ARRHYTHMIAS IN PROGRAMMED ELECTRICAL STIMULATION IN THE COURSE OF EXPERIMENTALLY INDUCED HYPERTHYROIDISM IN AN ANIMAL MODEL

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Abstract

To determine changes in physiological parameters of the myocardium in experimentally induced hyperthyroidism in an animal model, the occurrence and type of arrhythmias triggered during programmed electrical stimulation and changes in electrophysiological parameters of ventricular cardiomyocytes with hypertrophy due to hyperthyroidism were investigated.

Hyperthyroidism was induced experimentally in five pigs, which were orally administered L-thyroxine at a dose of 20 µg/kg. Five untreated pigs served as the control. Programmed electrical stimulation was performed before administration of L-thyroxine (EPS 1), four (EPS 2) and eight (EPS 3) weeks after the onset of thyroxine administration, and four weeks after drug withdrawal (EPS 4). After the last stimulation, the animals were sacrificed and necropsied, with particular regard to heart autopsy. During the EPS 2, VERP was decreased in the group treated with the hormone (P<0.05). The mean values of AERP and AVNERP in the group were decreased as well. Atrial flutter and atrial fibrillation were induced during stimulation of the experimental group. In the other pigs of the experimental group, singular and paired ventricular extrasystolic were observed. In the EPS 3, AERP and AVNERP were statistically shorter in pigs with hyperthyroidism. A significant difference in Wenckebach CL between the control and experimental groups was observed. SNRT was shorter in the group with hyperthyroidism. In all pigs with hyperthyroidism, atrial fibrillation was induced. In one pig, non-sustained ventricular tachycardia was observed. During EPS 4, AERP remained shorter in group with hyperthyroidism. In two pigs of the group, atrial fibrillation was induced during pacing, and in two pigs, ventricular fibrillation was observed. The assessment of the heart’s weight revealed a significant increase in its mass in pigs with hyperthyroidism. An increase in the thickness of the right and left ventricle free walls (P<0.01) and interventricular septum (P<0.01) was found in pigs with hyperthyroidism. At the same time, the inner diameter of the left ventricle was significantly smaller in this group (P<0.01) due to a concentric hypertrophy of the ventricle. In view of these findings, experimental hyperthyroidism caused shortening of refractory periods of different parts of the conducting system and enhanced susceptibility to supraventricular and ventricular arrhythmias, both spontaneous and induced during electrical stimulation. The mechanism of these arrhythmias can differ as a consequence of the hypertrophy of the left ventricle.

Key words: pigs, arrhythmias, hyperthyroidism.

Thyroid hormones exert a significant influence on the electrophysiological properties of cells forming the sinus node and other atrial cardiomyocytes, which is manifested by sinus tachycardia, numerous atrial extrasystolic beats, and susceptibility to atrial fibrillation (14, 15). Ventricular arrhythmias have also been described in patients with hyperthyroidism (14). The electrophysiological property responsible for arrhythmias associated with hyperthyroidism involves shortening of the refractory period of cardiomyocytes. Moreover, thyroid hormones cause cardiac hypertrophy (2, 8). The hypertrophy may lead to changes in the electrophysiological parameters of the myocardium. Studies in animals resembling humans, as far as the cardiovascular system and thyroid metabolism are concerned, can be useful for explaining the electrophysiological remodelling due to cardiac hypertrophy in the course of hyperthyroidism.

The aim of the study included:

1. Evaluation of the changes in the electrophysiological parameters of the myocardium in experimentally induced hyperthyroidism in an animal model (swine).
2. Evaluation of the incidence and types of the arrhythmias induced during programmed electrical stimulation.
3. Evaluation, whether the changes in the electrophysiological parameters of the ventricles can be associated with ventricular hypertrophy due to hyperthyroidism.
Material and Methods

Ten 12-week-old pigs of Polish breed, from two different litters were used in the study. Hyperthyroidism was induced in five pigs by oral administration of L-thyroxine at the daily dose of 20 µg/kg. The dose administered was recommended by Morvat and Dauncey (13). The control group consisted of five pigs without any medication.

The programmed electrical (EP) stimulation was performed with catheter electrodes and UHS 20 pacemaker. The electrophysiological study was conducted before the administration of the thyroxine (EPS 1), four (EPS 2) and eight (EPS 3) weeks after the onset of the drug administration, and four weeks after the drug withdrawal (EPS 4). The procedures were performed under intravenous general anaesthesia. After 24-h fasting, anaesthesia was induced by premedication with azaperone (2 mg/kg) and ketamine (10 mg/kg) followed by sodium pentobarbital (8-10 mg/kg). The anaesthetic agents used have minimal effect on the electrophysiological properties of cardiac myocytes and they are commonly applied during electrical stimulation in animals (19). The pigs were artificially ventilated through a cuffed endotracheal tube. After intubation, the animal was immobilised in the dorsal position. During the whole experiment, oxygen was administered through the endotracheal tube to maintain the saturation level above 95%. The saturation was checked by pulsoxymeter with the sensor placed on the tongue or the ear. Pacing protocol consisted of the ventricular effective refractory period (VERP), AV nodal effective refractory period (AVNERP), atrial effective refractory period (AERP) at underlying sinus rate and at fixed rate (100 bpm, 130 bpm, 150 bpm, 180 bpm), Wenckebach CL (cycle length), and sinus node recovery time (SNRT). The range of the procedure was actually different because some parameters were omitted, for example refractory periods at rate 100 bpm and 180 bpm.

After the last EP study, the animals were sacrificed and necropsied, with particular regard to heart autopsy. During the examination, the height, width, and mass of the heart, diameters of the right and left ventricles, and thickness of the ventricle walls and of interventricular septum were measured.

All the data are presented as a mean with standard deviation (± SD) and percentage. ANOVA or its non-parametric equivalent was used as appropriate to judge the significance of differences. Parametric or non-parametric tests for unpaired variables, depending on distribution, were applied to estimate the differences between groups. Statistical significance was considered to be at P≤0.05.

The study protocol was approved by regional ethics committee (No. 107/03).

Results

During the first EP examination (EPS 1), at baseline, we did not observe any statistically significant differences between the estimated parameters in both studied groups. At a rate >130 bpm the retrograde atrial conduction was present in six of eight pigs.

During the EPS 2 (4-week administration of L-thyroxine), a significant shortening of the VERP at a rate 100 bpm, 130 bpm, 150 bpm, and 180 bpm was noted in the group with hyperthyroidism in comparison to the control group. Shortening of AERP and AVNERP was also observed in the group with hyperthyroidism. There were no significant differences either in Wenckebach CL or in SNRT. During the EP stimulation, arrhythmias were induced only in the group with hyperthyroidism. Atrial flutter was induced at a rate 130 bpm with the coupling interval between S1 and S2 190 ms. In the same subject, the atrial fibrillation was induced at the rate 150 bpm with the coupling interval between S1 and S2 180 ms. The arrhythmias were terminated with cardioversion (CV) with a single 100 J electrical shock. The remaining pigs from the group with hyperthyroidism developed ventricular extrasystoles at the rate 150 bpm and 180 bpm. During the stimulation, no arrhythmias were observed in pigs from the control group. In six of the eight pigs, the retrograde atrial conduction was present as previously.

During the EPS 3 (8-week administration of L-thyroxine), the shortening of AERP and AVNERP in the pigs with hyperthyroidism was observed. In the group with hyperthyroidism, VERP increased as compared to the data of EPS 2 and was comparable in both groups. Wenckebach CL was different – the conduction block occurred in higher frequency of the heart rate (Table 1) in the tested groups and SNRT was significantly shorter in the group with hyperthyroidism. In every pig with hyperthyroidism, atrial fibrillation was induced by atrial stimulation, both during sinus rhythm and during atrial pacing at a fixed rate. Conversion to sinus rhythm was spontaneous or by electrical cardioversion (with energy levels from 100 to 150 J. During atrial stimulation, spontaneous sustained ventricular tachycardia was observed in one pig. In the control group, no arrhythmias were induced, either during atrial or ventricular pacing. In five of the seven pigs, the retrograde atrial conduction was present.

During the four EPS (four weeks after L-thyroxine withdrawal), statistically significant shortening of AERP was observed in the group with hyperthyroidism. AVNERP and VERP were comparable in both groups. The level of Wenckebach CL differed between the groups. The conduction block occurred in higher frequency of the heart rate (Table 1). No significant differences in SNRT were observed. In two pigs from the group with hyperthyroidism, atrial fibrillation was induced during the procedure. In the first one, the arrhythmia was triggered three times during atrial pacing at the rate 130 bpm and 150 bpm with an atrial extrastimulus (S2) at a coupling interval of 210 ms and 200 ms.
**Table 1**

Results of EPS

<table>
<thead>
<tr>
<th>EPS Group</th>
<th>HR (bpm)</th>
<th>VERP 100 (ms)</th>
<th>VERP 130 (ms)</th>
<th>VERP 150 (ms)</th>
<th>VERP 180 (ms)</th>
<th>AERP mean (ms)</th>
<th>AVNERP mean (ms)</th>
<th>SNRT (ms)</th>
<th>Wenckebach cycle (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>106±9.9</td>
<td>303.33±21.33</td>
<td>252.5±15</td>
<td>230.0±14.4</td>
<td>215±5.77</td>
<td>185.0±12.8</td>
<td>323.125±14.375</td>
<td>702.5±112.66</td>
<td>±17.72</td>
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<tr>
<td>B</td>
<td>92.7±7.6</td>
<td>335±50.66</td>
<td>282.50±9.57</td>
<td>210±14.14</td>
<td>192.5±9.57</td>
<td>200±30.5</td>
<td>313.125±14.375</td>
<td>880.0±105.83</td>
<td>±29.2</td>
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<tr>
<td>2 A</td>
<td>99±11.16</td>
<td>192.5±17.07</td>
<td>177.5±17.07</td>
<td>165.0±12.91</td>
<td>176.65*</td>
<td>258.33</td>
<td>680</td>
<td>263.7</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>±17.07</td>
<td>±17.07</td>
<td>±12.91</td>
<td>±5.14</td>
<td>±26.6</td>
<td>±58.88</td>
<td>±10.74</td>
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<tr>
<td>B</td>
<td>91.25±6.75</td>
<td>245.0±34.16</td>
<td>212.5±20.62</td>
<td>190±10.2</td>
<td>200.0±7.07</td>
<td>281.66</td>
<td>760</td>
<td>263.7</td>
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<tr>
<td>3 A</td>
<td>93.33±15.27*</td>
<td>310.0±25.17</td>
<td>243.33±10.0</td>
<td>220.0±5.77</td>
<td>186.67±14.43</td>
<td>183.3*</td>
<td>291.0*</td>
<td>691.25*</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>±34.2</td>
<td>±20.62</td>
<td>±10.2</td>
<td>±7.07</td>
<td>±30.33</td>
<td>±188.33</td>
<td>±25.17</td>
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<tr>
<td>B</td>
<td>66.50±4.65*</td>
<td>302.5±49.9</td>
<td>240.0±29.44</td>
<td>212.5±15.0</td>
<td>183.3±12.06</td>
<td>224.25*</td>
<td>367.75*</td>
<td>1015*</td>
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<tr>
<td>4 A</td>
<td>72.67±15.04</td>
<td>295.0±35.35</td>
<td>240.0±32.46</td>
<td>220.67±15.27</td>
<td>206.67±12.06</td>
<td>172.0*</td>
<td>283.3</td>
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<tr>
<td></td>
<td></td>
<td>±35.35</td>
<td>±20.82</td>
<td>±20.82</td>
<td>±12.91</td>
<td>±16.07</td>
<td>±25.16</td>
<td>±69.28</td>
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<tr>
<td>B</td>
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<td>276.67±77.67</td>
<td>242.5±79.32</td>
<td>227.50±12.91</td>
<td>175.0±9.44</td>
<td>210.62*</td>
<td>275.0</td>
<td>825</td>
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<tr>
<td></td>
<td></td>
<td>±77.67</td>
<td>±82.21</td>
<td>±12.91</td>
<td>±9.44</td>
<td>±26.46</td>
<td>±16.58</td>
<td>±18.04</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05

1 - EPS at start of the study (before administration of L-thyroxine), 2 - EPS after four weeks of L-thyroxine administration, 3 - EPS after eight weeks of L-thyroxine administration, 4 - EPS four weeks after L-thyroxine withdrawal; A – experimental group with hyperthyroidism, B - control group

![Fig. 1. Concentric hypertrophy of the left ventricle in the pig with hyperthyroidism.](image-url)
In the other pig, AF was induced twice during atrial pacing at the rate 100 bpm with an atrial extrastimulus (S2) at the coupling interval of 190 ms. For the restoration of the sinus rhythm, electrical cardioversion was performed (energy levels from 200 to 300 J). In two animals, ventricular fibrillation was induced during ventricular pacing at the rate 180 bpm. The sinus rhythm was restored after three consecutive shocks with the energy level 360 J. In one of the animals, non-sustained ventricular tachycardia was induced during atrial pacing at the rate 150 bpm for 30 s. In five of the seven pigs, the retrograde atrial conduction was present.

All the results are shown in Table 1.

Cardiac mass in the group with experimental hyperthyroidism was significantly higher in comparison to the control group (520±100 vs 380±40 g P<0.05) and the external dimensions were similar in both group (11.5 x 7.1 vs 10.6 x 6.8 cm). The right ventricle wall thickness was increased in the group with hyperthyroidism (9.0±2.0 vs 6.3±0.7 mm P<0.05) as well as that of the left ventricle (32.0±1.5 vs 18.0±1.5 mm P<0.01) and the interventricular septum (20.0±10.0 vs 17.0±2.0 mm P<0.01). The inner diameter of the left ventricle was significantly smaller in the group with hyperthyroidism (23.0±1.0 vs 36.0±3.0 mm P<0.01). The experimental group revealed a concentric hypertrophy of the left ventricle (Fig. 1).

Discussion

The results of the present study demonstrate shortening of atrial, ventricular, and AV nodal effective refractory period in the initial part of the experiment. We observed the arrhythmogenic effect of hyperthyroidism as a susceptibility to ventricular arrhythmias.

The shortening of the effective refractory period was described in previously published studies dealing with isolated animal heart, animals with experimentally induced hyperthyroidism, and humans (3, 4, 15). In the present study, we observed susceptibility to supraventricular and ventricular arrhythmias. Supraventricular arrhythmia is commonly observed in hyperthyroidism (1, 3, 7, 9, 11, 14-16).

Willems et al. (18) showed in in vivo experiments that structural remodelling of atrium and shortening of the AERP is the reason for episodes of atrial fibrillation in the sheep. However, Allessi et al. (1) see the cause of AF in sympathetic stimulation and premature atrial beats.

The decrease in Wenckebach cycle length (CL) was observed after 4-week administration of L-thyroxine. The observation was consistent with the results of obtained by Biondi et al. (4), who demonstrated the shortening of AV nodal effective refractory period and Wenckebach CL, in women taking thyroid hormones. In such conditions, there is a major risk of fast atrioventricular conduction of atrial fibrillation or flagellation. In euthyrosis, longer AV nodal effective refractory period prevents too fast conduction from the atrium to the ventricle. The shortening of AV nodal effective refractory period and Wenckebach CL caused by L-thyroxine could be expected to increase the propensity for tachyarrhythmias e.g. atrial fibrillation with fast ventricular rate. A significant shortening of the sinus node recovery time in the group with hyperthyroidism (EPS 3) indicates the enhancement of sinus node automatism due to thyroid hormones. Arrhythmia can be a consequence of the shortening of the refractory period as well as an increase in the dispersion of repolarisation observed in hypertrophied myocardium (2, 3, 16, 17).

Hyperthyroidism causes cardiac hypertrophy (2, 4, 8-10, 17), which was confirmed in the examined animals at the autopsy. The post-mortem examination showed concentric hypertrophy, typical of pressure-overload (6).

Our results are consistent with those presented by other authors. The experiments by Kuncova and Slavikova (12) in newborn rats with hyperthyroidism showed an increase in cardiac mass, mainly in the atria, due to hyperplasia. In adult rats, the changes were present usually in the ventricles and the mechanism was different; the mass increased by means of hypertrophy. A considerable change in the thickness of the cardiac walls, as observed in many studies in different animal models (17), must result in the modification of the intraventricular conduction. The manifestation of the above-mentioned disturbances includes lengthening of the ventricular effective refractory period revealed in electrophysiological tests after 8-week administration of L-thyroxine. Increased left ventricular mass and concentric hypertrophy observed in the experiment are the result of many coexisting processes, including haemodynamic overload as a consequence of enhancement of cardiac contractility observed in the initial phase of hyperthyroidism (4) and the overload due to activation of the renin-angiotensin-aldosterone system (2, 10). Another consequence of increased left ventricular mass is the deterioration of ventricular filling, which, accompanying atrial fibrillation can result in cardiomyopathy, as in our study (20), or even diastolic heart failure (8). An increase in the thickness of cardiac walls is associated with impairment of perfusion and hypoxaemia, which is enhanced by the shortening of the diastole and contributes to ischaemia, focal necrosis, and fibrosis. The presence of fibrotic elements can promote arrhythmias due to re-entry (3, 11). The left ventricular hypertrophy, along with the increased dispersion of repolarisation, correlate with the high risk of ventricular arrhythmias, including torsade de points (19). Ventricular fibrillation after ventricular stimulation, sustained and non-sustained ventricular tachycardia, observed during the EPS 4 (4 weeks after L-thyroxine withdrawal), point to an increase in the propensity for ventricular arrhythmias in a hypertrophic myocardium.

The obtained results offer an important insight into the mechanism underlying an increase in the
propensity for arrhythmias in hyperthyroidism in the experimental animal model under conditions very similar to those in humans, since only the cardiovascular system of primates resembles closely this in humans.

Further experimentation on the electrophysiological changes in hyperthyroidism will be required to elucidate the course of regression of changes due to hyperthyroidism and clarify whether the observations are typical of young organism in the growth stage only or can be generalised. The present study is limited by a small number of groups, which made it impossible to determine the statistical relationship of ventricular arrhythmias and cardiac hypertrophy.

The following conclusions can be drawn from the presented investigations:
- experimentally induced hyperthyroidism causes shortening of effective refractory periods of individual elements of the conduction system, particularly in its initial stage;
- hyperthyroidism increases the propensity for supraventricular and ventricular arrhythmias inducible during EPS, as well as for spontaneous arrhythmias;
- the mechanism underlying arrhythmias in hyperthyroidism can be different for atrial and ventricular arrhythmias because of ventricular hypertrophy present in the disease

References