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Extracellular Ascorbic Acid Increases in Striatum Following Systemic Amphetamine

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SALAMONE, J. D., L. S. HAMBY, D. B. NEILL AND J. B. JUSTICE, JR. *Extracellular ascorbic acid increases in striatum following systemic amphetamine.* PHARMACOL BIOCHEM BEHAV 20(4) 609–612, 1984.—Push-pull perfusion of the anterior striatum was performed in freely moving rats which were administered 4 mg/kg d-amphetamine sulfate. Ascorbic acid was measured in the perfusate using high performance liquid chromatography with electrochemical detection. Increased extracellular ascorbic acid resulted from the amphetamine, lasting over a period of two hours. The time course of the increase corresponded to the increased oxidation current measured by intrastriatal chonoamperometry under equivalent conditions.

Ascorbic acid Push-pull perfusion Amphetamine Striatum Dopamine Voltammetry Electrochemistry

AMPHETAMINE is a central nervous system stimulant and sympathomimetic agent whose neurochemical effects have been widely investigated. This drug increases release and blocks reuptake of dopamine (DA) and norepinephrine [5,10]. A recently demonstrated effect of amphetamine is an increased release of electroactive compounds from DA terminal areas of the brain as measured by in vivo voltammetric methods [1, 2, 6, 11, 15, 23]. Since DA is electroactive, this increase was initially attributed to amphetamine-induced release of DA [1,15]. However in addition to DA and its metabolites, there are a number of other easily oxidizable electroactive species present in the extracellular fluid of the striatum, including serotonin, 5-hydroxyindole acetic acid, and ascorbic acid.

The question of what compound or compounds contribute to the amphetamine-induced increases in electrochemical signals from the brain has generated conflicting evidence. In 1973, Kissinger *et al.* concluded that ascorbic acid was a principal source of the oxidation current observed during in vivo cyclic voltammetry [14].

Lane *et al.* [15] used an iodide-treated platinum electrode which distinguished between DA and ascorbic acid oxidation in the striatum. They reported that injection of amphetamine near the working electrode tip caused an increase in the DA, but not the ascorbic acid current. Amphetamine in concentrations from micromolar to millimolar was shown to induce release of DA, but not ascorbic acid, from striatal tissue slices as measured by liquid chromatography [1]. Gonon has developed an electrochemical method for differentiating ascorbic acid and DA [7,8]; his findings are in contrast to the earlier work [1,15]. Gonon *et al.* [8] reported that amphetamine increased the amplitude of the ascorbic acid peak obtained from striatum and suggested that since the extracellular concentration of ascorbic acid is much greater than that of DA, increases in signals obtained from nonselective electrochemical methods such as chronoamperometry [1, 6, 11, 23] are probably due to amphetamine-induced release of ascorbic acid. Support for this conclusion has come from Ewing *et al.* [4], who observed that amphetamine-induced increases in pulse polarographic signals obtained from striatum more closely resembled the contour of ascorbic acid oxidation than DA oxidation. Recently O'Neill *et al.* [21] summarized much of the information on in vivo voltammetry and experimentally resolved a number of points. In particular, they provided further voltammetric evidence that amphetamine increases extracellular ascorbic acid in the striatum.

In the present study, push-pull perfusion methods [3, 18–20] were used to determine the effect of amphetamine on endogenous extracellular ascorbic acid in striatum.

METHOD

Subjects

Eight male CFE rats (Charles River) weighing 300–350 g were used. The animals were housed individually and maintained on ad lib food (Rodent Lab Chow, Ralston Purina) and water. A 12 hour light/dark cycle (lights on 0800 hr) was

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maintained in the animal colony, and the temperature was kept at 72 degrees F. All testing was conducted during the light portion of the cycle (1200–1700 hr).

Surgery

Eight rats were each chronically implanted with a guide tube in the left anterior striatum at the following stereotaxic coordinates: AP 8.6, H 1.2, L 2.5 [22]. Rats were anesthetized with sodium pentobarbital (50 mg/kg) for this surgery. The guide tube was anchored to the skull using machine screws and dental cement. A dustcap with 23 gauge tubing in the center was used to plug the guide tube when the animal was not being perfused.

Push-Pull Perfusion

Repeated push-pull perfusions were obtained from rats by placing the removable push-pull cannula inside a chronically implanted guide cannula. The push-pull cannula was a concentric design similar to that previously described by Myers [18]. The inner push tube was 30 gauge stainless steel tubing, beveled at the tip. An outer pull side was 23 gauge stainless steel tubing. The inner tube protruded 0.5 mm beyond the outer tube. The guide tube consisted of 20 gauge tubing, the bottom of which was beveled to reduce tissue damage, while the top was cemented to the center of a 6-pin female connector (Plastic Products, Roanoke, VA). The push-pull cannula itself was cemented into the center of a complementary 6-pin connector. This allowed for firm attachment of the push-pull cannula to the guide tube.

Perfusion was performed with a Harvard Apparatus (Millis, MA) model 975 infusion pump, modified to accept two back-to-back 1 ml gastight syringes (Hamilton Co., Reno, NV). The syringes were attached to the push-pull cannula tubes with Teflon tubing (Small Parts Inc., Miami, FL). Perfusion fluid was artificial cerebrospinal fluid [20]. A flow rate of 17.1 μl/min was used during sample collection.

Six to eight days after surgery, rats were given a session of 3–5 small perfusions (about 100 μl) to clean out the area at the cannula tip. Two days later, rats were perfused to collect drug or control data. These animals were unanesthetized and freely moving within a 21 by 37 cm chamber on the treatment day. To collect a single sample, the push-pull cannula was placed in the guide tube and a perfusion at 17.1 μl/min was conducted for 10 minutes. The interval between the initiation of each sample perfusion was 25 minutes. After sample collection, 100 μl of perfusate were pipetted into an Eppendorf centrifuge tube containing 100 μl of 0.1 M perchlorate and 100 mg/liter EDTA. This solution was centrifuged for 2 minutes, then 100 μl of the sample were injected into the HPLC for measurement of the ascorbic acid content. Animals did not receive a drug treatment until a stable baseline was achieved. A baseline was considered established if, over three successive samples, the difference between each sample and the mean of all 3 was less than 25% of the mean. Four rats received 4 mg/kg d-amphetamine sulfate (Sigma) in a deionized water vehicle and 4 received injections of 1 ml/kg physiological saline (Abbott Laboratories) intraperitoneally. Ten minutes were allowed to elapse between injection and collection of the next sample. Nine samples were collected after injection.

Ascorbic Acid Analysis

The quantity of ascorbic acid in the samples was determined by using reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection. A C18 reverse phase column was used with an ISCO Model 825 HPLC pump. The detector consisted of a glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary electrode (Bioanalytical Systems, West Lafayette, IN). The potentiostat-amplifier was built in this laboratory and for all analyses the working electrode potential was maintained at +0.6 V relative to the reference electrode. Output of the detector was recorded on a strip chart recorder (Linear Instruments).

The mobile phase for the HPLC consisted of 0.1 M sodium acetate titrated to pH 4.0 with glacial acetic acid. Two hundred mg sodium EDTA was added per liter of mobile phase. Flow rate of the mobile phase was 1.0 ml/min. Standard solutions of ascorbic acid were prepared by dissolving ascorbic acid (L-(+)-ascorbic acid, Aldrich Chemical Company, Milwaukee, WI) in a 1:1 solution of 0.1 M perchloric acid and artificial cerebrospinal fluid (CSF; [18]). The perchlorate-CSF mixture was bubbled with nitrogen before ascorbic acid was added to remove dissolved oxygen. These standards gave a linear calibration curve with zero intercept over the range of interest (0–10 ng).

Ascorbic acid standards of 10 ng/100 μl were run before the first sample and between sample collections to ensure reproducibility. The ascorbic acid content of a sample was determined by comparing the peak height of sample ascorbic acid peak with that of the previous 10 ng standard.

Histology

After a day's perfusion, the animals were anesthetized with sodium pentobarbital and perfused with physiological saline followed by 10% formalin-saline. The brains were preserved in 10% formalin for several days, then blocked in the angle of the atlas [22]. Frozen sections 50 μm thick were cut through the area of the cannulas, mounted on microscope slides, and stained with thionin.

Data Analysis

The data are reported as nanograms of ascorbic acid per 100 μl of perfusate. The mean ascorbic acid content of the 3 samples prior to injection was considered the baseline. The mean ascorbic acid content of the first 5 samples after saline and amphetamine injection was also calculated and expressed as a percent of the baseline for each condition. The difference between saline and amphetamine treatments was compared using a two-tailed *t*-test.

RESULTS

Ascorbic acid was detected as a well resolved peak in the standard and sample solutions. The retention time for ascorbic acid was 4.9 minutes. Figure 1 contains chromatograms obtained under the following conditions: (a) 10 ng ascorbic acid in standard solution; (b) a sample obtained from perfusion of striatum, diluted 1:1 with 0.1 M perchloric acid and centrifuged for two minutes.

The average responses of all 8 freely moving animals to injections of saline or amphetamine are shown in Fig. 2. These data are expressed as the nanograms of ascrobic acid per 100 μl sample. Amphetamine injection was followed by an increase in ascorbic acid content of the perfusates to a level approximately twice that observed in the baseline

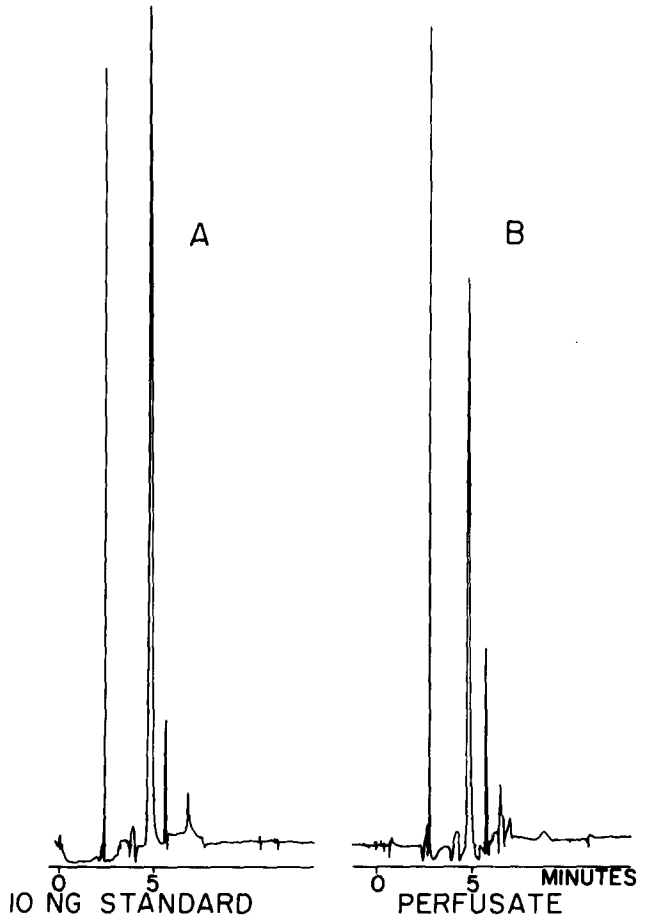

FIG. 1. Chromatograms of (A) 10 ng ascorbic acid standard and (B) perfusate. Retention time of ascorbic acid is 4.9 minutes. Conditions as described in text.

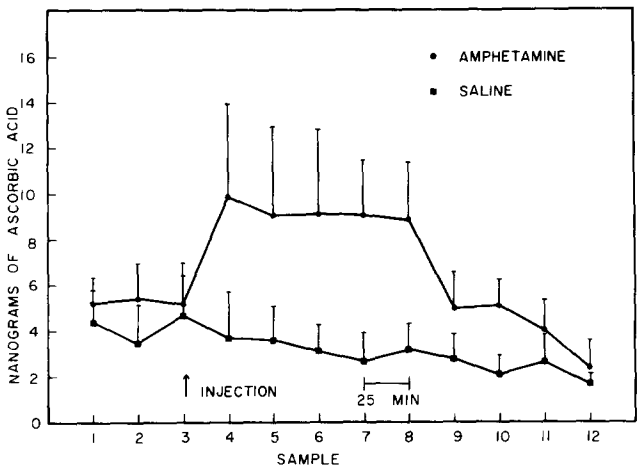

FIG. 2. Nanograms of ascorbic acid in saline (■) and amphetamine treated rats (●). There are four animals in each group. The data is expressed per 100 μl of perfusate.

period. This increase occurred in the first half hour following injection and lasted for about two hours. In contrast, injection of saline caused no increase in ascorbic acid levels. The ascorbic acid content of samples obtained from saline-injected animals showed a slight but consistent decline during the entire period of sample collection.

The changes in ascorbic acid content from the mean of the 3 baseline samples to the mean of the 5 samples after injection were compared for amphetamine and saline subjects. The percent of baseline ascorbic acid after injection was 169 percent (±27, S.E.M.) for the amphetamine group, and 66 percent of baseline (±16, S.E.M.) for the saline controls. The difference in these groups is statistically significant, $t(6)=3.27$, $p<0.02$, using a two-tailed t-test.

DISCUSSION

Injection of 4 mg/kg d-amphetamine resulted in an increase in extracellular ascorbic acid content in the striatum as sampled by the push-pull perfusion method. The time course of the increase was similar to that of the chronoamperometric signal observed with carbon epoxy electrodes following the same dose of amphetamine [23]. In the chronoamperometric data however, in which the sum of oxidation currents from all extracellular species oxidizing at the applied potential (+0.6 V vs. Ag/AgCl) is measured, the magnitude of the increase was about only 25 percent above baseline. The difference is due to the relatively poor response of ascorbic acid at the electrode and the effect of the other more responsive but less concentrated electroactive components, such as the dopamine metabolites DOPAC and HVA, which decrease over a similar time course following amphetamine (submitted for publication). The net effect is only a small increase in the chonoamperometric signal. The interpretation of in vivo voltammetric data using results obtained by perfusion methods is discussed more fully elsewhere [12].

The magnitude and time course of the increase is similar to the increase in ascorbic acid following 4 mg/kg methamphetamine observed with more selective in vivo electrochemical methods [8]. Our push-pull perfusion results confirm voltammetric results [4,8] that systemic amphetamine increases extracellular ascorbic acid in the striatum.

Other voltammetric studies have reported that amphetamine did not increase ascorbic acid release [1,15]. However, it is interesting to note methodological differences between the studies which report amphetamine-induced increases in ascorbic acid versus those that do not. In studies in which the amphetamine was administered systemically, an increased ascorbic acid release was found [4,8]. In the studies in which no increase was reported, the drug was applied directly to striatal tissue, either onto a tissue sample [1] or in vivo via cannula [15].

O'Neill *et al.* [21] have used linear sweep voltammetry at carbon paste electrodes to examine the chemical identity of the various voltammetric peaks which occur in recordings obtained from the striatum. They observed that amphetamine (5 mg/kg) caused an increase of 70 percent in the peak which they attribute to ascorbic acid when the amphetamine was administered IP, but that the ascorbate peak decreased when the drug was administered locally at the electrode tip through a cannula, a decrease which they attribute to dilution effects at the electrode tip. In the present

study, amphetamine was administered systemically, and an increased ascorbic acid release was found. Thus it appears that systemic or at least extrastriatal application of amphetamine is necessary to induce release of ascorbic acid from striatal tissue.

The relationship of ascorbic acid to amphetamine and the catecholamines is unclear. It has been shown to inhibit the specific binding of the dopamine agonists ADTN and apomorphine, but not the antagonists haloperidol and spiroperidol [13], and to have a strong, dose-dependent inhibitory effect on the binding of tritiated dopamine to neostriatal membrane preparations [9]. Ascorbic acid also blocks amphetamine-induced turning behavior in rats with unilateral nigrostriatal lesions [24,25]. The results of our experiment, coupled with the above reports, indicate that ascorbic acid may play a role in neostriatal dopaminergic transmission.

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