Physiol. Res. 52: 767-771, 2003

Vegetable Oils Used as Vitamin E Vehicle Affect the Electrical Activity of the Rat Heart

S. OZDEMIR, M. AYAZ, T. TUNCER, M. UGUR, B. TURAN

Department of Biophysics, Faculty of Medicine, Ankara University, Ankara, Turkey

Received December 10, 2001
Accepted August 29, 2002

Summary

The aim of this study is to define the possible effects of vegetable oils used as vitamin E vehicle on the electrical activity of the rat heart. To test the possible effects of vitamin E vehicles we studied the effect of i.p. injected corn oil, hazelnut oil or peanut oil on the action potential parameters recorded in both papillary and left atrial muscle strips. Four experimental groups were used. The control group was injected (i.p.) with distilled water, while the three remaining groups received injections of corn oil, hazelnut oil, or peanut oil for five weeks (in a dose of 0.4 ml/kg/day – minimum amount of oil in which vitamin E could be dissolved). We used borosilicated (15-20 MΩ) capillary electrodes and intracellular action potentials (AP) were recorded in isolated papillary and left atrium muscle strips. While administration of three different types of vegetable oil had no significant effect on AP parameters of papillary muscle, they significantly prolonged the repolarization phase of AP in atrial strips. These results show that vegetable oils used as vitamin E vehicles may alter the electrical activity of the heart in a tissue-dependent manner. The present data indicate that the possible effect of vegetable oil vehicles should be kept in mind while evaluating the possible effects of *in vivo* vitamin E administration.

Key words

Action potential • Vitamin E • Corn oil • Peanut oil • Hazelnut oil • Papillary muscle • Atrial muscle • Repolarization

Introduction

Vitamin E is a naturally occurring free radical scavenger and its most widely accepted biological function is its antioxidant property. Vitamin E is well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant, protecting cell membranes from peroxidative damage (Oski 1980). In addition to its antioxidant function, vitamin E influences the cellular response to oxidative stress through modulation of signal-transduction pathways (Azzi *et al.* 1992).

Vitamin E (α-tocopherol) has the highest biological activity (Burton *et al.* 1988, Gonzalez *et al.* 1991, Liebler *et al.* 1996, Weiser *et al.* 1996) and reverses vitamin E deficiency symptoms in humans (Brin *et al.* 1986, Sokol *et al.* 1988, Schuelke *et al.* 1999). Since its discovery, vitamin E has been mentioned as an essential nutrient for all animal species. It has been used in the treatment of diabetes mellitus, autoimmune diseases, and genetic disorders (for review see Tengerdy 1989, Packer and Landvik 1989). Epidemiological studies (Stampfer *et al.* 1993, Rimm *et al.* 1993) have reported that a high vitamin E intake correlates with a reduced risk of cardiovascular diseases and vitamin E also plays specific roles beyond that of its antioxidant function. In addition to the free phenolic form, oxidation-protected

© 2003 Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

forms such as α-tocopheryl acetate and α-tocopheryl succinate (commercially available vitamin E supplements) are also employed.

The esterified form of α-tocopherol, α-tocopheryl acetate, is dissolved in various vegetable oils for i.p. or i.v. injection in animals and humans (Armstrong *et al.* 1998, Dhalla *et al.* 1998, Elsayed *et al.* 2000). In some studies, the investigators have not mentioned which type of vehicle was used for vitamin E (α-tocopheryl acetate) administration (Bendich *et al.* 1984, Ozden *et al.* 1989, Venditti *et al.* 1997, 1998, Yilmaz *et al.* 1997, Dhalla *et al.* 1998).

The aim of this study was to define the possible effects of vegetable oils (used as vitamin E vehicle) on the electrical activity of the rat heart. In these experiments, we tested the effects of corn oil, hazelnut oil, and peanut oil (0.4 ml/kg/day, a minimum amount of oil in which vitamin E could be dissolved) on the intracellular action potential parameters of the rat papillary muscle. We also tested the effect of administration of these vegetable oils on the atrial action potential parameters. Surprisingly, while administration of these vegetable oil had no significant effect on the action potential parameters of rat papillary muscles, they significantly prolonged the action potential repolarization phase of the atrial strips. This result implies that vegetable oils used as the vitamin E vehicle may themselves alter the electrical activity of the heart.

Methods

Animal Care

Since no significant differences were found in the action potential parameters of male and female rat hearts (data not shown), Wistar rats of either sex, weighing 200-250 g, were used. Animals were separated depending on their sex and housed three rats per cage throughout the experiment. They were fed with standard rat chow and drank tap water *ad libitum*.

The first group of rats (control group, Group I) was injected (i.p.) with distilled water while the other groups (Groups II, III, IV) received injections (i.p) of 0.4 ml/kg/day corn oil, 0.4 ml/kg/day peanut oil, or 0.4 ml/kg/day hazel-nut oil for five weeks prior to heart excision.

Action Potential Recordings in Papillary and Atrial Muscle Strips

The rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and the hearts were rapidly removed and placed into a low-Ca^{2+}-containing ($CaCl_2$, 0.625 mmol/l) modified Krebs solution (mmol/l): NaCl 120; KCl 5; $MgCl_2$ 1.2; NaH_2PO_4 2; Na_2SO_4 1.2; $NaHCO_3$ 5; Glucose 10; HEPES 20, pH 7.4. The left ventricular papillary muscle and the left atrium were isolated in the same solution. For the electrophysiological recordings, the papillary muscle or the left atrium samples were investigated under similar experimental conditions. Briefly, the samples were pinned down with a stimulating electrode horizontally on a paraffin block placed in a 5 ml organ bath in the Krebs solution containing 2.5 mmol/l $CaCl_2$. The bathing solution was bubbled and circulated (3 ml/min) with oxygen mixture (95 % O_2 and 5 % CO_2). All experiments were carried out at 37 °C after an equilibration period (50-60 min). Inner filamented borosilicate glass capillaries (Clark Electromedical Instruments GCl50F-15) were used to make glass microelectrodes (10-15 MΩ) filled with 3M KCl. The output of the microelectrode recording preamplifier was stored on-line on the hard disk of a microcomputer at a sampling rate of 10 kHz. Muscle preparations were stimulated (Grass S48 stimulator) with a pinned down stimulating electrode (twice the threshold potential) and rectangular pulse (3 ms duration) at a constant frequency (1 Hz).

Data Analysis

Recorded action potential data were converted to an Excel worksheet with a specially prepared TurboPascal program. Since there was no significant difference between the implements, the average values of the data were used. The average action potential of each implement was used to calculate the time required to reach 25, 50 and 75 % of repolarization (APD25, APD50, and APD75). Mean values ± S.E.M. of the experimental groups were compared with the values of the control group using the one-way ANOVA test.

Results

The initial and final body weights as well as final heart weights of all animals studied were determined. Administration of corn oil (Group II), hazelnut oil (Group III), or peanut oil (Group IV) had no significant effects on the body weight and heart weight/body weight index when compared to the control group (Group I).

Effect of vegetable oils on electrical activity of papillary muscle

Time required to reach 25, 50 and 75 % of repolarization (APD25, APD50 and APD75) measured in papillary muscle strips was not different between the groups studied (Table 1), indicating that repolarization parameters of oil injected groups did not significantly differ from the control values ($p>0.05$).

Table 1. Action potential parameters recorded in papillary muscle strips.

Groups	APD25 (ms)	APD50 (ms)	APD75 (ms)
Group I (n=8)	8.1±0.7	15.7±1.7	32.8±3.8
Group II (n=8)	8.8±1.2	15.8±2.0	30.7±3.1
Group III (n=3)	9.3±1.2	17.0±2.4	32.8±4.3
Group IV (n=8)	9.7±0.6	18.5±1.4	37.2±3.2

The values are given as mean ± S.E.M., n indicates the number of animals used in the experiments. Group I: control group (injected with water). Group II: corn oil injected group. Group III: hazelnut oil injected group. Group IV: peanut oil injected group.

Table 2. The effect of injection of vegetable oils on the action potential parameters of left atria

Groups	APD25 (ms)	APD50 (ms)	APD75 (ms)
Group I (n=8)	5.3±0.6	10.0±1.0	19.5±1.8
Group II (n=6)	10.4±0.9*	22.4±2.2*	45.9±3.1*
Group III (n=6)	10.7±0.8*	21.3±1.4*	41.0±0.5*
Group IV (n=5)	10.9±1.1*	22.1±1.7*	40.8±2.3*

*The values are given as mean ± S.E.M., n shows the number of animals used in experiments. Group I: control group (injected with water). Group II: corn oil injected group. Group III: hazelnut oil injected group. Group IV: peanut oil injected group. APD25, APD50, and APD75 are the time required to reach 25, 50, and 75 % of repolarization. * Significantly different from group I ($p<0.001$).*

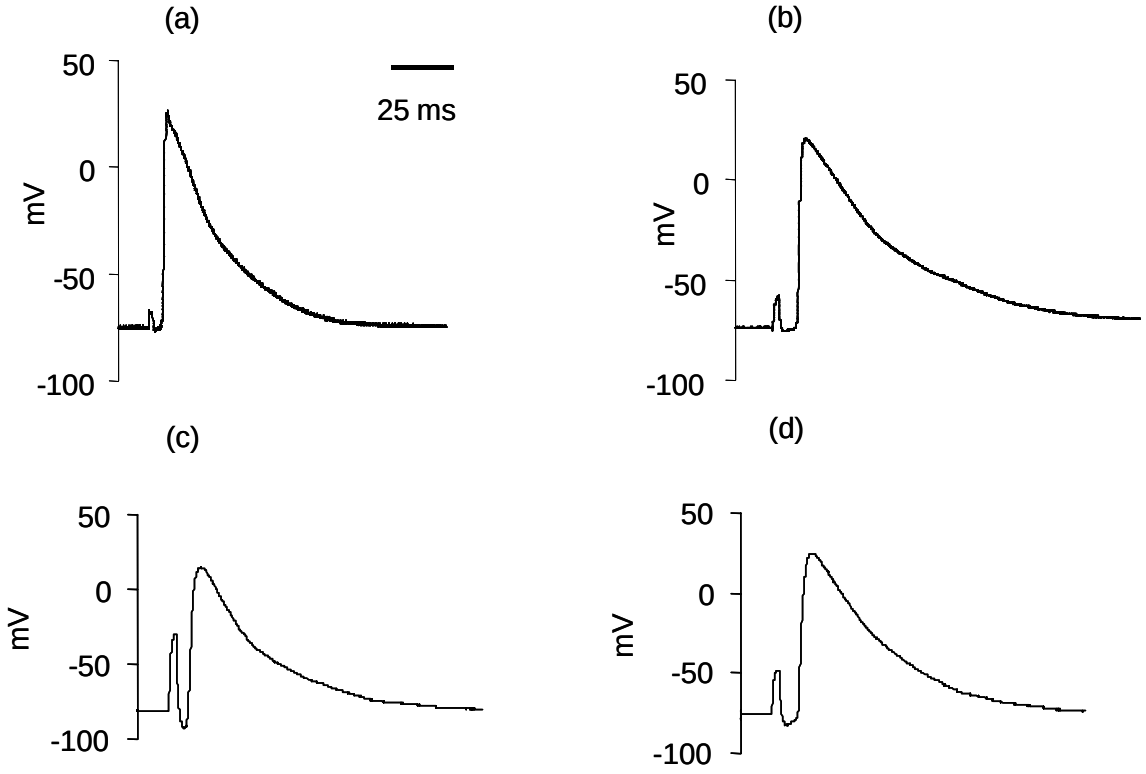

Fig. 1. *Representative action potential traces recorded in atrial muscle preparations. Intracellular action potential recorded with glass microelectrodes filled with 3M KCl (10-15 MΩ) in left atrial muscle strips from (a) control, (b) corn oil, (c) hazelnut oil, and (d) peanut oil injected rats*

Effect of corn oil on action potential of atrial muscle

Under exactly the same experimental conditions, we measured action potential parameters of left atria from rats injected with three different types of vegetable oils (Table 2). As can be seen from this table, injection of all three types of vegetable oils caused a significant ($p<0.001$) prolongation in the repolarization phase of atrial action potentials. Especially, the prolongation in the late phase of the action potential (APD75) is very prominent. Representative action potential recordings from control, corn oil, peanut oil, and hazel-nut oil injected groups are given in Figure 1.

Discussion

The main result of the present study concerns the finding that although administration of three different types of vegetable oils (corn oil, peanut oil, hazel-nut oil) did not alter the action potential parameters of rat papillary muscle preparations, the administration of these oils significantly prolonged the atrial action potential duration. This difference may arise from different functional and/or structural properties of ventricles and atria. Although many authors did not mention which type of vitamin E carrier was used in their experiments (Bendich *et al.* 1984, Ozden *et al.* 1989, O'Farrell and Jackson 1997, Yilmaz *et al.* 1997, Venditti *et al.* 1997, 1998, Dhalla *et al.* 1998), our present data suggest that vegetable oils used as vitamin E vehicle may significantly alter the various functions of organs including that of the cardiac tissue. One should check such effects before use and also consider that these effects may be tissue-dependent.

The effects of some dietary oils (soybean oil, olive oil and triolein) on lipid peroxidation and on antioxidant parameters of rat were studied (Scaccini *et al.* 1992, Skuladottir *et al.* 1994, Gerbi *et al.* 1999). It was shown that the optimal balance between the content of unsaturated fatty acids and natural antioxidants in dietary oils is of major importance. Recently, Armstrong *et al.* (1998) studied the effect of vitamin E in 2 ml corn oil on the improvement of blood oxygenation after high-energy impulse noise (blast) exposure of rats but, surprisingly, they did not test the effect of corn oil alone on these parameters under the same experimental protocol. A similar study by Hageman *et al*. (1999) can be accepted as support of our present data about corn oil effect on atrial contractility and the above comments related to the vitamin E vehicle, because in their study, the excess of vitamin E (with corn oil emulsion) decreased canthaxanthin absorption and consequently the bioavailability of carotenoid in the rat. This effect may arise because of the side effect of corn oil as vitamin E vehicle.

Acknowledgements

This work was partly supported by Ankara University Research Funds, projects 98-09-00-05 and 99-09-00-10, and DPT project 99K-120190.

References

ARMSTRONG KL, COOPER MF, WILLIAMS MT, ELSAYED NM: Vitamin E and lipoic acid, but not vitamin C improve blood oxygenation after high-energy impulse noise (blast) exposure. *Biochem Biophys Res Commun* **253**: 114-118, 1998.

AZZI A, BOSCOBONIK D, HENSEY C: The protein kinase C family. *Eur J Biochem* **208**: 547-557, 1992.

BENDICH A, D'APOLITO P, GABRIEL E, MACHLIN LJ: Interaction of dietary vitamin C and vitamin E on guinea pig immune responses to mitogens. *J Nutr* **114**: 1588-1593, 1984.

BRIN MF, PEDLEY TA, EMERSON RG, GOURAS P, MACKAY C, KAYDEN HJ, LEVY J, BAKER H: Electrophysiological features of abetalipoproteinemia: functional consequences of vitamin E deficiency. *Neurology* **36**: 669-673, 1986.

BURTON GW, INGOLD KU, FOSTER DO, CHENG SC, WEBB A, HUGHES L, LUSZTYK E: Comparison of free α-tocopherol and α-tocopheryl acetate as sources of vitamin E in rats and humans. *Lipids* **23**: 834-840, 1988.

DHALLA NS, LIU X, PANAGIA V, TAKEDA N: Subcellular remodelling and heart dysfunction in chronic diabetes. *Cardiovasc Res* **40**: 239-247, 1998.

ELSAYED NM, ARMSTRONG KL, WILLIAMS MT, COOPER MF: Antioxidant loading reduces oxidative stress induced by high-energy impulse noise (blast) exposure. *Toxicology* **155**: 91-99, 2000.

GERBI A, MAIXENT JM, ANSALDI JL, PIERLOVISI M, COSTE T, PELISSIER JF, VAGUE P, RACCAH D: Fish oil supplementation prevents diabetes-induced nerve conduction velocity and neuroanatomical changes in rats. *J Nutr* **129**: 207-213, 1999.

GONZALEZ-FLECHA BS, REPETTO M, EVELSON P, BOVERIS A: Inhibition of microsomal lipid peroxidation by alpha-tocopherol and alpha-tocopheryl acetate. *Xenobiotica* **21**: 1013-1022, 1991.

HAGEMAN SH, SHE L, FURR HC, CLARK RM: Excess vitamin E decreases canthanxanthin absorption in the rat. *Lipids* **34**: 627-631, 1999.

LIEBLER DC, BURR JA, PHILIPS L, HAM AJ: Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products. *Anal Biochem* **221**: 797-805, 1996.

O'FARRELL S, JACKSON MJ: Dietary polyunsaturated fatty acids, vitamin E and hypoxia/reoxygenation-induced damage to cardiac tissue. *Clin Chim Acta* **267**: 197-211, 1997.

OSKI F A: Vitamin E – a radical defense. *New Engl J Med* **303**: 454-455, 1980.

OZDEN I, DENIZ G, TASALI A, ULUSARAC A, ALTUG T, BUYUKDEVRIM S: The effect of vitamin E on glycosylated hemoglobin levels in diabetic rats: a preliminary report. *Diabetes Res* **12**: 123-124, 1989.

PACKER L, LANDVIK S: Vitamin E: introduction to biochemistry and health benefits. *Ann N Y Acad Sci* **570**: 1-6, 1989.

RIMM EB, STAMPFER M J, ASCHERIO A, GIOVANNUCCI E, COLDITZ GA, WILLETT WC: Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* **328**: 1450-1456, 1993.

SCACCINI C, NARDINI M, D'AQUINO M, GENTILI V, DI FELICE M, TOMASSI G: Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat. *J Lipid Res* **33**: 627-633, 1992.

SCHUELKE M, MAYATEPEK E, INTER M, BECKER M, PFEIFFER E, SPEER A, HUBNER C, FINCKH B: Treatment of ataxia in isolated vitamin E deficiency caused by α-tocopherol transfer protein deficiency. *J Pediatr* **134**: 240-244, 1999.

SKULADOTTIR GV, SHI-HUA D, BRODIE AE, REED DJ, WANDER RC: Effects of dietary oils and methyl ethyl ketone peroxide on in vivo lipid peroxidation and antioxidants in rat heart and liver. *Lipids* **29**: 351-357, 1994.

SOKOL RJ, KAYDEN HJ, BETTIS DB, TRABER MG, NEVILLE H, RINGEL S, WILSON WB, STUMPF DA: Isolated vitamin E deficiency in the absence of fat malabsorption-familial and sporadic cases: characterisation and investigation of causes. *J Lab Clin Med* **111**: 548-559, 1988.

STAMPFER MJ, HENNEKENS CH, MANSON JE, COLDITZ GA, ROSNER B, WILLETT WC: Vitamin E consumption and the risk of death from coronary artery disease in women. *N Engl J Med* **328**: 1444-1449, 1993.

TENGERDY RP: Vitamin E, immune response, and disease resistance. *Ann N Y Acad Sci* **570**: 335-344, 1989.

VENDITTI P, DE LEO T, DI MEO S: Vitamin E administration attenuates the triiodothyronine-induced modification of heart electrical activity in the rat. *J Exp Biol* **200**: 909-914, 1997.

VENDITTI P, DE LEO T, DI MEO S: Antioxidant-sensitive shortening of ventricular action potential in hyperthyroid rats is independent of lipid peroxidation. *Mol Cell Endocrinol* **142**: 15-23, 1998.

WEISER H, RISS G, KORMANN AW: Biodiscrimination of the eight alpha-tocopherol stereoisomers results in preferential accumulation of the four 2R forms in tissues and plasma of rats. *J Nutr* **126**: 2539-2549, 1996.

YILMAZ O, CELIK S, NAZIROGLU M, CAY M: The effects of dietary selenium and vitamin E and their combination on the fatty acids of erythrocytes, bone marrow and spleen tissue lipids of lambs. *Cell Biochem Funct* **15**: 1-7, 1997.

Reprint requests

Prof. Belma Turan, Ankara University, Faculty of Medicine, Department of Biophysics, Morfoloji Binasi, Sihhiye, 06100, Ankara, Turkey. Fax: +90 312 310 6370, E mail: bturan@medicine.ankara.edu.tr