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γ-Tocopherol Enhances Sodium Excretion as a Natriuretic Hormone Precursor

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Summary Endogenous natriuretic factors are believed to be responsible for extracellular fluid homeostasis in mammals. A new endogenous natriuretic factor, Loma Linda University-alpha (LLU-α) has recently been proven to be a 2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman (γ-CEHC), which is a metabolite of γ-tocopherol (γ-Toc). The purpose of this study was to investigate whether γ-Toc could accelerate sodium excretion into rat urine as a natriuretic hormone precursor. Male SD strain rats were divided into two groups; one was a control diet group, while the other was a high NaCl group (50 g/kg diet). Next, the two groups were each subdivided into two groups consisting of a placebo group and a γ-Toc group. After the oral administration of one experimental dose of 20 mg γ-Toc or placebo, rat urine was collected at 6 h intervals for 24 h, and then the urine volume, sodium and potassium and γ-CEHC content were determined. γ-Toc increased in the urine volume of the high-NaCl intake group. The sodium excretion in the high-NaCl group given γ-Toc was 8.29±2.20 g, while in the control group given γ-Toc it was 6.24±1.49 g from 12–18 h. In contrast, the potassium excretion in the rat urine did not change in any of the groups. Our findings suggested that γ-Toc accelerates the degree of sodium excretion in rats with a high sodium intake.

Key Words γ-tocopherol, γ-CEHC, natriuretic hormone, sodium excretion

Vitamin E has eight different natural forms: four tocopherols (α-, β-, γ-, δ-) and four tocotrienols (α-, β-, γ-, δ-) (*1*). α-Tocopherol (α-Toc) exists more abundantly in animal plasma and tissue than γ-tocopherol (γ-Toc) in spite of the fact that the animals were given higher amounts of γ-Toc than those on the α-Toc diet, and the mechanism for this action has recently been clarified (*2*, *3*). Vitamin E homologues are absorbed equally well from the small intestine without discrimination. After the uptake of vitamin E homologues into the intestine cells, they are secreted into chylomicrons.

Chylomicron remnants are subsequently catabolized during circulation by lipoprotein lipase. After the uptake of chylomicron remnants by the liver, α-Toc is preferentially secreted in association with VLDL, and only α-Toc circulates in the plasma and tissue. This is caused by the presence of α-Toc transfer protein (α-TTP) in hepatic cytoplasma, which clearly discriminates α-Toc from tocopherol homologues (*4*, *5*).

The biodiscrimination by α-TTP is another phenomenon related to the Toc bioavailability. The relative affinity for tocopherol is as follows: α-Toc 100%, β-Toc 38%, γ-Toc 9%, δ-Toc 2% (*6*). In addition, absorbed γ-Toc has been observed to disappear rapidly from plasma.

Vitamin E, α-Toc and γ-Toc are affected via the metabolite pathway. The metabolites of α-Toc and γ-Toc are 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) (*7*) and 2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman (γ-CEHC) (*8*). On the other hand, γ-Toc has recently been reported to play a more extensive role than just that of an antioxidant, because γ-Toc urinary metabolites, such as γ-CEHC play a role similar to that of natriuretic hormones (*8–10*). 'Natriuretic hormone', the putative controller of extracellular fluid volume (*11*), was first identified more than 30 y ago, and is believed to play a pathomimetic role in hypertension, congestive heart failure and other volume-expanded states. On the other hand, Wechter et al. reported a new endogenous natriuretic hormone, LLU-α, which was isolated from human uremic urine (*8*).

LLU-α later proved to be γ-CEHC, which is the metabolite of γ-Toc (*9*, *10*) (Fig. 1) and γ-tocotrienol (*12*, *13*).

Recently, Saito et al. (*14*) showed that γ-tocotrienol could cause natriuresis and diauresis in rats. Therefore, we presumed that γ-Toc could also have natriuretic potency as a netriuretic hormone precursor as well as γ-tocotrienol, because γ-CEHC is a metabolite of both γ-Toc and γ-tocotrienol.

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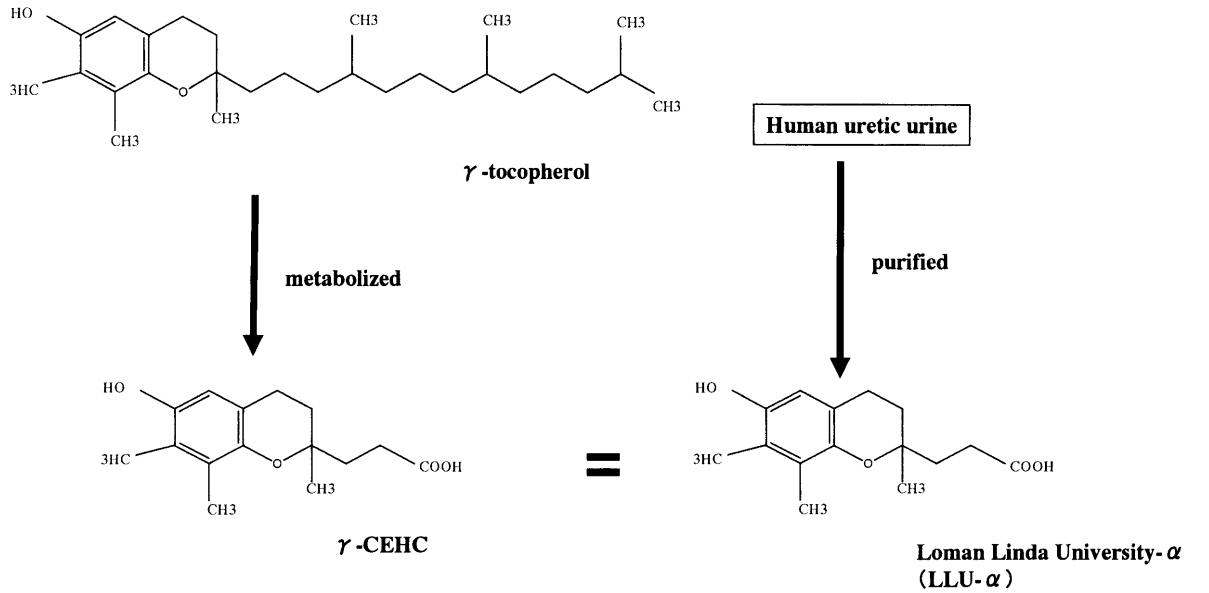

Fig. 1. Structures of γ-tocopherol and γ-CEHC;LLU-α. LLU-α, a new endogenous natriuretic factor proved to be a γ-CEHC, a metabolite of γ-tocopherol.

In this study, we investigated whether γ-Toc was able to accelerate sodium excretion into rat urine.

MATERIALS AND METHODS

Materials

γ-Tocopherol and γ-CEHC were donated by Eisai Co. (Tokyo, Japan). All reagents used in this study were either HPLC grade or reagent grade.

Animals

Male Sprague-Dawley strain rats (7-wk-old; $n=20$) were purchased from Clea Japan Co. Ltd. (Tokyo, Japan) and kept individually in stainless steel cages at 22±1°C and 55% humidity with a 12 h light/dark cycle. The rats were initially fed a commercial diet (CE-2; Clea Japan Co., Ltd.) for a week, to allow them to adapt to the new environment. The rats were divided into two groups; one was the control diet group, while the other was the high-NaCl diet group. Both groups were placed on a diet deficient in vitamin E (AIN-76 modified by Eisai Co.; Funabashi Noujyou, Chiba, Japan) for 4 wk.

This diet consisted of 236.8 g sucrose, 236.8 g glucose, 189.5 g casein (vitamin free), 142.1 g cornstarch, 47.4 g filter paper, 33.2 g mineral mixture, 9.5 g vitamin mixture except vitamin E, 2.8 g DL-methionine, 1.9 g choline bitartate, and 100 g stripped corn oil.

The high-NaCl diet was a diet deficient in vitamin E with 50 g NaCl added to it per kilogram. Both groups were given 20 g of rat chow, and 150 mL of distilled water per day.

Study population and design

These two groups were subdivided into two groups after 12 h of fasting; one was the placebo group while the other was the γ-Toc group.

The placebo group ($n=5$) was given a 0.5 mL dosage of stripped corn oil, while 0.5 mL of stripped corn oil containing 20 mg of γ-Toc was given to the γ-Toc group ($n=5$). After oral administration, the rats were housed individually in metabolic cages.

Collection of urine samples

Urine was collected in flasks and then cooled with dry ice at 6 h intervals after administration for 24 h. All urine samples were immediately stored at −20°C until further analyzed. The present study was approved by the Animal Committee of Ochanomizu University.

Determination of the urine volume, sodium and potassium content in rat urine

The urine volume was measured after urine collection and then standardized by referring to the creatinine concentration in the urine.

The creatinine concentrations in each urine sample were measured by the Jaffe method (*15*, *16*) (Hitachi auto analyzer 7011, Hitachi Medical Co., Tokyo, Japan), while the sodium and potassium content in the urine was determined by an electrode method (*17*, 18) (Hitachi auto analyzer 7011, Hitachi Medical Co., Tokyo, Japan).

Extraction of γ-CEHC from the rat urine

Next, 0.1 mL of ascorbic acid solution (0.5 g/mL) and 1 mL of EDTA solution (0.54 mM) were both added to the urine samples (0.5 mL).

All the urine samples were then immediately lyophilized. After 2 mL of 3 N methanolic HCl solution was added to each tube, the contents were methylated by shaking at 60°C for 1 h under N_2 gas.

After the methylated solution was cooled in ice water, 6 mL of water was added to each tube and the medium was shaken vigorously with 3 mL of *n*-hexane for 1 min. This mixture was thereafter centrifuged at 600×*g*, for 5 min, and the upper layer was collected and evaporated. The residue was dissolved in 100 mL of 40% acetonitrile-water to which was added 50 mM sodium perchlorate for the determination by HPLC-ECD (*19*).

Chromatographic apparatus and conditions

The HPLC system consisted of the Shiseido intelligent HPLC pump (SI-2) (Shiseido Co., Kyoto, Japan), JASCO

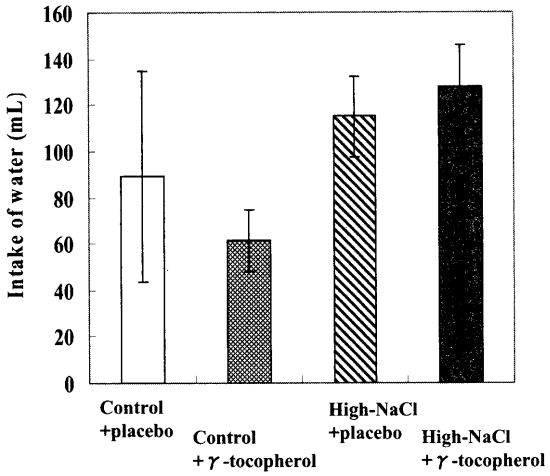

Fig. 2. Total volume of water intake per day. The rats were previously fed either a vitamin E-deficient diet (control) or a high-NaCl diet for 4 wk. In each group, one subgroup was administered a placebo, while the other was given γ-tocopherol. The values are the mean±SD of 5–6 rats.

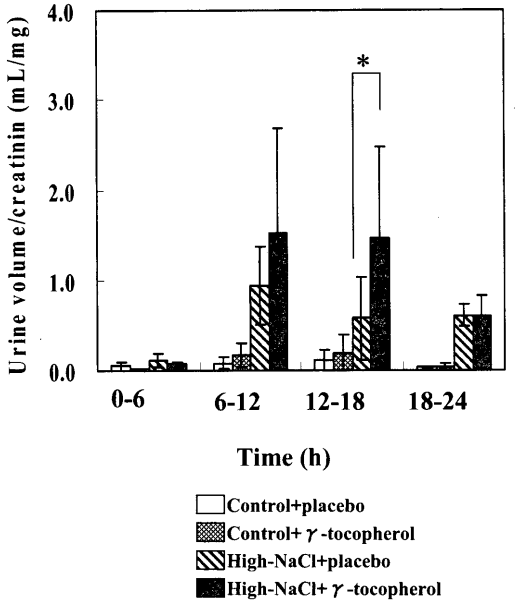

Fig. 3. Changes in the rat urine volume/creatinine level after the oral administration of a 20 mg of placebo and 20 mg of γ-tocopherol. The rats were previously fed a vitamin E-deficient diet (control) or a high-NaCl diet for 4 wk. In each group, one subgroup was administered a placebo, while the other was given γ-tocopherol. The significant differences between the experimental groups in every 6 h period were analyzed by multivariable ANOVA (MANOVA). The values are the mean±SD of 3–6 rats, * $p<0.05$.

intelligent sampler (AS-950-10), a column oven (860-10), and an integrator (807-IT) (JASCO Co., Tokyo, Japan) while applying a potential of +0.6 V vs. Ag/AgCl. The analysis of γ-CEHC was performed at 35°C using an RP-18T C18 column (250×2.0 mm I.D., IRICA Instruments Inc., Tokyo, Japan). The mobile phase was performed using acetonitrile–water (40 : 60, v/v) with 50 mM sodium perchlorate at a flow rate of 0.2 mL/min.

Statistics

Statistical analyses were performed using the Stat View Version 5.0 software package (SAS Institute Inc., Cary, NC, USA). All results were expressed as the means±S.D.

The significance between the four experimental groups was evaluated using the multivariate ANOVA (MANOVA).

RESULTS

Urine volume. The rats given high amounts of salt were observed to have a larger water intake (Fig. 2). Figure 3 shows the changes in the urine volume/creatinine after the oral administration of the placebo and γ-Toc.

The urine volume/creatinine level showed no significant differences between the control group given the placebo and γ-Toc.

In contrast, the urine volume of the high-NaCl group given γ-Toc (1.47±1.00 mL/creatinine mg; $p<0.05$) was significantly higher than that of the high-NaCl group given the placebo (0.58±0.46 mL/creatinine mg) from 12 h to 18 h after the oral administration of the placebo and γ-Toc. According to these findings, γ-Toc was shown to enhance the urine volume in rats consuming the high-NaCl diet.

Sodium and potassium excretion into rat urine. We next examined the effect of γ-Toc on sodium and potassium

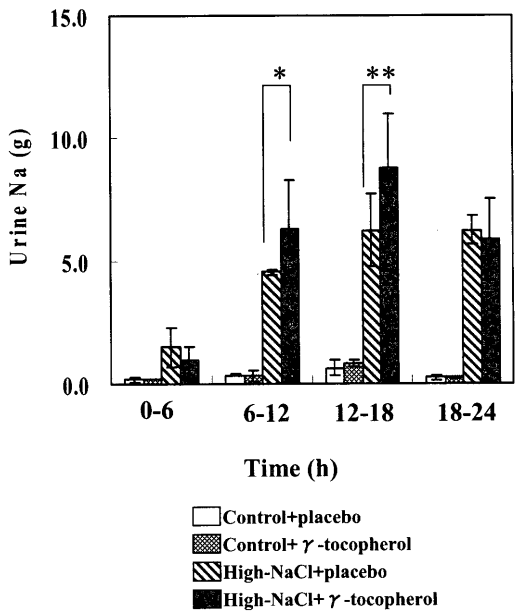

Fig. 4. Changes in the sodium of rat urine levels after the oral administration of a 20 mg placebo or 20 mg of γ-tocopherol. The rats were previously fed either a vitamin E-deficient diet (control) or a high-NaCl diet for 4 wk. In each group, one subgroup was administered a placebo, while the other was given γ-tocopherol. Significant differences between the experimental groups for every 6 h period were analyzed by the multivariate ANOVA (MANOVA). The values are the mean±SD of 3–6 rats, * $p<0.05$, ** $p<0.01$.

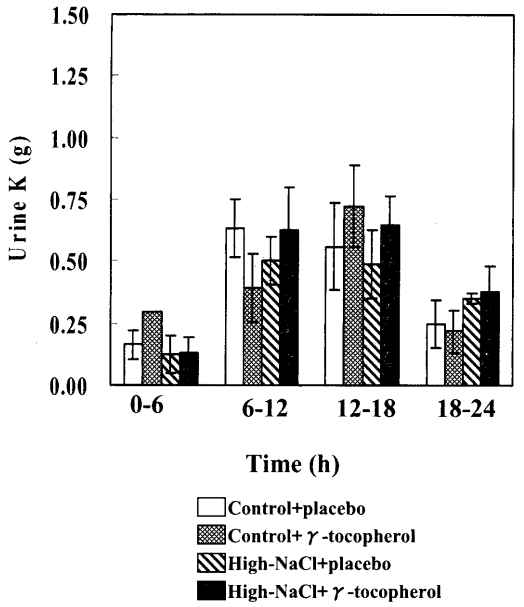

Fig. 5. Changes in the potassium content of rat urine after the oral administration of a 20 mg placebo or 20 mg of γ-tocopherol. The rats were previously fed a vitamin E-deficient diet (control) or a high-NaCl diet for 4 wk. In each group, one subgroup was administered a placebo, while the other was given γ-tocopherol. The values are the mean±SD of 3–6 rats.

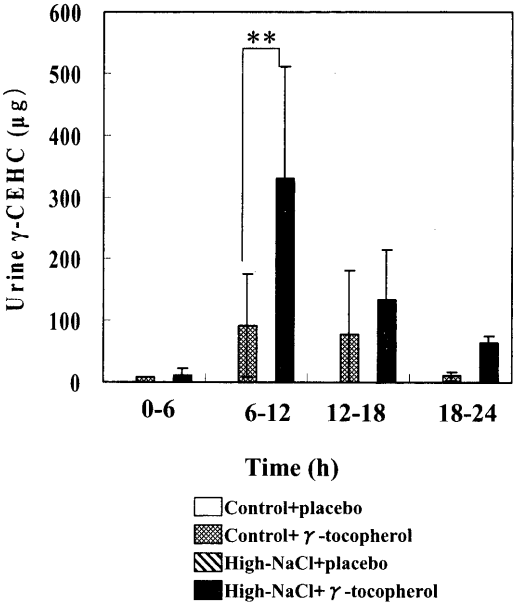

Fig. 6. Changes in γ-CEHC levels in rat urine after the oral administration of a 20 mg placebo or 20 mg of γ-tocopherol. The rats were previously fed either a vitamin E-deficient diet (control) or a high-NaCl diet for 4 wk. In each group, one subgroup was administered a placebo, the other γ-tocopherol. Significant differences between the experimental groups for every 6 h period were analyzed by multivariate ANOVA (MANOVA). The values are the mean±SD of 3–6 rats, ** $p<0.01$.

excretion into rat urine. Concerning sodium excretion, the sodium content of rat urine in the high-NaCl group given γ-Toc (8.79±2.20 g; $p<0.01$) was significantly higher than that in the high-NaCl group given a placebo (6.24±1.49 g) from 12 h to 18 h after the oral administration of the placebo and γ-Toc (Fig.4).

In contrast, the potassium excretion rates in the rat urine showed no substantial changes in all experimental groups (Fig.5). According to these findings, we presumed that the γ-Toc only accelerates sodium excretion in rats with a high sodium intake, while it has no effect on potassium excretion.

γ-CEHC excretion into rat urine. Figure 6 shows the change of γ-CEHC, after the oral administration of γ-CEHC in the high-NaCl group given γ-Toc (328.67±182.53 mg; $p<0.01$), to be significantly higher than that in the control group given γ-Toc (92.45±8.27 mg) from 6 h to 12 h after the oral administration of γ-Toc.

As a result, a high level of NaCl is thus thought to promote the γ-Toc metabolism of γ-CEHC in rats.

DISCUSSION

We investigated whether γ-Toc induced natriuresis in vivo, and thereby showed that γ-Toc enhanced sodium excretion while it also had a hormone-like function.

The urine volume/creatinine (Fig. 3) and sodium content of rat urine (Fig. 4) in the high-NaCl group given γ-Toc, were significantly higher than those in the high-NaCl group given a placebo from 12 h to 18 h after oral administration.

On the other hand, Saito et al. (*14*) reported the urine volume/creatinine and sodium content of the high-NaCl group given γ-tocotrienol to be significantly higher than that of the high-NaCl group given a placebo from 6 h to 12 h. When comparing the differences in the urine volume/creatinine and sodium content between γ-Toc and γ-tocotrienol, the peak levels in the high-NaCl group given γ-tocotrienol appeared 6 h more rapidly than in the high-NaCl group given γ-Toc. Regarding this time lag, we considered that γ-tocotrienol may thus metabolize more rapidly than γ-Toc.

However, the potassium content of the rat urine did not significantly change in any experimental group (Fig. 5), and this finding was similar to Saito's results (*14*).

From 6 h to 12 h after administration, the γ-CEHC level in the high-NaCl group orally given γ-Toc was significantly higher than that in the control group given γ-Toc (Fig. 6). According to this finding, we presumed that the metabolism of γ-Toc accelerates with a high sodium intake.

We therefore conclude that γ-Toc stimulates the urinary output only in the presence of a high sodium intake. It is assumed that there may be a relationship between sodium excretion and the production of γ-CEHC.

Our data showed that γ-Toc accelerates sodium excretion in rats given NaCl, but no effect was observed regarding potassium excretion. This is because LLU-α is an inhibitor of the 70 pS ATP-sensitive K^+(K_{ATP}) chan-

nel in the thick ascending limb of the loop of Henle (*10*).

Most search paradigms have focused on the inhibition of sodium transport, especially the inhibition of the Na^+/K^+-ATPase (sodium pump) to pump inhibitors, like ouabain or digoxin, as an assay to detect this compound during isolation (*20, 21*). This has led to the isolation of digoxin (*22*) and 'iso-ouabain' (*23–26*). However, digoxin and ouabain are kaliuretic and not natriuretic (*27–30*), in contrast to atrial natriuretic peptide, which is also not a sodium pump inhibitor (*31, 32*). Therefore, LLU-α is considered to play an important role in substances exhibiting a prolonged natriuresis, such as that described for 'natriuretic hormone'.

γ-Toc demonstrated natriuretic potency as a natriuretic hormone precursor as well as γ-tocotrienol (*14*). In addition, the formation of γ-CEHC, coming from γ-tocotrienol, may also prevent the development of increased blood pressure by natriuretic factors.

If γ-Toc only accelerates sodium excretion in rats with a high sodium intake, while having no effect on potassium, γ-Toc might therefore be important as a precursor to a natriuretic hormone.

γ-Toc would be a second example of a vitamin acting as a precursor for a hormone, similar to vitamin D. It is necessary to further investigate the long-term administration of γ-Toc in the future.

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