ANTIHYPERTENSIVE ACTION OF \textit{d,l}-ALPHA-TOCOPHERYL NICOTINATE IN RATS

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Summary The effects of \textit{d,l}-alpha-tocopheryl nicotinate (EN) on model hypertension in rats were studied in comparison with \textit{d,l}-alpha-tocopheryl acetate (EA). The progress of hypertension in young SHR during the 9th to 15th weeks after birth was markedly accelerated by replacing their drinking water with 1\% saline. The highly-developed hypertension in old SHR (9 months of age) was further advanced by salt-loading. Oral administration of 20 or 100 mg/kg of EN or 88 mg/kg of EA, once a day, delayed the progress of hypertension in young SHR and reduced advanced hypertension in old SHR. An antihypertensive effect of tocopheryl esters was also found in DOCA-salt hypertensive rats. The treatment with EN or EA definitely reduced the incidence of pathological changes accompanying model hypertension such as suppressed weight gain, pulmonary edema, myocardial fibrosis, cerebral hemorrhage and protected the animals from death. In antihypertensive effect, EN was about 5 times more active than EA in molecular base, and the effects of EN protecting from pathological changes associated with model hypertension were more definite than those of EA.

The treatment with EN or EA reduced water and sodium retention in the DOCA-salt hypertensive animals. This fact may suggest the implication of a mechanism through electrolyte metabolism in the antihypertensive action of these tocopheryl esters.

Keywords tocopheryl nicotinate, tocopheryl acetate, spontaneously hypertensive rat, DOCA/salt hypertension, rat, cerebral hemorrhages, pulmonary edema, myocardial fibrosis, mortality, sodium balance

Clinical evidence that tocopheryl esters, especially \textit{d,l}-alpha-tocopheryl nicotinate (EN), are beneficial in treating some kinds of cardiovascular disturbances
has been increasing (1–5). Recently, two clinical evaluations of EN in the treatment of certain pathological conditions accompanied by hypertension and/or cerebral arteriosclerosis were made using double blind methods in Japan: Inno et al. studied 94 patients and reported that administration of 600 mg/day of EN for 4 to 6 weeks significantly improved the general condition and relieved patients from subjective symptoms such as insomnia, dizziness, heavy feeling in the head, numbness in the limbs and stiffness in the neck (6). Inagaki et al. also obtained similar results from clinical studies of 74 patients and, in addition, they found that administration of EN significantly reduced blood pressure in hypertensive patients (7).

For the purpose of elucidating the pharmacological nature related to the above-mentioned clinical effects of EN, we studied the effects of EN and d,l-alpha-tocopheryl acetate (EA) on model hypertension in rats.

METHODS

Two types of hypertension models were used: One model of hypertensive animals was spontaneously hypertensive rats (SHR). To induce more serious hypertension, the animals were given 1% saline instead of drinking water. The other model was desoxycorticosterone acetate (DOCA)-salt hypertensive rats. Besides measurement of blood pressure, morphological changes in the tissues were grossly and microscopically examined in both types of model hypertension, and sodium and potassium metabolism were studied in DOCA-salt hypertensive rats.

Genetic hypertension. Experiments were performed using male SHR of two different ages.

One experiment was carried out with 72 young male SHR (7 weeks of age). Sixteen of them were fed a commercially-prepared pellet diet (Japan CLEA Chow) and tap water ad libitum, and served as non-loaded controls. The remaining 56 rats were given 1% saline instead of drinking water. The saline-loaded rats were divided into four groups of 14 animals per group. The animals in the first three groups were given by gavage 20 or 100 mg/kg of EN or 88 mg/kg of EA (equivalent to 100 mg/kg of EN in molecular base) once a day for 6 weeks from the 9th week of age. The fourth group was given gum arabic solution and served as saline-loaded controls.

In the other experiment, 40 old male SHR (37 weeks of age) with advanced hypertension above 200 mmHg of systolic blood pressure were divided into four groups of 10 animals per group. Drinking water was replaced by 1% saline during the experiment. The animals were given by gavage 20 or 100 mg/kg of EN, 88 mg/kg of EA or gum arabic solution once a day for 4 weeks.

Systolic blood pressure was measured weekly. At the end of experimental period, the animals were killed by exsanguination and submitted to gross and microscopic examinations. The animals which died during the experiment were autopsied as soon as possible after death.

DOCA-hypertension. Male Wistar rats (6 weeks of age) were unilaterally nephrectomized and fed the pellet diet (Japan CLEA Chow) and 1% saline ad
Forty-eight animals were divided into four groups of 12 animals per group and the animals in the first three groups were given by gavage 20 or 100 mg/kg of EN or 88 mg/kg of EA once a day for 7 weeks. The fourth group was given gum arabic solution and served as controls. All animals were given 12.5 mg/kg of DOCA intramuscularly once a week. Systolic blood pressure was measured weekly. After respective injections of DOCA at the 1st, 3rd and 7th weeks of the experiment, saline consumption and urinary electrolyte excretion during a 12-hr period were measured.

**Determination of blood pressure and pulse rate.** Blood pressure and heart rate were measured by the tail plethysmography-cuff method using an automatic blood pressure monitor (SCS-301, Shimadzu Seisaku-sho Co., Tokyo). Rats were

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Fig. 1. Diagram of the mechanism of automatic determination of systolic blood pressure. Two detectors, P and D, of plethysmographs are set on the tail proximal and distal to the occluding cuff. Air pressure in the cuff is automatically adjusted by signals of blood flow at detector D. The cuff pressure increases as long as the sphygmogram leading from detector D synchronizes with that from detector P, and decreases if the cuff is inflated up to such a level that blood flow at the distal portion of the tail is obstructed and the signals from detector D disappear. The maximum cuff pressure which allows blood flow, i.e., systolic blood pressure, is continuously recorded on a pen-writing oscillograph. Pulse rate is measured through a tachometer which is triggered by the sphygmogram leading from detector P.
individually housed in plastic holders (KN325B, Natsume Seisaku-sho Co., Tokyo) and blood pressure and heart rate were measured in a prewarmed box (30°C) (8–10). The mechanism of blood pressure determination is illustrated in Fig. 1.

**Histopathological examination.** At the end of the experimental period, the animals were autopsied by routine procedure. Especially, hemorrhages in the brain were carefully examined by frontal dissection. The brain, lung, heart, kidneys, adrenals and pituitary gland were removed and weighed. For microscopic observation, the organs were fixed in 10% neutral buffered formalin and then embedded in paraffin to prepare 5μm thick histological sections, which were stained with hematoxylin-eosin (H.E.). For histologic details, renal specimens were stained with Mallory’s method for collagen and periodic acid schiff (PAS). Microscopic examination was carried out by one of the authors in a form of blind test.

**Electrolyte metabolism.** Saline consumption and sodium and potassium excretions into the urine during the 12 hr after the administration of DOCA were measured at the 1st, 3rd and 7th weeks of the experiment. The animals were individually housed in metabolism cages after the injection of DOCA and the urine voided was sampled. Sodium and potassium in the urine were measured by a flame photometer (205D, Hitachi Co., Tokyo).

**Tocopherol contents in the liver.** In experiments with old SHR and DOCA-salt hypertensive rats, tocopherol contents in the liver were determined. Tissue samples from 2 animals per group were taken at the end of the experiment. Tocopherol was extracted from liver homogenate with n-hexane and determined by the high-speed liquid chromatography method (11). As an internal standard, tocol was used.

**Drugs used.** Desoxycorticosterone acetate (Takeda Chemical Industries Co., Osaka), d,l-alpha-tocopheryl nicotinate and d,l-alpha-tocopheryl acetate (Eisai Co., Tokyo) were used.

**RESULT**

**Effects on blood pressure**

Figure 2 shows the progress of hypertension in young SHR. Drinking water was replaced by 1% saline at the 7th week of age, excepting one group which served as the non-loaded control group. Systolic blood pressure was about 150 mmHg at the 7th week of age, which increased up to 200 mmHg on the average at the end of experiment in the non-loaded control animals. During the first few weeks after replacing drinking water by saline, there was no significant difference in the progress of hypertension between both control groups with and without saline-loading. However, as the animals adapted to drinking saline and the consumption of saline markedly increased, the development of hypertension in the saline-loaded control animals rapidly accelerated and reached a level above 230 mmHg at the 14th week of age.

The treatment with 20 or 100 mg/kg of EN or 88 mg/kg of EA delayed the progress of hypertension in SHR loaded with saline to a level comparable to the
Progress of hypertension in male SHR during the 7th to 14th weeks after birth. Except for the group of non-loaded controls (---), the animals were given 1% NaCl instead of drinking water. Tocopheryl esters were orally administered once a day during the 9th to 14th weeks after birth. ---, Saline-loaded controls; ---, EN 20 mg/kg; ---, EN 100 mg/kg; ---, EA 88 mg/kg. Each point indicates the mean of 6-15 animals and vertical bars represent the S.E.M.

Figure 3 shows changes in systolic blood pressure during 4 weeks of saline loading in old SHR. The animals were 37 weeks old at the beginning of the experiment and their blood pressure was about 220 mmHg. The saline consumption was in the range of 10 to 20 ml/day at the 1st week of experiment, which increased up to 20 to 40 ml/day at the 2nd week and 40 to 80 ml/day thereafter. In the control animals, systolic blood pressure remained unchanged during the first 2 weeks but markedly increased to above 240 mmHg in the 3rd week.

The administration of 100 mg/kg of EN reduced blood pressure by 15 mmHg in a week, which remained at a lowered level thereafter. Blood pressure in the animals receiving 20 mg/kg of EN or 88 mg/kg of EA was reduced at the 1st week, which increased again to a level exceeding the pre-dosing level at the 2nd and 3rd weeks.

Figure 4 (right) shows the development of hypertension in rats unilaterally nephrectomized 6 weeks after birth and given DOCA and saline as drinking water. In control animals, systolic blood pressure, which was 135 mmHg at the 7th week after birth, increased to 182 mmHg at the 13th week. Although both tocopheryl
Changes in blood pressure in male SHR when drinking water was replaced by 1% NaCl in the 37th week after birth. The animals were orally administered tocopheryl esters once a day for 4 weeks from the 37th week after birth. -○-, EN 20 mg/kg; -●-, EN 100 mg/kg; -△-, EA 88 mg/kg; -×-, controls. Each point indicates the mean of 6-10 animals and vertical bars represent the S.E.M.

Effects on pathological changes accompanying model hypertension

In the experiment with young SHR, body weight in the non-loaded control animals increased, on the average, from 160 g at the 7th week to 231 g at the 15th week of age. The rate of weight gain in the saline-loaded control animals was suppressed during the latter half of the experimental period and the animals weighed 202 g at the 15th week of age. The animals treated with tocopheryl esters had favorable weight gains; they weighed 220 g, 217 g and 223 g on the average in groups of 20 mg/kg and 100 mg/kg of EN and 88 mg/kg of EA, respectively, at the 15th week of age. Body weight of the old SHR at the 37th week of age was in the range of 380 to 450 g, which remained unchanged throughout the period of the 4-week experiment.
Figure 4 (left) shows changes in body weight in DOCA-salt hypertensive rats during 7 weeks of the experiment. Weight gain in the control animals stopped at the 9th week after birth, when their blood pressure increased above 170 mmHg. On the other hand, body weight in the animals treated with EN or EA continued to increase throughout the period of the experiment.

Table 1 shows the number of animals which died during the course of the experiment. Thirty of 72 young SHR died, most of which died during the first few weeks of the experiment. In these animals, bloody rhinorrhoea, roughened coat and loss of body weight were remarkable, and pulmonary edema and hemorrhage were found grossly and microscopically. Some animals died during the latter half of the experiment but the causes of death in these animals were not definite. They were seriously hypertensive, but did not exhibit any abnormal signs which were observed in the animals which died during the first few weeks of the experiment. Although scattered foci of round-cell infiltration in the myocardium and sclerotic changes in the glomerulus were found in these animals, we could not detect hemorrhages in the brain in any animal.

![Graph showing changes in body weight and blood pressure](https://example.com/graph.png)

Fig. 4. Changes in body weight (left) and blood pressure (right) in DOCA-salt rats. The animals unilaterally nephrectomized at the 6th week after birth were given, instead of drinking water, 1% NaCl and DOCA (12.5 mg/kg, i.m., once a week). EN, EA or gum arabic was orally administered once a day for 7 weeks. ○ -- , EN 20 mg/kg; • -- , EN 100 mg/kg; △ -- , EA 88 mg/kg; × × × × × × , control. Each point indicates the mean of 6–12 animals and vertical bars represent the S.E.M.
Table 1. Effect of tocopheryl esters on the mortality during the experiment.
The number of surviving animals at the end of the weeks is shown.

A) Experiment with young SHR: For the non-loaded control group, drinking water was replaced by 1% NaCl at the 7th week after birth. EN, EA or gum arabic was orally administered once a day from the 9th week on.

| Weeks after birth | Start | 7th | 8th | 9th | 10th | 11th | 12th | 13th | 14th |
|-------------------|-------|-----|-----|-----|------|------|------|------|------|
| Non-loaded controls | 16    | 15  | 13  | 12  | 11   | 11   | 11   | 11   | 11   |
| EN 20 mg/kg       | 14    | 13  | 13  | 12  | 11   | 11   | 11   | 10   | 10   |
| EN 100 mg/kg      | 14    | 12  | 11  | 10  | 8    | 7    | 7    | 7    | 7    |
| EA 88 mg/kg       | 14    | 13  | 11  | 11  | 9    | 9    | 8    | 8    | 8    |
| Saline-loaded controls | 14 | 12  | 11  | 10  | 10   | 9    | 7    | 7    | 6    |

B) Experiment with old SHR: Drinking water was replaced by 1% NaCl at the 37th week after birth and EN, EA or gum arabic was orally administered once a day from the 37th to 40th week after birth.

| Weeks after birth | Start | 37th | 38th | 39th | 40th |
|-------------------|-------|------|------|------|------|
| Controls          | 10    | 10   | 9    | 7    | 6    |
| EN 20 mg/kg       | 10    | 10   | 10   | 10   | 10   |
| EN 100 mg/kg      | 10    | 10   | 10   | 10   | 10   |
| EA 88 mg/kg       | 10    | 10   | 10   | 10   | 10   |

C) Experiment with DOCA-salt rats: The animals unilaterally nephrectomized at the 6th week after birth were given 1% NaCl and DOCA (12.5 mg/kg, i.m., once a week). From the 7th week on, EN, EA or gum arabic was orally administered once a day.

| Weeks after birth | Start | 7th | 8th | 9th | 10th | 11th | 12th |
|-------------------|-------|-----|-----|-----|------|------|------|
| Control           | 12    | 12  | 11  | 11  | 10   | 8    | 6    |
| EN 20 mg/kg       | 12    | 10  | 10  | 10  | 10   | 10   | 10   |
| EN 100 mg/kg      | 12    | 12  | 12  | 12  | 12   | 11   | 11   |
| EA 88 mg/kg       | 12    | 12  | 12  | 12  | 12   | 12   | 11   |

Fig. 5. Microscopic changes observed in male SHR which were given, instead of drinking water, 1% NaCl for three weeks from the 37th week after birth. 1) Focal hemorrhages in the brainstem. H.E., ×200. 2) Pulmonary edema with focal hemorrhages. H.E., ×200. 3) Scattered fibrotic foci accompanied by round-cell infiltration in the apical myocardium. H.E., ×200. 4) Sclerotic glomerulus and hyaline casts in the dilated renal tubules. PAS, ×200.
Fig. 5.
In the experiment with old SHR, 4 of 12 control animals died, whereas all of 36 animals treated with EN or EA survived the experiment. The animals which died during the experiment were apparently healthy, but died of shock when the animal was taken out of the cage to measure body weight, or during handling for determination of blood pressure. Although massive hemorrhages in the brain were not found by gross examination, petechial hemorrhages were microscopically detected. Cerebral hemorrhages, as shown in Fig. 5, were found in 3 of 4 animals which died and exclusively in 1 of 36 animals which survived throughout the period of the experiment. The animals in which cerebral hemorrhages were detected were 3 of 10 control animals and 1 of 10 animals treated with 88 mg/kg of EA and none of 20 animals treated with 20 or 100 mg/kg of EN.

Besides hemorrhages in the brain, remarkable changes were found in the heart, lung and kidneys. Figure 5 shows some typical microscopic findings in the experiment with old SHR. In the control animals, scattered foci of round-cell infiltration consisting of lymphocytes and mononuclear histiocytes were found in the myocardial interstitium. However, as shown in Fig. 6, these pathological changes were definitely slight in the animals receiving 20 or 100 mg/kg of EN. The major changes in the lung were congestion and edema in the control animals. They were less distinct in the animals receiving EN. In the kidneys, there were focal areas of round-cell infiltration and congestion in the cortex, scattered glomerular sclerosis and dilation of Bowman’s capsules with accumulation of protein-like exudate. Furthermore, occasional dilation of the cortical tubules and thickening of the tubular basement membranes were seen. Among these changes, dilation of Bowman’s capsules and sclerotic changes in the glomerulus were not evident in animals treated with the tocopheryl esters, especially in the group receiving 100 mg/kg EN.

In the experiment with DOCA-salt hypertensive rats 10 animals died during the experiment. They were 6 of 12 control animals, 2 of 12 animals in the group receiving 20 mg/kg of EN, 1 of 12 animals receiving 100 mg/kg of EN and 1 of 12
animals receiving 88 mg/kg of EA. Suppressed weight gain, bloody rhinorrhoea and pulmonary hemorrhage were remarkable in the animals which died.

**Effects on water and electrolyte metabolism in DOCA-salt hypertensive rats**

Table 2 summarizes saline consumption, urine volume and sodium and potassium excretions during the 12 hr following administration of DOCA (12.5 mg/kg, i.m.) in the 1st, 3rd and 7th weeks of the experiment.

After the 1st administration of DOCA, the control animals drank, on the average, 32.5 ml of 1% saline during 12 hr, whereas the animals treated with EN or EA drank about 20 ml. Although insensitive water loss through respiration and sweating was not measured, it was estimated from the balance between saline consumption and urine volume the amount of water retained by the control animals due to administration of DOCA. The water retention in the animals treated with EN or EA was less than half that in the control animals. Sodium balance was calculated from the consumption of saline and excretion into urine. As shown in Fig. 7, 4.2 mEq of sodium was retained during the 12 hr after the 1st administration of DOCA in the control animals, whereas the sodium retention in the animals given 20 and 100 mg/kg of EN, and 88 mg/kg of EA were, on the average, 3.2, 2.6 and 2.6 mEq, respectively.

| Treatment | Week | No. rats | B.W. | Saline intake (ml) | Urine (ml) | Na (mEq) | K (mEq) |
|-----------|------|----------|------|------------------|-----------|----------|---------|
| Controls  | 1st  | 12       | 200  | 32.5±4.9         | 11.4±3.3  | 1.05±0.33 | 0.68±0.10 |
|           | 3rd  | 11       | 235  | 33.6±5.3         | 25.5±5.6  | 4.79±1.12 | 1.36±0.11 |
|           | 7th  | 8        | 245  | 33.8±7.3         | 33.9±7.7  | 4.75±1.15 | 1.21±0.18 |
| EN 20 mg/kg | 1st | 10       | 200  | 22.9±2.6         | 7.8±2.0   | 0.79±0.21 | 0.66±0.10 |
|           | 3rd  | 10       | 250  | 33.6±5.5         | 16.5±3.6  | 2.79±0.74 | 1.35±0.18 |
|           | 7th  | 10       | 300  | 35.1±8.3         | 17.7±5.7  | 2.27±0.79 | 1.11±0.22 |
| EN 100 mg/kg | 1st | 12       | 200  | 20.4±1.8         | 7.7±1.6   | 0.86±0.22 | 0.73±0.08 |
|           | 3rd  | 12       | 260  | 26.7±1.7         | 10.8±1.3  | 1.66±0.21 | 1.35±0.13 |
|           | 7th  | 11       | 320  | 32.2±5.4         | 23.9±5.4  | 3.04±0.74 | 1.50±0.22 |
| EA 88 mg/kg | 1st | 12       | 200  | 19.2±1.3         | 6.8±1.6   | 0.61±0.17 | 0.81±0.20 |
|           | 3rd  | 12       | 250  | 22.5±2.4         | 10.8±1.3  | 1.81±0.21 | 1.11±0.08 |
|           | 7th  | 12       | 310  | 31.6±8.0         | 23.5±6.3  | 3.06±0.93 | 1.43±0.17 |

B.W.: body weight (g).
Fig. 7. Change in sodium-retaining effect of DOCA in DOCA-salt rats. The animals unilaterally nephrectomized at the 6th week after birth were given, instead of drinking water, 1% NaCl and DOCA (12.5 mg/kg, i.m., once a week). Individual data of sodium balance (consumption of salt-urinary excretion) during the 12 hr after the 1st, 3rd and 7th administrations of DOCA are plotted. Horizontal bar indicates the mean of the group.

After repeated administration of DOCA, urine volume and sodium excretion were markedly increased in the control group, although the amount of saline intake remained unchanged. As a result, water and sodium balances became seriously negative in some animals in the control group. The amount of saline intake increased nearly parallel with the weight gain in the animals treated with EN or EA. As shown in Fig. 7, sodium balance between intake from 1% saline and excretion into urine remained unchanged through the period of the experiment in the animals given tocopheryl esters. There was no definite difference in urinary excretion of potassium among the groups.

Tocopherol content in the liver
At the end of the experiment with old SHR and DOCA-salt hypertensive rats, tocopherol contents in the liver were determined in 2 animals selected at random from each group of control, 20 or 100 mg/kg of EN or 88 mg/kg of EA. The contents in the control animals of old SHR and DOCA-salt hypertensive rats were within normal range and those in the animals given EN or EA were 1.5 to 6.0 times higher than normal.

DISCUSSION
The results of the present studies demonstrate the antihypertensive effect of
tocopheryl esters. In our own studies with normotensive animals and hypertensive animals, a single dose of EN or EA either by intravenous injection or oral administration did not change cardiac output, peripheral blood flow or blood pressure (unpublished data). However, repeated administration of tocopheryl esters delayed the progress of hypertension in saline-loaded young SHR and DOCA-salt hypertensive rats, and reduced the seriously advanced hypertension in old SHR. From these results, the mechanism of the antihypertensive action of tocopheryl esters appears not to be from any direct effect on the vascular muscle tone or from secondary effects through the sympathetic nervous system. The facts that the administration of EN or EA delayed the progress of hypertension in saline-loaded young SHR to the level of non-saline-loaded SHR, and that water and sodium retention after the 1st administration of DOCA in unilaterally nephrectomized and saline-loaded rats was reduced by the administration of EN or EA may imply that in the antihypertensive action of these tocopheryl esters, there is a mechanism relying on electrolyte metabolism. The administration of EN or EA did not merely prevent the development of hypertension but protected the animals from some morphological changes in tissues, weight loss and death. From observations of animal condition and histopathological examination of the tissues, pneumonia, cardiac shock and stroke were considerable as causes of death in the experimental animals. The stroke-prone SHR which were developed by successive selective breeding by OKAMOTO et al. (12) have been described as characteristic of more rapid increase of blood pressure than the usual SHR. Although we did not detect such massive hemorrhages as have been shown by OKAMOTO et al., some animals in the present studies probably died of stroke during the course of the experiment, since they were apparently healthy but seriously hypertensive, and petechial hemorrhages were found in 3 of 4 animals which died but exclusively in 1 of 36 animals which survived in the experiment with old SHR.

To discuss the clinical or pharmacological effects of tocopherols, their effect on membrane peroxidation (13–16) seems to be most important. However, the hypertensive animals in the present studies appeared not to suffer from tocopherol deficiency, although we determined tocopherol contents in the liver only in a restricted number of animals. Tocopherol contents in the liver from animals given EN or EA were markedly higher than normal. Regarding antihypertensive effect, EN was about five times more active than EA. The protecting effects of EN from pathological changes associated with model hypertension were more definite than those of EA. EN is chemically an ester of tocopherol with nicotinic acid, and it has been shown that most of EN orally given undergoes hydrolysis in the gastrointestinal tract and enters the blood stream through the lymph (17). Although pharmacological effects of nicotinic acid which is formed by hydrolysis of EN should be taken into consideration for the specific effects of EN, we did not detect any nicotinic acid-like actions such as flushing and vasodilation after either oral or parenteral administration of EN, even at extremely high doses (unpublished data). ADACHI et al. reported that administration of EN
increases the biosynthesis of NAD; they found that NAD levels in the liver of mice which were orally given EN were markedly high and such an increased NAD level lasted for more than 24 hr, whereas after oral administration of nicotinic acid, NAD levels only transiently increased (18). The vasodilating property of exogenous NAD is well known (19, 20), and there is some evidence suggesting that myocardial dysfunctions result from decreased NAD-biosynthesis (21, 22). Although the specific nature of EN among the tocopheryl esters is not yet completely clarified, there are several pieces of evidence suggesting a higher therapeutic activity of EN compared with other tocopheryl esters, e.g., on skin microcirculation on patients (5), capillary fragility in diabetes (23) and in the aftereffect of cerebral apoplexy (24), pulmonary edema induced by norepinephrine-infusion in mice (25), alloxan diabetes in rats (26) and platelet aggregation (27). Regarding the result of the present studies that the administration of EN markedly reduced the incidence of fibrotic change in the myocardium, the finding by Yasue et al. that the administration of EN accelerated the formation of collateral vessels in experimentally-induced myocardial infarction in dogs (28) should be referred to.

In conclusion, the findings of the present studies in model hypertensive animals may support the beneficial clinical effects of EN on symptoms associated with hypertensive disease and other related cardiovascular disturbances.

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