Age-Related Changes in Sympathetic Nervous Activity of Rats Receiving Vitamin E-Deficient Diet

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Summary

To study the relationship between age-related stimulation of sympathetic nervous activity and vitamin E, excretion of urinary catecholamine and the contents of organ catecholamine were measured in rats receiving a vitamin E-deficient or control diet for 95 weeks. Rats exhibited about 95% hemolysis after 4 weeks on the vitamin E-deficient diet and this value remained the same for 95 weeks. α-Tocopherol in plasma was not detectable in the deficient diet-fed rats, and lipid peroxide concentrations in the plasma, liver and adrenal glands of rats receiving the vitamin E-deficient diet for 95 weeks were 3- to 30-fold higher than those of control rats. Urinary excretion of catecholamine (norepinephrine (NE), epinephrine (E) and dopamine (DA)) increased with age. Excretion of NE in 24-h urine of rats receiving the vitamin E-deficient diet for 50 and 95 weeks was 2- to 3-fold higher than that of control rats, although no significant difference was observed at week 12. Contents of NE and E in the adrenal glands and of NE in the heart from the deficient rats were significantly lower than those of control rats at week 95. These results suggest that sympathetic nervous activity is enhanced in aged rats and that the sympathetic nervous activity in vitamin E-deficient rats is greater than in control rats.

Key Words vitamin E, catecholamine, sympathetic nervous activity

Vitamin E plays an important role in the maintenance of normal neural structure and function. Several workers have reported on neurological abnormalities responsive to vitamin E in abetaproteinemia, chronic liver disease and cystic fibrosis in which fat absorption was blocked and steatorrhea was present (1–6). The neurological changes in vitamin E-deficient humans are similar to those in rats (7–9). Chronic vitamin E deficiency in rats resulted in remarkable alterations of axon terminals in the central and peripheral nervous systems referred to as dystrophic axonal changes (1, 10–12). These changes have also been reported to increase with age in normal humans and animals (12, 13).

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The plasma catecholamine concentration and urinary excretion of catecholamine are considered to be general markers of the sympathetic nervous system activity level (14). Several studies have reported that catecholamine metabolisms were altered with the aging process (14–18). There is a tendency for organ NE content to decline in ad libitum-fed 24-month-old rats (15). These findings agree with earlier anatomical descriptions of age-related degeneration of the nervous system (19, 20).

In order to clarify the role of vitamin E in age-related changes of sympathetic nervous activity, urinary excretion of catecholamine was compared in rats receiving a vitamin E-deficient diet for 12, 50 and 95 weeks. In this report we describe the age-related increase in activity of the sympathetic nervous system and increased sympathetic nervous activity of rats receiving a vitamin E-deficient diet.

METHODS

Diet. AIN-76 purified diet with vitamin E omitted (vitamin E-deficient diet) was prepared as described by the Council of the American Institute of Nutrition (21, 22). The composition of the vitamin E-deficient diet is shown in Table 1. Control rats were fed an AIN-76 purified diet.

Animals. The experiment was conducted using 4-week-old male Sprague-Dawley rats, each weighing about 60 g. They were housed in plastic cages with sawdust floors in a temperature-regulated (24 ± 2°C), light-controlled (light on 0700–1900 h) room. They were divided into two groups of 15 animals per group. One group was fed the vitamin E-deficient diet and the other group received the AIN-76 purified diet (control diet). All of the animals received food and water ad libitum. Weights were recorded weekly during the experimental period.

To evaluate the vitamin E status, rats receiving the vitamin E-deficient diet and control diet were bled from the tail vein at weeks 1, 2, 3, 4, 5, 7, 50, and 95. Two to five drops of blood were mixed with citrate-NaCl solution (0.5 g of sodium citrate and 0.45 g of NaCl per 100 ml). The solution was centrifuged at 3,000 rpm for 3 min.

Table 1. Composition of vitamin E-deficient diet.

| Ingredient                        | %    |
|-----------------------------------|------|
| Casein (vitamin-free)             | 20.0 |
| DL-Methionine                     | 0.3  |
| Sucrose                           | 50.0 |
| Cornstarch                        | 15.0 |
| Cellulose powder                  | 5.0  |
| Corn oil (tocopherol-stripped)    | 5.0  |
| Mineral mix.                      | 3.5  |
| Vitamin mix. (tocopherol-free)    | 1.0  |
| Choline bitartrate                | 0.2  |

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and red blood cells (RBC) were collected and used for evaluation of hemolysis.

At weeks 12, 50 and 95, both groups of rats were transferred to metabolic cages and 24-h urine was collected in 5 ml of 3 N HCl solution for 5 days. Urine was centrifuged at 5,000 × g and then the supernatant fluid was stored at −20°C after adding EDTA (disodium ethylene diamine tetraacetate) (36 µg/ml urine) and reduced glutathione (24 µg/ml urine). An aliquot of each urine specimen was used to determine the catecholamine content. At week 96, the rats were sacrificed by decapitation, and the whole brain, adrenal glands, heart and liver were immediately removed and homogenized in ice-cold 0.4 N perchloric acid with EDTA (0.5 g/liter) and sodium metabisulfate (1 g/liter) added as antioxidants and dihydroxybenzylamine (DHBA) added as an internal standard.

Analytical procedure. 0.4 N perchloric acid homogenates of the adrenal glands, brain and heart were centrifuged at 12,000 × g for 15 min and the supernatants were collected. Two molar Tris-HCl (pH 8.6) and acid-washed alumina (23) were added to the supernatant fluid to adsorb catecholamine and DHBA. After washing with water, catecholamine and DHBA were eluted with 0.1 N perchloric acid, and quantitated by high performance liquid chromatography (HPLC) (Zorbax ODS, Dupon Instruments) with electrochemical detection (LC-304, Bioanalytical System, Inc.).

The onset of vitamin E deficiency was evaluated by the hemolysis test. RBC were assayed for H2O2 peroxidative hemolysis by the partially modified method of Lubin et al. (24).

To measure the concentration of α-tocopherol and lipoperoxide, the brain, liver and adrenal glands of 99-week-old rats were homogenized in 5 volumes of ice cold water. Tocopherols were extracted from homogenate with n-heptan. α-Tocopherol contents in the plasma and tissues were determined by HPLC as described by Bieri et al. (25).

The concentration of lipid peroxide in plasma and tissues was determined by the thiobarbituric acid test as described by Yagi (26).

RESULTS

Effect of vitamin E on growth of rats

The change in body weight of rats over the 95-week period is shown in Fig. 1. There were no appreciable differences in body weight and general appearance between the rats receiving the vitamin E-deficient diet and the control diet for 30 weeks. After 30 weeks, the body weight of rats receiving the vitamin E-deficient diet showed a slight decrease, but rats receiving the control diet steadily gained weight throughout the experimental period. Although no significant difference was observed in body weight between these two groups for 50 weeks, the final body weight of rats receiving the vitamin E-deficient diet for 95 weeks was significantly lower than that of rats receiving the control diet.

After 32 weeks, almost complete paralysis of the hind legs developed in rats...
Fig. 1. Growth of rats receiving vitamin E-deficient (■) or control diet (●). Values are means for 12 rats. * Values are means for 8 rats.

Fig. 2. Hemolysis of rats receiving vitamin E-deficient (■) or control diet (●). Values are means for 12 rats. * Values are means for 8 rats.

receiving the vitamin E-deficient diet. At the same time, the rats appeared unkempt and emaciation was usually noticeable.

*Evaluation of vitamin E deficiency*

The onset of vitamin E deficiency was detected by the peroxide hemolysis test. The animals receiving the vitamin E-deficient diet exhibited about 95% hemolysis after 4 weeks on the diet, which remained for 95 weeks. Erythrocytes from rats receiving the control diet exhibited approximately 10% to 20% hemolysis throughout the study (Fig. 2).

The concentrations of \( \alpha \)-tocopherol in the plasma, liver, brain and adrenal glands of both group of rats are presented in Fig. 3. In rats receiving the vitamin E-
deficient diet, no $\alpha$-tocopherol was detected in the plasma, liver or adrenal glands. However, in control rats, the $\alpha$-tocopherol concentration was 0.28 mg/100 ml in plasma, 2.9 mg/100 g in liver and 29.3 mg/100 g in adrenal glands. The $\alpha$-tocopherol concentration in the brain of rats receiving the vitamin E-deficient diet was about

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25% that of rats receiving the control diet.

The lipid peroxide concentration in the plasma and tissues of rats after receiving the vitamin E-deficient diet for 95 weeks is shown in Fig. 4. The plasma lipid peroxide concentration was 10-fold higher in rats receiving the vitamin E-deficient diet as compared to rats receiving the control diet. Liver and brain lipid peroxide concentrations in rats receiving the vitamin E-deficient diet was about 3-fold higher than that of rats receiving the control diet. Lipid peroxide concentration in adrenal glands of rats receiving vitamin E-deficient diet increased 22-fold compared to that in rats receiving the control diet.

**Urinary output of catecholamine**

Urinary output of catecholamine was determined in 24-h urine samples obtained from both groups of rats at 12, 50 and 95 weeks (Fig. 5). In control rats, urinary excretion of NE in aged rats (54 and 99 weeks old) was higher than in young rats. However, there was no significant difference in urinary NE excretion between the rats receiving the control diet for 50 and 95 weeks. In rats receiving the vitamin E-deficient diet, urinary NE excretion increased with age, and excretion at weeks 50 and 95 was 3- to 4-fold higher than that of rats which had received the diet for 12 weeks. No significant difference was observed in 24-h NE excretion between rats receiving the vitamin E-deficient diet or control diet for 12 weeks. However, at 50 and 95 weeks, rats receiving the vitamin E-deficient diet had 2- to 3-fold higher NE urine values than rats on the control diet.

**Fig. 5.** Urinary excretion of norepinephrine, epinephrine and dopamine of rats receiving vitamin E-deficient or control diet for 12, 50 and 95 weeks. Twenty-four-h urine was collected for 5 days. Values are mean ± SE for 5 days, 8 animals per group.
Analysis of E and DA contents in 24-h urine in both groups revealed a
significant increase with age (Fig. 5, B and C). It was found that E and DA levels in
24-h urine in 54-week-old rats were significantly higher than those of 16-week-old
rats. Moreover, E and DA contents in 24-h urine of 99-week-old rats were higher
than those of 54-week-old rats in both groups. In rats receiving the vitamin E-
deficient diet for 95 weeks, excretion of E in the urine was significantly higher than
that of control rats. However, no significant difference in urinary E excretion was
observed between rats receiving the vitamin E-deficient diet or the control diet for
12 and 50 weeks. There was no significant difference in urinary DA excretion
between the two groups of rats at 12, 50 and 95 weeks.

Catecholamine content in tissues

Table 2 shows the catecholamine contents in the adrenal glands, heart and
brain in vitamin E-deficient rats and control rats at 95 weeks. There was no
significant difference in adrenal gland or brain weight between the rats receiving the
vitamin E-deficient diet and the control diet, although the heart weight of vitamin
E-deficient rats was lower than that of control rats. However, as the body weight of
rats receiving the vitamin E-deficient diet for 95 weeks was significantly lower than
that of control rats, the relative weights of adrenal glands and brain of vitamin E-
deficient rats were larger than those of control rats. No significant difference was
observed in heart weight/body weight between the two groups.

Contents of NE and E in the adrenal glands, and of NE in the heart, of rats

Table 2. Catecholamine content in adrenal glands, heart and brain of rats receiving
vitamin E-deficient diet or control diet for 95 weeks.

| Vitamin E in diet |   -   |   +   |
|------------------|-------|-------|
| Adrenal glands   |       |       |
| Weight (mg)      | 57 ± 6| 63 ± 5|
| (mg/100 g body weight) | 16 ± 2| 10 ± 2|
| NE (µg/adrenal glands) | 11.6 ± 0.8| 15.7 ± 1.1|
| E (µg/adrenal glands) | 55.4 ± 7.2| 73.9 ± 6.0|
| Heart            |       |       |
| Weight (g)       | 5.3 ± 0.4| 6.7 ± 0.4|
| (g/100 g body weight) | 1.3 ± 0.3| 1.1 ± 0.4|
| NE (µg/heart)    | 0.37 ± 0.05| 0.53 ± 0.04|
| Brain            |       |       |
| Weight (g)       | 2.1 ± 0.2| 2.3 ± 0.2|
| (g/100 g body weight) | 0.50 ± 0.3| 0.38 ± 0.2|
| NE (µg/brain)    | 0.60 ± 0.08| 0.58 ± 0.09|
| DA (µg/brain)    | 1.72 ± 0.21| 1.93 ± 0.24|

Values are mean ± SE, 8 animals per group.

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receiving the vitamin E-deficient diet for 95 weeks were significantly lower than those of rats receiving the control diet. However, there was no significant difference in NE and DA contents in the brain between the vitamin E-deficient and control rats.

**DISCUSSION**

Assessment of sympathoadrenal medullary activity in animals is usually made by determining serum and urinary free catecholamine levels (14). Adrenal medullary secretion is assessed in rats by measuring urinary excretion of E (27, 28). Moreover, Young and his co-worker have demonstrated that urinary excretion of NE reflects the activity of the sympathetic nervous system under ordinary conditions (29, 30). The results of this study indicated that there was no significant difference in urinary excretion of NE, E and DA between rats receiving the vitamin E-deficient and the control diets at 12 weeks. However, urinary excretion of NE in rats receiving the vitamin E-deficient diet at 50 weeks was higher than that of rats receiving the control diet. Urinary excretion of NE and E in rats receiving the vitamin E-deficient diet at 95 weeks was also higher than those of rats receiving the control diet. The most interesting finding obtained here was that aged rats receiving the vitamin E-deficient diet excreted a large amount of NE into the urine compared with rats receiving the control diet (Fig. 5). Moreover, NE and E contents in adrenal glands and NE in the heart slightly but significantly decreased in rats receiving the vitamin E-deficient diet for 95 weeks. Therefore, it is considered that the activity of sympathetic nervous system was enhanced by chronic vitamin E deficiency in aged rats.

Several recent reports have suggested that chronic vitamin E deficiency in the rat produces a remarkable alteration of axon terminals in the central and peripheral nervous systems referred to as dystrophic axonal changes (10–12). Nelson et al. suggested that chronic vitamin E deficiency in rats, monkeys and humans leads to degeneration and loss of sensory axons in the posterior columns, sensory roots and peripheral nerves (7). The degenerative changes in axons are considered to alter nervous function in vitamin E-deficient animals. Several nervous disorders such as loss of reflexes, unsteady gait, loss of position sense, deposition of position sense and paralysis have been reported during vitamin E deficiency (1–6). Increased sympathetic nervous activity observed here may be due to alteration of the nervous function in vitamin E-deficient animals.

The mechanism of two axonal changes induced by vitamin E deficiency is not known. As shown in this paper, rats receiving the vitamin E-deficient diet had almost no detectable levels of α-tocopherol in plasma and tissues (Fig. 3). Lipid peroxide levels in the plasma and tissues were significantly higher in vitamin E-deficient rats compared to control rats (Fig. 4). As vitamin E appears to have a protective role for membrane integrity (31, 32), it has been suggested that this membrane effect is mediated by two distinct functions of vitamin E: free radical
scavenging and structure stabilization. In vitro, and probably in vivo, vitamin E acts as an antioxidant and may maintain the stability of the biological membrane. The mode of action of vitamin E on the nervous system has not been established. However, it would be interesting to examine why the axonal membrane or myelin sheath is particularly susceptible to vitamin E deficiency.

The results obtained in the present study have clearly demonstrated that stimulation of the sympathetic nervous system was observed in aged rats, and particularly in aged rats receiving the vitamin E-deficient diet. Sharma et al. and Pentschew and Schwartz have demonstrated that the axonal changes of the central and peripheral nervous systems described in vitamin E deficiency are similar to those seen in aging animals (12, 33). Therefore, it is reasonable to assume that the axonal changes result from the lack of the protective effect of vitamin E on nerve structures with premature aging. Paralysis of the hind legs was observed in rats receiving the vitamin E-deficient diet over 32 weeks and degenerative changes in nerve fibers have been reported in vitamin E-deficient animals (10–12). Further study is necessary to determine the relationship, if any, between neuropathological changes and neurological function.

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