoxicity caused by methamphetamine and 3,4-methylenedioxymethamphetamine has been studied. Schmidt et al. (1990) demonstrated that 3,4-methylenedioxymethamphetamine-induced neurochemical changes observed 1 week later are blocked by maintaining animals at reduced ambient temperature and suggested that hyperthermia may contribute to the development of the drug's long-term neurochemical effects. Other investigators found that lowering ambient temperature to 4°C during treatment with methamphetamine blocks hyperthermia and the long-term changes in 5-hydroxytryptamine neurons (Bowyer et al., 1992,1993). These reports

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prompted us to study the effect of body temperature on the early, rapid decrease of tryptophan hydroxylase activity induced by 3,4-methylenedioxymethamphetamine in order to determine the relationship of the early serotonergic changes to neurotoxicity and elucidate the mechanism of these immediate effects.

A possible mechanism responsible for neurotoxicity caused by amphetamine analogues is the formation of free radicals. Quinone species formed via oxidative metabolism of either the parent amphetamine compound or from drug-liberated catecholamines, as well as superoxide anions and hydroxyl radicals formed via intracellular redox cycling may play a major role in the induction of serotonergic neurotoxicity (Stone et al., 1989a,b; Hiramatsu et al. 1990; Cohen and Heikkila, 1974). The observation that the free radical scavenging agent, *N-tert*-butyl-α-phenylnitrone protects against 3,4-methylenedioxymethamphetamine-induced neurotoxicity supports this hypothesis (Lim and Foltz, 1992). It is possible that 3,4-methylenedioxymethamphetamine-induced hyperthermia favors the formation of free radicals. The possibility that free radicals also contribute to the rapid initial decrease in tryptophan hydroxylase activity was investigated in this study by determining the effect of *N-tert*-butyl-α-phenylnitrone on this response. In addition, the effect of *N-tert*-butyl-α-phenylnitrone with or without 3,4-methylenedioxymethamphetamine on body temperature was also measured.

2. Materials and methods

2.1. Animals and drugs

Male Sprague-Dawley rats (170–220 g) were housed 4–6 per cage in a temperature-controlled room (24°C) with a 12 h alternating light-dark cycle. They were allowed free access to standard laboratory food and water.

3,4-Methylenedioxymethamphetamine was supplied by the National Institute of Drug Abuse. The following compounds were purchased from Sigma Chemical Co.: L-tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxyindole-3-acetic acid, DL-6-methyl-5,6,7,8-tetrahydropterine (6-MPH_4), Hepes, *m*-hydroxybenzyl-hydrazine dihydrochloride (NSD 1015) and *N-tert*-butyl-α-phenylnitrone. Dithiothreitol (dithiothreitol) was obtained from Calbiochem. Co.; EDTA from Fisher Scientific Co.; monochloroacetic acid (monochloroacetic acid) from Mallinckrodt, and 1-octanesulfonic acid sodium salt from Eastman Kodak.

2.2. Experimental procedure

Rats were weighed and injected with a single dose of 3,4-methylenedioxymethamphetamine (20 mg/kg, dissolved in 0.9% saline, s.c.) and maintained at 25°C or 6°C with their respective control groups for 2 h. In separate experiments, rats were treated with *N-tert*-butyl-α-phenylnitrone (100 mg/kg or 200 mg/kg, dissolved in 0.9% saline, i.p.) alone or 15 min prior to 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.) and maintained at 25°C for 2 h. After this period of time, rectal temperatures were measured and the rats were killed by decapitation. Immediately after decapitation, the brain was placed on ice and bilateral striata, hippocampi and frontal cortices were excised. Tissues were stored at −80°C until assayed.

Tryptophan hydroxylase activity was determined with high performance liquid chromatography with electrochemical detection (HPLC-EC) by measuring the formation of 5-hydroxy-L-tryptophan resulting from the hydroxylation of tryptophan according to a modification of the methods described by Boadle-Biber et al. (1986) and Hotchkiss et al. (1979). One of the bilateral tissues was weighed and homogenized in 100–130 μl of 50 mM Hepes buffer (pH 7.4), containing 0.2% Triton X-100 and 6 mM dithiothreitol using a Potter-Elvejhem homogenizer. After centrifugation at 40 000 × *g* for 15 min (4°C), 7.5 μl of supernatant was transferred into 6 × 50 mm glass tubes. 5 μl of reaction mixture containing 2 mM tryptophan, 1.25 mM NSD 1015 and 3.5 mM 6-MPH4 were added to the supernatant and incubated for 30 min at 37°C. The reaction was terminated by placing tubes in ice and by adding 500 μl monochloroacetic acid buffer (150 mM monochloroacetic acid, 201.6 μM EDTA, 115.7 μM octanesulfonic acid and 12.5% methanol, pH 2.9) containing 63.2 ng/ml 5-hydroxyindole-3-acetic acid internal standard. After centrifugation at 1000 × *g* for 15 min, the supernatant was assayed for 5-hydroxy-L-tryptophan by HPLC-EC.

2.3. Statistical analyses

Statistical analyses of the data were performed by ANOVA (analysis of variance) followed by Fisher's PLSD (precise least significant difference) multiple comparison test or by unpaired two-tail Student's *t* test comparison.

3. Results

3.1. Effect of 3,4-methylenedioxymethamphetamine on body temperature

2 h after a single injection of 3,4-methylenedioxymethamphetamine, the body temperature of rats maintained at 25°C was increased by an average of 1.2°C when compared to the control group, whereas 3,4-methylenedioxymethamphetamine decreased the body temperature of rats maintained at 6°C by 1.4°C (Fig. 1). The cold environment alone had no effect on body temperature of control animals (Fig. 1).

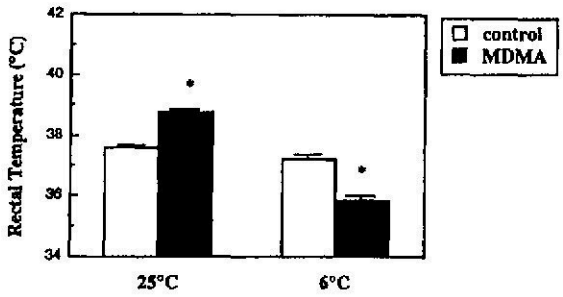

Fig. 1. Body temperatures of rats maintained at 25°C or 6°C for 2 h after injection of either 0.9% saline or 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.). Data are presented as the means ± S.E.M. for 18 animals in each group. * indicates difference from respective control groups ($P<0.05$). White columns: control; black columns: 3,4-methylenedioxymethamphetamine.

3.2. Effect of body temperature on the rapid decrease in tryptophan hydroxylase activity induced by 3,4-methylenedioxymethamphetamine

Tryptophan hydroxylase activity measured 2 h after 3,4-methylenedioxymethamphetamine injection in rats kept at 25°C was decreased significantly to 61%, 65% and 71% of control levels in the hippocampus, striatum and frontal cortex, respectively (Fig. 2). However, under hypothermic conditions, tryptophan hydroxylase activity was not altered by 3,4-methylenedioxymethamphetamine treatment compared to the respective control values in any of the three tissues examined.

3.3. Effect of body temperature alone on tryptophan hydroxylase activity

In light of the above observations, it was important to determine the effect of hyperthermia or hypothermia on tryptophan hydroxylase activity in non-3,4-methylenedioxymethamphetamine treated rats. Rats were maintained at 32°C for 2 h or at 6°C for 3 h to achieve hyperthermia or hypothermia. These respective conditions increased or decreased body temperature by 1.2°C. Neither hyperthermia nor hypothermia alone had an effect on tryptophan hydroxylase activity (data not shown).

3.4. Effect of N-tert-butyl-α-phenylnitrone on body temperature and tryptophan hydroxylase activity

The prevention of the 3,4-methylenedioxymethamphetamine-induced decrease on tryptophan hydroxylase activity under hypothermic conditions suggested that this effect may be due to a reduced formation of free radicals, since hypothermia can decrease metabolic rate. *N-tert*-butyl-α-phenylnitrone was administered either alone or 15 min prior to 3,4-methylenedioxymethamphetamine injection. At a dose of 100 mg/kg, *N-tert*-butyl-α-phenylnitrone alone decreased body temperature by 1.3°C compared to the control group (37.8°C), whereas preadministration of *N-tert*-butyl-α-phenylnitrone did not block the hyperthermia (39.0°C) caused by 3,4-methylenedioxymethamphetamine (Fig. 3A). *N-tert*-butyl-α-phenylnitrone alone had no direct effect on tryptophan hydroxylase activity in any of the three tissues examined. 3,4-Methylenedioxymethamphetamine alone decreased tryptophan hydroxylase activity significantly and *N-tert*-butyl-α-phenylnitrone did not protect against the decrease of tryptophan hydroxylase activity caused by 3,4-methylenedioxymethamphetamine (Fig. 3B).

Since the dose of 100 mg/kg might be inadequate to block 3,4-methylenedioxymethamphetamine-induced hyperthermia, even though it caused hypothermia by itself, a dose of 200 mg/kg of *N-tert*-butyl-α-phenylnitrone was administered. This dose of *N-tert*-butyl-α-phenylnitrone alone decreased body temperature by 2.7°C compared to the control group (37.6°C) but had no effect on tryptophan hydroxylase activity. *N-tert*-butyl-α-phenylnitrone (200 mg/kg) completely prevented 3,4-methylenedioxymethamphetamine from raising the temperature above normal (Fig. 4A) and attenuated the rapid decrease in tryptophan hydroxylase activity caused by 3,4-methylen-

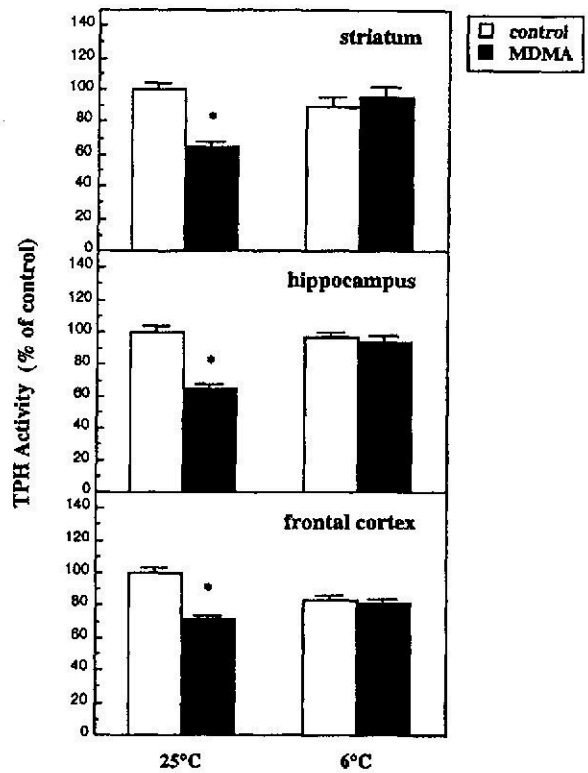

Fig. 2. Prevention of 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.)-induced early decrease in tryptophan hydroxylase activity by lowering body temperature of rats. The experimental conditions were the same as those described in the legend of Fig. 1. Data are presented as the means ± S.E.M. for 18 animals in each group. * indicates significantly different from control group ($P<0.05$). White columns: control; black columns: 3,4-methylenedioxymethamphetamine.

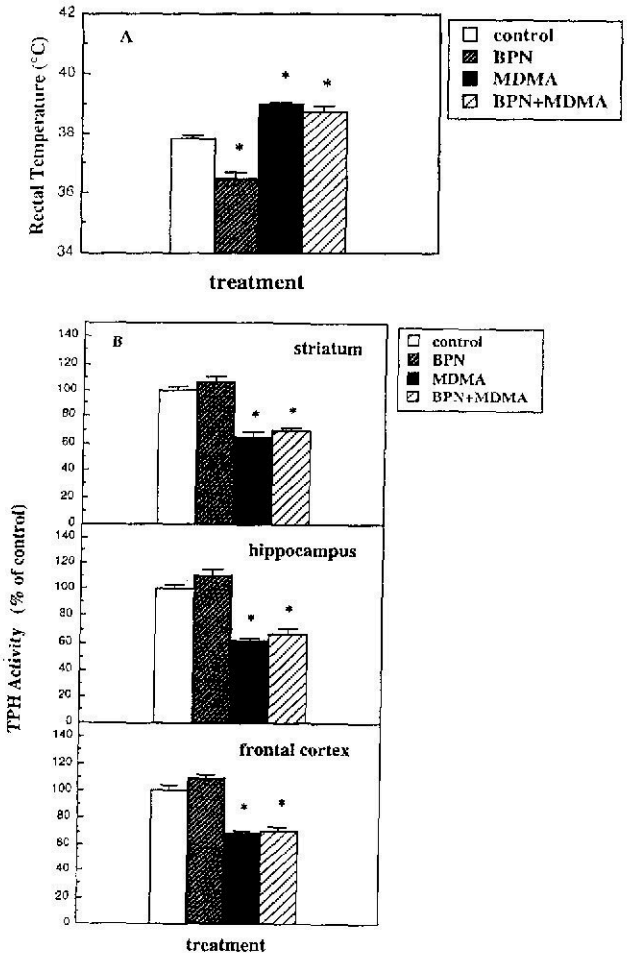

Fig. 3. Effect of *N-tert*-butyl-α-phenylnitrone (100 mg/kg, i.p.) on body temperature and tryptophan hydroxylase activity. *A*: body temperatures of the rats treated with saline, *N-tert*-butyl-α-phenylnitrone, 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.) or both *N-tert*-butyl-α-phenylnitrone and 3,4-methylenedioxymethamphetamine, measured 2 h after 3,4-methylenedioxymethamphetamine injection. *B*: tryptophan hydroxylase activity in brain tissues of the rats treated with saline, *N-tert*-butyl-α-phenylnitrone, 3,4-methylenedioxymethamphetamine, or both *N-tert*-butyl-α-phenylnitrone and 3,4-methylenedioxymethamphetamine, measured 2 h after the injection of 3,4-methylenedioxymethamphetamine. Data are presented as means ± S.E.M. for 12 animals in each group. * indicates significantly different from control group ($P<0.05$). White columns: control; striped columns: *N-tert*-butyl-α-phenylnitrone; black columns: 3,4-methylenedioxymethamphetamine; hatched columns: *N-tert*-butyl-α-phenylnitrone + 3,4-methylenedioxymethamphetamine.

edioxymethamphetamine in hippocampus, striatum and frontal cortex (Fig. 4B).

In order to determine whether the protective effect of *N-tert*-butyl-α-phenylnitrone against the 3,4-methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity is due to its effect on body temperature or its free radical scavenging effect, we examined the effect of *N-tert*-butyl-α-phenylnitrone on the 3,4-methylenedioxymethamphetamine-induced decrease in tryptophan hydroxylase activity at hyperthermic conditions. A group of rats was treated with *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) prior to 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.) injection and maintained at 28°C to obtain hyperthermia. 2 h after 3,4-methylenedioxymethamphetamine injection, the average body temperature of the rats was 39.2°C, which was comparable to that of the group treated with 3,4-methylenedioxymethamphetamine alone (Fig. 5A). *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) did not protect against the 3,4-methylenedioxymethamphetamine-induced decrease in

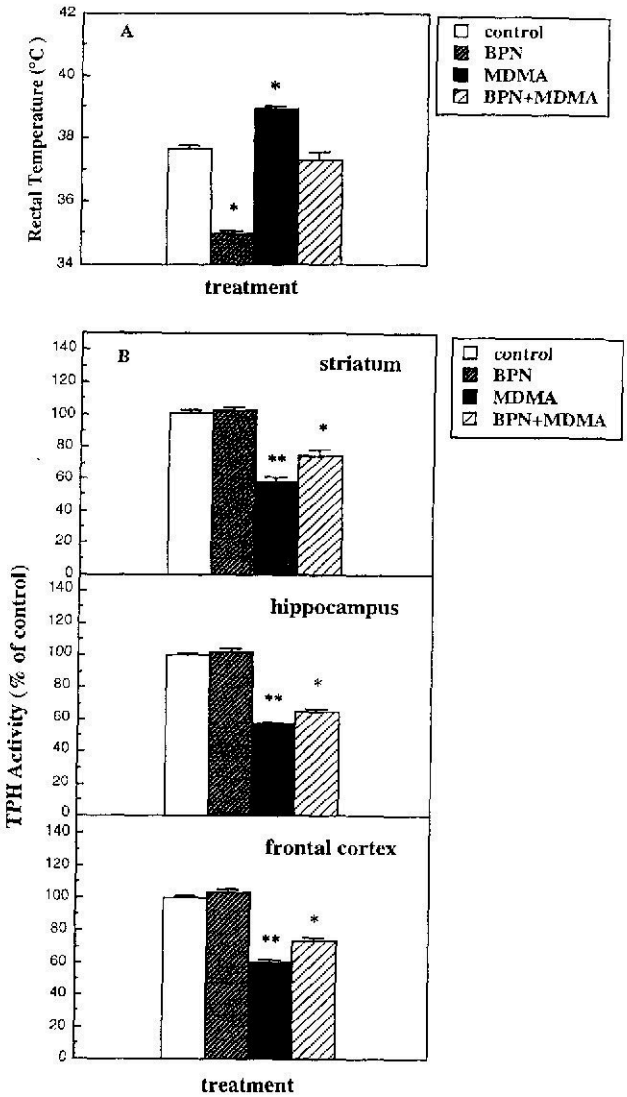

Fig. 4. Effect of *N-tert*-butyl-α-phenylnitrone (200 mg/kg) on body temperature and tryptophan hydroxylase activity. The experimental conditions were the same as those described in the legend of Fig. 3 except that a higher dose of *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) was administered. Data are presented as the means ± S.E.M. for 12 animals in each group. * indicates significantly different from control; ** in *B* indicates significantly different from both control and *N-tert*-butyl-α-phenylnitrone + 3,4-methylenedioxymethamphetamine ($P<0.05$). White columns: control; striped columns: *N-tert*-butyl-α-phenylnitrone; black columns: 3,4-methylenedioxymethamphetamine; hatched columns: *N-tert*-butyl-α-phenylnitrone + 3,4-methylenedioxymethamphetamine.

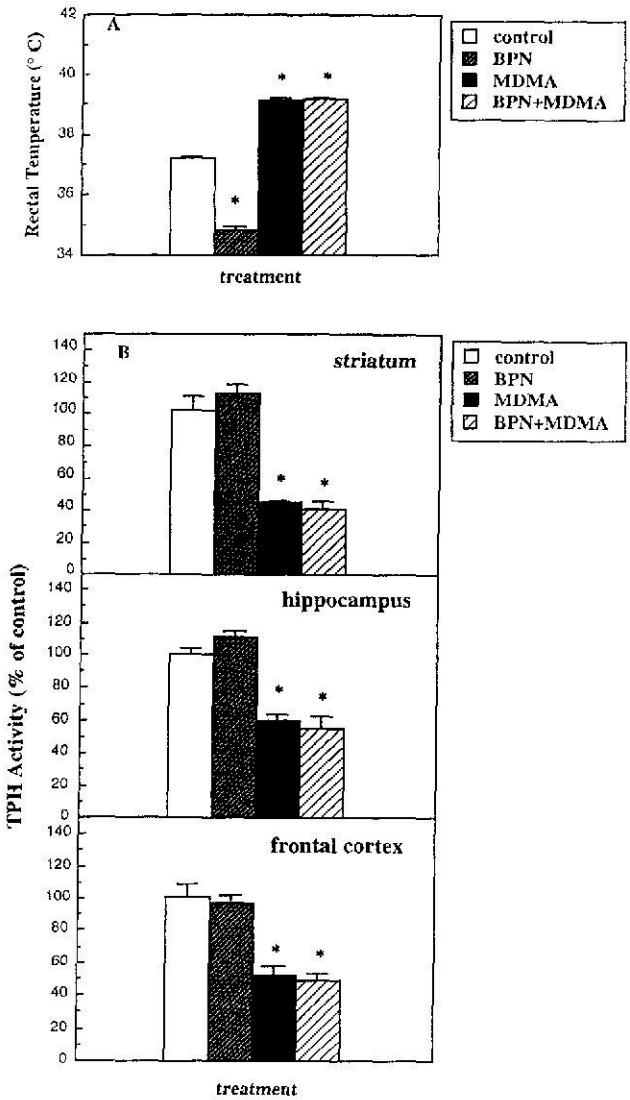

Fig. 5. Effect of *N-tert*-butyl-α-phenylnitrone (200 mg/kg) on body temperature and tryptophan hydroxylase activity at an altered ambient temperature. The experimental conditions were the same as those described in the legend of Fig. 4 except that the rats treated with the combination of *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) and 3,4-methylenedioxymethamphetamine (20 mg/kg, s.c.) were maintained at 28°C to produce hyperthermia. Data are presented as the means ± S.E.M. for six animals in each group. * indicates significantly different from control ($P<0.05$). White columns: control; striped columns: *N-tert*-butyl-α-phenylnitrone; black columns: 3,4-methylenedioxymethamphetamine; hatched columns: *N-tert*-butyl-α-phenylnitrone + 3,4-methylenedioxymethamphetamine.

tryptophan hydroxylase activity when the rats were hyperthermic in any of the three tissues examined (Fig. 5B).

4. Discussion

3,4-Methylenedioxymethamphetamine causes an immediate, followed by a long-term, neurochemical change in the serotonergic system (Stone et al., 1987). Brain tryptophan hydroxylase activity decreases within 15 min after injection of 3,4-methylenedioxymethamphetamine (10 mg/kg) and multiple doses of 3,4-methylenedioxymethamphetamine cause long-term deficits in tryptophan hydroxylase activity, and 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid content in serotonin-containing axon terminals (Stone et al., 1987). 3,4-Methylenedioxymethamphetamine also causes hyperthermia that appears to influence the neurochemical response to this drug. Schmidt et al. (1990) demonstrated that maintaining animals at reduced ambient temperature protected against 3,4-methylenedioxymethamphetamine-induced neurochemical changes observed 1 week later, suggesting that hyperthermia may play a role in the drug-induced long-term neurotoxicity. In our study, maintaining the animals in a cold environment prevented both the early 3,4-methylenedioxymethamphetamine-induced hyperthermia and the acute decrease in enzyme activity.

Monoaminergic pathways in the central nervous system play a major role in systemic thermoregulation (Lin, 1994). Administration of 5-hydroxytryptamine or 5-hydroxytryptamine receptor agonists causes hypothermia due to decreased metabolic rate and cutaneous vasodilation. Administration of 5-hydroxytryptamine receptor antagonists causes hyperthermia due to increased metabolism and cutaneous vasoconstriction (Lin, 1994). The hyperthermia and hypothermia induced by 3,4-methylenedioxymethamphetamine when rats were maintained at 25°C or 6°C, respectively, indicate that the drug alters thermoregulation in the animals.

It is not clear why lowering body temperature blocks the effect of 3,4-methylenedioxymethamphetamine on the serotonergic system. Previous studies demonstrated that tryptophan hydroxylase activity is increased by the stress caused by sound (Singh et al., 1994) or electrical stimulation (Boadle-Biber et al., 1986). It is possible that tryptophan hydroxylase activity is increased by the stress of the cold environment and this increase offsets the response of tryptophan hydroxylase to 3,4-methylenedioxymethamphetamine. But our observation that hypothermia alone did not alter tryptophan hydroxylase activity argues against this explanation.

The involvement of free radicals in long-term neurotoxicity caused by amphetamine analogues has been suggested by several investigators (Friedman et al., 1972; Hiramatsu et al., 1990; Houslay and Tipton, 1973; Lim and Foltz, 1992; Stone et al., 1989a). The nature of the tryptophan hydroxylase inactivation induced by amphetamine analogues suggests that oxidative stress plays a major role in the induction of serotonergic neurotoxicity (Kappus and Sies, 1981; Stone et al., 1989a). 3,4-Methylenedioxymethamphetamine induces a transient release of dopamine and 5-hydroxytryptamine; monoamine oxidase (MAO) metabolizes intracellular monoamines and generates H_2O_2 (Houslay and Tipton, 1973), an oxidant suggested to inactivate tryptophan hydroxylase (Friedman et

al., 1972). Cytochrome P450 oxidizes 3,4-methylenedioxymethamphetamine to a catechol and superoxide oxidizes catecholamines to quinones which can react with thiol groups of the enzyme (Hiramatsu et al., 1990). Lim and Foltz (1992) reported that *N-tert*-butyl-α-phenylnitrone protected against the decrease in tryptophan hydroxylase activity 1 week after 3,4-methylenedioxymethamphetamine injection. In our study, 100 mg/kg *N-tert*-butyl-α-phenylnitrone did not block the 3,4-methylenedioxymethamphetamine-induced hyperthermia and had no effect on the acute 3,4-methylenedioxymethamphetamine-induced decrease in tryptophan hydroxylase activity 2 h after 3,4-methylenedioxymethamphetamine administration. A higher dose of *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.), however, prevented 3,4-methylenedioxymethamphetamine from raising the temperature above normal and partially attenuated the drug-induced decrease in tryptophan hydroxylase activity. In addition to preventing the 3,4-methylenedioxymethamphetamine effects on hyperthermia this attenuation could also be attributed to the free radical scavenging by *N-tert*-butyl-α-phenylnitrone. When the ambient temperature was elevated to 28°C, the partial protection of the higher dose of *N-tert*-butyl-α-phenylnitrone disappeared; however, the rats again became hyperthermic. The ability of *N-tert*-butyl-α-phenylnitrone (200 mg/kg, i.p.) to prevent 3,4-methylenedioxymethamphetamine from raising the temperature above normal and attenuate the acute 3,4-methylenedioxymethamphetamine-induced decrease in tryptophan hydroxylase activity suggests that factors other than elevated temperature are also involved in the rapid neurochemical change induced by 3,4-methylenedioxymethamphetamine. *N-tert*-butyl-α-phenylnitrone is reported to protect against neurodegenerative disease such as ischemia due to its free radical scavenging effect (Hearse and Tosaki, 1988). The hypothermic property of *N-tert*-butyl-α-phenylnitrone could possibly contribute to its protection against this type of neurodegeneration.

Lowering body temperature may interfere with the changes in the serotonergic system by reducing the metabolism of 3,4-methylenedioxymethamphetamine to active metabolites, including free radicals. Lim and Foltz (1988) and Lim et al. (1991) demonstrated that 3,4-methylenedioxymethamphetamine was metabolized to 17 different metabolites by *N*-methylation, *O*-dealkylation, aromatic hydroxylation, deamination and conjugation. Although the role of the metabolites in mediating central neurotoxicity has not been well established, some of the metabolites are known to be active (Elayan et al., 1993). It has been suggested that the peripheral generation of active metabolites could contribute to the acute neurochemical effect of 3,4-methylenedioxymethamphetamine (Schmidt and Taylor, 1988; Johnson et al., 1991; Cho et al., 1990). Because of slower metabolism at lower body temperature, active metabolites formed may be inadequate to alter tryptophan hydroxylase activity acutely.

Even though the mechanism of the protection of hypothermia against the 3,4-methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity is unclear, this observation suggests that hyperthermia plays an important role in both the immediate and long-term response of tryptophan hydroxylase to 3,4-methylenedioxymethamphetamine. To compare tryptophan hydroxylase activity in 3,4-methylenedioxymethamphetamine-treated rats with enzyme activity in non-treated rats at the same body temperature, hyperthermia or hypothermia were achieved by maintaining rats at ambient temperatures of 32°C or 6°C. Interestingly, while elevated 5-hydroxytryptamine or depressed 5-hydroxytryptamine are known to cause hypothermia or hyperthermia (Lin, 1994), respectively, hyperthermia and hypothermia alone did not alter tryptophan hydroxylase activity. From these observations, it is apparent that the 3,4-methylenedioxymethamphetamine-induced decrease in tryptophan hydroxylase activity cannot be attributed to hyperthermia alone, and that other factors play a role in this complex response.

In conclusion, lowering body temperature protects against the immediate decrease in tryptophan hydroxylase activity induced by 3,4-methylenedioxymethamphetamine, suggesting that hyperthermia plays a prominent role in the acute as well as the long-term decrease in enzyme activity.

Acknowledgements

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