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Thyroid Hormones Modulate Occurrence and Termination of Ventricular Fibrillation by both Long-Term and Acute Actions

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Summary

Thyroid hormones (TH) are powerful modulators of heart function, but their arrhythmogenic effects are less elucidated. We have examined both acute and long-term action of TH on the heart susceptibility to the ventricular fibrillation (VF) and on the heart ability to terminate VF and restore a sinus rhythm. Triiodothyronine (T_3) was applied in the range of 10^{-9}-10^{-6} mol/l in acute experiments using isolated perfused aged (14-month-old) guinea pig hearts. L-thyroxine (T_4) was applied in the dose of 50 μg/100g/day to young (3-month-old) and aged (20-month-old) rats for 2 weeks. The T_4 treatment resulted in an increased susceptibility of young, but not adult rat hearts to a hypokalemia-induced VF and facilitated a spontaneous sinus rhythm (SSR) restoration in the latter group. The acute T_3 administration in the range of 10^{-9}-10^{-7} mol/l significantly decreased the susceptibility of an isolated heart to an electrically induced VF and also facilitated the sinus rhythm restoration**.** The SSR restoration was, however, not affected by 10^{-6} mol/l concentration of T_3, which also led to an increased VF susceptibility. Results indicate that TH can affect the susceptibility of the heart to VF and its ability to restore the sinus rhythm *via* acute (non-genomic) and long-term (genomic) actions. Furthermore, an anti- and pro-arrhythmic potential of TH appears to be age- and dose-dependent.

Key words

Rat hearts • Ventricular fibrillation • Sinus rhythm restoration • Thyroid hormone • Genomic and non-genomic effects

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Introduction

The cardiovascular system is sensitive to the effect of thyroid hormones (TH) and deficiency or excess in hypo- or hyperthyroid status may significantly alter the cardiac function (Černohorský *et al.* 1998, Fadel *et al.* 2000, Yen 2001). Beneficial effects of TH are associated with positive inotropic actions and a therapeutic potential of TH as a cardioactive agent has been addressed recently (Hamilton *et al.* 1998, Klemperer 2002). Adverse effects are linked with arrhythmogenicity although the ventricular arrhythmias incidence in clinic is not usually attributed only to hyperthyroidism itself (Aronow 1995). Most of the prominent effects of TH is thought to be mediated by thyroid nuclear receptors leading to changes at the transcription level, however, there is some evidence for their non-genomic action (not related to transcription) (Davis and Davis 2002, Dillman 2002).

We suggest that both the beneficial and adverse effects may be due to either extranuclear (acute) or nuclear (prolonged) TH action. We have shown recently that TH in a supra-physiological concentration (10^{-8} and 10^{-7} mol/l) rapidly attenuated a Ca^{2+} overload in a neonatal heart-cell culture (Zinman *et al.* 2006). Likewise TH in concentration of 10^{-5} mol/l decreased a previously elevated cytosolic free Ca^{2+} in an isolated heart preparation, although in higher concentration (10^{-4} mol/l) further abnormal Ca^{2+} increase was detected (Tribulová *et al*. 2004). Alterations of the myocardial Ca^{2+} handling (in particular function of sarcoplasmic reticulum – SR) due to the long-term effect of pharmacological dose of TH and/or of hyperthyroidism are well established (Aronow 1995, Khoury *et al*. 1996, Černohorský *et al*. 1998).

Since high diastolic Ca^{2+} has been shown to be a key arrhythmogenic factor involved in the triggering as well as in the maintenance of malignant arrhythmias (Kihara and Morgan 1991, Merrilat *et al*. 1990, Tribulová *et al*. 2002), it can be expected that TH may modulate the arrhythmia susceptibility via modulation of the cytosolic free Ca^{2+}.

TH, in addition, can affect the expression of the intercellular gap junction channel protein, connexin-43 (Stock and Sies 2000, Tribulová *et al*. 2004). Connexin channels are responsible for a cell-to-cell electrical and metabolic coupling enabling the electrical and molecular signals propagation and hence the maintenance of a myocardial synchronization (Dhein 1998). On the other hand, an impaired coupling due to changes in distribution and/or the expression of connexin-43 (Severs 2001, Salameh and Dhein 2005, Fialová *et al*. 2008), or closing of connexin channels due to an intracellular Ca^{2+} overload (de Mello 1986, Dhein 1998) is believed to be arrhythmogenic (Manoach and Watanabe 1995, Manoach *et al*. 1997, Tribulová *et al*. 2003). Taken into consideration of all mentioned facts, this study was delineated to examine both the acute and long-term effects of increased TH levels on the susceptibility of the heart to the malignant arrhythmias and on its ability to restore the sinus rhythm in order to contribute to a further elucidation of beneficial and/or adverse effects of TH.

Methods

Two series of experiments were performed. First, Wistar female rats at the age of 3 and 20 months received 50 μg/100g/day of T_4 with the food for two weeks. Hearts excised from T_4 treated (n = 8) and from untreated age-matched control (n = 8) rats were perfused *via* cannulated aorta with an oxygenated Krebs-Henseleit (K-H) solution at the constant pressure and temperature. ECG, the left ventricular pressure and the coronary flow were continuously monitored. Upon 20-min stabilization, the hearts were subjected to a K^+ deficient K-H solution to induce a sustained, two-minute lasting VF, which was followed by a perfusion with a standard K-H solution to restore the sinus rhythm. At the end of experiments each heart was fixed by a perfusion with a buffered glutaraldehyde and processed for an electron microscopy.

Second, the hearts of the 14-month-old male guinea pigs (n = 18) were excised and perfused via the cannulated aorta for 20 min at the constant flow with the oxygenated K-H solution. ECG and functional parameters were continuously monitored. T_3 (in the range of 10^{-9}-10^{-6} mol/l) was administered to the perfusate 5 min prior to testing of a VF inducibility using a 1-sec burst of electrical rectangular pulses, 100 pps, duration 1 ms and 2x threshold voltage using Elektrostimulator ST-3“ (made in Hungary). When the sustained VF was induced, the time to the spontaneous sinus rhythm restoration was examined.

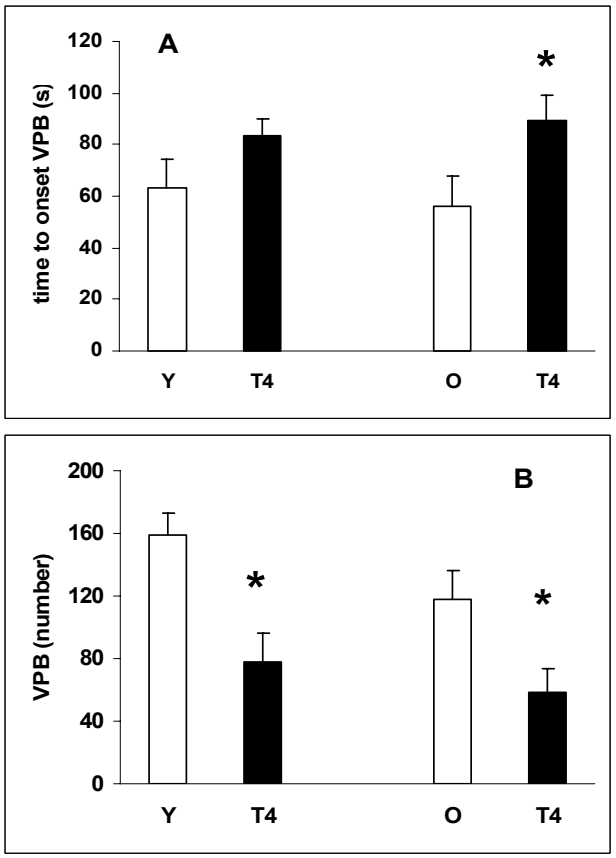

Fig. 1. Time to the onset (A) and number of hypokalemia-induced ventricular premature beats (B) in the isolated hearts of young (Y, n=6) and old (O, n=6) rats treated with L-thyroxine (T4) for two weeks. Note that TH suppressed significantly the incidence of VPB recorded during 5 min of hypokalemic perfusion in both young and old rat hearts as well as the onset of VPB in the latter ones. The results are expressed as means ± S.E.M, $p<0.05$.

Statistics

Results are given as means ± S.E.M. Statistical significances were ascertained by using the Student's two-tailed test for unpaired observations. Only corrected $p<0.05$ values were considered as significant.

Results

Total T_3 serum levels increased in 3-month-old rats (after application of T_4) by 69 % (from 0.80±0.03

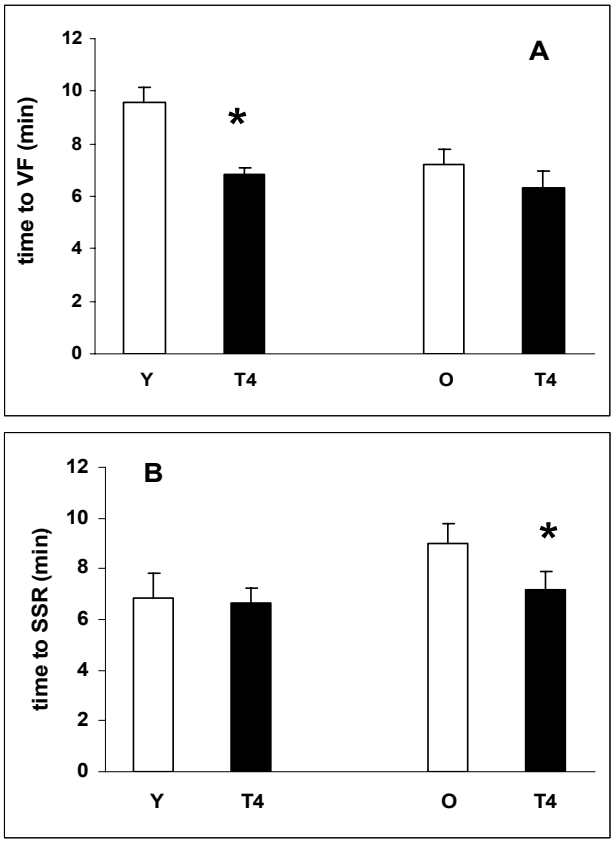

Fig. 2. Time to hypokalemia-induced VF (A) and time to the spontaneous sinus rhythm restoration (B) due to normokalemia in the isolated hearts of young (Y) and old (O) rats treated with L-thyroxine (T4) for two weeks. Note that TH significantly ($p<0.05$) facilitated the occurrence of VF in young, but not in old rat hearts (A) in which, in addition, TH accelerated the termination of VF and sinus rhythm restoration (B).

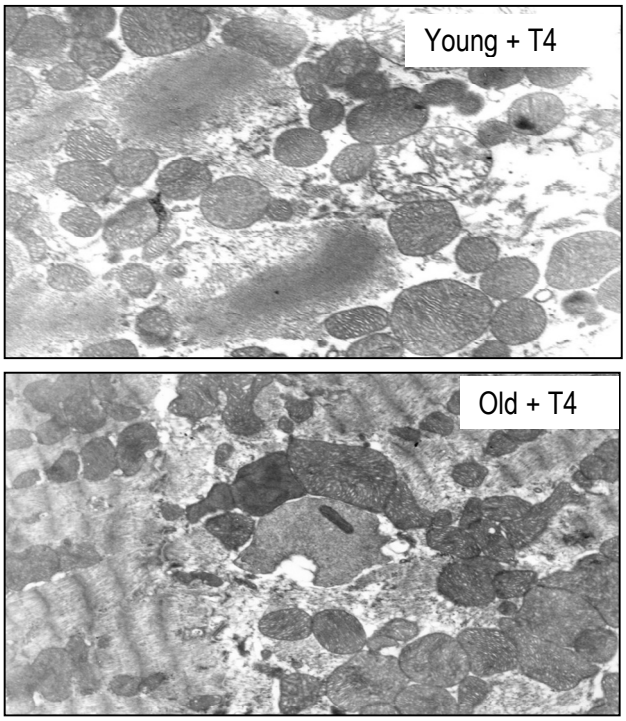

Fig. 3. Ca^{2+} overload-related subcellular injury of the cardiomyocytes in the hearts of young and old L-thyroxine-treated rats detected upon the termination of VF and the restoration of sinus rhythm. Note more pronounced deterioration of mitochondria (mi) ultrastructure and contracture (CB) of myofibrils in young compared to old rat hearts. Magnification x14 000.

ng ml^{-1}), in old rats by 105 % (from 0.56±0.05). Levels of T_4 were not significantly changed. All T_4 treated rats exhibited a delay in the onset of low K^+-induced ventricular premature beats (VPBs) (Fig. 1A), as well as a decreased number of VPB in their hearts (Fig. 1B). The susceptibility to VF (given by shorter time of VF onset) was, however, significantly increased in hearts of young 3-month-old rats, but not in hearts of old T_4-treated rats (Fig. 2A) compared to hearts of age-matched untreated rats. In contrast, T_4 facilitated the spontaneous sinus rhythm restoration in old rats, but not in hearts of young rats (Fig. 2B) in comparison to untreated rats of the same age. Moreover, the 2-week administration of T_4 improved significantly a mechanical function of the heart upon VF (not shown), which was particularly evident in old-rat hearts. The 20-month-old T_4-treated rats exhibited also much less pronounced Ca^{2+} overload injury (due to hypokalemia and VF) of the cardiomyocytes and their intercellular junctions compared to young T_4-treated rat hearts, as shown by the electron microscopic examinations (Fig. 3).

In the acute experiments, the administration of T_3 in the range of 10^{-9}-10^{-7} mol/l significantly decreased an inducible VF of the isolated guinea pig hearts, in contrast to the higher 10^{-6} mol/l concentration that increased the sensitivity to VF (Fig. 4A). Besides, T_3 in lower (10^{-9}-10^{-7} M) concentrations, but not in a higher (10^{-6} mol/l) concentration, facilitated the restoration of the sinus rhythm (Fig. 4B).

Discussion

Available literature data suggest that the effects of TH can be mediated by both the genomic (Dillman 2002) and non-genomic manner (Davis and Davis 2002). In agreement with it, we have found for the first time that long-term (two weeks) administration of TH and acute exposure to TH affects the susceptibility of the heart to fatal arrhythmias. Accordingly, the hearts of young rats treated with T_4 during two weeks and guinea pig hearts subjected for 5 min to 10^{-6} mol/l of T_3 were significantly much prone to develop VF compared to untreated controls.

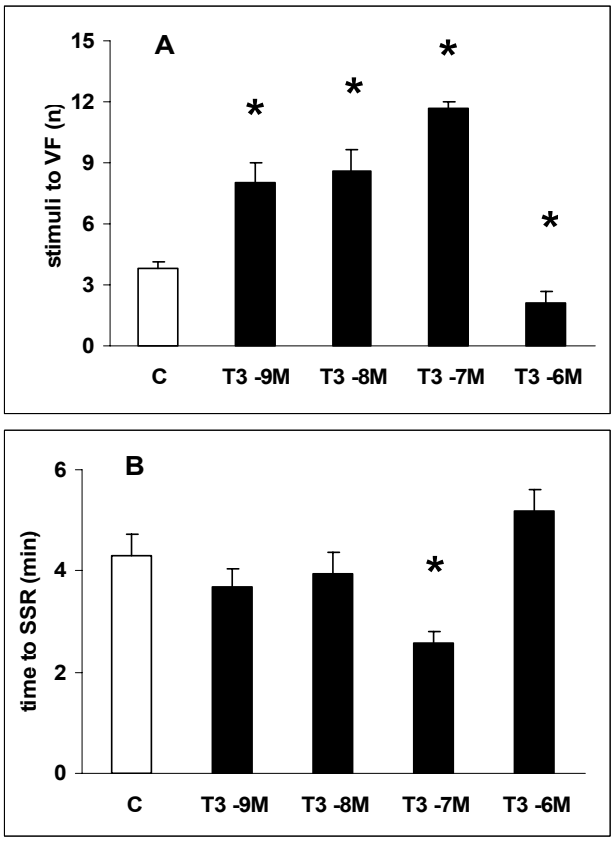

Fig. 4. Acute dose-dependent effects of triiodothyronine (T3) on the occurrence of electrically-induced VF (A) and spontaneous sinus rhythm restoration (B) in isolated guinea pig hearts. Note that T_3 in range of 10^{-9}-10^{-7} mol/l delayed the occurrence of VF, while in the higher (10^{-6} mol/l) concentration it facilitated it (A). In addition, T_3 facilitated the termination of VF and sinus rhythm restoration that was significant upon 10^{-7} mol/l, while the (10^{-6} mol/l) higher concentration had the opposite effect (B).

It should be noted that differently to young rats there was no difference in susceptibility to VF of old rats treated with T_4 when compared to the untreated ones. Unlike to the hyperthyroidism, the hypothyroidism (often accompanying the aging) contribute to decreased vulnerability to VF (Liu *et al*. 1996). Thus, the treatment of old rats with T_4 did not affect their vulnerability to malignant arrhythmia but, in contrast, it resulted in an earlier spontaneous sinus rhythm restoration when compared to the untreated littermates. Young rats (exhibiting higher natural levels of thyroid hormones) were able to restore the sinus rhythm more quickly compared to old rats, regardless the treatment. Notably, both young and old T_4-treated-rat hearts exhibited a significantly better mechanical recovery upon the restoration of the sinus rhythm compared with untreated rats. Since TH enhance the Ca^{2+} uptake by SR (Černohorský *et al*. 1998, Khoury *et al*. 1996) we suggest that it can help in the recovery from cardio-depression resulting from the VF-induced Ca^{2+} overload. This effect can be important under experimental as well as clinical conditions (Holland *et al*. 1991, Factor *et al*. 1993).

As far as we know, the data dealing with acute effects of TH in the context of their arrhythmogenicity are still missing. We used guinea pig hearts instead of rats to examine acute effects of TH because there are species-related differences as for Ca^{2+} handling, i.e. cell membrane predominates in the guinea pig, while sarcoplasmic reticulum predominates in the rat heart. Our acute experiments have revealed that in contrast to higher (10^{-6} mol/l) T_3 concentration that facilitated the occurrence of electrically-induced VF, the lower T_3 concentrations (10^{-9}-10^{-7} mol/l) decreased susceptibility of the heart to the malignant arrhythmia. It indicates that TH can exert both arrhythmogenic and antiarrhythmic effects, but a "therapeutic window" seems to be quite narrow.

Acute excess of cytosolic free Ca^{2+} ($[Ca^{2+}]i$) or Ca^{2+} overload plays a crucial role in the initiation of VF (Kihara and Morgan 1991, Tribulová *et al*. 2002) due to spontaneous Ca^{2+} oscillations that can trigger ventricular premature beats (Lakata and Guarnieri 1993). Accordingly, it seems very likely that sudden increase of $[Ca^{2+}]i$ due to the higher concentration of T_3 (Zinman *et al*. 2006, Tribulová *et al*. 2004) can be arrhythmogenic. On the other hand, we have shown previously that administration of lower concentrations of T_3 can prevent and/or attenuate the Ca^{2+} overload (Zinman *et al*. 2006, Tribulová *et al*. 2004), likely due to an acceleration of SR Ca^{2+} uptake and can be thus anti-arrhythmic. These mechanisms can explain, at least in part, a dose-dependent effect of acute TH administration on the heart susceptibility to VF. It should be noted that TH-induced effects on Ca^{2+} handling are similar to the catecholamine-induced ones, therefore, we suggest that the mechanisms involved in well known pro- and anti-arrhythmic effects of these compounds maybe similar. This assumption is supported by findings that acute prevention and/or attenuation of the Ca^{2+} overload by other compounds (Manoach *et al*. 1997, Seki *et al*. 2003) was associated with a decreased susceptibility of the heart to VF (Merrilat *et al*. 1990, Manoach and Watanabe 1995).

TH modulate an intracellular free Ca^{2+} not only under acute, but also under chronic conditions. Our results suggest that a TH supplementation (but not overdose) could be beneficial for the hearts with a decreased function of SR (old animals) to support the

contractility of the heart as well as to prevent $[Ca^{2+}]i$ disorders (by enhancement of SR function). In contrast, an over-stimulation of SR by excess of TH may induce the Ca^{2+} overload of SR and spontaneous Ca^{2+} leakage triggering arrhythmias.

In addition to effects of TH mentioned above, they can in both acute and long-term conditions modulate the intercellular communication via gap junction connexin-43 channels. While an acute elevation of TH can inhibit connexin-43 channels and coupling due to an excess of $[Ca^{2+}]i$, a lower concentration of TH can be beneficial via prevention of the Ca^{2+} overload. Long-term elevation of TH can be arrhythmogenic due to a down-regulation of connexin-43 (decreased expression and phosphorylation of connexin-43) detected in the young rat heart ventricles (Lin *et al*. to be published). Interestingly, there were no significant alterations in the expression of connexin-43 in aged TH-treated rat hearts (Tribulová *et al*. 2005).

In conclusion, our results indicate that the susceptibility of the heart to VF and its ability to restore the sinus rhythm can be affected by both acute TH application, suggesting the presence of non-genomic (not related to transcription) events and by genomic (transcription related) actions, resulting from the chronic TH application. The anti-arrhythmic and pro-arrhythmic potential of TH seems to be age- as well as dose-dependent.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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