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EFFECTS OF METHAMPHETAMINE ON CENTRAL MONOAMINERGIC SYSTEMS IN NORMAL AND ASCORBIC ACID-DEFICIENT GUINEA PIGS*

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Abstract—Repeated injections (s.c.) of methamphetamine (METH) were administered to normal and ascorbic acid-deficient (scorbutic) guinea pigs to assess a potential role for ascorbic acid in the METH-induced effects in central monoaminergic systems. The ascorbic acid-deficient condition differentially influenced the METH-induced responses of dopaminergic and serotonergic variables in the striatum: drug-induced changes in dopaminergic variables were identical in normal and scorbutic animals; METH-induced decreases in serotonergic variables [tryptophan hydroxylase activity, serotonin (5-HT) and 5-hydroxyindoleacetic acid concentrations], however, were prevented in scorbutic animals. The scorbutic condition did not alter significantly the distribution of METH in the brain, nor were striatal concentrations of dopamine (DA) or 5-HT affected. *In vitro*, ascorbic acid increased significantly DA-mediated [^{3}H]5-HT release from striatal slices, thus suggesting a potential role for ascorbate in DA-mediated actions of METH on serotonergic systems. Although supplemental ascorbate failed to restore the METH-induced serotonergic effects in scorbutic guinea pigs, these data suggest that, in a normal animal, the effects of multiple injections of METH, on serotonergic systems, involve ascorbic acid.

Repeated administration of amphetamine (AMPH§) or methamphetamine (METH) causes long-lasting changes in monoaminergic transmitter systems in various regions of the brain [1–3]. Notably, multiple doses of METH significantly decrease the activities (V_{max}) of the rate-limiting enzymes responsible for the synthesis of dopamine (DA) and serotonin (5-HT), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) respectively [4–7]. These effects on TH and TPH activities persist for 110 days after treatment with METH [3] and are presumed to result from a neurotoxic action of the drug [8]. In addition to these changes in enzyme activities, DA and 5-HT concentrations, as well as reuptake sites for these two neurotransmitters, remain decreased significantly for weeks after treatment with multiple doses of METH [8, 9]. Although the classification of AMPH and METH as indirectly-acting sympathomimetic amines is well established, it is unclear how the ability of these compounds to release monoaminergic transmitters produces long-lasting alterations in dopaminergic and serotonergic systems in the CNS.

Various lines of evidence implicate the neurotransmitter, DA, as an essential mediator for the persistent neurochemical changes observed in animals treated with METH [10, 11]. Although this transmitter has been described as a potential mediator of neurotoxicity in a number of situations [12, 13], it appears that the release of DA may not be solely responsible for the long-lasting neurochemical changes observed in animals treated with METH. Indeed, other agents (uptake blockers or L-DOPA) which should increase the release of monoamines (i.e. DA) fail to produce long-lasting changes in DA concentrations or in TH and TPH activities [11, 14]. Along with the release of monoamines, however, systemically administered AMPH also increases the release of ascorbic acid in the rat CNS [15–17]. Although a number of physiological functions of ascorbic acid are known, a well defined role for this compound in the CNS has not yet been established [18, 19]. With regard to the CNS effects of METH, however, it is possible that ascorbic acid contributes to the long-lasting neurochemical changes which appear to be mediated by DA. The auto-oxidation of dopamine to cytotoxic substances such as 6-OHDA and/or quinone-type molecules has been proposed as a mechanism for the neurotoxic effects of METH [20]. Indeed, the cytotoxic mechanisms of either 6-OHDA or related quinone-type substances can be influenced by the presence of ascorbic acid [21, 22].

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§ Abbreviations: AMPH, amphetamine; COMT, catechol-*O*-methyltransferase; DOPAC, dihydroxyphenylacetic acid; DA, dopamine; HPLC-EC, high performance liquid chromatography-electrochemical detection; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 6-OHDA, 6-hydroxydopamine; KRB, Krebs-Ringer buffer; L-DOPA, 1-dihydroxyphenylalanine; METH, methamphetamine; MAO, monoamine oxidase; 5-HT, serotonin; TPH, tryptophan hydroxylase; and TH, tyrosine hydroxylase.

Therefore, to determine if ascorbic acid plays a role in the dopaminergic and serotonergic effects of METH, we examined the neurochemical responses of normal and ascorbic acid-deficient (scorbutic) guinea pigs treated with multiple doses of METH.

In addition to examining monoaminergic changes, forebrain drug concentrations from drug-treated animals were also measured. Due to the altered nutritional status of the scorbutic guinea pigs, the levels of both METH and its N-demethylated metabolite, AMPH, were determined to verify that the brain distribution of METH was not significantly different between normal and ascorbate-deficient animals. In addition to the above-mentioned experiments, the effect of ascorbate on DA-induced release of 5-HT, *in vitro*, was examined. These experiments were conducted to determine if ascorbate could be involved in DA-mediated mechanisms which specifically alter serotonergic systems in a methamphetamine-treated animal.

METHODS

Drug administration. Female Hartley guinea pigs (450–550 g) were exposed to a 12-hr light–dark cycle and provided free access to food and water. The scorbutic condition was induced by maintaining animals on an ascorbate-free diet (ICN Nutritional Biochemicals, Inc.) for 21 days prior to METH treatment. Guinea pigs that received this diet displayed signs (alopecia, petechiae, weakness) which typically indicate an ascorbic acid deficiency. Although not included in the present paper, ascorbic acid concentrations in the striata of guinea pigs fed on similar diets and feeding schedules decrease to approximately 25% of normal levels [23]; ascorbate in the cerebral cortex also decreases in a similar manner (data not shown). Animals received saline or METH (7.5 mg/kg) once every 6 hr for a total of five doses and, for enzyme activity and transmitter studies, were decapitated 18 hr after the final injection (s.c.). Immediately following decapitation, brains were removed, and tissues from the cerebral cortex and neostriatum were dissected out and frozen on dry ice. Tissues were stored at −70° until assayed. In experiments conducted to determine forebrain drug concentrations, both normal and scorbutic animals were treated with METH, as described, but were killed 2 hr after the fifth injection. In addition to treatment with saline or METH, some scorbutic animals received supplemental ascorbic acid (145 mg/kg, pH 7.0) which was prepared just prior to administration (i.p). This supplemental administration of ascorbic acid restored both the striatal and cortical ascorbate concentrations to normal in scorbutic animals (data not shown).

Determination of monoaminergic variables. TH activity (V_{max}) was determined using a tritium release assay as described by Nagatsu *et al.* [24], while TPH activity (V_{max}) was measured by a modified $^{14}CO_2$ trapping method as described by Hotchkiss *et al.* [7]. The concentrations of striatal monoamines (DA and 5-HT) and their major metabolites were determined using high performance liquid chromatography with electrochemical detection (HPLC-EC). Briefly, tissues were homogenized in 0.5 ml of mobile phase containing monochloroacetic acid (0.2 M), EDTA (2.0 mM) and octane sulfonic acid (25 mg/L) in 12.5% methanol, pH 2.9. Samples were then centrifuged and filtered through a 0.2-μm Microfilter system (Bioanalytical Systems, Inc.). An aliquot (50 μl) of the sample was then injected onto a 3-μm Microsorb C-18 column (Rainin Instrument Co.) on a model 5000 Liquid Chromatograph (Varian) equipped with a model LC-4B Amperometric Detector (Bioanalytical Systems, Inc.).

Determination of forebrain drug concentration. Forebrain concentrations of METH and AMPH were measured by capillary gas chromatography-electron impact mass spectrometry using a Hewlett–Packard 5970A Gas Chromatograph-Mass Selective Detector*. Forebrains, rostral to the hypothalamus, were homogenized in 0.1 M perchloric acid and centrifuged. An aliquot of the supernatant fraction was then diluted to 2 ml and combined with 0.5 g sodium carbonate:sodium chloride (4:1) prior to extraction into *n*-butyl chloride. *N*-Propylamphetamine (0.4 μg) was used as an internal standard. After centrifuging, the organic layer was removed, 0.2 ml trifluoroacetic anhydride (TFA) was added, and the mixture was heated at 70° for 15 min. After cooling, the organic layer was evaporated to dryness (room temperature), 0.05 ml ethylacetate was added, and 1–2 μl was injected onto a 12.5-meter cross-linked dimethylsilicone (0.2 mm) capillary column. The ions monitored for the TFA derivatives were as follows: AMPH, 140 m/z; METH, 154 m/z; and *N*-propylamphetamine, 182 m/z. The sensitivity of this assay has been reported previously as approximately 0.17 ng/mg for 300 mg brain tissue for each compound [25].

Influence of ascorbic acid on DA-induced 5-HT release. Striatal slices were taken from 1-mm coronal sections of rat brain (male, Sprague–Dawley) and immediately incubated in 10 ml Krebs–Ringer buffer (KRB) containing 100 μM pargyline at 37°. After 10 min, the slices were transferred to 5 ml of KRB containing 10^{-7} M [^{3}H]5-HT (NEN, 6 Ci/mmol) for 15 min. After washing, the slices were sandwiched between nichrome screens and placed individually in eight scintillation vials containing KRB with 5 mM sodium ascorbate in half of the vials. After 20 min each slice was transferred to a new vial containing fresh KRB and 1 μM DA plus or minus ascorbate for an additional 20 min; a third 20-min incubation, in the presence or absence of ascorbic acid, followed. The slices were then removed, solubilized in Protosol (NEN), and counted to determine residual radioactivity. Release of radioactivity during each 20-min incubation was then corrected for and expressed as a fraction of total tissue radioactivity. Using the first incubation period as baseline, the percentage release of radioactivity above baseline in the second and third incubation were combined for slices either in the presence or absence of ascorbate. The efflux of radioactivity was verified to be authentic [^{3}H]5-HT by HPLC-EC. This *in vitro* demonstration of DA-induced release of 5-HT has been described previously [26] and was effectively anta-

* M. A. Peat, preliminary communication, cited with permission.

gonized by several agents which selectively inhibit 5-HT uptake systems. This DA-induced release of tritium was not affected by compounds that block the reuptake of catecholamines, thus demonstrating minimal amounts of release occurring from dopaminergic terminals.

Statistical analysis. Data were analysed using either a two-tailed Student's *t*-test or a one-way ANOVA followed by Duncan's analysis for multiple comparisons (0.05 alpha level).

RESULTS

METH-induced effects on striatal and cortical TPH activities in normal and ascorbic acid-deficient animals. The effect of repeated administration of METH (7.5 mg/kg, five doses) on striatal TPH activity in normal and ascorbic acid-deficient (scorbutic) guinea pigs is shown in Fig. 1. In saline-treated (control) scorbutic animals, striatal TPH activity did not vary significantly from that in normal control animals; however, in animals treated with METH, the drug-induced decrease in TPH activity (50%), which occurred in normal animals, was not observed in the ascorbic acid-deficient group. Striatal TPH activity in the METH-treated scorbutic guinea pigs was not reduced significantly (92%) compared to that in scorbutic controls.

METH-induced decreases of cortical TPH activity also differed between normal and scorbutic animals (Fig. 1). However, in contrast to striatal enzyme activities, these data were more difficult to interpret because, in two separate experiments, the scorbutic condition itself reduced cortical TPH activity, whereas striatal TPH, in these same animals, was unaffected. In the cortex, TPH activity was decreased in both normal and ascorbate-deficient animals treated with METH compared to their respective controls. In scorbutic animals, however, the METH-induced decrease of cortical TPH activity (65% of scorbutic controls) was markedly less than that observed in normal animals (23% of normal controls).

Effect of the scorbutic condition on METH-induced decreases of striatal 5-HT and 5-HIAA. In scorbutic controls, striatal concentrations of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), varied insignificantly from that in normal controls (Fig. 2). Treatment with METH, however, markedly decreased striatal concentrations of both 5-HT and 5-HIAA in normal animals yet had no significant effect on the concentrations of these substances in scorbutic animals. In normal animals treated with METH, striatal 5-HT and 5-HIAA concentrations were 46 and 51% of control respectively; in scorbutic animals, however, the administration of METH only decreased striatal 5-HT to 87% and 5-HIAA to 90% of their respective scorbutic controls. Although the METH-induced effects on striatal 5-HT concentrations have been reported previously [23], these data are included in the present paper to provide a more complete description of the response of the serotonergic system in METH-treated scorbutic animals.

Effects of multiple dose METH treatment on striatal concentrations of DA, its metabolites and TH activity. Unlike the effect of METH to influence differentially TPH activity, 5-HT and 5-HIAA concentrations in normal and scorbutic guinea pigs, striatal dopaminergic variables were affected similarly in both groups of METH-treated animals (Table 1). Comparisons between normal and scorbutic control animals did not reveal any significant difference in TH activity or concentrations of DA or its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Similarly, in treated animals, METH-induced reductions of TH activity or transmitter and metabolite concentrations were not markedly different in scorbutic compared to normal guinea pigs.

Effect of supplemental ascorbic acid in control and METH-treated scorbutic animals. To determine if the absence of METH-induced effects in the serotonergic systems in scorbutic animals was due specifically to the ascorbic acid deficiency, two groups of scorbutic animals received supplemental

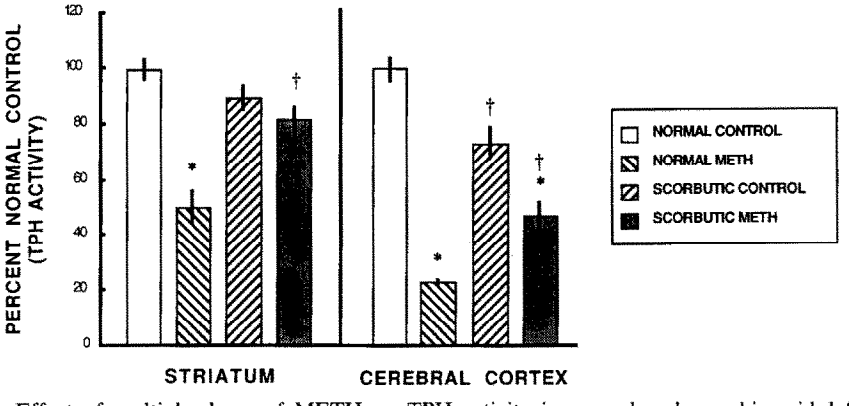

Fig. 1. Effect of multiple doses of METH on TPH activity in normal and ascorbic acid-deficient (scorbutic) guinea pigs. Animals (N = 4–9) received saline or METH (7.5 mg/kg, s.c.) every 6 hr for a total of five doses and were killed 18 hr after the final injection. Columns represent the average enzyme activity as a percentage of normal controls ± SEM. Control TPH activities in the striatum and cerebral cortex were 29.6 ± 1.0 and 19.9 ± 0.8 nmol tryptophan oxidized/g tissue/hr respectively. Key: (*) $P < 0.05$ (Duncan's test) compared to respective controls, and (†) $P < 0.05$ (Duncan's test) compared to corresponding non-scorbutic (normal) groups.

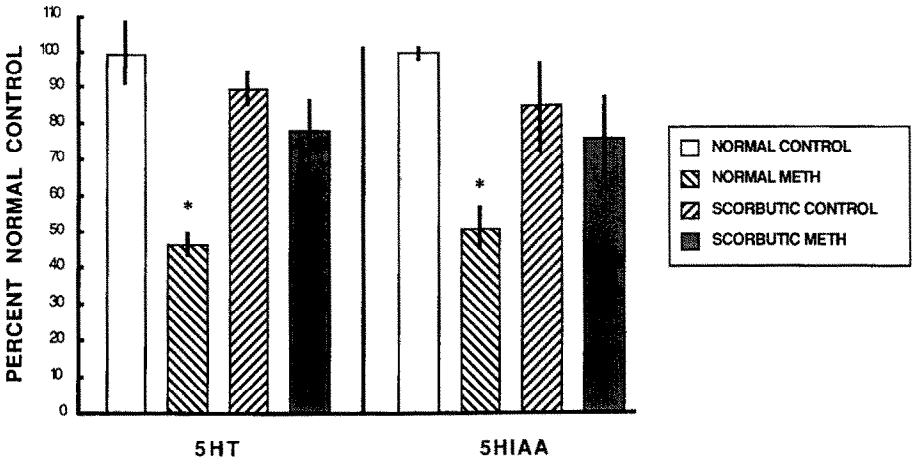

Fig. 2. Striatal 5-HT and 5-HIAA concentrations from METH-treated normal and scorbutic guinea pigs. Data were obtained from the same animals for which enzyme data are given in Fig. 1. Columns represent the mean concentrations of 5-HT (left panel) or 5-HIAA (right panel) as a percentage of normal controls ± SEM. Average values for 5-HT and 5-HIAA concentrations in normal control animals were 0.298 and 0.124 μg/g respectively. Key: (*) $P < 0.05$ (Duncan's test) compared to respective controls.

ascorbic acid (145 mg/kg, i.p.) at 12-hr intervals for a total of three doses. One group of these scorbutic animals also received treatment with METH as described above (7.5 mg/kg, five doses). In scorbutic animals, the supplemental ascorbic acid alone did not alter cortical or striatal enzyme activities (data not shown); nor were there significant changes in striatal 5-HT, 5-HIAA, DOPAC or HVA concentrations. Supplemental ascorbate did, however, significantly reduce striatal DA to 78% of that in scorbutic control animals (Fig. 3). With regard to the effects of METH on striatal transmitter concentrations, supplemental ascorbate did not alter significantly the METH-induced response of either 5-HT or DA in scorbutic animals (Fig. 3).

Although the ascorbate repletion process occurs gradually (ascorbate levels increase after each successive dose of ascorbate), three doses of ascorbate fully replete both striatal and cortical ascorbic acid concentrations [23, *] in scorbutic guinea pigs (this repletion process is not impeded by concurrent treatment with METH). However, to determine if METH-induced actions on serotonergic systems required normal concentrations of brain ascorbic acid throughout the entire treatment (METH) period, an additional experiment was performed. Scorbutic animals received six doses of supplemental ascorbic acid (12-hr intervals) such that three ascorbic acid doses were given prior to the co-administration of three more ascorbic acid doses along with METH; however, in METH-treated animals again, striatal TPH activity did not differ significantly from scorbutic controls (data not shown).

* L. A. Matsuda, unpublished observations.

Table 1. Effect of METH (7.5 mg/kg × 5) on striatal dopaminergic variables in normal and scorbutic animals

Treatment	TH activity	DA	DOPAC	HVA
	(%)	(%)		
Normal control (N = 4)	100 ± 5.1 (8328)	100 ± 7.3 (12.4)	100 ± 6.1 (2.29)	100 ± 9.5 (3.06)
Normal METH (N = 5)	58.8 ± 5.2*	40.3 ± 7.3*	62.0 ± 17.9*	50.0 ± 11.1*
Scorbutic control (N = 4)	99.1 ± 2.4	106.5 ± 2.4	95.2 ± 3.9	79.1 ± 6.5
Scorbutic METH (N = 9)	65.6 ± 1.9*	33.5 ± 3.2*	46.7 ± 5.2*	43.1 ± 5.2*

Numbers represent average values ± SEM as a percentage of normal control values and were obtained from the same animals used in Figs. 1 and 2. Absolute values for TH activity (nmol/g/hr) and transmitter or metabolite concentrations (μg/g tissue) are provided in parentheses.

* $P < 0.05$ (Duncan's test) compared to respective controls.

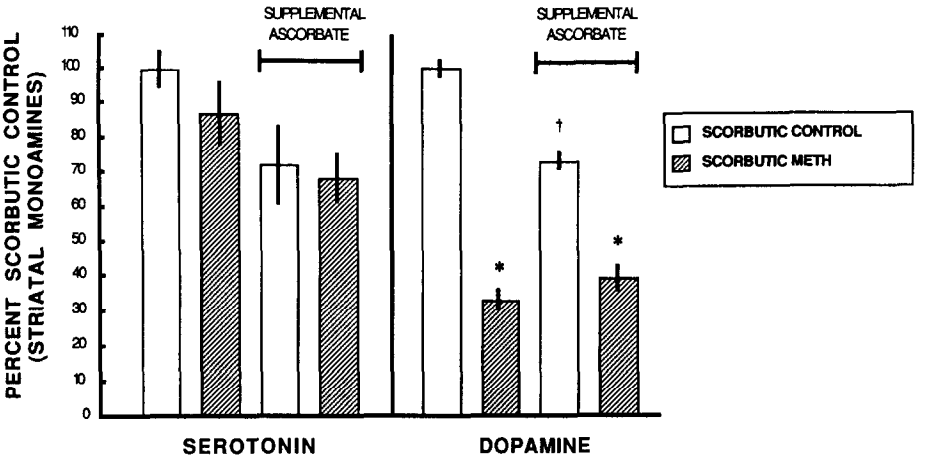

Fig. 3. Effect of supplemental ascorbic acid in saline- and METH-treated scorbutic animals. Guinea pigs (N = 4–9) were treated with saline or METH (7.5 mg/kg, five doses) with or without supplemental ascorbate (145 mg/kg, i.p.) every 12 hr for a total of three doses (the first dose of ascorbate was given 6 hr prior to the first dose of METH). Animals were killed 18 hr after treatment. Columns represent the average concentration of striatal 5-HT (left panel) and DA (right panel) as a percentage of scorbutic controls ± SEM. Mean values for striatal monoamine concentrations in control animals were 0.268 ± 0.015 and 13.2 ± 0.3 μg/g for 5-HT and DA respectively. Key: (*) $P < 0.05$ (Duncan's test) compared to respective controls, and (†) $P < 0.05$ (Duncan's test) compared to scorbutic controls.

Effect of ascorbate-deficiency on forebrain concentrations of METH and AMPH. A significant alteration of the distribution of METH in the brain, due to the ascorbate-deficiency in scorbutic animals, could account for the altered neurochemical response of the serotonergic system in METH-treated scorbutic animals; therefore, forebrain concentrations of METH and its demethylated metabolite, AMPH, were determined in normal and scorbutic animals treated with METH (7.5 mg/kg, five doses). Two hours after the last injection, average concentrations of METH did not differ significantly between the two groups (Fig. 4). Concentrations of AMPH, however, were dissimilar, levels in scorbutic animals being approximately 30% less than levels in normal animals. Addition of the concentrations of METH and AMPH for an individual animal revealed the total amount of drug in the forebrains of each guinea pig and, when averaged, demonstrated no significant difference between the distribution of drug into the brains of normal and scorbutic animals treated with METH.

Effect of ascorbic acid on DA-induced release of 5-HT in vitro. To determine more directly if ascorbic acid could influence the actions of DA on serotonergic systems, we observed the DA-induced release of 5-HT *in vitro* in the presence and absence of ascorbic acid. The release of [^{3}H]5-HT from pre-

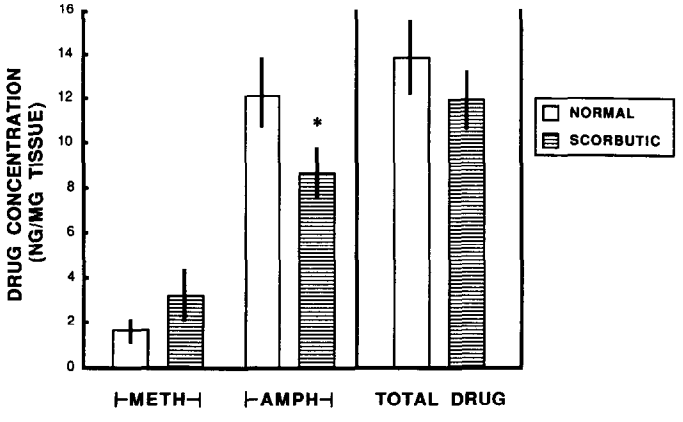

Fig. 4. Forebrain concentrations of METH and AMPH (ng drug/mg tissue) observed after repeated administration of METH to normal (control) and scorbutic guinea pigs. Animals received multiple doses of METH (7.5 mg/kg), as described previously, and were killed 2 hr after the fifth injection. Columns represent the average concentrations (N = 6) of METH, AMPH or total drug (METH + AMPH) ± SEM. Key: (*) $P < 0.05$ (two-tailed Student's *t*-test) compared to corresponding normal groups.

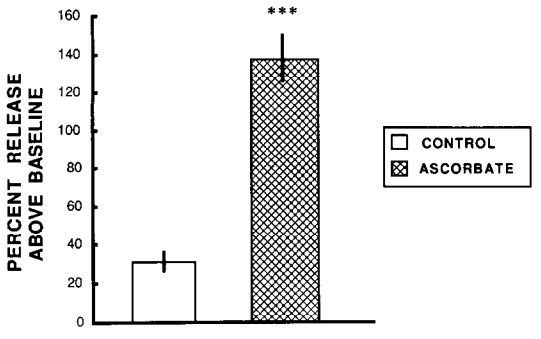

Fig. 5. Effect of ascorbic acid on DA-induced release of [^{3}H]5-HT. Striatal slices preloaded with [^{3}H]5-HT were exposed to DA (1 μM) in the presence (N = 8) or absence (control, N = 7) of ascorbate (5 mM). The release of tritium is expressed as the percent increase of fractional release above baseline (release in the absence of DA) from two identical experiments. Each value represents the average fractional release ± SEM. Baseline release values (percentage of total radioactivity in tissues) from the two experiments were 2.62 ± 0.24 (N = 7) and 2.60 ± 0.30 (N = 8). Key: (***) $P < 0.001$ (two-tailed Student's t-test) compared to control.

loaded striatal slices was increased significantly in tissues that were exposed to DA (1 μM) along with 5 mM ascorbate (Fig. 5). Although DA alone increased release by 33% above baseline (release in the absence of DA), in the presence of ascorbate DA-induced release of [^{3}H]5-HT was increased to 137% above baseline release. This DA-induced release of [^{3}H]5-HT was not affected by the dopaminergic receptor antagonist, fluphenazine, and in preloaded tissues exposed only to KRB containing ascorbic acid (data not shown), the fractional release did not differ significantly from release in the absence of ascorbic acid.

DISCUSSION

The present study revealed that dopaminergic and serotonergic systems responded very differently to treatment with METH in scorbutic compared with normal animals. In both normal and scorbutic METH-treated guinea pigs, decreases in dopaminergic variables were virtually the same (Table 1); however, the effects of METH on striatal and cortical TPH activity (Fig. 1) and striatal concentrations of 5-HT and 5-HIAA (Fig. 2) were attenuated significantly in scorbutic compared to normal animals. These findings suggest that different mechanisms are responsible for the effects of METH on dopaminergic and serotonergic systems.

It is noteworthy that the METH-induced serotonergic changes can be antagonized by several types of pharmacological strategies [1, 27, 28]; consequently, a scorbutic condition could possibly block the effects of METH by several different mechanisms. First, in order for METH to decrease serotonergic neurochemical parameters, DA must be present and released [10, 11, 27, 29, 30]. Thus, if the scorbutic condition interfered with METH-induced release of DA, attenuation of the serotonergic changes would occur. However, DA release is also necessary for the dopaminergic changes which occur following METH administration [27], and because this METH effect was not influenced by the scorbutic condition (Table 1) this is an unlikely explanation for these observations. Second, METH-induced changes in the serotonergic system are effectively antagonized by concurrent administrations of dopaminergic receptor antagonists [27, 30, 31]. Therefore, another possible mechanism for the blockade of METH-induced serotonergic changes in scorbutic animals is that ascorbate deficiency interferes with DA receptor activation. An argument against this possibility is that DA receptor antagonists also block the METH-induced changes in the dopaminergic systems, whereas the scorbutic condition in guinea pigs had no such effect. Finally, blockade of the 5-HT uptake system also prevents METH-induced serotonergic changes, but not corresponding dopaminergic changes [27, 28, 32]. This is because DA released by METH administration must be taken into serotonergic terminals in order to cause the decreases in TPH activity as well as the 5-HT and 5-HIAA concentrations [11, 26]. Consequently, if ascorbate deprivation selectively interfered with the 5-HT uptake carrier mechanism, the effect of METH on serotonergic parameters would be blocked without altering METH-related dopaminergic changes [33]. Our observations were consistent with this explanation (Table 1, Figs. 1 and 2). Further support of this possibility is our finding that ascorbate substantially enhanced the DA-mediated release of 5-HT from rat striatal slices while having no effect of its own on this system (Fig. 5). If the lack of ascorbate blocks the 5-HT uptake mechanism, the presence of ascorbate would be expected to stimulate this uptake carrier which would increase the movement of DA into 5-HT terminals [34] and increase the 5-HT release.

Another factor which might contribute to the present findings is the oxidative property of ascorbate. Although this is an area of some controversy, ascorbate has anti-oxidant action *in vitro* and might influence the *in vivo* oxidation and metabolism of substances such as DA [22, 35]. If this is true, the lack of ascorbate in scorbutic animals could cause more rapid oxidation and metabolism of DA, thereby interfering with those METH effects that are DA-mediated. However, the lack of an effect of ascorbate depletion on METH-related dopaminergic changes, which is also a DA-mediated phenomenon, argues against this possibility.

The inability to reverse the blockade of METH-induced serotonergic changes with supplemental ascorbate was unexpected. In a previous communication, we reported that METH-induced increases in the nigral concentration of the neuropeptide, substance P, do not occur in scorbutic animals. However, the METH effect on levels of substance P is restored in scorbutic animals treated with supplemental ascorbate [23]. Like the serotonergic responses, the METH-induced changes in substance P concentrations are mediated by DA

[36, 37]. The absence of a methamphetamine response by the 5-HT system in scorbutic animals, to ascorbate supplementation, was not due to a lack of ascorbic acid in the brain, as we found that such supplementation (three doses of 145 mg/kg ascorbate concurrent with METH treatment) fully restored ascorbic acid levels in the striatum and cerebral cortex. We also found that treating animals with three doses of ascorbate prior to METH administration (thereby replenishing ascorbic acid before drug treatment) failed to restore a drug-induced decrease in TPH activity (data not shown). Although the explanation is not presently clear, these results suggest that ascorbic acid has unique roles relative to DA, 5-HT and substance P transmitter systems and that attenuation of METH-induced serotonergic changes in scorbutic animals may be due to additional or secondary alterations that are not readily reversible with supplemental ascorbate treatment.

The observations presented in this paper suggest that ascorbic acid may maintain or facilitate some of the *in vivo* effects of METH. This notion, however, is inconsistent with other studies which suggest that ascorbate antagonizes effects of the amphetamines [38–40]. Such conflicting results may be due to differences in study designs (ascorbate administration versus depletion) which may result in qualitatively different ascorbate-induced effects in the CNS. Gardiner *et al.* [41] have reported varying effects of iontophoretically-applied ascorbate on striatal neuronal activity depending on the ejection currents used; these findings, therefore, suggest that the functional significance of brain ascorbic acid is highly concentration dependent. One other possible explanation for the conflicting data between these studies and the present findings is that the distribution of drugs in the brain may be altered significantly by the administration or depletion of ascorbate. However, in the present study, forebrain concentrations of total drug (METH + AMPH) did not vary significantly between normal and scorbutic METH-treated animals (Fig. 4), although the reduced levels of AMPH found in scorbutic guinea pigs suggest that the metabolism (N-dealkylation) of METH in ascorbate-deficient animals was decreased. While these data are consistent with reports of decreased cytochrome P-450 activity in scorbutic guinea pigs [42], it is not clear whether the lower concentration of AMPH could be responsible for the attenuated effects of METH in the serotonergic systems of scorbutic animals. Indeed, Warren *et al.* [43] reported that METH itself, rather than AMPH, is responsible for the long-term effects in the serotonergic systems of METH-treated rats. However, it is well known that the rat and guinea pig metabolize METH and AMPH very differently [44, 45]. Therefore, although the monoaminergic systems of these two species appear to respond similarly to treatment with METH, the consequences of reduced AMPH levels in the CNS of scorbutic guinea pigs could be significant and require further investigation.

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