Hyper- and Hypothyroidism Affect Myocardial Connexin-43 Expression and Susceptibility of the Rat Heart to Malignant Arrhythmias

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Abstract. *We aimed to explore relationship between myocardial electrical coupling protein connexin-43 (Cx43) and susceptibility of rats with altered thyroid status to malignant arrhythmias and to examine possible beneficial effect of red palm oil (RPO). We used as experimental model euthyroid (EU), hypothyroid (HY) and hyperthyroid (TH) rats, which were fed RPO (100μl/100g b.w./day) for 6 weeks. Left ventricular tissue was taken for determination of mRNA and protein expression of Cx43 and PKCε. Sustained ventricular fibrillation (sVF) induction was estimated using isolated perfused heart. Results showed that the threshold for sVF was significantly decreased in the TH, in which Cx43 and PKCε expression was down-regulated in comparison with the EU. On the contrary, sustained VF was not induced even by the highest stimulus strength in the HY, in which Cx43 and PKCε expression was up-regulated. RPO intake partly, although significantly, increased VF threshold in TH as well as Cx43 and PKCε proteins expression. We conclude that there is an inverse relationship between myocardial Cx43 expression and heart susceptibility of rats with altered thyroid status to VF.*

Keywords: thyroid hormones, cardiac arrhythmias, connexin-43

1. Introduction

Mammalian heart is a major target organ for thyroid hormone (triiodo-L-thyronine, T_3) action. T_3 plays an important role in cardiac electrophysiology and Ca^{2+} handling through both genomic and nongenomic mechanisms [1, 2]. Thyroid dysfunction is classified as hyperthyroidism and hypothyroidism. Chronic changes in the amount of plasma concentration of thyroid hormones result in structural, electrophysiological and Ca^{2+} handling remodeling of the heart, while acute changes may influence basal metabolic activity of cardiomyocytes [3]. Consequently, these alterations may affect heart function as well as its susceptibility to arrhythmias [1]. We have previously shown that increased propensity to arrhythmias was associated with T_3-induced down-regulation of atrial and ventricular connexin-43 (Cx43) [4, 5]. Defects in expression and/or distribution of Cx43 have been established to impair Cx43 channels mediated cell-to-cell communication and electrical coupling that is highly arrhythmogenic [6, 7]. Because the fundamental role of Cx43 in intercellular communication and action potential propagation, it seems likely that it might be a molecular target for novel approaches to prevent life-threatening cardiac arrhythmias.

We aimed to explore our hypothesis that different propensity to malignant arrhythmias in rats with altered thyroid status is most likely inversely associated with up- or down-regulation of myocardial Cx43 (in relation to PKCε expression).

2. Subject and Methods

The experiments were carried out on 3-month-old male Wistar rats. Rats were randomly divided into 3 groups: 1) euthyroid (EU), 2) hypothyroid (HY) and 3) hyperthyroid (TH). HY status was induced and maintained with a 0.05 % solution of methimazole in drinking water and TH status by intraperitoneal injection of T_3 (0.15 mg/kg body weight) three times a week. Red palm oil (RPO) was administered orally daily to half of animals a week before and during six week thyroid hormone alteration period (100 µl/100g BW/day). At the end of experiment body weight (BW), blood glucose (BG) as well as the levels of T_3 and T_4 were registered. The heart was excised into ice-cold saline and both heart (HW) and left ventricular weight (LVW) were quickly registered. Snap frozen left ventricular (LV) tissue was taken for Cx43, PKCε protein analysis using western blots [8] and for mRNA determination by Real Time PCR. Threshold to induce VF in fibrillating heart were estimated using isolated heart preparation as described elsewhere [8]. Statistical evaluations was performed using ANOVA and Bonferroni's Multiple Comparison Test. Data were expressed as mean ± SD to analyze the statistical significance determined at $P < 0.05$.

3. Results

As shown in Table 1 the administration of T_3/T_4 or methimazole caused TH or HY status of experimental rats. RPO significantly increased T_3 level in the HY rats, even above the level of the EU rats. Compared to EU, TH status was characterized by significant increase of HW, LVW, while HY status led to a decrease of LVW. BG was increased in the TH group and it was further enhanced by RPO. Compared to EU rats the threshold to induce sustained VF was significantly lower in TH group and this decrease was partly compensated by RPO intake. In contrast to TH rats, it was not possible to induce VF in the HY and HY+RPO rats even by the highest stimulus strengths (50 mA). There was no significant difference in myocardial mRNA levels for Cx43 in the TH versus EU rats unlike the HY and HY+RPO rats, in which Cx43 gene transcription was markedly increased (Fig. 1). RPO intake did not affect Cx43 mRNA expression in either group. As shown by WB (Fig. 2A), expression of total Cx43 protein (Fig. 2A, B) as well as its phosphorylated forms (Fig. 2A, C) were significantly decreased in TH and increased in the HY compared to the EU rats. Intake of RPO enhanced levels of both total and phosphorylated forms of Cx43 in TH compared to EU status and suppressed, although not significantly, elevation of Cx43 in the HY rats. Comparing to EU rats, mRNA (Fig. 3A) as well as protein expression for PKCε (Fig.3B,C) were significantly increased in the HY unlike TH rats, in which both parameters were significantly decreased. RPO intake had no effect on Cx43 mRNA levels, but it partly normalized changes in rats with altered thyroid status.

Table. 1 Main characteristics of rats with altered thyroid status without and with RPO intake

	EUc	EUrpo	THc	THrpo	HYc	HYrpo
T3 (nmol/l)	1.2 ± 0.3	1.4 ± 0.2	3.1 ± 1.3*	3.1 ± 1.8	0.85 ± 0.07*	1.9 ± 0.2#
T4 (nmol/l)	49 ± 18	71 ± 10	122 ± 40*	107 ± 22	14 ± 2*	14 ± 1,5
BG(mmol/l)	3.33 ± 1.05	4.43 ± 0.38	5.23 ± 0.06*	6.18 ± 0.22#	3.87 ± 0.40	4.03 ± 0.49
BW (g)	379 ± 88	411 ± 7	419 ± 50	414 ± 52	406 ± 14	346 ± 49#
HW (g)	1.04 ± 0.18	1.15 ± 0.08	1.35 ± 0.1*	1.18 ± 0.12#	0.84 ± 0.08	0.81 ± 0.03
LVW (g)	0.75 ± 0.14	0.82 ± 0.01	0.97 ± 0.04*	0.84 ± 0,13#	0.59 ± 0.04*	0.57 ± 0.02
Threshold for sVF	31.2 ± 9.7	45.1 ± 7.8	14.2 ± 3.7*	23.3 ± 7.6#	50 ± 0*	50 ± 0

Abbreviations: Euthyroid control rats (Euc, n = 6), EU rats treated with RPO (Eurpo, n = 6), hyperthyroid rats (THc, n = 6), TH rats treated with RPO (THrpo, n = 6), hypothyroid rats (HYc, n = 6), HY rats treated with RPO (HYrpo, n = 6), BG-blood glucose, BW-body weight, HW-heart weight, LVW- left ventricular weight, sVF - sustained ventricular fibrillation. Data are means ± SD, $^{*}P < 0.05$ *vs* EUc, $^{\#}P < 0.05$ untreated *vs* treated

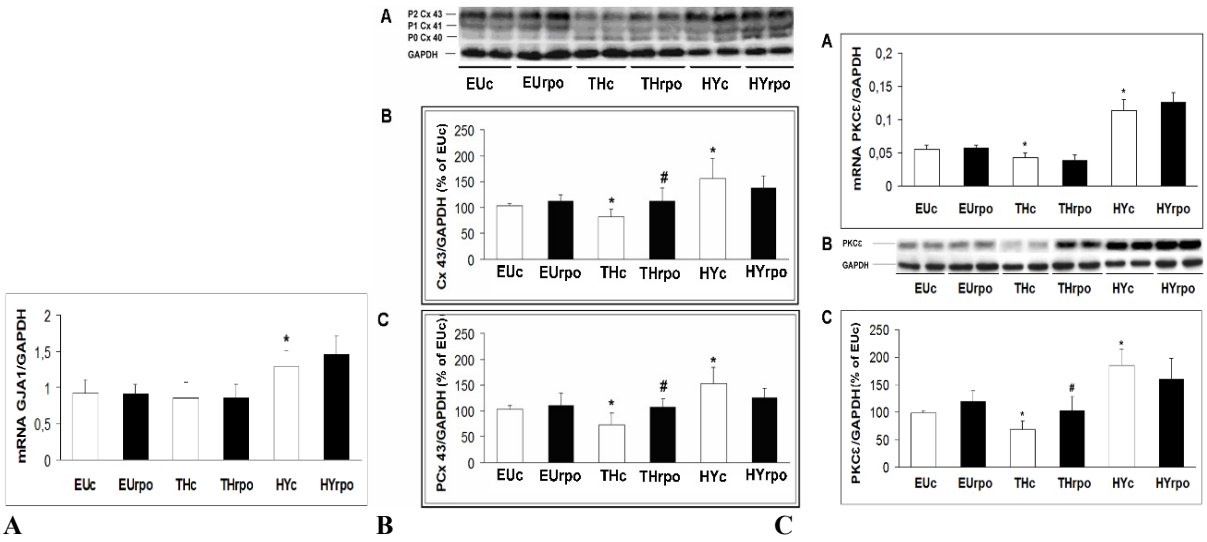

Fig. 1: A. Myocardial expression of mRNA CXA1 normalized to GAPDH in rats with altered thyroid status, B. Expression of myocardial total Cx43 protein (A, B) and its phosphorylated forms (A, C) normalized to GAPDH in rats with altered thyroid status. C. Myocardial expression of PKCε mRNA (A) and protein (B, C) normalized to GAPDH in rats with altered thyroid status. Abbreviations: Euthyroid control rats (Euc, n = 6), EU rats treated with RPO (Eurpo, n = 6), hyperthyroid rats (THc, n = 6), TH rats treated with RPO (THrpo, n = 6), hypothyroid rats (HYc, n = 6), HY rats treated with RPO (HYrpo, n = 6). Data are means ± SD, $^{*}P < 0.05$ *vs* EUc, $^{\#}P < 0.05$ untreated *vs* treated with RPO.

4. Discussion

According to the general classification of cardiac arrhythmias all disturbances of rhythm result from one of two primary abnormalities in electrical activity. The first is an abnormality in impulse initiation and the second, an abnormality in impulse propagation, whereby both may co-exist [9]. The former abnormality is associated particularly with triggered activity and/or abnormal automaticity, whereas the latter with block of conduction and re-entry. In this context it should be noted that down-regulation of Cx43 in TH rats is most likely crucial in the process of arrhythmogenesis due to promoting of re-entry mechanism and hence VF [6], while an increase of K^{+}, Ca^{2+} and RyR currents in TH rats [10] can contribute to development of transient arrhythmias by promoting triggered activity [1]. In contrast, suppression of these channels activity in HY status can decrease this risk. Transient arrhythmias in the setting of impaired Cx43-mediated cell-to-cell coupling often lead to VF. We have further demonstrated that RPO intake resulted in increase of threshold to induce VF and shortening the time to sinus rhythm restoration in the TH rats. Antiarrhythmic effect of RPO was associated predominantly with up-regulation of Cx43 protein together with an increase of its functional phosphorylated forms. This fact points out the implication of Cx43 in prevention of malignant arrhythmias. PKCε has been shown to be involved in Cx43 phosphorylation and to be implicated in influence of conductivity and permeability of Cx43 channels [11]. Noteworthy, the protein as well as mRNA levels for PKCε were up-regulated in HY but down-regulated in TH status. It appears that thyroid hormones affect PKC signaling pathway in the heart by modulation of PKC gene transcription. Positive correlation between myocardial expression of PKCε and phosphorylated forms of Cx43 suggests its implication in Cx43 phosphorylation. RPO intake resulted in up-regulation of both PKCε and Cx43 protein expression in the TH rats that can explain its antiarrhythmic effects. Besides, antioxidant-rich RPO can via inhibition of oxidative stress prevent Cx43 degradation [12]. However, it is not clear yet how the enhancement and suppression of Cx43 phosphorylation by PKCε in rats with altered thyroid status would affect Cx43 channel function. Data dealing with direct Cx43 channel

related conductivity in the TH or HY rat heart are missing. Nevertheless, we have shown previously that down-regulation of PKCε and Cx43 proteins was associated with faster myocardial conduction velocity (CV) in the the TH comparing to EU rats [4]. Taken together, we can speculate that partial slowing of Cx43 channels conduction (in case of the diabetic and the HY rats as well as the RPO-treated TH rats) can make the heart more electrically stable likewise slowing of voltage-dependent Na^+ channels (involved in conduction of electrical signals) by antiarrhythmic agents. In conclusion, our findings indicate that there is an inverse relationship between myocardial Cx43 expression and susceptibility of the heart of rats with altered thyroid status to VF. RPO intake increased threshold for induction of VF in hyperthyroid rats apparently expressed due to up-regulation of Cx43.

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