In Vivo Effect of Triiodo-L-Thyronine on Sarcolemmal Ca^{2+}, Mg^{2+}-Adenosine Triphosphatase in rats

Judith Zs. Szabó, Klára Nosztray, Edith Varga, Klára Guba, József A. Szentmiklósi, József Szegi

Department of Pharmacology, Medical University of Debrecen, Debrecen, Hungary

Abstract

The Ca^{2+}-activated portion of Ca^{2+}, Mg^{2+}-ATPase of rat cardiac sarcolemma has been stimulated in vivo by 3, 5, 3'-triiodo-L-thyronine (T_3). This effect was more pronounced in rats made hypothyroid by methimazole treatment. The more pronounced inhibition of Ca^{2+}, Mg^{2+}-ATPase in hyperthyroid rats compared to euthyroid controls by propanolol indicates that hyperthyroidism may modify the effect of lipophylic β blockers.

Key words: hyperthyroidism, Ca^{2+}, Mg^{2+}-ATPase, β blockers.

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Enhanced myocardial contractility observed in hyperthyroidism may be mediated by extrinsic (neurogenic, humoral and peripheral circulatory) and intrinsic factors which include intracellular changes in the synthetic and modulatory functions of the cardiac tissue itself (e.g. number and affinity of adrenergic receptors [3] myosin ATPase and myosine isoenzyme distribution [4], calcium pump of sarcoplasmic reticulum [4] and the Na^+, K^+-ATPase of cardiac sarcolemma [10]). The study of functional alternations of sarcolemmal Ca^{2+}, Mg^{2+}-adenosine triphosphatase (Ca^{2+}, Mg^{2+}-ATPase) responsible for the export of Ca^{2+} from the cells [2] at different levels of thyroid state has still remained a neglected field, though an in vitro stimulation by physiologic concentrations of L-thyroxine (T_4) and T_3 has been observed [11, 19]. The present study was therefore undertaken to examine the effect of in vivo T_3 treatment on rat cardiac sarcolemmal Ca^{2+}, Mg^{2+}-ATPase. The inhibition of Ca^{2+}, Mg^{2+}-ATPase by β antagonist propranolol in eu- and hyperthyroid rats has also been compared.

Materials and Methods

Male CFY rats were randomly assigned to control and treated groups (in each group n = 20 unless otherwise stated). All the animals were fed ad libitum with standars rat chow (LATI, Gödöllö, Hungary) and tap water during the course of experiment. Hyperthyroidism was induced by daily intraperitoneal injection of 300 μg/kg/day T_3 for 6 days. To induce hypothyroidism, 100 mg/kg/day methimazole was given intraperitoneally for 14 days. T_3 (300 μg/kg/day) was also given to hypothyroid rats for two days after discontinuing methimazole treatment. Control animals received similar volume (1 ml/100 g) of vehicle parenterally. The animals were weighed daily. At the end of the treatments in randomly selected rats rectal temperature, oxygen consumption and serum total T_4 (RIA) and total T3 (RIA) were measured.

24 hours after the last injection the animals were sacrificed by decapitation. The beating heart was immediately removed and cooled; the blood vessels and the atria were cut off; the ventricles were cut open, washed, blotted dry and weighed. All subsequent steps were performed at 0-4°C.

Sarcolemma fractions were isolated by the differential centrifugation procedure of Velema and Zaagsma [18] the only difference being the addition of protease inhibitor phenylmethanesulfonylfluoride (PMSF) in 0.1 mmol/l to homogenization medium. Briefly, pooled (2-4) ventricles were minced and homogenized in ten volumes of 20 mmol/l Tris-HC1 solution containing 1 mmol/l EDTA and 0.1 mmol/l PMSF, pH 7.0 in a glass, motor (Universal Laboratory Aid, type MP W-309)-driven Potter homogenizer with a teflon pestle, during 3 periods of 30 s with a rheostat setting of maximum rpm and rest intervals of 15 s. The homogenate was next centrifuged at 8.800 xg to remove mitochondria and cell debris, and the supernatant was recentrifuged at 12.500 xg for 20 min. The supernatant from the second centrifugation contained the crude membrane vesicles. These vesicles were sedimented at 44.000 xg and subsequently resuspended in 0.6 mol/l KC1, 20 mmol/l Tris-oxalate and 1 mmole/1 EDTA, pH 6.8 to extract contractile proteins. Resedimentation at 44.000 xg yielded the membrane vesicles used in this study. The

Table I. Body weight, ventricle weight and protein content in control, T_3- and methimazole-treated rats

Parameters	Control[x] (n = 20)	T_3-treated* (n = 20)	Control[x] (n = 20)	Methimazole-treated** (n = 20)	Methimazole + T_3-treated*** (= 20)
Body weight (g) initial	191.45 ± 5.74	195.16 ± 5.81	201.43 ± 5.21	198.67 ± 5.97	195.06 ± 4.72
final	236.00 ± 7.08	214.76 ± 6.44^{+}	288.42 ± 6.47	226.12 ± 5.03^{+++}	220.48 ± 6.13^{+++}
Wet ventricle weight (mg)	746.65 ± 18.66	842.22 ± 16.00^{++}	785.57 ± 17.07	559.56 ± 14.36^{+++}	604.89 ± 25.40^{+++}
Protein content (mg)	89.60 ± 2.95	101.07 ± 2.88^{+}	98.19 ± 2.06	71.06 ± 2.13^{+++}	75.01 ± 2.87^{+++}

Values are means ± SEM.
[x]Age-matched, because the duration of treatments was different.
* 300 µg / kg / day for 6 days.
** 100 mg / kg / day for 14 days.
*** 100 mg / kg / day methimazole for 14 days + 300 µg / kg / day T_3 for 2 days after discontinuing methimazole treatment.
Significantly different from the age-matched control: $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

Table II. Effect of triiodothyronine treatment on rat cardiac sarcolemmal Ca^{2+}, Mg^{2+}-ATPase

Thyroid state	n°	Total	Basal	Ca^{2+}-activated*	ATPase Total in %	Basal of	Ca^{2+}-activated* control
		µmole P_i / mg protein / hour					
Euthyroid control	3	28.73 ± 1.75	24.83 ± 1.74	3.90 ± 0.19	100	100	100
Hyperthyroid	4	21.39 ± 0.35^{++}	17.00 ± 0.21^{++}	4.39 ± 0.24	74	68	113
Hypothyroid control	3	30.85 ± 1.45	26.89 ± 0.95	3.96 ± 0.45	100	100	100
T_3-treated hypothyroid	3	26.36 ± 1.45$^{\#}$	19.54 ± 0.39$^{+\#\#}$	6.82 ± 0.46$^{++\#}$	85	73	172

* Difference between total and basal ATPase activities.
Values are means ± SEM; ° number of individual determination made on pooled hearts.
Significantly different from euthyroid control: $^{+}p < 0.05$; $^{++}p < 0.01$.
Significantly different from hypothyroid control: $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

pellet was resuspended in a small volume of a buffer solution consisting of 20 mmol/l Tris-HCl, pH 7,2 and 10.7 mmol/l $MgCl_2$, kept on ice and used for determination of ATPase activities immediately after preparation. The protein content of the samples was determined according to Lowry et al [7] and protein recovery in sarcolemmal fraction was calculated.

For characterization of sarcolemmal membranes Na^+, K^+-ATPase activity and the sialic acid in homogenate and sarcolemmal fraction were determined. The total content of membrane sialic acid liberated after heating the membranes at 80°C for 1 h in 0.05 mol/l H_2SO_4 was determined spectrophotometrically by the procedure of Warren [20]. The Na^+, K^+-ATPase activity was assessed according to the method of Akera [1].

The yield of membrane protein, 0.5-0.7 mg/g wet ventricle weight and the protein recovery, 0.46-0.48% (expressed in percent of protein content of original homogenate) did not change significantly in the three thyroid states. The membrane fraction was shown to have Na^+, K^+-ATPase activity [16] that was increased about 5-fold over that of the homogenate (3.10 ± 0.13 µmol Pi/mg protein/hour, homogenate vs. 16.55 ± 0.48 µmol Pi/mg protein/hour, membrane fraction). The azide sensitive-portion of ATPase in homogenate was 66-72% and in membrane fraction showed a significant reduction by 20-25% in samples originating from both control and treated animals. The sialic acid content in homogenate of heart ventricles of control rats was 7.25 ± 0.18 nmol/mg protein and in both control and treated groups a 3-4-fold increase could be detected.

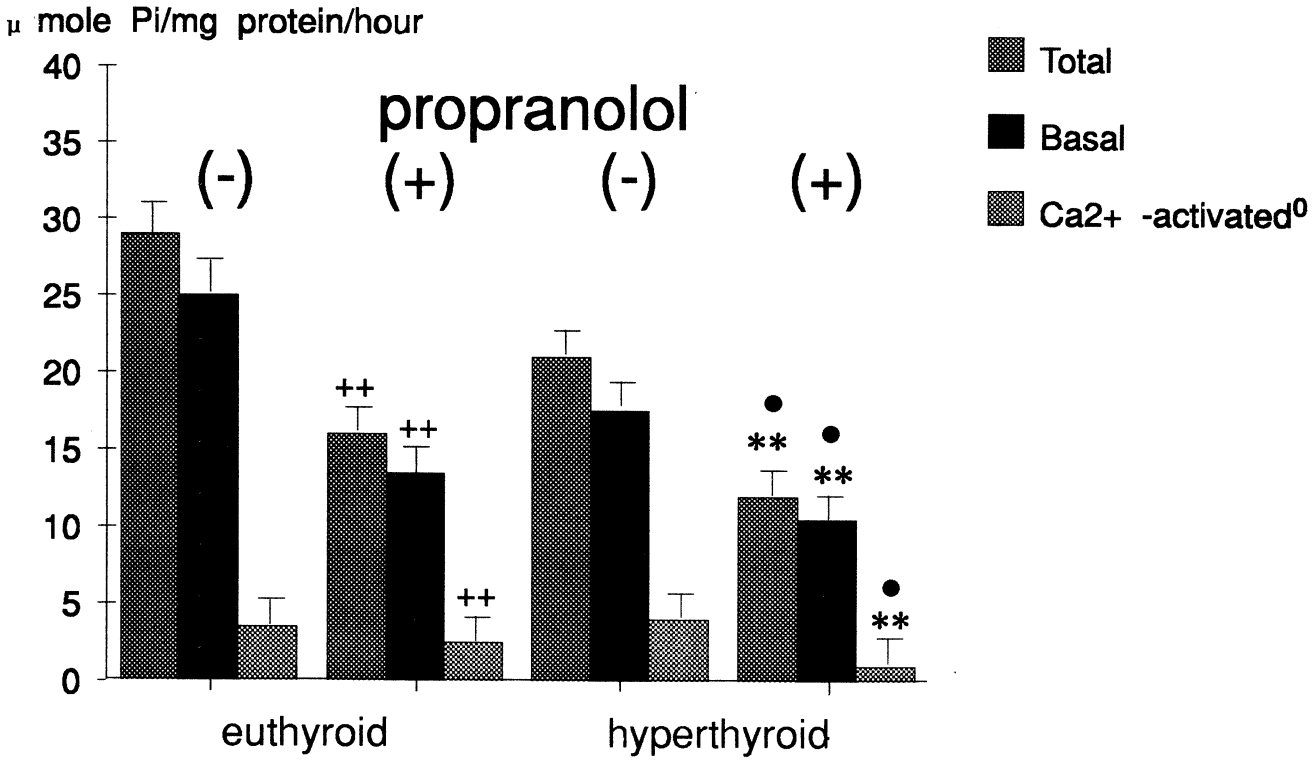

*Figure 1. Effect of 3 x 10^{-3} mol/l propranolol on sarcolemmal Ca^{2+}, Mg^{2+}-ATPase. The Ca^{2+}, Mg^{2+}-ATPase activity was estimated as described in the text. ^{0} Difference between total and basal ATPase activities. Results shown are means ± SEM from four experiments. Significantly different from euthyroid: $^{++}$ $p < 0.001$. Significantly different from propranolol-treated euthyroid: • $p < 0.01$. Significantly different from hyperthyroid: ** $p < 0.001$.*

Ca^{2+}, Mg^{2+}- ("total") ATPase activity was determined in a medium contained (in mmol/l) Tris 25, NaCl 75, KCl 25, $CaCl_2$ 0.15, EGTA 0.10, $MgCl_2$ 1, NaN_3 5, Na_2ATP 5, pH 7.4 and 40 ng exogenous calmodulin/μg membrane protein. The reaction volume was 1.0 ml containing 50 μg membrane protein. To measure "basal" (Mg^{2+}-) ATPase activity $CaCl_2$ was omitted. Assays were run for 15 minutes at 37°C. During this period, the rate of ATP hydrolysis was linear. Propranolol was dissolved in the incubation medium and preincubated with protein for 5 minutes. The reaction was initiated by addition of ATP and stopped by adding 1 ml 0.8 N perchloric acid. The precipitated protein was removed by centrifugation. Phosphate liberated from ATP was quantitated by the method of Taussky and Shorr [17]. Enzyme activity was expressed as μmol P/mg protein/hour. Ca^{2+}-activated portion was calculated by subtracting the "basal" from the "total" ATPase activity.

The activity of the enzyme in the presence of several concentrations of vanadate was measured to confirm the sarcolemmal origin of the Ca^{2+}, Mg^{2+}-ATPase [2]. Sodium-o-vanadate (0.2, 0.6 and 2.0 μmol/l) reduced the enzyme activity by 12.1, 19.6 and 24.3%, respectively. The relative low sensitivity of these preparations to vanadate means that the sarcolemmal vesicles may be contaminated with vesicles of sarcoplasmic reticulum origin.

Statistical analysis. Significance of changes in mean values for different parameters induced by hyper- or hypothyroid state or propranolol treatment was estimated by Student's t-test for unpaired data. Mean differences with a p value less then or equal to 0.05 were considered significant. All data are expressed as the mean ± SEM.

Materials: bovine albumin powder, fraction V (used as standard for protein determination) was a Fluka preparation. 3, 5, 3'-triiodo-L-thyronine (T_3), sodium-o-vanadate and DL-propranolol-HCl were Sigma preparations. Radioimmunoassay (RIA) kits used for measurement of serum T_3 and T_4 levels were obtained from Institute of Isotopes, Hungarian Academy of Sciences. All other chemicals were purchased from Reanal (Hungary) and were of analytical grade.

Results and discussion

The T_3-treated rats used in this study were clearly hyperthyroid; they failed to gain weight properly and their rectal temperature (37.67 ± 0.20°C / n = 5 / vs. 38.56 ± 0.12°C / n = 5 /; $p < 0.01$), oxygen consumption (1.969 ± 0.024 / n = 5 / vs. 2.442 ± 0.048 / n = 5 / O_2 ml / dm^2 / min; $p < 0.001$) and total T_3 (nmol/l: 6.65 ± 0.91 / n = 4 / vs. 10.20 ± 0.60

/ n = 2 / and in 5 rats > 12.00 /above measurable level/) were significantly elevated. At the same time, total T_4 decreased in all rats examined / n = 7 / below detectable level (< 17.5 nmol/l) in comparison with controls (81.20 ± 10.27 / n = 5/). T_3 treatment, - in a negative feedback fashion, - could result in inhibition of thyroid hormone synthesis. In addition, the animals developed cardiomegaly (Table I).

On the effect of methimazole treatment the rats also failed to gain weight properly (Table I). Their rectal temperature did not change significantly (37.67 ± 0.20°C / n = 5 / vs. 37.57 ± 0.15°C / n = 5 /), the oxygen consumption (1.969 ± 0.024 / n = 5 / vs. 1.480 ± 0.084 / n = 5 / O_2 ml / dm^2 / min, $p < 0.001$), total T_3 (6.65 ± 0.91 / n = 4 / vs. 1.91 ± 0.32 / n = 6 / nmol/l, $p < 0.001$) and total T_4 levels, however, (81.20 ± 10.27 / n = 5 / vs. < 17.5 (below detectable level) / n = 6 /) were significantly decreased. Ventricle weight and protein have also decreased (Table I). Spontaneous regression of hypothyroidism was significantly accelerated by T_3 treatment and the decrease in serum total T_3 level was counteracted (1.91 ± 0.32 / n = 6 / vs. > 12.0 (above measurable level) / n = 7 /). Serum T_4 level, however, in methimazole + T_3-treated group remained below detectable level (< 17.5 / n = 7 /) as it was in T_3-treated group.

In euthyroid rats only 13% of the total ATPase activity was the Ca^+-activated portion. The relatively high basal ATPase activity is a species-dependent characteristic of a rat cardiac membranes observed by others, too [8, 9] (Table II). As a consequence of in vivo T_3 treatment, total and basal ATPase activities were significantly decreased and the Ca^{2+}-activated portion was slightly increased. This increase was, however, more pronounced in hypothyroid rats compared to the euthyroid controls. T_3 treatment could also counteract the hypothyroidism induced elevation of total and basal ATPase activities (Table II).

To remove the adrenergic contribution to the enhanced myocardial contractility of hyperthyroidism β adrenergic blockade is used. Lipophylic β blockers - in addition to their specific drug-receptor binding, however - can interact with the sarcolemmal membrane bilayer [6] which is often referred as specific membrane activity [12] and includes the inhibition of sarcolemmal Ca^{2+}, Mg^{2+}-ATPase [15]. To ascertain that hyperthyroidism-induced alteration of membrane phospholipid constituents [13] cause any change in specific membrane activity of a lipophylic β blocker, cardiac sarcolemmal Ca^{2+}, Mg^{2+}-ATPase activities of eu- and hyperthyroid rats in the presence and in the absence of propranolol were also compared (Fig. 1).

Propranolol at a concentration of 3×10^{-3} mol/l decreased the Ca^{2+}, Mg^{2+}-ATPase activity in both control and T_3-treated rats; the inhibition of the Ca^{2+}-activated portion in T_3-treated rats was, however, more pronounced (Fig. 1). The threshold concentration of propranolol for observing an enhanced inhibition of ATPase activity in hyperthyroid rats was 5×10^{-4} mol/l. The inhibition may be due to some non-receptorial action of this agent on the membrane [6] and may have some significance in cardiodepressive effect of propranolol. This is why higher concentration of propranolol was necessary than the clinically effective plasma levels exerting specific blockade at adrenergic receptors [5].

A significantly lower unsaturation of fatty acids has been demonstrated in heart microsomes from T_4-treated rats to untreated animals [13]. Further experiments are needed to decide whether a similar alteration in the unsaturated fatty acid composition of cellular membranes could be responsible for the more pronounced inhibitory action of propranolol in hyperthyroid rats.

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Address correspondence to:

Dr. J. Szabo, Department of Pharmacology, Medical University of Debrecen, H-4012 Debrecen, Hungary.

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