# **Physiological Research Pre-Press Article**

Thyroid hormones modulate occurrence and termination of ventricular fibrillation by both long-term and acute actions.

V. Knezl, T. Soukup<sup>1</sup>, Ľ. Okruhlicová<sup>2</sup>, J. Slezák<sup>2</sup>, N. Tribulová<sup>2</sup>

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovak Republic

<sup>1</sup>Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

<sup>2</sup>Institute for Heart Research, Slovak Academy of Sciences, Bratislava,

Short title: Thyroid hormones and ventricular fibrillation

Corresponding author:

Vladimír Knezl, PhD.

Institute of Experimental Pharmacology, Slovak Academy of Sciences

Dubravska cesta 9, POBOx 104, 840 05 Bratislava, Slovak Republic

E-mail: exfaknem@savba.sk

## 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<sub>3</sub>) 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. Lthyroxine  $(T_4)$  was applied in the dose of  $50\mu g/100g/day$  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<sub>3</sub> administration in the range of 10<sup>-9</sup>-10<sup>-7</sup> 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<sub>3</sub>, which in addition, 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

## 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<sup>2+</sup> 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<sup>2+</sup> in an isolated heart preparation, although in higher concentration ( $10^{-4}$  mol/l) further abnormal Ca<sup>2+</sup> increase was detected (Tribulová *et al.* 2004). Alterations of the myocardial Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup>.

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.* in press), or closing of connexin channels due to an intracellular Ca<sup>2+</sup> 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. 1) Wistar female rats at the age of 3 and 20 months received  $50\mu g/100g/day$  of T<sub>4</sub> with the food for two weeks. Hearts excised from T<sub>4</sub> 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<sup>+</sup> 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 microscopic examination. 2) 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, 100pps, duration 1ms and 10 mA 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.

*Statistics:* Results are given as means  $\pm$  SEM. Statistical significances were ascertained by using the Student's two tailed test for unpaired observations. Only corrected *p* values < 0.05 were considered as significant.

## Results

Total T<sub>3</sub> serum levels increased in 3-month-old rats (after application of T<sub>4</sub>) by 69 % (from  $0.80 \pm 0.03$  ng ml<sup>-1</sup>), in old rats by 105 % (from  $0.56 \pm 0.05$ ). Levels of T<sub>4</sub> were not significantly changed. All T<sub>4</sub> treated rats exhibited a delay in the onset of low K<sup>+</sup>-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<sub>4</sub>-treated rats (Fig. 2A) compared to hearts of age-matched untreated rats. In contrast, T<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>-treated rats exhibited also much less pronounced Ca<sup>2+</sup> overload injury (due to hypokalemia and VF) of the cardiomyocytes

and their intercellular junctions compared to young T<sub>4</sub>-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 (2 week) 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 minutes to  $10^{-6}$  mol/l of  $T_3$  were significantly much prone to develop VF compared to untreated controls.

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 hypothyroidims (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<sup>-6</sup> mol/l) T<sub>3</sub> concentration that facilitated the occurrence of electrically-induced VF, the lower T<sub>3</sub> concentrations (10<sup>-9</sup>-10<sup>-7</sup> 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 Guarneri 1993). Accordingly, it seems very likely that sudden increase of [ $Ca^{2+}$ ]i due to the higher concentration of T<sub>3</sub> (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<sub>3</sub> 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.*, ) 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 proarrhythmic potential of TH seems to be age- as well as dose-dependent.

## **Conflict of Interest**

There is no conflict of interest.

#### Acknowledgement

This study was realized by support of APVV 51-059505, VEGA 6064/26, 2/5021/26, 2/7094/27, GAČR 304/05/0327 grants and by the Research project AV0Z 50110509.

## References

ARONOW WS: The heart and thyroid disease. Clin Geriatr Med 11: 219-229, 1995.

ČERNOHORSKÝ J, KOLÁŘ F, PELOUCH V, KORECKÝ B, VETTER R: Thyroid control sarcolemmal Na/Ca exchanger and SR Ca-ATPase in developing rat heart. *Am J Physiol* **2751**: H264-H273, 1998.

DAVIS PJ, DAVIS FB: Nongenomic actions of thyroid hormone on the heart. *Thyroid* **12**: 453-457, 2002.

DE MELLO WC: Interaction of cyclic AMP and Ca<sup>2+</sup> in the control of electrical coupling in the heart fibers. *Bioch Biophys Acta* **888**: 91-99, 1986.

DHEIN S: Gap junction channels in the cardiovascular system: pharmacological and physiological modulation. *Trends Pharm Sci* **19**: 229-241, 1998.

DILLMAN WH: Cellular action of thyroid hormone on the heart. *Thyroid* **12**: 447-466, 2002. FACTOR MA, MAYOR GH, NACHREINER RF AND D'ALECY LG: Thyroid hormone loss and replacement during resuscitation from cardiac arrest in dogs. *Resuscitation* **26**, 141-62, 1993.

FADEL BM, ELLAHHAM S, RIGEL MD, LINDSAY J, WARTOFSKY L, BURMAN KD.: Hyperthyroid heart disease. *Clin Cardiol* **23**: 402-8, 2000.

FIALOVÁ M, DLUGOŠOVÁ, OKRUHLICOVÁ L, KRISTEK F, MANOACH M, TRIBULOVÁ N. Adaptation of the heart to hypertension is associated with maladaptive gap junction connexin-43 remodelling. *Physiol Res* 2007 in press.

HAMILTOM MA, STEVENSON LW, FONAROW GC, STEIMLE A, GOLDHABER JI, CHILD JS, CHOPRA IJ, MORIGUCHI JD, AND HAGE A: Safety and hemodynamic effects of intravenous triiodothyronine in advanced congestive heart failure. *Am J Cardiol* **81**: 443-447, 1998.

HOLLAND FW, BROWN OS, WEINTRAUB BD AND CLARK RE: Cardiopulmonary bypass and thyroid function: a "euthyroid sick syndrome". *Ann Thorac Surg* **52**: 46-50, 1991.

KIHARA Y, MORGAN JP: Intracellular calcium and ventricular fibrillation. *Circ Res* 68: 1378-89, 1991.

KHOURY SF, HOIT BD, DAVE V, PAWLOSKY-DAHM CM, SHAO Y, GABEL M, PERIASAMY M, WALSH RA: Effects of thyroid hormone on left ventricular performance and regulation of contractile and Ca –cycling proteins in the baboon. *Circ Res* **79**: 727-735, 1996.

KLEMPERER JD: Thyroid hormone and cardiac surgery. Thyroid 12: 517-21, 2002.

MANOACH M, WATANABE Y: How can we facilitate spontaneous termination of ventricular fibrillation and prevent sudden cardiac death? *J Cardiovasc Electrophysiol* **6**: 584-590, 1995.

LAKATTA EG, GUARNIERI T. Spontaneous myocardial calcium oscillations: are they linked to ventricular fibrillation? *J Cardiovasc Electrophysiol* **4**: 473-489, 1993.

LIU P, FEI L, WU W, LI J, WANG J, ZHANG X: Effects of hypothyroidism on the vulnerability to ventricular fibrillation in dogs: a comparative study with amiodarone. *Cardiovasc Drugs Ther* **10**: 369-378, 1996.

MANOACH M., WATANABE Y: How can we facilitate spontaneous termination of ventricu-lar fibrillation and prevent sudden cardiac death? *J Cardiovasc Electrophysiol* **6**: 584-590, 1995.

MANOACH M., VARON D., SHAINBERG A., ZINMAN T., ISSACK A., HALLILI-

RUTMAN I., KAPLAN D., TRIBULOVA N: The protective effect of class III antiarrhythmic drugs against calcium overload in cultured myocytes. *Life Sci* **61**: PL227-P234, **1997**.

MERRILAT JC, LAKATTA EG, HANO O, GUARNIERI T: Role of calcium and the calcium channel in the initiation and maintenance of ventricular fibrillation. *Circ Res* **67**: 115-1123, 1990.

SALAMEH A, DHEIN S: Pharmacology of gap junctions. New pharmacological targets for treatment of arrhythmia, seizure and cancer?. *Biochim Biophys Acta*: 36-58, 2005.

SEKI S, TRIBULOVÁ N, MANOACH M, MOCHIZUKI S.: Modulation of intracellular Ca+ concentration by tedisamil, a class III antiarrhythmic agent, in isolated heart preparation. *Life Sci* **73**:1805-1811, 2003.

SEVERS NJ: Gap junction remodelling and cardiac arhythmogenesis: cause or coincidence? *J Cell Mol Med* **5:** 355-366, 2001.

STOCK A, SIES H: Thyroid hormone receptors bind to an element in the connexin43 promoter. *Biol Chem* **381**: 973-979, 2000.

TRIBULOVÁ N, OKRUHLICOVÁ Ľ, NOVÁKOVÁ S, PANCZA D, BERNÁTOVÁ I, PECHANOVÁ O, WEISMANN P, MANOACH M, SEKI S, MOCHIZUKI M: Hypertension-related intermyocyte junction remodeling is associated with higher incidence of low K<sup>+</sup>- induced lethal arrhythmias in isolated rat heart. *Exp Physiol* **87**: 195-205, 2002.

TRIBULOVÁ N, OKRUHLICOVÁ Ľ, VARON D, MANOACH M, PECHANOVÁ O, BERNATOVÁ I, WEISMANN P, BARANČIK M, STYK J, SLEZÁK J: Structural substrates involved in the development of severe arrhythmias in hypertensive rat and aged guinea pig hearts. In: *Cardiac Remodeling and failure*, P. Singal, I Dixon, L Kirschenbaum, NS Dhalla (eds), Kluwer Acad Publishers, Boston, USA 2003. TRIBULOVÁ N, HAILIN H, IMANAGA I, KNEZL V, SEKI S, MANOACH M: Factors involved in the antiarrhythmic and proarrhythmic effects of thyroid hormones. *J Mol Cell Cardiol* **37**: 310-311 abstract, 2004.

TRIBULOVÁ N, DUPONT E, SOUKUP T, OKRUHLICOVÁ L, SEVERS NJ: Sex differences in connexin-43 expression in left ventricles of aging rats. *Phys Res* **54**: 705-708, 2005.

YEN PM: Physiological and molecular basis of thyroid hormone action. *Physiol Reviews* **81**: 1097-10142, 2001.

ZINMAN T, SHNEYVAYS V, TRIBULOVÁ N, MANOACH M, SHAINBER A: Acute, nongemonic effect of thyroid hormones in preventing calcium overload in newborn rat cardiocytes. *J Cell Physiol* **207**: 220-231, 2006.

## Legends to the figures.

**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  $\pm$  S.E.M, p < 0.05.

**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.

**Fig. 4**. Acute dose-dependent effects of triiodotyronine (T3) on the occurrence of electricallyinduced 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).



















