Effect of Diet on Sympathetic Nervous System Activity in Chronic Vitamin E-Deficient Rats

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Summary This study was undertaken to examine the responsiveness of the sympathetic nervous system of chronic vitamin E-deficient rats to dietary changes. Rats receiving a vitamin E-deficient diet exhibited about 95% hemolysis after 4 weeks on the vitamin E-deficient diet and this value was maintained up to 18 months. α-Tocopherol in serum was not detectable in rats receiving the vitamin E-deficient diet for 18 months. Lipid peroxide concentration in serum of rats receiving the vitamin E-deficient diet for 18 months was 10-fold higher than that of control rats. Basal levels of urinary norepinephrine (NE) and epinephrine (E) excretion in chronic vitamin E-deficient rats was 2- to 3-fold higher than those of control rats. In control rats, urinary NE excretion declined during fasting and this decline was reversed upon refeeding. Urinary excretion of NE in control rats increased upon glucose feeding. However, in chronic vitamin E-deficient rats, no change was observed upon fasting and glucose feeding in either urinary NE or E excretion. These results suggest that the increase in basal levels of urinary NE excretion in chronic vitamin E-deficient rats was not influenced by dietary manipulation.

Key Words vitamin E-deficient, catecholamine, sympathetic nervous activity, dietary manipulation

Previous studies from our laboratory measuring urinary catecholamine excretion have demonstrated that stimulation of sympathetic nervous system activity was observed in aged rats receiving a vitamin E-deficient diet (1). Plasma catecholamine concentration and urinary excretion of catecholamine are considered to be general markers of the activity level of the sympathetic nervous system (2). Several studies have reported that catecholamine metabolism are altered by the aging process (2-6). There is a tendency for the organ NE content to decline in ad lib-fed 24-month-old rats (3). These findings agree with earlier anatomical descriptions of age-related degeneration of the nervous system (7, 8).
Alteration in dietary intake exert an important influence on the activity of the sympathetic nervous system in experimental animals and humans (9). Sympathetic nervous system activity is suppressed by fasting or caloric restriction (10–14). Whereas overfeeding of sucrose and glucose stimulate the sympathetic nervous system activity in experimental animals and humans (12–15). The studies described in this report were designed to examine the functional state of sympathetic nervous system in chronic vitamin E-deficient rats. The experiment was undertaken to determine whether fasting or glucose feeding affected urinary excretion of catecholamine in chronic vitamin E-deficient rats.

METHODS

Diet. The 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 (16, 17). The composition of the vitamin E-deficient diet is shown in Table 1. Control rats were fed the AIN-76 purified diet supplemented with 50 IU of DL-α-tocopherol acetate per kg.

Animals. Four-week-old Sprague-Dawley male albino rats, each weighing about 60 g at the start of the experiment were used. They were separated into two groups and fed the vitamin E-deficient diet and the AIN-76 purified diet, respectively. They were housed in plastic cages with sawdust floors in a temperature-regulated (22 ± 2°C), light-controlled (light on 0700–1900 h) room. All of the animals received food and water ad libitum. Weights were recorded weekly during the experimental period.

To evaluate plasma vitamin E states, rats receiving the vitamin E-deficient and control diets were bled from the tail vein at weeks 2, 4, 6 and 8. Two to five drops of blood were dropped into 2 ml of sodium 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, and red blood cells (RBC) were collected and used for evaluation of hemolysis.

Table 1. Composition of vitamin E-deficient diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (vitamin-free)</td>
<td>20.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>15.0</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn oil (tocopherol-stripped)</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral mix.</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix. (tocopherol-free)</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Eighteen months after the initiation of feeding on the experimental diets, both groups of six rats each were transferred to other clean, stainless-steel wire cages and 24-h urine was collected. After a 5-day acclimatization period in the metabolic cages when the urinary excretion of catecholamine had become constant, the experiment on fasting and glucose feeding started.

The two groups of rats receiving the vitamin E-deficient and control diets were fasted for 3 days and refed the same diet thereafter. Five days after refeeding, glucose solution (4 mmol/100 g body weight/day) was given intragastrically to both groups of rats for 3 days. Collection of 24-h urine continued during these experimental period as described previously (1). An aliquot of each urine specimen was used for analysis of catecholamine.

At the end of the glucose feeding experiment, all of the rats were decapitated. Four to five drops of blood were dropped into 2 ml of sodium citrate-NaCl solution and remaining trunk blood was collected in another centrifuge tubes. The sodium citrate-NaCl solution was centrifuged and red blood cells were used for evaluation of hemolysis. The serum obtained after centrifugation was used for the analysis of α-tocopherol and lipid peroxide. For use in the analysis of catecholamine, the adrenal glands, brain and heart were quickly removed and immediately homogenized in ice-cold 0.4 M perchloric acid with dihydroxybenzylamine (DHBA) added as an internal standard as described previously (1).

Analytical procedure. Urinary catecholamine was determined in 24-h urine samples obtained from the rats while in metabolic cages as described previously (1). To measure catecholamine concentration in tissues, catecholamine and DHBA in the perchloric acid supernatant were adsorbed into acid-washed alumina (18) at pH 8.6 and eluted with 0.1 M perchloric acid. Catecholamines and DHBA in the eluate were isolated using high performance liquid chromatography (HPLC) (Zorbax ODS, Dupon Instruments) and quantitated by 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 methods of Lubin et al. (19). Tocopherols extracted from serum were determined by HPLC as described by Bieri (20). The concentration of lipid peroxide in serum was determined by the thiobarbituric acid test as described by Yagi (21).

RESULTS

Effect of vitamin E on growth of rats

The body weight of rats over an 18-month period are shown in Fig. 1. There was no appreciable difference in body weight and general appearance observed between the rats receiving the vitamin E-deficient diet and those receiving the control diet for 8 months. After feeding for 8 months, the body weight of rats on the vitamin E-deficient diet showed a slight decrease, but rats on the control diet showed a steady body weight gain throughout the experimental period. Therefore,
the final body weights of rats receiving the vitamin E-deficient diet for 18 months were significantly lower than those of rats receiving the control diet.

After feeding for 8 months, almost complete paralysis of the hind legs developed in rats receiving the vitamin E-deficient diet. At about the same time the rats appeared unkempt and emaciation was usually prominent.

**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, and that hemolysis value was maintained up to 18 months. Erythrocytes from rats receiving the control diet exhibited approximately 10% to 20% hemolysis throughout the study under identical conditions.

The concentrations of \( \alpha \)-tocopherol and lipid peroxide in the serum of rats receiving the vitamin E-deficient or control diet for 18 months are presented in Table 2. In rats receiving the vitamin E-deficient diet, \( \alpha \)-tocopherol in serum was not
detectable. However, in control rats, the concentration of α-tocopherol was 0.33 mg/100 ml. The serum lipid peroxide concentration was about 10-fold higher in rats receiving the vitamin E-deficient diet compared to rats receiving the control diet.

**Urinary output of catecholamine during fasting**

The activity of the sympathetic nervous system in rats is markedly reduced during fasting, although this reduction is reversed by refeeding (22, 23). Studies of urinary output of catecholamine in fasting rats have corroborated this finding. Therefore, the present study was undertaken to examine the changes of urinary output of catecholamine of chronic vitamin E-deficient rats during fasting (Fig. 2).

Basal levels of urinary NE and E excretion in chronic vitamin E-deficient rats were significantly higher than those in control animals. In control rats, urinary NE excretion declined 40% below basal values over a 2-day fast. This decline was reversed by refeeding and exceeded baseline levels in the second day of refeeding. However, no significant difference was observed in urinary NE excretion in vitamin E-deficient rats during the experimental period. In both groups of animals, no significant changes were observed in urinary E excretion with fasting. However, upon refeeding, urinary E excretion of control rats rose to a level significantly higher than the basal level. There was no significant difference in urinary DA excretion between the rats receiving control or the vitamin E-deficient diet during
Stimulation of sympathetic nervous system activity is observed when experimental animals and humans are fed supplemental glucose or sucrose solution (12, 13, 15, 22, 24). In many of these studies, when the total caloric intake was increased by 20% to 30%, the NE turnover rate is accelerated in some tissues including the heart, liver, pancreas and kidney. In order to estimate the effect of glucose feeding on sympathetic nervous system activity of chronic vitamin E-deficient rats, we measured urinary output of catecholamine. As shown in Fig. 3, control rats exhibit a 180% increase in urinary NE excretion and a 30% increase in urinary E excretion when fed supplemental glucose solution for 3 days. This decreases toward the basal level when the supplemental glucose solution is withdrawn. However, in chronic vitamin E-deficient rats, no such change is observed in either urinary output of NE or E with glucose feeding. However, the basal levels of urinary output of NE and E in chronic vitamin E-deficient rats were significantly higher than in control animals fed a supplemental glucose solution.

**Urinary output of catecholamine during glucose feeding**

The endogeneous catecholamine levels in the brain, adrenal glands and heart of...
VITAMIN E AND NERVOUS ACTIVITY

Rats receiving the vitamin E-deficient diet and control diet for 18 months are shown in Table 2. There was no significant difference in the weight of adrenal glands and brain between rats receiving the vitamin E-deficient diet and those on the control diet. The heart weight of vitamin E-deficient rats was lower than that of control rats. However, since the body weight of rats receiving the vitamin E-deficient diet for 18 months was significantly lower than that of control rats, the adrenal gland weight/body weight and brain weight/body weight ratios of vitamin E-deficient rats were larger than those of control rats. No significant difference was observed in the heart weight/body weight ratio between the two groups.

Contents of NE and E in the adrenal glands, and NE in the heart of rats receiving the vitamin E-deficient diet for 18 months were significantly lower than those of rats receiving the control diet. However, there was no significant difference in the NE and DA contents in the brain between the vitamin E-deficient and control rats.

DISCUSSION

The effect of dietary intake on sympathetic nervous system activity is well established. Sympathetic nervous system activity is suppressed by fasting or caloric restriction, and stimulated by glucose or sucrose feeding (9–15). In this study, sympathetic nervous system activity was assessed by measurement of urinary output of catecholamine. Previous studies from this laboratory have demonstrated that aged rats that chronically received a vitamin E-deficient diet excreted large amounts of NE and E in the urine, as compared with rats receiving the control diet (1). As shown in this study, the increase in basal levels of urinary output of NE and E in chronically vitamin E-deficient rats was not influenced by fasting and glucose load. Thus, sympathetic nervous system activity of aged rats chronically receiving the vitamin E-deficient diet was not influenced by dietary manipulation, even when their sympathetic nervous system activity is stimulated.

Data from the present study (Table 3) indicate that the NE and E contents in adrenal glands and the NE content in the heart slightly decreased in rats receiving the vitamin E-deficient diet. Since the endogeneous organ content of catecholamine synthesis is maintained at steady state levels by catecholamine synthesis (25), an increased urinary catecholamine excretion is probably due to increased catecholamine turnover in vitamin E-deficient rats associated with stimulation of the sympathetic nervous system.

Chronic vitamin E deficiency in rats is known to produce a remarkable alteration of axon terminals in the central and peripheral nervous systems, referred to as dystrophic axonal changes (26–28). The mechanism of the nervous changes induced by vitamin E deficiency is not known. However, these axonal changes are not unique to vitamin E-deficient rats. Similar axonal changes have also been observed in various regions of the nervous system of normal aging humans and animals (28, 29). As shown in this paper, rats receiving the vitamin E-deficient diet
Table 3. Catecholamine content in adrenal glands, heart and brain of rats receiving the vitamin E-deficient diet and control diet for 18 months.

<table>
<thead>
<tr>
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<th>Vitamin E in diet</th>
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<tr>
<td></td>
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<tr>
<td>Adrenal glands</td>
<td></td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>(mg/100 g body weight)</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>NE (µg/adrenal glands)</td>
<td>10.8 ± 1.0</td>
</tr>
<tr>
<td>E (µg/adrenal glands)</td>
<td>53.3 ± 6.3</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>(g/100 g body weight)</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>NE (µg/heart)</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
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<tr>
<td>Weight (g)</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>(g/100 g body weight)</td>
<td>0.55 ± 0.2</td>
</tr>
<tr>
<td>NE (µg/brain)</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>DA (µg/brain)</td>
<td>1.61 ± 0.20</td>
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Values are means ± SE of 6 animals per group.

had almost no detectable levels of plasma α-tocopherol (Table 2). Lipid peroxide levels in the plasma were significantly higher in vitamin E-deficient rats compared to the control. On the other hand, Diplock et al. proposed that vitamin E stabilizes membranes by virtue of specific physicochemical interactions between its phytanyl side chain and the fatty acyl chains of polyunsaturated phospholipids (30, 31). It has thus been suggested that its membrane effect is mediated by two distinct functions of vitamin E: free radical scavenging and structure stabilization. It is generally accepted that in vitro, and probably in vivo, vitamin E acts as an antioxidant and may maintain the stability of the biological membrane. However, the mode of action of vitamin E on the nervous system has not yet been established. However, it would be interesting to examine why the axonal membrane or myelin sheath is particularly susceptible to vitamin E deficiency.

Adrenal medullary function is assessed by quantitating changes in urinary E excretion and in adrenal E content, and sympathetic nervous system activity is estimated by measurement of the urinary NE level. The present study confirmed earlier reports by the other authors demonstrating that urinary output of catecholamine is decreased by fasting and increased by glucose feeding in normal rats (9–15). However, in chronic vitamin E-deficient rats these dietary manipulations were not shown on changing patterns of urinary output of catecholamine. Presumably as chronically vitamin E-deficient rats had to maintain a stimulated level of sympathetic nervous activity, no decrease in urinary output of catecholamine was observed in response to fasting. We also postulate that vitamin E-
deficient rats were not able to produce further increases in their higher levels of urinary output of catecholamine in response to glucose feeding.

The results in the present study have demonstrated that chronic vitamin E deficiency causes a significant increase in urinary output of NE. These results indicated that vitamin E deficiency stimulates the sympathetic nervous system in the body. Chronic vitamin E deficiency in the central and peripheral nervous system are referred to as dystrophic axonal changes (26–28, 32). Pathologic changes, such as demyelination, scattered fat droplets, increased intestinal fibers and accumulation of lipofusctine have been noted in the nervous system (axonal ending and nuclei) of vitamin E-deficient rats. Sung et al. proposed that the axonal dystrophic lesions found in the nervous system are at least partially due to vitamin E deficiency (32). The mechanism by which a vitamin E-deficient diet produces degeneration of the nervous system is not known. However, paralysis of the hind legs was observed in rats receiving the vitamin E-deficient diet for over 8 months and degenerative changes in the nerve fibers of chronically vitamin E-deficient rats may be unrelated to the stimulation of sympathetic nervous activity. Further study is necessary to determine the relationship between the neuropathological changes observed in a vitamin E-deficient state in the nervous system and changes in neurological function.

REFERENCES


