Failure of ascorbic acid to influence brain catecholamines in the guinea-pig[1]

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Summary. Lack of dietary ascorbic acid lowered plasma levels of ascorbic acid but failed to change levels of brain norepinephrine or dopamine.

Norepinephrine (NE) is regarded as a neurotransmitter in the central nervous system and ascorbic acid (AA) serves as a cofactor in the synthesis of NE[3]. In the adrenal gland, a deficiency of AA does not alter dopamine β-hydroxylase activity[4] nor lower NE levels[5]. Following changes in AA levels investigators[6,7] using the trihydroxyindole (THI) technique have reported changes in brain catecholamine (CA) levels. In a recent paper, Mells and Gustafson[8] reported that the administration of AA interfered with the measurement of NE if one is using the THI method. The brain contains a greater quantity of AA than it does CAs and the investigators using the THI technique with differing endogenous levels of AA may have incorporated errors in the measurement of CAs which were not identified. By using a weak cationic exchange column and high pressure liquid chromatography analysis, we were able to measure the levels of dopamine (DA) and NE without interference by AA. In this experiment NE and DA were studied in guinea-pigs under 2 dietary regimens to determine whether or not AA influences steady-state brain levels of these putative neurotransmitters.

Methods and materials. Male Hartley guinea-pigs (Charles River, Wilmington, Mass.) with an initial weight of 200–250 g were maintained on specific diets and water ad libitum. The basal diet was a pelleted AA free diet made according to specifications developed by Reid and Briggs[9]. Guinea-pigs were fed either the basal diet alone or a basal diet supplemented daily with an oral dose of AA (25 mg/100 g b.wt). Animals were sacrificed by decapitation after either 18 or 25 days. Their brains were quickly removed and frozen. Brains were stored at −50 °C until used in the assay. 2 brains were pooled for each assay of DA and NE. These brains were weighed and homogenized in 0.4 N perchloric acid. The supernatant was centrifuged, brought to pH 6.5 with K_2CO_3, and placed on a commercially available weak-cationic exchange column[10]. After 2 washings of the column with distilled water, the CAs were eluted with 4% boric acid, the eluate was titered to pH 2.2 with NaOH and injected in 100 µl portions into a high pressure liquid chromatography system. A reverse phase separation technique was used on a C_{18} μ Bondapak column (Waters); acetic acid (0.17 M, pH 3.2) served as the mobile phase. Peak heights were measured in UV absorbance at 280 nm and calibrated by internal standards in each assay run. Measurements of AA content in plasma and brain were performed on animals maintained on the diet for 18 days. Overall recovery values were determined at the end of the experiment by the addition of NE and DA standards to brain homogenates. Recoveries of CAs were 47.0+1.4% ($\bar{X}$+SEM, N=4) for DA and 45.5±2.4% ($\bar{X}$±SEM, N=4) for NE. Data are corrected for these recoveries. The AA was determined with a procedure similar to that of Roe[11]. Statistical significance was established by Student's t-test.

Results and discussion. Although plasma and brain AA levels decreased in guinea-pigs fed a deficient diet (table 1), no differences were found in the levels of CAs between deficient (0 mg/100 g, b.wt) and AA fed (25 mg/100 g, b.wt) groups (table 2); no difference in brain weights were observed in ascorbic acid fed and deficient guinea-pigs (3.57±0.29 g vs 3.60±0.9, g respectively). Deana et al.[7] reported that in the guinea-pig brain AA depletion decreased the level of NE and Izquierdo et al.[6] reported that in the rat the i.p. injection of AA increased the level of NE in the brain. Since both authors used a version of the THI technique the validity of their findings must be questioned in view of the fact that AA may interfere with the accurate determination of NE[8]. Our findings demonstrate that no changes occur in the steady-state levels of CAs in AA-deficient guinea-pigs.

This report presents results demonstration that no changes occur in brain CA levels during AA deficiency and lends no support to the concept of a unique function of AA in brain CA metabolism.

Table 1. Tissue levels of ascorbic acid in guinea-pigs fed for 18 days

AA (mg/100 g b.wt per day)	Plasma (µg/ml)	Brain (µg/g)
0	1.8 ±0.13 (7)	82.9±7.12 (7)
25	6.68±0.33* (5)	285.9±9.57* (5)

Values are expressed as means ± SEM, number of animals per group is indicated in parenthesis. Plasma AA and brain AA values are significantly different between 0 and 25 mg/100 g b.wt per day AA groups (* $p<0.001$).

Table 2. Effect of ascorbic acid deficiency on plasma ascorbic acid and brain dopamine and norepinephrine levels following 25 days of dietary regimen

AA (mg/100 g b.wt per day)	Plasma AA (µg/ml)	DA (ng/g)	NE (ng/g)
0	2.36±0.32 (8)	629±25 (8)	394±42 (8)
25	10.65±1.16* (8)	626±37 (7)	454±55 (7)

Values are expressed as means ± SEM, number of assays is indicated in parenthesis. Plasma AA values are significantly different between 0 and 25 mg/100 g b.wt per day AA groups (* $p<0.001$).

1 Investigators adhered during the research described in this report to the policy set forth in the 'Guide for the Care and Use of Laboratory Animals' by the Committee on Revision of the Guide for Laboratory Animal Resources, National Research Council.

2 The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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