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Effects of Salicylate on 3,4-Methylenedioxymethamphetamine (MDMA)-Induced Neurotoxicity in Rats

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YEH, S. Y. *Effects of salicylate on 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity in rats.* PHARMACOL BIOCHEM BEHAV **58**(3) 701–708, 1997.—The drug 3,4-methylenedioxymethamphetamine (MDMA) is a serotonergic neurotoxicant that causes hyperthermia and depletion of serotonin (5-HT) and 5-hydroxy-indole-3-acetic acid (5-HIAA) in the central nervous system. Formation of neurotoxic metabolites of MDMA, e.g., 2,4,5-trihydroxy-methamphetamine and 2,4,5-trihydroxyamphetamine, involves hydroxyl and/or superoxide free radicals. The present study was designed to determine whether the hydroxyl free-radical-trapping agent salicylate could provide protection against MDMA neurotoxicity in rats. In the acute studies, sodium salicylate (12.5–400 mg/kg, calculated as free acid) was injected interperitoneally (IP) 1 h before subscutaneous (SC) injections of MDMA (20 mg/kg as base). In the chronic studies, sodium salicylate (3.1–100 mg/kg) was injected IP 1 h before repeated SC injections of MDMA (10 mg/kg as base, twice daily, at 0830 and 1730 h for 4 consecutive days). Repeated MDMA administration depleted contents of 5-HT and 5-HIAA in the frontal cortex, hippocampus and striatum. Coadministration of salicylate plus MDMA did not significantly alter MDMA-induced depletion of 5-HT and 5-HIAA in these tissues. Thus, salicylate, a hydroxyl free-radical-trapping agent, does not protect against MDMA-induced hyperthermia and depletion of 5-HT and 5-HIAA. These observations suggest that MDMA-induced neurotoxicity may occur mainly through the production of superoxide or other radicals rather than hydroxyl free radicals. Salicylate actually potentiated MDMA-induced hyperthermia and lethality, findings that might be of clinical relevance. Published by Elsevier Science Inc.

3,4-Methylenedioxymethamphetamine (MDMA) Neurotoxicity Salicylate
3,4-Methylenedioxyamphetamine (MDA) Hydroxyl radicals Superoxide radicals

THE drug 3,4-methylenedioxymethamphetamine (MDMA), a substitute amphetamine, is well known as a drug of abuse in the United States and Europe. We and others have shown that MDMA is a serotonergic neurotoxicant (1,10,18). MDMA causes hyperthermia in rats and in humans (3,20,21) and decreases in tryptophan (TPH) and tyrosine hydroxylase (TYH) activity, concentrations of serotonin (5-HT) and 5-hydroxy-indole-3-acetic acid (5-HIAA), 5-HT transporters, 5-HT uptake sites and 5-HT neuronal terminals in several brain regions of rodents and nonhuman primates (1,10,18,23,27,47,52,54,63). MDMA-induced neurotoxicity can last for several months (2). MDMA-induced neurotoxic effects were increased by a hyperthermic environment and decreased by a hypothermic environment (15,40,45).

Intracerebroventricular (ICV) administration of MDMA does not produce neurotoxicity (47,49,56), suggesting that MDMA-induced neurotoxicity might be related to its metabolites and prompted to search for toxic metabolites. Results of metabolic studies of MDMA have indicated that dihydroxymethamphetamine (DHMA), dihydroxyamphetamine (DHA), 6-hydroxy-MDMA (6-OH-MDMA) and 6-hydroxy-MDA (6-OH-MDA) can be detected in the brain, liver and plasma of rats treated with MDMA (35). Formation of DHMA, DHA (22,35), trihydroxymethamphetamine (THMA) and trihydroxyamphetamine (THA) (36) requires cytochrome P-450 enzymes and NADPH-generating systems (22,35,36). This observation suggests that superoxide and/or hydroxyl free radicals are involved. Hydroxyl free radicals mediate en-

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zymatic *O*-demethylenation of MDMA and MDA (31). This idea is supported by the observation that benzoate, a hydroxyl radical scavenging agent, can inhibit the *O*-demethylenation of MDMA and MDA in vitro (37). Incubation of MDMA and MDA with rat microsomes yielded DHMA and DHA, which, after conversion to a quinone, formed an adduct with glutathione and other thiol compounds (22,43). The adduct formation was inhibited by superoxide dismutase, suggesting that superoxide radicals were involved in these reactions (22, 43).Of the identified MDMA metabolites (34–36,41,42,70–72), THMA, THA, (13,14,72), 5-(glutathione-S-yl)-α-methyldopamine (44), MDA, 4-hydroxy-3-methoxyamphetamine, and α-methyldopamine (70) are neurotoxicants. In contrast, ICV administration of the precursors of THMA and THA, DHA, 2-OH-MDMA and 2-OH-MDA failed to induce neurotoxicity (13,14,24).

MDMA-induced depletion of 5-HT and 5-HIAA and inactivation of TPH activity was attenuated by L-cysteine (55) and other reducing agents, e.g., dithiothreitol plus Fe^{2+} (64), suggesting either oxygen–radical-mediated oxidation of the sulfhydryl (−SH) residues of TPH or interaction of such radicals with MDMA metabolites. MDMA-induced depletion of 5-HT and 5-HIAA also was attenuated by the monoamine oxidase inhibitor (MAOI) L-deprenyl (62). This finding suggests that inhibiton of deamination of MDMA and the formation of hydrogen peroxide are involved. Hydrogen peroxide in the presence of Fe^{2+} forms hydroxyl free radicals and hydroxyl ions via the Fenton reaction (67). The role of hydroxyl radicals on MDMA-induced neurotoxicity has not been studied. The present study was undertaken to investigate whether a well-known hydroxyl free-radical-trapping agent, salicylate, would protect against MDMA-induced neurotoxic effects in vivo.

METHODS

Subjects

Male Sprague–Dawley rats (245–275 g; Harlan Industries, Indianapolis, IN) were housed, 3 per polycarbonate cage (18 × 8.5 × 8 in.), bedded with corn chips. They were provided with free access to Purina Chow and water in an air-conditioned room (22 ± 1°C) with 12-h–12-h light–dark cycles (lights on: 7:00–19:00). Rats were allowed at least a 1-week adjustment period before being used in any experiment. Animals used in this study were maintained in facilities accredited by the Association for the Accreditation of Laboratory Animal Care, and the experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH.

Drugs

Sodium salicylate, sodium 1-heptanesulfonate, perchloric acid, citric acid, sodium acetate, and acetonitrile (Sigma, St. Louis, MO) and dl-MDMA (Research Triangle Park, N.C. via NIDA) were used in these studies.

Neurochemical Experiments

In the acute studies, rats, housed 3 per cage, were injected interperitoneally (IP) with sodium salicylate 1 h before subcutaneous (SC) injection of 20 mg/kg of MDMA as the base (53). The doses of sodium salicylate were 12.5, 25, 50, 100, 200 and 400 mg/kg, calculated as free acid. In the chronic studies, rats were injected IP with salicylate (3.1, 6.3, 12.5, 25, 50 and 100 mg/kg) 1 h before SC injection of 10 mg/kg of MDMA (twice daily, at 0830 and 1730 h for 4 consecutive days). The doses of salicylate were reduced because of toxic effects observed in the acute studies. These doses of salicylate trap free hydroxyl radicals (6,61). Control rats were injected with salicylate and saline. All rats were decapitated 1 week after the last injection. Frontal cortex, brainstem, hippocampus and striatum were dissected over ice according to the method of Glowinski and Iversen (17) and frozen in liquid nitrogen. Tissues were stored at −75°C for the assay of monoamines and their metabolites with high performance liquid chromatography, using a refrigerated auto-sample injector, a dual pistol pump (Water Associates, Marlborough, MA) and an electrochemical detector with apply potential set at 0.69 mV (Bioanalytic Systems, West Lafayette, IN).

Norepinephrine (NE), 5-HT, 5-HIAA, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT) and DHA (internal standard) were separated on a C18 reverse guard column and a 5-μ spherical C18 reverse column (Resolve, Water Associates) with a mobile phase of citrate buffer at pH 4.0. The mobile phase consisted of 0.3 g disodium EDTA, 1 g sodium 1-heptanesulfonate, 14 g citric acid.H_2O, 11.4 g sodium acetate trihydrate and 1 ml triethylamine per 1000 ml deionized water, adjusted to pH 4.0 with 10 N NaOH or acetic acid, filtered through a 0.2-μm Nylon-66 filter (Rainin, Woburn, MA), and degassed. Twenty-five to forty milliters of acetonitrile were added to 960 ml of the degassed buffer solution to yield an optimal separation.

Temperature Experiments

These experiments were undertaken separately to examine the effects of salicylate on MDMA-induced hyperthermia because (a) MDMA-induced hyperthermia plays a role in its neurotoxicity (15,18,45) and (b) in the acute studies, we had observed that rats sweated profusely after coadministration of salicylate and MDMA and that salicylate potentiated the lethality of MDMA.

On the day of the experiment, rats were transferred to an air-conditioned room (22 ± 1°C), weighed and housed in hanging metal cages (10 × 8.5 × 7 in.) without bedding for at least 1 h before temperature measurement. Rats were provided with chow and water ad libitum through the experiment. Core temperature was measured with a probe lubricated with mineral oil and a digital readout (Yellow Spring, Columbus, OH) before injection of sodium salicylate (12.5, 25, 50, 100 and 200 mg/kg) and hourly following the injection of MDMA for 5 h. MDMA (20 mg/kg) was injected SC 1 h after the injection of sodium salicylate. The probe was inserted about 7 cm into the colon until a stable temperature reading was obtained (about 1 min). Control rats were injected with saline.

Data Analysis

A two-way analysis of variance (ANOVA; BMDP program 4V) (12) was used to assess the overall significance of the results of body temperature. A two-way ANOVA (BMDP program 3D) was used to assess the overall significance of the results of core temperature after drug administration with that of 0 h control temperature, and Student's *t*-test was used to assess the differences between individual pairs of means. A two-way ANOVA (BMDP program 7D) was used to assess the overall significance of the results of contents of monoamine in various brain tissue regions and of core temperature. A multiple *t*-test with Bonferroni adjustment was used to assess differences between individual pairs of means. Criteria for significance were set at $p < 0.05$.

TABLE 1

EFFECTS OF MULTIPLE DOSES OF SALICYLATE (SA) ON MDMA (10 mg/kg, TWICE/DAY FOR 4 DAYS)-INDUCED DEPLETION OF 5-HT AND 5-HIAA IN THE FRONTAL CORTEX AND HIPPOCAMPUS OF RATS

	Frontal Cortex (% Saline ± SEM)		Hippocampus (% Saline ± SEM)	
Treatment (mg/kg)	5-HT	5-HIAA	5-HT	5-HIAA
SAL + SAL	100.00 ± 2.69	100.00 ± 6.81	100.00 ± 4.23	100.00 ± 5.78
SAL + MDMA	51.52 ± 9.28***	35.53 ± 4.35**,++	64.08 ± 6.81	56.44 ± 7.83*
SA(3.1) + SAL	104.97 ± 2.74	79.13 ± 2.48	107.24 ± 9.30	79.52 ± 6.41
SA(3.1) + MDMA	65.02 ± 3.39***,++	55.05 ± 3.32**,++	71.47 ± 8.92	62.55 ± 11.78
SA(6.3) + SAL	93.91 ± 5.26	91.97 ± 2.76	113.22 ± 2.30	100.34 ± 7.14
SA(6.3) + MDMA	47.54 ± 6.24***,++	43.44 ± 6.13**,++	48.52 ± 8.76**,++	42.51 ± 7.45**,+
SA(12.5) + SAL	90.59 ± 5.88	71.48 ± 3.24**	94.40 ± 8.39	85.18 ± 5.09
SA(12.5) + MDMA	46.97 ± 5.67***,++	35.57 ± 3.65**,++	50.49 ± 6.23**,+	53.01 ± 8.27**
SA(25) + SAL	90.98 ± 3.94	73.53 ± 2.89*	93.56 ± 9.82	99.95 ± 13.94
SA(25) + MDMA	45.35 ± 5.33***,++	37.95 ± 5.76**,++	47.81 ± 6.33**,+	42.30 ± 4.79***,++
SA(50) + SAL	86.53 ± 8.86	69.79 ± 2.10	109.34 ± 8.21	84.90 ± 7.54
SA(50) + MDMA	42.98 ± 5.00***,++	25.29 ± 1.99**,++	51.48 ± 10.74**,++	46.06 ± 6.02**
SA(100) + SAL	97.37 ± 6.64	74.29 ± 3.52**	98.62 ± 11.33	92.09 ± 6.42
SA(100) + MDMA	39.72 ± 2.82***,+++	29.94 ± 4.80**,++	65.76 ± 9.36	44.65 ± 10.13**

Concentrations (pg/mg wet tissue) of 5-HT and 5-HIAA were 515.67 ± 25.90 and 287.08 ± 12.69 in the frontal cortex of saline-saline (SAL-SAL) control rats and 359.68 ± 23.86 and 412.59 ± 32.63 in the hippocampus, respectively.

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. saline control. $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ vs. vehicle control.

The values for SAL-SAL groups were obtained from 10–12 rats and for treatment groups from 5–6 rats.

RESULTS

Effects of MDMA and Salicylate on Monoamine Contents in Various Brain Regions

Table 1 shows that chronic treatment with MDMA or salicylate (3.1–100 mg/kg, IP) plus MDMA (10 mg/kg, SC, twice daily for consecutive 4 days) significantly decreased 5-HT and 5-HIAA contents in the frontal cortex of rats vs. those of saline controls ($p < 0.01$) or the respective salicylate–saline controls ($p < 0.01$). Similar results were observed in the hippocampus (Table 1), brainstem and striatum (Table 2). Chronic administration of salicylate (>12.5 mg/kg) decreased 5-HIAA level in the frontal cortex. NE content was not affected by any of the treatments.

Acute MDMA administration did not cause significant decreases in the levels of 5-HT and 5-HIAA contents in the frontal cortex, hippocampus, brainstem and striatum. However, rats treated with 100 mg/kg of salicylate plus MDMA showed significant decreases in 5-HT levels in the frontal cortex (Table 3). Treatment with 50 mg/kg of salicylate plus MDMA significantly decreased 5-HT levels in the hippocampus (Table 3)

TABLE 2

EFFECTS OF MULTIPLE DOSES OF SALICYLATE (SA) ON MDMA (10 mg/kg, TWICE/DAY FOR 4 DAYS)-INDUCED DEPLETION OF 5-HT AND 5-HIAA IN THE BRAINSTEM AND STRIATUM OF RATS

	Brainstem (% Saline ± SEM)		Striatum (% Saline ± SEM)	
Treatment (mg/kg)	5-HT	5-HIAA	5-HT	5-HIAA
SAL + SAL	100.00 ± 1.67	100.00 ± 3.57	100.00 ± 5.90	100.00 ± 4.76
SAL + MDMA	83.25 ± 6.93	82.12 ± 4.86*	49.16 ± 5.36**	48.17 ± 4.52**
SA(3.1) + SAL	94.79 ± 2.14	95.68 ± 5.16	84.89 ± 6.36	82.10 ± 6.81
SA(3.1) + MDMA	90.19 ± 5.06	78.67 ± 3.94**	53.47 ± 8.28*	59.17 ± 8.34*
SA(6.3) + SAL	97.07 ± 5.11	88.32 ± 2.64	91.04 ± 8.91	76.08 ± 6.12
SA(6.3) + MDMA	83.52 ± 4.29	72.83 ± 4.19***	47.46 ± 11.27**	38.93 ± 7.83***
SA(12.5) + SAL	109.83 ± 7.08	102.03 ± 4.33	80.99 ± 15.92	82.49 ± 14.13
SA(12.5) + MDMA	84.21 ± 3.731	89.40 ± 4.78	65.81 ± 12.39	69.41 ± 15.85
SA(25) + SAL	98.46 ± 5.38	97.25 ± 6.11	83.86 ± 13.10	86.54 ± 13.43
SA(25) + MDMA	72.18 ± 9.48**,+	93.69 ± 5.99	46.39 ± 7.25**	63.91 ± 6.24
SA(50) + SAL	77.05 ± 9.87*	96.99 ± 4.09	108.09 ± 5.04	127.01 ± 19.71
SA(50) + MDMA	61.32 ± 6.65***	83.95 ± 4.28	31.75 ± 4.13***,+++	42.51 ± 8.95**
SA(100) + SAL	77.67 ± 4.47*	94.61 ± 3.05	81.28 ± 12.93	94.54 ± 15.41
SA(100) + MDMA	70.38 ± 2.88**	87.34 ± 3.42	32.64 ± 9.29***,+	59.53 ± 19.23

Concentrations (pg/mg wet tissue) of 5-HT and 5-HIAA were 744.34 ± 21.61 and 744.01 ± 51.38 in the brainstem and 441.21 ± 18.72 and 570.39 ± 21.95 in the stiatum, respectively. Keys for statistics are listed in Table 1.

TABLE 3

EFFECTS OF A SINGLE DOSE OF SALICYLATE (SA) (12.5–400 mg/kg, 1 H PRIOR TO MDMA) ON MDMA (20 mg/kg),-INDUCED DEPLETION OF 5-HT AND 5-HIAA IN THE FRONTAL CORTEX AND HIPPOCAMPUS OF RATS

		Frontal Cortex (% Saline ± SEM)		Hippocampus (% Saline ± SEM)	
Treatment (mg/kg)	*n*	5-HT	5-HIAA	5-HT	5-HIAA
SAL + SAL	6	100.00 ± 3.45	100.00 ± 7.25	100.00 ± 5.76	100.00 ± 3.92
SAL + MDMA	6	87.14 ± 3.64	91.76 ± 3.33	82.15 ± 4.58	83.12 ± 3.13
SA(12.5) + SAL	6	87.96 ± 4.91	89.37 ± 1.77	98.40 ± 5.64	102.92 ± 7.98
SA(12.5) + MDMA	6	74.81 ± 3.14*	85.93 ± 2.96	76.27 ± 5.29	84.58 ± 3.89
SA(25) + SAL	6	91.59 ± 5.49	98.35 ± 5.86	83.71 ± 5.56	90.62 ± 2.30
SA(25) + MDMA	6	89.22 ± 8.49	84.19 ± 9.56	78.47 ± 11.97	68.35 ± 8.36*
SA(50) + SAL	6	92.59 ± 4.86	85.15 ± 3.27	82.65 ± 5.27	86.16 ± 3.73
SA(50) + MDMA	5	73.84 ± 7.3	66.87 ± 6.33*,+	51.39 ± 7.15**	57.32 ± 4.23**
SA(100) + SAL	6	86.54 ± 5.48	88.68 ± 4.72	90.53 ± 3.72	92.44 ± 7.00
SA(100) + MDMA	2	76.01, 44.19*	75.11, 62.86	92.66, 59.34	108.08, 69.70
SA(200) + SAL	6	94.28 ± 5.58	96.55 ± 4.94	88.78 ± 5.61	90.57 ± 4.14
SA(400) + SAL	6	90.17 ± 4.02	93.45 ± 4.40	85.86 ± 5.74	86.60 ± 5.04

Concentrations (pg/mg wet tissue) of 5-HT and 5-HIAA in the frontal cortex and hippocampus of saline-saline (SAL-SAL) control rats were in the same ranges as stated in Table 1.

*$p < 0.05$ and **$p < 0.01$ vs. saline control. $^{+}p < 0.05$ vs. vehicle control.

The values for each treatment groups were obtained from 5–6 rats, except the group of SA(100) plus MDMA, in which only 2 rats survived.

but not in the frontal cortex. Treatment with 25 mg/kg or 50 mg/kg of salicylate plus 20 mg/kg of MDMA decreased levels of 5-HIAA in the frontal cortex and hippocampus (Table 3) but not in the brainstem and striatum (Table 4). Other doses of salicylate in combination with MDMA had no significant effects on monoamine contents in these brain regions.

Effects of Salicylate and MDMA on Lethality

In the acute studies, rats sweated profusely when housed 3 per cage 1 h after the injection of MDMA plus a large dose of salicylate (100 or 200 mg/kg). Six of 6, 4 of 6 and 1 of 6 rats died 2–24 h after injections of 200, 100 and 50 mg/kg of salicylate plus 20 mg/kg of MDMA, respectively. The lethality of MDMA plus salicylate was significantly higher than that of MDMA plus saline (200 mg/kg, $p < 0.01$; 100 mg/kg, $p < 0.05$). In the chronic studies, 2 of 6 and 1 of 6 rats died 35–48 h after MDMA plus 100 and 50 mg/kg of salicylate, respectively. Sodium salicylate, LD_{50} = 1200 mg/kg (891 mg/kg calculated as free acid) (33) appeared to potentiate the lethality of MDMA (LD_{50} = 49 mg/kg) (11,19). When rats were housed in the hanging metal cage, 3 rats died 2–3 h after MDMA (20 mg/kg) plus salicylate (200 mg/kg); all rats survived after MDMA with 100 or 50 mg/kg of salicylate. The lower lethality of MDMA plus salicylate observed in these experiments appeared to be due to the hanging metal mesh cages with the

TABLE 4

EFFECTS OF A SINGLE DOSE OF SALICYLATE (SA) (12.5–400 mg/kg, 1 H PRIOR TO MDMA) ON MDMA (20 mg/kg),-INDUCED DEPLETION OF 5-HT AND 5-HIAA IN THE BRAINSTEM AND STRIATUM OF RATS

		Brainstem (% Saline ± SEM)		Striatum (% Saline ± SEM)	
Treatment (mg/kg)	*n*	5-HT	5-HIAA	5-HT	5-HIAA
SAL + SAL	6	100.00 ± 8.99	100.00 ± 8.89	100.00 ± 6.04	100.00 ± 19.97
SAL + MDMA	6	98.19 ± 4.24	90.93 ± 9.26	93.05 ± 10.05	97.99 ± 21.34
SA(12.5) + SAL	6	91.51 ± 6.63	126.39 ± 17.38	93.48 ± 5.61	100.18 ± 18.69
SA(12.5) + MDMA	6	95.36 ± 4.65	86.26 ± 9.54	82.75 ± 12.64	93.97 ± 21.28
SA(25) + SAL	6	114.67 ± 8.71	110.39 ± 17.71	97.49 ± 7.51	96.54 ± 20.07
SA(25) + MDMA	6	107.58 ± 3.88	83.70 ± 5.83	102.46 ± 17.66	82.31 ± 20.83
SA(50) + SAL	6	105.82 ± 7.60	83.96 ± 14.53	84.72 ± 5.93	87.15 ± 16.78
SA(50) + MDMA	5	94.81 ± 8.03	91.23 ± 17.33	93.35 ± 7.73	83.61 ± 20.31
SA(100) + SAL	6	120.50 ± 10.44	98.91 ± 15.87	115.16 ± 15.71	115.60 ± 26.52
SA(100) + MDMA	2	98.82, 109.08	76.21, 100.81	76.49, 101.72	155.00, 103.58
SA(200) + SAL	6	114.02 ± 7.13	107.52 ± 12.58	86.05 ± 8.50	94.56 ± 20.04
SA(400) + SAL	6	102.75 ± 7.96	103.01 ± 11.36	96.39 ± 12.30	91.75 ± 18.19

Concentrations (pg/mg wet tissue) of 5-HT and 5-HIAA in the brainstem and striatum of saline-saline (SAL-SAL) control rats were in the same ranges as stated in Table 2. Keys for statistics are listed in Table 3.

opening at the top and chicken wire at the bottom and front panel. This construction provides better ventilation to the animals.

Effects of Salicylate and MDMA on Core Temperature

Core temperature of rats treated with saline and MDMA (20 mg/kg, SC) was significantly elevated by 1°C 2 h after injection of MDMA (Fig. 1). Treatment of MDMA plus 200 mg/kg of salicylate increased core temperature by 2.5°C and 3.5°C at 1 and 2 h after drug injection, respectively. Treatment of MDMA plus 100 mg/kg or 25 mg/kg of salicylate increased core temperature by 1°C at 3–6 h after drug injection (Fig. 1). Core temperature of rats treated with 20 mg/kg of MDMA plus 50 mg/kg of salicylate was not significantly different from that of saline controls. Administration of salicylate alone had no effect on core temperature.

DISCUSSION

The present results indicate that (a) a single injection of a moderate dose of salicylate potentiates MDMA-induced depletion of 5-HT and 5-HIAA in the frontal cortex and hippocampus and that (b) salicylate also potentiates MDMA-induced hyperthermia and lethality. These results have clinical relevance by highlighting the potential danger involved in using salicylate antipyretics for treatment of MDMA-induced hyperthermia. However, in a clinical setting, humans are treated post-MDMA ingestion; in the present study, rats were pretreated with salicylate. Hence, its possible effects on MDMA-induced hyperthermia are difficult to predict in humans.

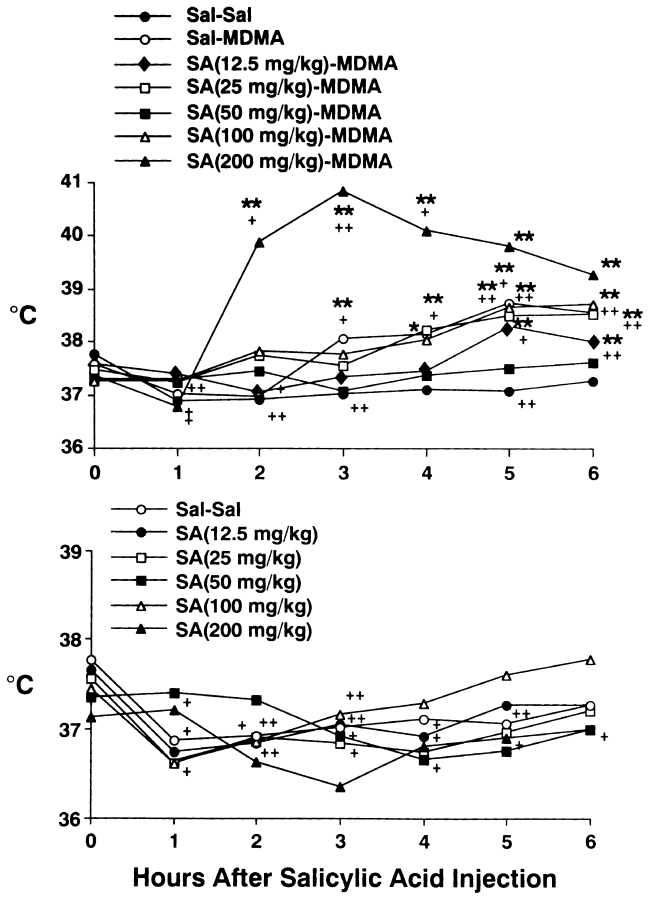

FIG. 1. Effects of MDMA (20 mg/kg, SC) and salicylate (SA) on the core temperature of rats ($n = 6$). MDMA was injected 1 h after the injection of salicylate. *$p < 0.05$, **$p < 0.01$ vs. saline; +$p < 0.05$, ++$p < 0.01$ vs. zero time.

Possible Mechanisms of MDMA-Induced Neurotoxic

The present results indicate that salicylate does not provide protection against MDMA-induced depletion of 5-HT and 5-HIAA contents. However, the effects of salicylate on MDMA-induced neurotoxicity might be complicated by hyperthermic effects of the drug combination. Lack of protection by salicylate on MDMA-induced neurotoxic effects suggests that MDMA-induced neurotoxic effects may not be due to hydroxyl free radicals because salicylate is a scavenger of hydroxyl radicals (6,31,61). Toxic metabolites of MDMA itself may be important factors in causing 5-HT depletion in rat brain. Possible ways by which MDMA might produce specific neurotoxicants are discussed in the following paragraphs.

Formation of neurotoxic metabolites THMA and THA from MDMA and MDA involves *O*-demethylenation of MDMA, MDA, 6-OH-MDMA and 6-OH-MDA. *O*-demethylenation of these compounds involves either superoxide or hydroxyl radicals (22,37). Because salicylate failed to protect against the MDMA-induced neurotoxic effects, formation of THMA and THA from MDMA may occur through auto-oxidation of DHMA and DHA, which is analogous to the auto-oxidation of phenylalanine (48). Auto-oxidation of DHA or DHMA produces a quinone that forms an adduct with glutathione (22). The adducts of DHMA and DHA with glutathione or other thiol compounds may cause depletion of 5-HT and 5-HIAA because ICV administration of 5-(glutathione-S-yl)-α-methyldopamine depleted of 5-HT, 5-HIAA, DA and NE (44). The 5-(glutathione-*S*-yl)-α-methyldopamine was rapidly metabolized to 5-(*N*-acetyl-*L*-cysteine-S-yl)-DHA, which has been implicated for MDMA-induced long-term neurotoxicity (43).

An alternative hypothesis for the formation of the oxidative metabolites of MDMA, THMA and THA from MDMA and MDA is via superoxide radicals. This idea is supported by the observation that CuZn-superoxide dismutase (CuZn-SOD) transgenic mice were protected from MDMA-induced depletion of 5-HT and 5-HIAA and lethal effects (4,5). We and others have demonstrated that MDMA-induced neurotoxic effects are blocked by the spin trapping agent, *n*-tert-butyl-α-phenylnitrone (PBN) (9,68,69), by lowering of body temperature, trapping of free radicals of reactive oxygen species and of MDMA-related metabolites. In addition, PBN also can increase glutathione peroxidase and reductase activity in vitro (65), glutamine synthetase (66) and protease activity in vivo (7). PBN scavenges hydroxyl, superoxide (8,25,26) and nitroxide radicals (29,32,46). Adducts of PBN with free radical metabolites of norcocaine, (e.g., norcocaine nitroxide) (39), ethanol and chloralkanes, such as CCl_3 (60), $CClCCl_2$ (50) and free radicals of halothane (28) and of 3-methylindole (30) have been detected in the bile after administration of these compounds. In addition, PBN attenuates 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) and 1-methyl-4-phenyl-pyridinum (MPP^+)-induced neurotoxicity in mice (59). Because PBN antagonizes MDMA-induced hyperthermia, slowing of MDMA metabolism could result in lower concentrations of MDMA toxic metabolites. Further discussion of mechanism involved in PBN protection against MDMA-induced neurotoxicity is presented elsewhere (69).

In addition to the possible free radical of metabolites of MDMA, those generated from catabolism of DA and 5-HT also could contribute to the MDMA-induced neurotoxicity. In vitro, oxidation of DA produces a quinone and an adduct with glutathione (20,44). This finding is supported by experiments that depletion of brain DA attenuates the toxic effects of MDMA (16,63). In addition, replacement of DA reinstituted the toxic effects of MDMA (58). Moreover, 5-HT reuptake blockers also block the toxic effects of this drug (53,54,57), suggesting that the toxic agents are taken up into 5-HT neurons via 5-HT transporters. The hydroxyl radical generated from the catabolism of DA and 5-HT may accelerate the demethylenation of MDMA and MDA to form their toxic metabolites.

The mechanisms involved in the potentiation of MDMA-induced depletion of 5-HT and 5-HIAA, hyperthermia and lethality by salicylate are not clear. The formation of glutathione adducts with salicylate metabolite 2,5-dihydroxybenzoic acid and MDMA metabolites DHMA and DHA is one possibility. Salicylate reacts with hydroxyl radicals to form 2,3- and 2,5-dihydroxybenzoic acids (6,61), which oxidize to a quinone and form an adduct with glutathione (38). The glutathione conjugate of 2,5-dihydroxybenzoic acid may cause depletion of 5-HT and 5-HIAA in the frontal cortex and hippocampus because such conjugates of DA and DHA deplete contents of 5-HT and 5-HIAA in the brain (44). Repeated administration of salicylate (12.5–100 mg/kg) decreased contents of 5-HIAA in the frontal cortex, sugesting that salicylate may inhibit metabolism of 5-HT to 5-HIAA.

CONCLUSION

Salicylate potentiated MDMA-induced hyperthermia. Repeated injections of MDMA or salicylate plus MDMA (10 mg/kg, twice daily for consecutive 4 days) decreased 5-HT and 5-HIAA contents in the frontal cortex, hippocampus and brainstem. These data suggest that salicylate affords no protection against MDMA-induced neurotoxic effects. Acute administration of salicylate (12.5–100 mg/kg) and repeated administration of salicylate (12.5–100 mg/kg) decreased levels of 5-HT and 5-HIAA in the frontal cortex. MDMA-induced neurotoxicity could be due to metabolites formed through interaction with superoxide rather than with hydroxyl free radicals.

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