Alterations of Contractility and Sarcoplasmic Reticulum Function of Rat Heart in Experimental Hypo- and Hyperthyroidism

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Abstract. Myocardial contractility and Ca^{2+}-pump function of sarcoplasmic reticulum (SR) were studied on hearts of untreated, thyroidectomized and thyroxine-treated rats. In hypothyroid rats the conctractile force, the maximum velocity of tension development and relaxation significantly decreased (by 73.2%, 68.2%; and 67.8%, respectively), while the time to peak tension was prolonged (by 25.9%) as compared with the control group. In hyperthyroidism opposite changes were found. Since the transport of calcium opposite changes were found. Since the transport of calcium by SR plays an important role in controlling contraction and, first of all, relaxation of muscle, function of the sarcoplasmic reticulum was also investigated under the above experimental conditions. In thyroidectomized rats the rate of Ca^{2+}-uptake and Ca^{2+}-activated ATPase activity of SR significantly decreased (by 31.7% and 61.0%, respectively), while Ca^{2+}-binding remained unchanged. After thyroxine treatment both the Ca^{2+}-uptake and binding capacity of SR were even decreased (by 25.6% and 12.9%, respectively), in spite of an increase in Ca^{2+}-activated ATPase activity (by 67.3%). These changes in Ca^{2+} transport function of cardiac SR may only partially be responsible for the abnormities in contraction and relaxation observed in hearts from hypo- and hyperthyroid rats.

Key words: Sarcoplasmic reticulum — Calcium transport — Rat heart — Thyroidectomy — Thyroxine treatment — Myocardial contractility

Introduction

It is well known that changes in the thyroid state are associated with marked alterations in cardiac growth (Bonnin et al. 1983; Cohen 1974; Sanford et al. 1978; Zähringer and Klaubert 1982) and contractile function of the heart (Buccino et al. 1968; Taylor et al. 1969). However, the mechanism responsible for the altered contractility of heart associated with hypo- and hyperthyroidism has not

been fully elucidated. The effects of the thyroid hormone, including those exerted upon the cardiovascular system, were initially thought to be mediated through the sympathetic nervous system (Cohen et al. 1966). Recent studies have cast doubt upon this view and have suggested that thyroid hormone enhances myocardial contractility by a direct action on the heart (Conway et al. 1976; Hjalmarson et al. 1970; Suko 1973).

In our previous work we observed alterations in the cardiac myosin ATPase activity in hyper- and hypothyroid rats (Nosztray et al. 1980; Szabó et al. 1979). These findings could partially explain abnormal cardiac contraction, but not altered relaxation. Since changes in Ca^{2+} transport in the sarcoplasmic reticulum may represent the major mechanism by which thyroxine modulates cardiac relaxation and contraction, the purpose of the present work was to investigate this problem by measuring myocardial contractility and SR function of hearts of hypo- and hyperthyroid rats.

Materials and Methods

Chemicals

L-thyroxine sodium salt was purchased from BDH Chemicals Ltd. Bovine serum albumin powder, fraction V, was a Fluka preparations. $^{45}CaCl_2$ was purchased from Institute of Isotopes, Hungary.

Animals and procedures

Male CFY: LATI rats (Gödöllő, Hungary) weighing 220 to 250 g were used in all experiments. One group of rats was made hypothyroid by thyroidectomy. One week after thyroidectomy, the animals received 2 Mbq of ^{131}I-labelled NaI peritoneally. Twenty days after the surgical removal of the thyroid glands, the animals were killed by decapitation. Control animals were sham operated. Another group was made hyperthyroid by daily peritoneal injections of 1 mg/kg body weight of l-thyroxine for 14 days. The control animals were injected with physiological saline. The rats were decapitated 24h after the last injection. The ventricular myocardium of all animals was used for experiments. Serum thyroxine levels were estimated from blood samples obtained at the time of sacrifice, to control the extent of hypo- and hyperthyroidism. The values obtained by radioimmunassay were as follows: controls: 56.4 ± 1.55 nmol-/l; thyroxine-treated: 959.8 ± 185.3 nmol/l; and thyroidectomized: <17.6 nmol/l (below detectable levels).

Studies on contractility of the ventricular myocardium

Trabecular muscles derived from the left ventricles isolated by the method of Ullrick and Whitehorn (1956) were mounted vertically in a jacketed, constant temperature bath of 10 ml at 37 °C (Krebs solution, pH 7.4; gase phase 95% O_2 and 5% CO_2). One end of the muscle was fixed, the other end was tied to a strain gauge of an isometric force-displacement transducer. The muscles were stimulated electrically by applying square-wave pulses of 1 ms duration via two punctate platinum electrodes. The frequency of stimulation was 2 Hz and the intensity twice the excitation threshold (1.4 ± 0.5 V). Electronic differentation was used to obtain dT/dt values. After an equilibration period lasting 40 to 60 min, the contractile force (CF), maximum velocity of tension development ($+dT/dt_{max}$), maximum velocity of relaxation ($-dT/dt_{max}$) and time to peak tension (TPT) were determined.

Isolation of the membrane sarcoplasmic reticulum fraction

Membrane fraction enriched in fragmented SR was prepared from ventricular myocardium according to the method of Harigaya and Schwartz (1969) with a slight modification as described previously (Takács et al. 1980). The homogenate was centrifuged at $8700 \times g$ for 40 min, and the supernatant was centrifuged at $3700 \times g$ for 30 min. The pellets were resuspended and washed with the following medium (in mmol/l): KCl 600; dithiothreitol 1; sucrose 300; and Tris-maleate 20 (pH 6.8); and centrifuged at $3700 \times g$ for 30 min. Microsomes were suspended in the above medium without dithiothreitol, stored on ice and used within several hours after the preparation. Protein was determined by the biuret method (Layne 1957) using bovine serum albumine as standard. The yields of SR protein from control and hypo- and hyperthyroid rat hearts were similar (about 1 mg SR prot./g heart weight). Succinate dehydrogenase (SDH) activity for the mitochondrial contamination was determined by the method of Slater and Bonner (1952), SDH activities in several SR fractions were the same (about 0.250 mmol $K_3Fe/CN/_6$/mg prot./min).

Assay of calcium accumulation and ATPase activity

Ca^{2+}-uptake and Ca^{2+}-binding by SR were determined using the Millipore filtration technique according to the method of Takeo et al. (1980). Ca^{2+}-uptake: the membranes (50—100 μg protein/ml) were incubated at 25 °C in the following medium (in mmol/l): Tris-maleate 20; KCl 100; $MgCl_2$ 5; potassium oxalate 5; Na_2ATP 5; NaN_3 5; $^{45}CaCl_2$ 0.1. Ca^{2+}-binding activity was measured in the same medium without potassium oxalate. Mg^{2+}-ATPase, or "basal", and $Mg^{2+} + Ca^{2+}$-ATPase, or "total", ATPase activities were determined according to the method of Drabikowski et al. (1972). The inorganic phosphate liberated was determined by the method of Taussky and Shorr (1953). The activity of Ca^{2+}-activated ATPase was calculated by subtracting the basal from the total ATPase activity. Calcium uptake and ATPase activities were measured during the initial phase of the reaction, during which the time course of the activity was linear.

Data analysis

The results are expressed as mean ± S.E. Statistical significance was evaluated by Student's t test. $P < 0.05$ was taken as the level of significance.

Results

The characteristics of mechanical activity in electrically driven left ventricular trabeculae taken from eu-, hypo- and hyperthyroid rats are summarized in Table 1. In hypothyroid rats CF, $+dT/dt_{max}$ and $-dT/dt_{max}$ decreased significantly, and TPT was prolonged. After thyroxine treatment, CF and $+dT/dt_{max}$ increased significantly, while the enhancement in $-dT/dt_{max}$ was insignificant. TPT was shortened.

In the following series of experiments, we investigated the function of SR by measuring Ca^{2+}-uptake, Ca^{2+}-binding and ATPase activities (Table 2). It should be pointed out that our control values for Ca^{2+}-uptake, Ca^{2+}-binding and ATPase activities were similar to those reported in the literature for the rat heart (Penpargkul et al. 1977; Penpargkul 1979; Narayanan 1983; Takeo et al. 1980). Table 2 shows that, after thyroidectomy, the total, Ca^{2+}-activated ATPase activity and rate of Ca^{2+}-uptake decreased significantly, while the basal ATPase activity and Ca^{2+}-binding remained unchanged. In hyperthyroid rats, the Ca^{2+}-activated ATPase activity increased by about 70%. This increase originates from a diminu-

Table 1. Effects of hypo- and hyperthyroidism on the rat heart contractility

Treatment	CF		$+dT/dt_{max}$		$-dT/dt_{max}$		TPT	
	mg/mm^2	per cent change	$g/s/mm^2$	per cent change	$g/s/mm^2$	per cent change	ms	per cent change
Control rats ($n = 14$)	204.40 ± 21.9		3.77 ± 0.46		2.02 ± 0.28		96.00 ± 1.56	
Thyroidectomized rats ($n = 5$)	54.80 ± 11.5**	−73.2	1.20 ± 0.20**	−68.2	0.65 ± 0.20**	−67.8	122.00 ± 5.02**	+25.9
Thyroxine-treated[□] rats ($n = 7$)	332.80 ± 28.9*	+62.8	6.66 ± 1.21*	+76.2	2.85 ± 0.92	+41.1	71.70 ± 5.25**	−25.3

[□] i.p. injections of 1 mg/kg l-thyroxine daily, for 14 days.

Each value is a mean ± S.E. Significance levels: $*p < 0.01$; $**p < 0.001$.

CF = contractile force; $+dT/dt_{max}$ = maximum velocity of tension development; $-dT/dt_{max}$ = maximum velocity of relaxation; TPT = time to peak tension; n = number of animals

tion of basal ATPase activity, since the total ATPase activity remained unchanged. In spite of an increased Ca^{2+}-activated ATPase activity, Ca^{2+}-uptake and binding activities decreased.

Similar significant changes of Ca^{2+}-uptake, binding and Ca^{2+}-activated ATPase activity in hypo- and hyperthyroid rat hearts were also found during the non-linear phase of the reaction (after 15 min).

Discussion

Hyperthyroidism results in an increase in the intensity of the active state of myocardium as measured by an increase in the rate of tension development; at the same time, it shortens the duration of the active state, as reflected by the time to peak tension. Hypothyroidism induces changes in the opposite direction.

Although it is clear that changes in the thyroid state can affect myocardial performance significantly, independent on changes in catecholamine concentration or adenylate cyclase system (Nayler et al. 1971), the mechanism underlying these changes remains unclear. Alterations in cardiac myosin ATPase activity (Nosztray et al. 1980; Szabó et al. 1979) may partially explain abnormal myocardial contractility in hypo-and hyperthyroid states.

An alternative point of this control would be the changes in Ca^{2+} accumulation by cardiac SR. Calcium sequestration and release by the sarcoplasmic reticulum is a major mechanism by which the duration of active state, relaxation, and rate of tension development are regulated in the myocardium.

In the present work we found a decrease in Ca^{2+}-uptake and Ca^{2+}-activated ATPase activities in hypothyroidism. Our results are in good agreement with results of Suko (1973), who described similar changes in cardiac SR function in hypothyroid rabbits. A diminution in velocity of Ca^{2+} transport observed in hypothyroidism might partially be responsible for a slower reduction in the free Ca^{2+} in the sarcoplasma and thus for a decreased rate of relaxation.

In hyperthyroidism, the Ca^{2+}-activated ATPase activity increased, and this enhancement resulted from a diminution in basal ATPase activity, the latter being unusually high in rat hearts (Narayanan 1983; Nayler et al. 1975; Penpargkul et al. 1977; Penpargkul 1979; Takács et al. 1981). Also, Nayler et al. (1971) described reduced basal ATPase activity in cardiac SR isolated from thyroxine-treated dogs, and unchanged Ca^{2+}-activated ATPase activity with an increased Ca^{2+}-exchanging activity.

However, in our present experiments, Ca^{2+}-uptake and binding activities in thyroxine-treated rats were even smaller, despite an increased Ca^{2+}-activated ATPase activity.

The reason for this phenomenon is not clear. Ash et al. (1972) found that

Table 2. Effects of hypo- and hyperthyroid state on Ca^{2+}-uptake, Ca^{2+}-binding and ATPase activities of cardiac SR

Treatment	Ca^{2+}-binding	Ca^{2+}-uptake	ATPase		
	nmol Ca^{2+}/mg protein/5 min		Basal	Total nmol P_i/mg protein/min	Ca^{2+}-activated
Control rats	12.70 ± 0.380	188.85 ± 6.50	1164.4 ± 12.80	1515.7 ± 11.20	352.3 ± 15.90
Thyroidectomized rats	12.80 ± 0.120	129.07 ± 5.90**	1196.9 ± 32.20	1333.8 ± 31.20**	137.4 ± 11.40***
Thyroxine-treated□ rats	9.48 ± 0.460*	164.53 ± 5.00**	793.5 ± 79.7**	1385.7 ± 134.6	589.6 ± 123.2**

□ i.p. injections of 1 mg/kg l-thyroxine daily, for 14 days.

For conditions of incubation in measuring Ca^{2+}-uptake and binding, are Materials and Methods.

Ca^{2+}-activated ATPase activity: difference between total ATPase activity (conditions as in Ca^{2+}-uptake) and basal ATPase activity (medium as above without Ca^{2+}, with 0.4 mmol/l EGTA added). The results shown are mean ± S.E. of 4—5 experiments using different membrane preparations. *Significance levels*: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

chronic administration of thyroxine reduced Ca^{2+}-uptake and Ca^{2+}-binding by skeletal muscle SR due to an increase in passive permeability of SR vesicles to Ca^{2+}.

It is possible that the increase in Ca^{2+}-uptake becomes not manifested due to some structural alteration (lipid-protein ratio) in SR membranes; as a results, SR is less efficent in retaining the newly accumulated Ca^{2+} in hyperthyroid rat hearts. Also, it is likely that other mechanisms are operative as well: Ca^{2+}-binding and uptake by SR may be compensated by a calmodulin-mediated increase in sarcolemmal Ca-extrusion via Ca-pump. Further extensive studies are required to identify the molecular mechanisms by which thyroxine controls the intracellular Ca transport and myocardial contractility in rat hearts.

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