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VITAMIN E ADMINISTRATION ATTENUATES THE TRI-IODOTHYRONINE-INDUCED MODIFICATION OF HEART ELECTRICAL ACTIVITY IN THE RAT

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Summary

This work was designed to determine whether the thyroid-hormone-induced modifications of heart electrical activity are, at least in part, due to increased free radical production. For this study, 60-day-old euthyroid, hyperthyroid and hyperthyroid vitamin-E-treated rats were used. Hyperthyroidism, elicited by a 10 day treatment with tri-iodothyronine, induced an increase in lipid peroxidation without changing the level of antioxidants. Intraperitoneal vitamin administration to hyperthyroid rats led to a reduction in lipid peroxidation and a non-significant increase in antioxidant level.

The hyperthyroid state was also associated with an increase in heart rate measured *in vivo* and a decrease in the duration of the ventricular action potential recorded *in vitro*. Administration of vitamin E attenuated the thyroid-hormone-induced changes in heart rate and action potential duration, which were, however, significantly different from those of the control euthyroid rats. These results suggest that vitamin E protects hyperthyroid heart against lipid peroxidation by mechanisms that may be independent of the changes in antioxidant systems. Moreover, the reduction in the tri-iodothyronine effects on heart electrophysiological properties indicates that such effects are mediated, at least in part, through a membrane modification, probably related to increased lipid peroxidation, involving a free radical mechanism.

Key words: antioxidants, hyperthyroidism, vitamin E, cardiac action potential, lipid peroxidation, rat, tri-iodothyronine, heart.

Introduction

Thyroid hormone has been known for many years to exert direct chronotropic effect through a modulation of cardiac electrophysiological properties. Intracellular electrode recordings from both rabbit sinoatrial node (Johnson *et al.* 1973) and ventricular preparations from several mammalian species (Sharp *et al.* 1985; Binah *et al.* 1987; Di Meo *et al.* 1991) have shown that the action potential duration is shortened in hyperthyroidism and lengthened in hypothyroidism. However, the mechanism for thyroid hormone action on heart electrophysiology has not been studied extensively. Experimental evidence indicates that many of the actions of thyroid hormone are mediated by controlling the expression of specific genes (Samuels *et al.* 1988) initiated through binding to nuclear receptors, which have been identified as the products of the c-erbA proto-oncogenes (Evans, 1988; Samuels *et al.* 1988). It is, therefore, possible that thyroid hormone may influence transmembrane conductance through its interactions with nuclear binding sites to produce ionic channels with modified gating and kinetic properties. In heart, thyroid hormone induces increases in the level of several mRNAs (Rohrer and Dillmann, 1988; Arai *et al.* 1991; Kamitani *et al.* 1992; Ram and Waxman, 1992), but no evidence has been found that the hormone induces increases in the level of specific mRNAs coding for proteins that are part of ionic channels. Moreover, recent findings indicate that thyroid hormone increases the L-type Ca^{2+} current ($I_{Ca,L}$) through a modulation of the channel protein phosphorylation state mediated by activation of the adenylate cyclase cascade (Mager *et al.* 1992). However, the electrophysiological effects of thyroid hormone could be mediated in part by modifying membrane lipids and lipid–protein interactions. In fact, reports are available indicating the dependence on thyroid state of determinants of membrane fluidity, such as the cholesterol/phospholipid ratio and the composition of phospholipid acids (Szymańska *et al.* 1991), and lipid peroxidation (Asayama *et al.* 1987). The acceleration of heart lipid peroxidation, which occurs in experimental hyperthyroidism, suggests the contribution of reactive oxygen species to the cardiomyopathy usually found in this pathological state. These results also suggest the possible involvement of free radicals in the modifications of the electrophysiological properties of the sarcolemmal membrane in hyperthyroid heart. To substantiate this hypothesis, studies determining the effect of the administration of antioxidant to hyperthyroid animals are required. We designed the following study to examine whether the electrophysiological

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modifications produced by treatment with thyroid hormone were attenuated by the administration of vitamin E to rats. To assess the effectiveness of hormone and vitamin administration in, respectively, inducing or preventing oxidative alterations in the animals, we determined the cardiac level of lipid peroxidation and antioxidants.

Materials and methods

Animals

Male Wistar rats (60 days old) were used in the experiments. The animals, purchased at weaning from Nossan (Correzzana, Italy), were housed one or two per cage in a temperature-controlled room at 24±1 °C and maintained on a 12 h:12 h light:dark photoperiod. All animals were provided with the same diet, a commercial rat chow purchased from Nossan, containing 105 i.u. kg^{-1} of vitamin E and water on an *ad libitum* basis. From day 50, animals were randomly assigned to one of three groups: C, control rats; H, rats made hyperthyroid by treatment with daily intraperitoneal injections of T_3 (10 μg 100 g^{-1} body mass) for 10 days; H+VE, rats treated for 10 days with daily intraperitoneal injections of 10 μg 100 g^{-1} body mass of T_3 and 10 mg 100 g^{-1} body mass of vitamin E. These T_3 and vitamin doses are able to induce hyperthyroidism (Kolář *et al.* 1992) and prolong endurance (Novelli *et al.* 1990), respectively, in euthyroid animals.

Experimental procedure

After a 12 h overnight fast, the animals were subjected to the measurement of resting metabolic rate (RMR) by an open-circuit indirect calorimetry system (Columbus Instruments International Corp., Columbus, Ohio, USA). Before being killed, the animals were anaesthetized with Ethrane (Abbot, Aprilia, Italy) and subjected to electrocardiographic recording, as previously reported (Valente *et al.* 1989). Rats, still under anaesthesia, were killed by decapitation and the hearts were rapidly excised and placed in cold oxygenated Krebs solution (135 mmol l^{-1} NaCl, 5 mmol l^{-1} KCl, 1 mmol l^{-1} $MgCl_2$, 2 mmol l^{-1} $CaCl_2$, 13 mmol l^{-1} $NaHCO_3$, 1 mmol l^{-1} NaH_2PO_4, 11 mmol l^{-1} glucose; pH 7.4). The great vessels and valves of the heart were trimmed away, the ventricles and atria were cut open and rinsed free of blood, and the right ventricular papillary muscles were removed to be used in the electrophysiological determinations. After the hearts had been weighed, 20 % (w/v) homogenates were prepared using a Potter–Elvehjem homogenizer set at a standard velocity (500 revs min^{-1}) for 2 min in ice-cold medium consisting of 0.175 mol l^{-1} KCl, 15 mmol l^{-1} Tris, pH 7.4.

Level of tissue antioxidants

The homogenates were diluted with equal volume of 15 mmol l^{-1} Tris containing 0.2 % Lubrol PX (Sigma Chimica, Milano, Italy) at pH 8.5, so that the concentration of the homogenate was 10 % (w/v). These preparations were used to determine the level of heart antioxidants by a method based on the enhanced luminescence technique (Venditti *et al.* 1995). In this method, the ability of the 10 % homogenates to quench the light emission from a peroxidase-induced luminescent reaction was compared with that of antioxidant solutions. As the more reproducible results were obtained by using desferrioxamine (Venditti *et al.* 1995), in this study the activity scale was obtained using this compound and the heart antioxidant capacity was expressed as an equivalent concentration of desferrioxamine.

Measurement of lipid peroxidation

As a measure of lipid peroxidation, the malondialdehyde (MDA) content of 10 % homogenates in 0.175 mol l^{-1} KCl, 15 mmol l^{-1} Tris, pH 7.4, was determined using the thiobarbituric acid reaction, according to the method of Buege and Aust (1978).

Transmembrane potential determination

Right ventricular papillary muscles were mounted horizontally in a chamber between bipolar silver electrodes and superfused, at a rate of 11 ml min^{-1}, with Krebs solution gassed with 95 % O_2/5 % CO_2 and warmed to 26±0.4 °C. The preparations were allowed to equilibrate for 1 h under stimulation at 0.1 Hz before measurements were taken. During measurements, muscles were stimulated at 1 Hz. Transmembrane potentials were measured by cellular impalement using glass capillary microelectrodes filled with 3 mol l^{-1} KCl and standard microelectrode techniques (Di Meo *et al.* 1991). The action potential signal was displayed and monitored on an oscilloscope (Tektronix 502A) throughout the experiment. Each action potential was recorded on-line in digital form at 80 100 μs intervals on an IBM PC AT and stored on hard disk for subsequent processing. The transmembrane potentials were analysed using a computer program for the following characteristics: resting membrane potential, depolarization time, action potential amplitude, area above 20 % depolarization and recovery times from 10 to 90 % of repolarization.

Statistical analyses

As impalements in approximately 20 cells of each preparation were performed, mean values of the electrical parameters were calculated for each preparation, and the sample means were averaged. The resulting means were used to provide traces of action potential characteristic for each group. Values are reported as means ± S.E.M. in the tables and indicated by vertical bars in the figures. The data were analyzed using one-way analysis of variance. When a significant F ratio was found, Student–Newman–Keuls multiple range test was used to assess differences among group means. In all instances, the values were considered significantly different at $P<0.05$.

Results

Indicators of thyroid state

The T_3 treatment is associated with a decrease in body mass and increases in heart mass and the heart mass/body mass ratio,

Table 1. *Body parameters*

Group	Body mass (g)	Heart mass (g)	Heart mass/ body mass (mg g^{-1})
C	292±13	0.79±0.03	2.70±0.08
H	245±10*	0.94±0.02*	3.89±0.15*
H+VE	251±8*	0.97±0.04*	3.89±0.21*

Values are means ± S.E.M. of eight different experiments.

C, control rats; H, hyperthyroid rats; H+VE, hyperthyroid vitamin-E-treated rats.

*Significant (P<0.05) difference compared with the C group.

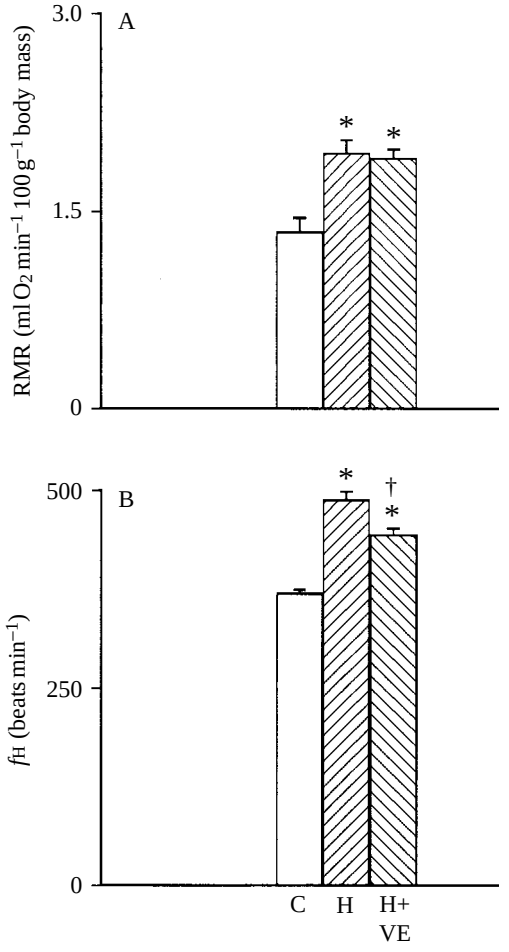

Fig. 1. Effect of vitamin E and/or T_3 administration on (A) resting metabolic rate (RMR) and (B) heart rate (f_H) of rats. C, control rats; H, hyperthyroid rats; H+VE, hyperthyroid vitamin-E-treated rats. *Significantly (P<0.05) different from the value for the C group; †significantly (P<0.05) different from the value for the H group. Values are means + S.E.M., N=8.

and the administration of vitamin E to hyperthyroid rats has little effect on these changes (Table 1). The RMR and the heart rate (f_H) of hyperthyroid animals are higher than those of control rats. Administration of vitamin E attenuates the effect on f_H but not that on RMR (Fig. 1).

Table 2. *Level of antioxidants and lipid peroxidation in rat heart*

Groups	Antioxidants	Lipid peroxidation
C	0.47±0.09	75.9±3.1
H	0.35±0.08	93.1±4.0*
H+VE	0.62±0.11	75.5±2.6†

Values are the means ± S.E.M. of eight different experiments.

Antioxidant levels are expressed as equivalent concentration of desferrioxamine (mmol l^{-1}). Lipid peroxidation levels are expressed as nmol malondialdehyde g^{-1} wet mass.

C, control rats; H, hyperthyroid rats; H+VE, hyperthyroid vitamin-E-treated rats.

*Significant (P<0.05) difference compared with the C group.

†Significant (P<0.05) difference compared with the H group.

Antioxidants and lipid peroxidation

Notwithstanding its limits, the measurement of MDA using the thiobarbituric acid reaction is a useful index of lipid peroxidation. The data in Table 2 indicate that T_3 treatment significantly increases peroxidative processes, whereas administration of vitamin E to hyperthyroid rats protects against this increase.

We also measured the antioxidant levels in heart homogenates. Our results show that neither the T_3 treatment nor the combined treatment with hormone and vitamin produced significant changes in the levels (Table 2).

Ventricular action potential

The effect on heart rate was consistent with those on action potential characteristics, clearly shown by the superimposed recordings of the averaged action potentials of papillary muscles in Fig. 2. These recordings demonstrate that the treatments are associated with marked alterations in action potential configuration, the most pronounced change being a decrease in action potential duration in hyperthyroid rats; vitamin E treatment lessens this decrease.

An examination of the related quantitative values shows that the resting potential, action potential amplitude and depolarization time are not affected by either T_3 or vitamin E treatment. The repolarization phase of the action potential is significantly accelerated in H animals, as the recovery time from 30% to 90% depolarization is markedly shortened. Furthermore, the area of the action potential is decreased. Administration of vitamin E attenuates the T_3-induced modifications of the repolarization phase of the action potential. Although the recovery times in the H+VE rats are longer than those in the H rats, they remain shorter than those in the control rats (Table 3).

Discussion

It has recently been suggested that the hypermetabolic state brought about by hyperthyroidism accelerates free radical production and induces changes in the antioxidant defence

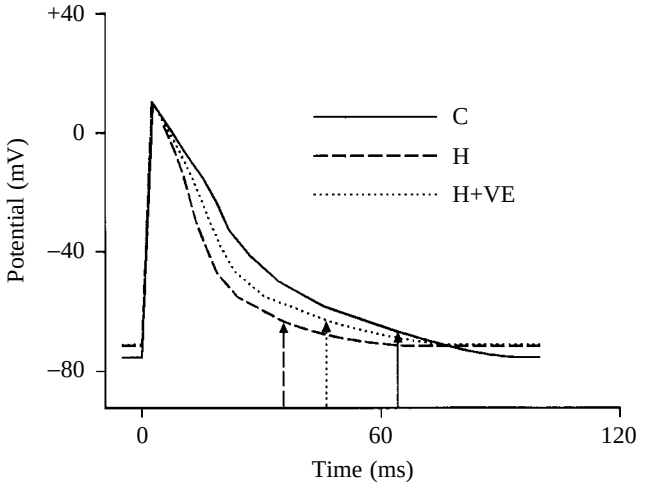

Fig. 2. Effect of vitamin E and/or T_3 administration on action potentials recorded from rat papillary muscle fibres. The working frequency was 1 Hz, the temperature was 26 °C. The arrows indicate the action potential duration at 90 % repolarization. C, control rats; H, hyperthyroid rats; H+VE, hyperthyroid vitamin-E-treated rats. The action potentials are computer-averaged traces from *N*=8 preparations (see Materials and methods).

Table 3. *Electrical properties of rat papillary muscle fibres*

	Group		
Variable	C	H	H+VE
Resting potential (mV)	–75.2±1.2	–71.4±1.2	71.0±1.4
Action potential (mV)	85.1±1.3	81.9±1.1	81.6±0.8
DT (ms)	2.8±0.3	2.5±0.3	2.5±0.6
A_{20} (mV ms)	1304±76	727±68*	959±89*,†
RT_{30} (ms)	7.9±1.0	12.8±1.2*	9.8±1.1
RT_{40} (ms)	16.3±1.3	9.6±1.1*	12.3±1.8
RT_{60} (ms)	24.4±1.5	13.7±1.2*	17.4±2.4*,†
RT_{70} (ms)	31.5±1.8	16.3±1.2*	20.8±2.6*
RT_{80} (ms)	42.9±2.3	21.5±1.3*	28.1±2.8*,†
RT_{90} (ms)	61.5±3.3	33.1±1.7*	43.8±4.0*,†

Values are the mean ± S.E.M. of eight different experiments.

DT, depolarization time; A_{20}, integrated area above 20 % depolarization; RT_{30}, RT_{40}, RT_{60}, RT_{70}, RT_{80}, RT_{90}, recovery time at 30, 40, 60, 70, 80 and 90 % repolarization, respectively.

C, control rats; H, hyperthyroid rats; H+VE, hyperthyroid vitamin-E-treated rats.

*Significant (P<0.05) difference compared with the C group.

†Significant (P<0.05) difference compared with the H group.

system in skeletal and heart muscle (Asayama and Kato, 1990). In effect, it has been found that mitochondrial oxidative metabolism and the level of lipid peroxides increase in a parallel manner in the heart of hyperthyroid rats (Asayama *et al.* 1987, 1989*a*,*b*), but the information on heart antioxidants is poor and inconclusive. The activity of mitochondrial superoxide dismutase increases, whereas the activities of cytoplasmic superoxide dismutase, glutathione peroxidase and catalase decrease (Asayama *et al.* 1987, 1989*a*,*b*). Coenzyme Q concentration decreases, whereas α-tocopherol concentration, which increases after 10–30 days of thyroxine treatment (Neradilová *et al.* 1973; Hrubá *et al.* 1976; Mano *et al.* 1995), declines gradually during more prolonged treatment (Hrubá *et al.* 1976). It is evident that measurement of the activities of the various scavenger systems does not allow the effect of thyroid hormone on the heart antioxidant status to be determined. Therefore, we have used a method which supplies a quantitative evaluation of the overall antioxidant capacity of the tissue (Venditti *et al.* 1995). Our results show that T_3 treatment increases the cardiac production of thiobarbituric-acid-reactive substances and produces no significant changes in the antioxidant defences. This indicates that, in hyperthyroidism, the antioxidant level is not related to oxidative metabolism. The administration of vitamin E to hyperthyroid rats reinstates lipid peroxidation in the heart to a normal level without significantly modifying the antioxidant capacity of the tissue. The protection offered against tri-iodothyronine-induced acceleration of lipid peroxidation is consistent with several reports showing a therapeutic function for vitamin E in experimental hyperthyroidism. In fact, vitamin E seems to protect against both the reduction in glycogen concentration (Postelnicu, 1972) and the acceleration of oxidative phosphorylation in the brain (Uzebekova, 1970) of hyperthyroid rats. Furthermore, tocopherols are the most important scavenging factors for lipid peroxides *in vivo* (Burton *et al.* 1987).

It is possible to offer some explanation for the discrepancy between the above effects of vitamin E and its inability to increase heart antioxidant capacity. Vitamin E appears to have an important protective role towards the polyunsatured fatty acid components of highly organized membrane structures (Abrams *et al.* 1973), but to have a weak preventive effect for the oxidation of substances in the aqueous phase (Chen *et al.* 1993). Furthermore, thyroid hormone can induce a decrease in the levels of components of the antioxidant defence system which directly or indirectly serve to regenerate vitamin E. This idea is supported by results showing that levels of glutathione (Fernández *et al.* 1988) and glutathione peroxidase (Asayama *et al.* 1987) decrease in hyperthyroidism and that in euthyroid vitamin-E-supplemented diet-fed rats heart antioxidant capacity is higher than in rats fed a control diet (Di Meo *et al.* 1997).

The acceleration of heart lipid peroxidation and the protection offered by vitamin E suggest that reactive oxygen species contribute to the heart injury occurring in hyperthyroidism. The oxygen free radicals also seem to play a role in the mediation of heart injury elicited by other stress conditions. Reperfusion of heart muscle after prolonged ischaemia (Downey, 1990), strenuous physical exercise (Kumar *et al.* 1992), hyperoxia and stimulation of intracellular oxidases (Cohrane *et al.* 1988) leads to the generation of oxidant radicals, resulting in structural and functional alterations. Some research has focused on arrhythmias and

related alterations in the electrophysiological properties as manifestations of myocardial injury. The *in vitro* effects of free radicals on cardiac electrophysiology have been widely studied (Tarr and Valenzeno, 1989; Beresewicz and Horackova, 1991; Matsuura and Shattock, 1991; Jabr and Cole, 1993), whereas very little information is available on electrophysiological modifications related to the accelerated production of reactive oxygen species *in vivo*. We have recently shown that procedures leading to increased production of free radicals, such as hydroperoxide administration (Ji and Fu, 1992) and physical exercise (Davies *et al.* 1982), are associated with shortening of the ventricular action potential (Di Meo *et al.* 1996; Venditti *et al.* 1996). The similarity between the changes in action potential configuration found in hyperthyroidism and in the aforementioned conditions suggests the possible involvement of free radicals in the modifications of electrical properties of the sarcolemmal membrane of the hyperthyroid heart. Evidence for this involvement of free radicals is based on the ability of an antioxidant to remove or ameliorate the alteration. Therefore, the reduction of the T_3-induced modifications in ventricular action potential duration after vitamin E administration indicates that such modifications are attributable to the production of large quantities of free radicals. Moreover, the attenuation of the heart rate suggests that free radicals are also involved in the hyperthyroidism-induced reduction of action potential duration in the pacemaker cells (Johnson *et al.* 1973). Because the recovery of action potential duration and heart rate is incomplete, it is likely that the electrophysiological effects of thyroid hormone are in part mediated by other nuclear or extranuclear mechanisms.

The basis of the T_3-induced electrical alterations in terms of the underlying changes in membrane ionic currents is poorly defined. However, some results cause us to suspect that an increased K^+ current is responsible for the early fast phase of repolarization (Binah *et al.* 1987; Sharp *et al.* 1985). The changes in membrane structure by which the radicals modify membrane permeability are also still to be defined. Because protection against changes in heart electrical activity and against peroxidative reactions is afforded by vitamin E, it is possible that the above modification is produced by the peroxidation of membrane lipids. However, alternative mechanisms, such as an effect on the ionic channel, cannot be excluded because of the lack of a full understanding of the mechanism of action of vitamin E in the membrane. It has been suggested that the membrane effect is mediated by two distinct functions of the vitamin: (i) free radical scavenging, and (ii) a stabilizing structural role (Lucy, 1978). This view is supported by the observation that changes in the composition of the membranes which affect their fluidity also alter their susceptibility to peroxidative reactions. In fact, substances lacking the structural features of an antioxidant stabilize the membrane against lipid peroxidation through a mechanism involving a decrease in membrane fluidity (Wiseman *et al.* 1990, 1993). Studies using such substances could help to determine whether antioxidant-sensitive shortening of action potential duration is due to an attack by free radicals on lipid or protein components of the myocardial membrane.

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