

Thyroxine Induced Transformation in Sarcoplasmic Reticulum of Rabbit Soleus and Psoas Muscles

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Z. Naturforsch. **40c**, 726–734 (1985); received July 11, 1985

Sarcoplasmic Reticulum, Thyroxine, Fast and Slow Twitch Muscles

The properties of the sarcoplasmic reticulum membranes isolated from slow-twitch type I soleus and fast-twitch type II psoas muscles of control and thyroxine treated rabbits were comparatively studied. Membrane yield, maximal calcium storing capacity, ATP-supported calcium uptake, calcium-dependent ATPase activity and calcium-dependent phosphoprotein formation were found to be 3–10 fold higher in psoas than in soleus preparations. Membrane yield, calcium-dependent ATPase activity, ATP-supported calcium transport and calcium-dependent phosphoprotein are at least twice enhanced in the membranes from soleus muscles of animals treated for 14–21 days with thyroxine. The corresponding capacities of the membranes from psoas muscles are not further augmented by the same thyroxine treatment. The maximal calcium storing capacity of the psoas membranes is their sole specific property which is significantly increased. The changes in the properties of the soleus muscles' sarcoplasmic reticulum membranes are engendered by an increase from 5 to 30–50% in the number of type II fibres. Since the calcium transporting properties of the sarcoplasmic reticulum membranes from type II fibres qualitatively differ from those of type I fibres, thyroxine does not only affect quantitative but also qualitative parameters of the muscles' sarcoplasmic reticulum membrane system.

Introduction

The adaptation occurring in muscle contractility in response to modification of the functional state of the thyroid gland is an interesting example of muscle plasticity. The changes in skeletal muscle mechanical activity mainly concern peak twitch tension, contraction and especially relaxation time [1, 2]. These effects of thyroxine were found to be correlated with changes in the enzymatic activity of the contractile proteins as well as of mitochondrial and glycolytic enzymes [3, 4]. The changes in the contractile behaviour require that beside the energy consuming and providing systems the regulation of contractile activity by the sarcoplasmic reticulum membranes is likewise affected. In fact, from hyperthyroid animals sarcoplasmic reticulum membranes were obtained the calcium transport activity of which is considerably higher than that from control animals [2]. Conversely, calcium transport activity of isolated sarco-

plasmic reticulum vesicles from hypothyroid animals is sub-normal. Suko [5, 6] and Limas [7] described relatively small changes in the activity of cardiac sarcoplasmic reticulum. Thyroxine application increased the activity by 20% while the activity of membranes from hypothyroid animals was depressed by 20%. On the other hand, Fitts *et al.* [2] reported that the calcium uptake by sarcoplasmic reticulum membranes isolated from the soleus muscle of hyperthyroid rats was enhanced by a factor of two and that more membranes could be extracted from these animals. Yet, in a later study Kim *et al.* [8] did not observe any increase in the specific calcium uptake activity of sarcoplasmic reticulum membranes isolated after thyroxine treatment and explained their results by a thyroid hormone mediated increase in the quantity of the sarcoplasmic reticulum only. The reported differences of the effect of thyroxine on the activity of the sarcoplasmic reticulum calcium transport system of different muscles suggest that considerable differences in the membrane's responsiveness to the hormone might exist. Furthermore, the question whether the hormone might affect the muscles' content of sarcoplasmic reticulum membranes and/or

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0009–0726 \$ 01.30/0



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their specific properties must be considered to be unresolved [2, 9]. In the present study mainly the latter problem is addressed by comparing the effect of thyroxine on the calcium transport activity of sarcoplasmic reticulum membranes isolated from slow and fast twitch muscles of the rabbit, its soleus and psoas muscles respectively.

Materials and Methods

Animals and thyroxine treatment

Male rabbits weighing 2300–2800 g were housed together and divided randomly in two groups: control group and thyroxine treated group (l-thyroxine, Serva, Heidelberg, 100 µg/kg body weight ip daily during 2 or 3 weeks). The dosis corresponds to that applied to rats to produce thyrotoxicosis [4]. Rabbits treated with higher doses lost more weight and frequently died. Rabbits were fed pellets and drank water ad libitum. Body weight was regularly controlled during the experiment. At the end of the experimental period, the animals were killed by stunning and bled through the carotid arteries.

Levels of T_3 (triiodothyronine) and T_4 (thyroxine) were measured 10–20 days after thyroxine treatment in the serum after protein precipitation with acetonitrile using a radio-immunoassay (Radio-Chemical, Amersham).

Isolation of sarcoplasmic reticulum vesicles

The hind leg muscles soleus and psoas were used as slow and fast twitch muscles respectively. The muscles were quickly excised, pooled and stored on ice prepared from distilled water. The muscles were freed of connective tissue and fat and homogenized with an Omni-mixer (Ivan Sorval, Inc., USA) in a 10-fold volume of extracting medium for 120 s at 15700 rpm in a freshly prepared extraction solution containing 0.1 M KCl, 10 mM imidazole, pH 7.4, 0.3 M sucrose and 0.1 mM PMFS. Thereafter the homogenate was centrifuged for 30 min. (SS 34 Servall rotor \times 9000 \times g). The supernatant was passed through cheesecloth and then centrifuged for 30 min \times 113000 \times g (Ti 60, Beckman rotor). The sediment was resuspended in 0.1 M KCl, 0.2 mM Tris buffer, pH 7 to give a protein concentration of approximately 10–15 mg/ml. Mitochondrial contaminations monitored by azide inhibition of the mitochondrial ATPase was less than 10% of the mitochondrial fraction. This is in agreement with the

residual cytochromoxidase activity found by Berne [10] in the same membrane fraction. The $(Na^+ + K^+)$ -ATPase activity of three preparations from control and thyroxine treated animals determined according to Jørgensen [11] was less than 3% of the activity of that of skeletal muscle sarcolemma [12]. The protein concentration was determined by the Biuret method.

Assays of calcium uptake and ATPase activity

The rate of calcium uptake was estimated as described previously [13] using the following standard conditions: 5 mM NaN_3 , 5 mM $MgCl_2$, 20 mM histidine pH 7.0, 0.1 mM $^{45}CaCl_2$, 40 mM KCl, 5 mM ATP, 5 mM potassium oxalate and 0.1 mg vesicular protein/ml. The uptake reaction was stopped by filtration through Millipore filter (45 µm). The radioactivity in the filtrate was measured by liquid scintillation counting (4 g Omnifluor, New England Nuclear; 260 ml Triton X-100, 740 ml Toluene). Storage capacity of the preparations was measured after 25 min incubation at a series of protein concentrations declining from 0.1 mg/ml to 0.01 mg/ml. The assay mixture for determining ATPase activity contained 5 mM $MgCl_2$, 20 mM histidine pH 7.4, 0.1 mM $CaCl_2$, 40 mM KCl, 20 µM \times 537A, 5 mM ATP. For assaying Mg^{2+} -ATPase activity 2 mM EGTA was added to the medium instead of Ca^{2+} . The reactions were started by addition of the membranes (0.1 mg protein/ml). After 1, 2, 5, 10 min incubation aliquots were taken and mixed with an equal volume of 6% trichloroacetic acid. The mixture was filtered and P_i was determined in the supernatant according to Rockstein and Herron [14]. Calcium uptake and ATPase activity were measured at room temperature (20–22 °C).

Phosphoprotein formation

Vesicles (0.4 mg/ml) were incubated at 0 °C in a solution containing 2 µM $[^{32}P]$ ATP, 0.1 mM $MgCl_2$, 40 mM KCl, 50 mM histidine and 0.1 mM $CaCl_2$. Phosphorylation was stopped by the addition of 10 vol. of a cold solution of 10% TCA, 0.05 M H_3PO_4 to aliquots taken at 0, 2, 5, 10, 30, 60 and 120 s. After centrifugation for 4 min at 4000 rpm at 4 °C the pellets were resuspended with glass pearls deposited on glass filters and washed with at least 100 ml of cold stop solution. Subsequently, each filter was heated in 3 ml of 0.1 M NaOH for 3 min in a boiling water

bath, then 1 ml of 0.1 M H_3PO_4 was added, the samples were filtered and the radioactivity in aliquots were counted in a liquid scintillation counter.

Gel electrophoresis

The sarcoplasmic reticulum vesicular preparations were dialysed for 2 h against 0.01 M Tris-Bicin pH 8.2 at 4 °C. Subsequently, 1 mg SDS per 1 mg vesicular protein and bromophenol blue were added. Samples were run in polyacrylamid gels in 0.1 M Tris, 0.1 M Bicin, 0.1% SD pH 8.2. For calibration the high molecular weight protein standard from BioRad Lab. Richmond Calif. was used. The gels were fixed in sulphosalicylic acid 20% for 15 min, then stained with coomassi blue 0.25%. Destaining was performed in 7% acetic acid 5% methanol. The densitometric tracings of the protein bands were performed with a Hirschmann densitometre, München, FR Germany, at 601 nm.

Histochemistry

Six control rabbits and two groups of 8 animals each daily treated with thyroxine for 14 respectively 21 days were used. Three animals of both thyroxine groups were sacrificed 4 weeks after suspension of the treatment.

Psoas and soleus muscles were removed in a slightly stretched position and frozen in melting methylbutane at -160 °C as composite blocks.

Serial 8 and 15 μm sections were cut at -20 °C in a cryostate microtome and usually stained 60 to 90 min after warming at room temperature on the day of cutting or after overnight storing at -30 °C.

Sections were usually assayed for the myofibrillar myosin ATPase activity at pH 9.4 after formaldehyde fixation and alkaline preincubation at pH 10.4 or after acid preincubation at pH 4.35 without previous fixation according to Guth and Samaha [15, 16]. To determine fibre subtypes, several unfixed sections were preincubated at various pH's (9.4, 4.6, 4.3 and 3.9) using the method of Brooke and Kaiser [17, 18]. Sections from control and thyroxine treated animals were usually incubated together in the same solution.

Results

Serum thyroid hormone and body weight changes

Rises of T_4 from 15 to 50 nmol/l and of T_3 from 0.9 to 8 nmol/l were observed in the serum in the course of thyroxine treatment.

The thyroxine treated animals lost $20 \pm 2\%$, $\pm \text{SE}$ ($n = 8$) of their body weight, while the body weight of the control animals increased by $15 \pm 1.2\%$, $\pm \text{SE}$ ($n = 10$). The substantial weight loss of the thyroxine treated animals as well as the elevation of thyroid hormone in the serum indicate the efficacy of the applied régime.

Extraction yield of sarcoplasmic reticulum protein

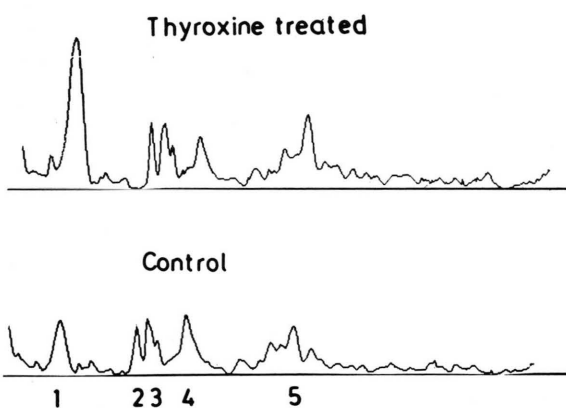
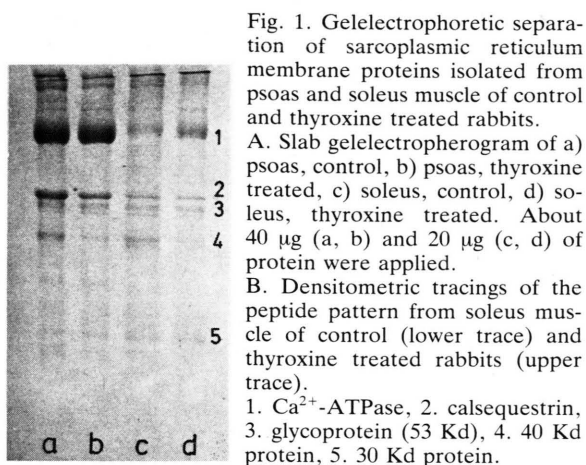
The thyroxine treatment results in a significant increase in the amount of membrane protein that can be isolated from psoas and soleus muscles. The extraction yields from soleus muscles rise from $1.4 \pm 0.1 \text{ mg/g}$ to $4.8 \pm 0.2 \text{ mg/g} \pm \text{SE}$ ($n = 6$) while the extraction yields from psoas muscles ($6.0 \pm 0.35 \text{ mg/g} \pm \text{SE}$ ($n = 6$)) increase only relatively little. These numbers for the soleus preparations fairly agree with those obtained for rat soleus muscles by Fitts *et al.* [2], but are considerably greater than corresponding values reported by Kim *et al.* [8]. Yet, statements concerning extraction yields must be considered with care as long as the completeness of the extraction and the purity of the extracted material remain uncertain (*cf.* [19]).

Gel electrophoresis

The electropherogrammes of all preparations show the major protein bands characteristic for sarcoplasmic reticulum membranes – the calcium transport protein and the calcium binding protein calsequestrin. Densitometric evaluation reveals that after thyroxine treatment the relative protein content of the ATPase in the membranes from psoas muscle rises by $20 \pm 5\%$ ($n = 4$). A much larger increase of up to $96 \pm 15\%$, $\pm \text{SE}$ ($n = 6$) occurs in the membranes from soleus muscle (Fig. 1). The relative calsequestrin content of $16 \pm 2\%$ ($n = 4$) of the membranes from both muscles is not significantly affected by thyroxine treatment. The relatively low ATPase content of 50% found even in psoas preparations is due to the fact that total unfractionated membrane preparations were used in this study.

Calcium-dependent and calcium-independent ATP-hydrolysis

The data collected in Table I illustrate the profound differences in the calcium-dependent and the calcium-independent ATPase activity of sarcoplasmic reticulum membranes isolated from psoas and



soleus muscles and the changes of these activities produced by the thyroxine treatment. The membranes isolated from the psoas muscles of the control animals exhibit high calcium-dependent ATPase activity ($0.7 \mu\text{mol}/\text{mg}\cdot\text{min}$; 20°C) while the calcium-independent activity of these preparations is quite low ($0.06 \mu\text{mol}/\text{mg}\cdot\text{min}$; 20°C). The same activity pattern is found in the routinely used preparations

from thigh and back muscles of the rabbit. The thyroxine treatment produces a small enhancement of the calcium-dependent ATPase activity while the calcium-independent activity is significantly enhanced by approximately 40%. In agreement with previous findings the membranes isolated from the soleus muscle are characterized by a very high activity of the calcium-independent magnesium ATPase [20]. Thyroxine treatment produces a significant decrease of this activity from 470 to 300 $\text{nmol}/\text{mg}\cdot\text{min}$. The calcium-dependent enzymatic activity concomitantly increases by 30% from 230 to 320 $\text{nmol}/\text{mg}\cdot\text{min}$, resulting in a small decline of the total enzymatic activity of the preparation. The observed activity changes of calcium-dependent ATPase are in line with those reported by Fitts *et al.* [2] for sarcoplasmic reticulum membranes isolated from rat soleus. The described activities could only be ascertained when the enzymatic activity was determined shortly after membrane isolation. Reported low or lacking calcium-dependent activity is most likely due to the enzyme's fast decay [20].

Calcium uptake and calcium storing capacity of the sarcoplasmic reticulum vesicles

The sarcoplasmic reticulum vesicles isolated from psoas muscle accumulated calcium from the oxalate supported uptake medium with an initial rate of approximately $1 \mu\text{mol}/\text{mg}\cdot\text{min}$ at 20°C (Fig. 2). The uptake proceeds nearly linearly with time and the medium calcium has completely been stored by the vesicles after 2 min. A virtually superimposable time course of the calcium uptake is displayed by the vesicle preparation obtained from the psoas muscle from thyroxine animals. As first reported by Sréter and Gergely [21], slow muscles yield vesicular preparations which take up calcium much more slowly than preparations from fast muscles. Fig. 2 shows that even after an incubation period of 20 min, vesicular

Muscle fibre	ATPase		Total [nmol/mg·min]
	Ca^{2+} dependent [nmol/mg·min]	Mg^{2+} dependent [nmol/mg·min]	
Psoas	733.2 ± 83.4	66.7 ± 15.3	799.8 ± 80.2
Soleus	$234.3 \pm 13.2^*$	$472.2 \pm 27.5^*$	706.5 ± 32.4
Psoas + T_4	866.5 ± 89.2	100.0 ± 26.0	966.4 ± 82.3
Soleus + T_4	$320.0 \pm 17.6^*$	$300.0 \pm 13.3^*$	620.0 ± 21.5

* Values are means of 3 determinations \pm SD. $p < 0.05$.

Table I. ATPase activity of sarcoplasmic reticulum vesicles isolated from psoas and soleus muscles of control and thyroxine treated rabbits. The calcium dependent and the magnesium dependent ATPase were determined as described in Materials and Methods at room temperature $20\text{--}22^\circ\text{C}$.

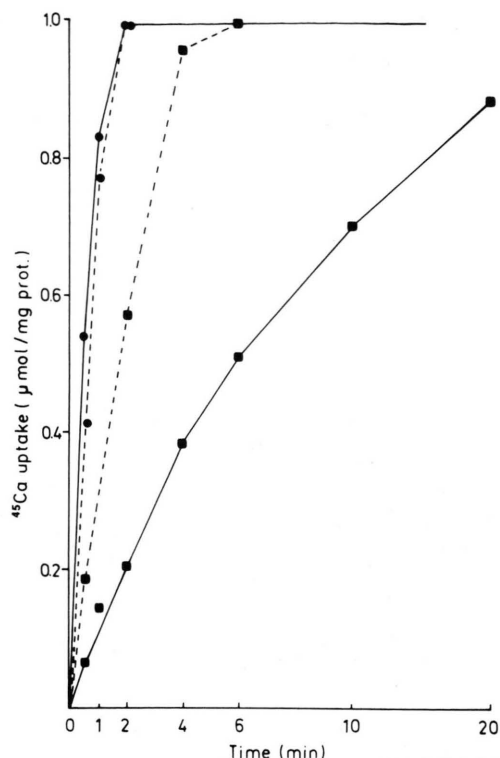


Fig. 2. Time course of ATP supported calcium uptake of sarcoplasmic reticulum vesicles isolated from psoas and soleus muscles from control and thyroxine treated rabbits. Calcium uptake from media containing 5 mM oxalate was determined as described in Materials and Methods at room temperature 20–22 °C.

●—● M. psoas, control; ●—●—● M. psoas, thyroxine treated; ■—■ M. soleus, control; ■—■—■ M. soleus, thyroxine treated.

preparations from control soleus muscles have not completely taken up the calcium present in the incubation medium, although the calcium to protein ratio was kept comparatively low. Yet, thyroxine treatment resulted in an enormous increase of the calcium uptake activity of the slow muscle membranes. The linearly proceeding uptake reached its maximum already after 6 min. Fig. 3 illustrates the increase of the maximal calcium load when the calcium/protein ratio in the medium is elevated from 1 to 10 μmol calcium/mg protein. At a calcium/protein ratio (μmol Ca^{2+}/mg) of 10 in the medium, the maximum storing capacity of psoas preparations reach 5–6 μmol Ca^{2+}/mg protein. The vesicles isolated from the slow muscle were characterized by a much lower uptake capacity. It is considerably enhanced to 3 μmol

Ca^{2+}/mg protein after thyroxine treatment. Thus, thyroxine treatment enhances both the activity of the calcium transport system as well as its storing capacity.

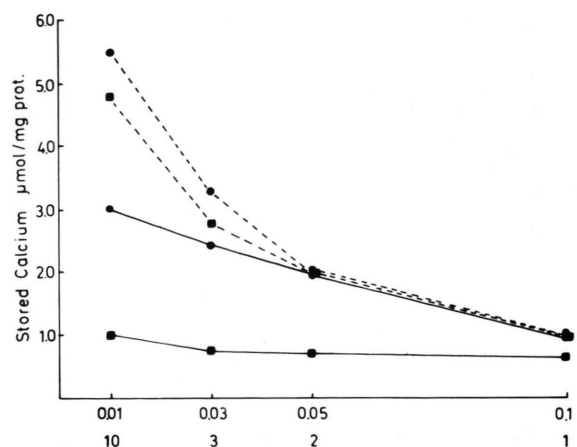


Fig. 3. Calcium storing capacity of sarcoplasmic reticulum vesicles isolated from control and thyroxine treated rabbits. Calcium storage in the presence of 5 mM oxalate was measured as described in Materials and Methods in suspension of sarcoplasmic reticulum vesicles containing 0.01–0.1 $\text{mg}\cdot\text{ml}^{-1}$. On the second abscissa the ratio of calcium to protein in the medium is given ($\mu\text{mol}\cdot\text{mg}^{-1}$). The amount of calcium stored per mg protein is plotted on the abscissa.

●—● M. psoas, control; ●—●—● M. psoas, thyroxine treated; ■—■ M. soleus, control; ■—■—■ M. soleus, thyroxine treated.

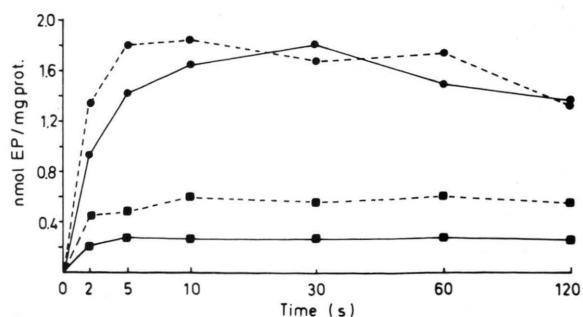


Fig. 4. Time course of phosphoprotein formation of sarcoplasmic reticulum vesicles isolated from control and thyroxine treated rabbits. Phosphoprotein formation was determined as described in Materials and Methods at 0 °C.

●—● M. psoas, control; ●—●—● M. psoas, thyroxine treated; ■—■ M. soleus, control; ■—■—■ M. soleus, thyroxine treated.

Phosphoprotein formation

Sarcoplasmic reticulum vesicles isolated from the psoas muscle of control and thyroxine treated animals are rapidly phosphorylated by ATP at 0 °C reaching a maximal phosphorylation level of 2 nmol/mg. In the following 2 min, a slow decay of the phosphoprotein level took place (Fig. 4). Under the same conditions the vesicles isolated from the soleus muscle of control animals accept only 0.25 nmol P/mg. Thyroxine treatment results in an increase of the phosphoprotein level to 0.5 nmol/mg. The phosphoprotein level of the controls from soleus muscle well match the data obtained by Heilmann and Pette [20] and Damiani *et al.* [22] under similar conditions. The calcium-dependent phosphoprotein rapidly decays on addition of EGTA to the low level phosphoprotein formed in the absence of calcium amounting to less than 2% of the calcium-dependent intermediate. On addition of ADP phosphoprotein likewise rapidly disappears.

Histochemistry

Control psoas muscle stained for the myofibrillar ATPase according to Guth and Samaha [15] almost exclusively consists of fibres with high alkali-resistant ATPase activity as it is characteristic for a fast twitch muscle; thyroxine treatment does not change this uniform fibre pattern. On the opposite (Fig. 5a), the slow twitch soleus muscle of euthyroid rabbits usually contains solely fibres with alkali-labile myofibrillar ATPase activity (*cf.* [23, 24]); in a few rabbits, however, a low amount of fibres with weak acid-stable myosin ATPase is also found (*cf.* [25]).

By contrast, the soleus muscle of animals treated with thyroxine displays numerous fibres with alkali stable myofibrillar ATPase activity. Fig. 5b shows that depending on the intensity of the stain – dark (*) or moderate (**) – at least two subtypes of fast fibres irregularly mixed with the unstained fibres are recognizable. Dark and moderately stained fibres are usually represented in about the same proportion.

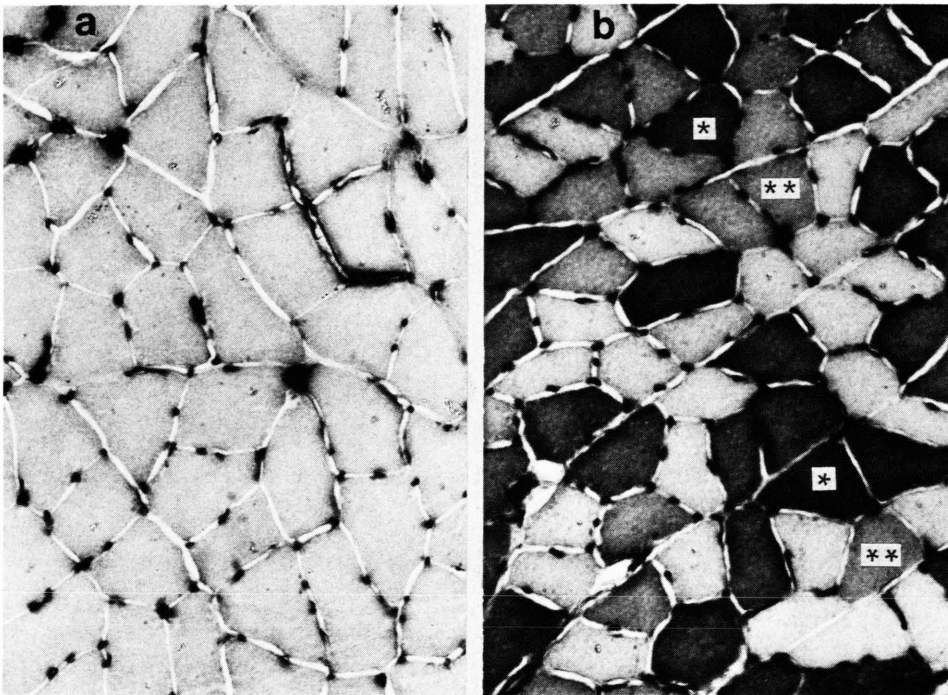


Fig. 5. Representative cross-sections through the soleus of a control (a) and of a 21 days thyroxine treated rabbit (b) stained for the myofibrillar ATPase after formaldehyde fixation and alkaline preincubation (pH 10.4) according to Guth and Samaha (1970). Only unstained fibres are seen in this control soleus (a). By contrast, more than one half of the fibres of the thyroxine soleus (b) are intensively (*) or moderately (**) stained like fast fibres (X 140).

However, their average ratio versus the unstained fibres varies remarkably in the various rabbits.

Correspondingly, staining of unfixed sections from thyroxine soleus by the method of Brooke and Kaiser [17] following alkaline preincubation at pH 9.4 shows a large amount of fibres – up to 50% in the whole muscle three weeks after thyroxine treatment – with positive ATPase reaction like fast type II fibres. Comparison with serial sections reveals that in part of the latter the enzyme reaction is already inhibited after preincubation at pH 4.6.

When sections were preincubated at pH 4.3 several fibres very weakly stained like the so-called fast transitional subtype II C fibres (*cf.* [26]) scattered among dark stained type I fibres and unstained type II fibres are seen (Fig. 6). The average proportion of subtype II C fibres varies in the various soleus examined and may reach values of about 30% of all soleus fibres.

Change in fibre pattern caused by thyroxine is reversible. Soleus sections examined four weeks after suspension of thyroxine treatment display a myofibrillar ATPase reaction undistinguishable from that of the control.

Discussion

The results of the present investigation clearly demonstrate that thyroid hormones are able to rapidly induce significant quantitative and qualitative alterations in the sarcoplasmic reticulum calcium transport system of rabbit's slow twitch, soleus muscle. The alterations detected in fast twitch, psoas muscle are less pronounced. The observations that

thyroxine treatment considerably elevates the membrane yield from soleus muscle while the yield obtained from psoas muscle remains unchanged, is in line with the effect of thyroxine on slow and fast twitch muscles of the rat [8]. The increase of the calcium storing capacity of fast as well as of slow twitch muscles' sarcoplasmic reticulum vesicles is another pronounced effect of the thyroxine treatment. This finding is in apparent disagreement with the observation of Fitts *et al.* [2] who measured total calcium uptake of sarcoplasmic reticulum vesicles isolated from rat soleus muscle. Yet, the reported conditions for measuring calcium uptake do in fact not allow to estimate calcium uptake capacity because the maximal load was limited by the calcium/protein ratio in the assay medium to 1.67 $\mu\text{mol/mg}$. The reported increase in the storing capacity indicates that a larger fraction of the vesicular membranes has become able to store calcium or, what appears less likely, that the calcium storing capacity of the storing competent fraction has increased. The increase in the storing capacity is only partially related to the observed increase in the rate of calcium uptake. The fourfold increase in the uptake capacity of slow muscle vesicles corresponds to only a twofold enhancement of the calcium uptake rate. For the fast muscle, too, the increase in the rate of uptake remains much smaller than the increase in the uptake capacity. This disparity indicates that the storing capacity and calcium pump properties are not directly related to each other. As is to be expected, the pump activity is more closely related to the activity of the preparation's calcium-dependent ATPase. As far as the properties of individual pump molecules and

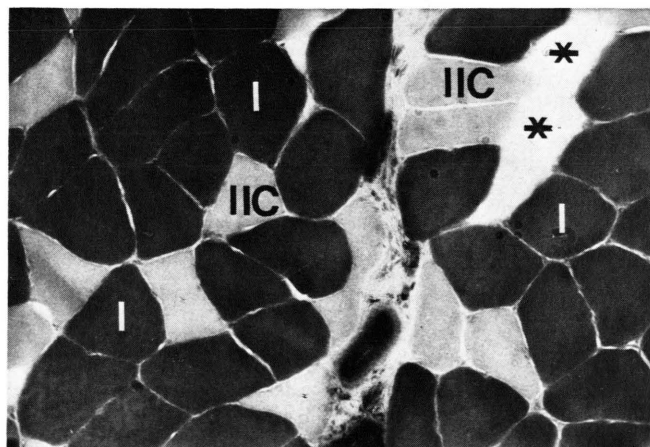


Fig. 6. Unfixed soleus section from a rabbit, treated with thyroxine for 14 days, stained for the myofibrillar ATPase after acid preincubation (pH 4.3) according to Brooke and Kaiser (1970). Several fibres moderately stained like subtype II C fibres scattered among dark stained type I fibres and two unstained type II fibres (*) are seen (X 130).

their possible alteration are concerned, the activity related to the preparation's total protein content might give misleading results. The concentration of the calcium transport ATPase in the various preparations is different as shown by the protein pattern of the gelelectropherogram. The steady state phosphoprotein level reached during calcium-dependent phosphorylation by radioactive ATP must be considered to be the most reliable reference for the quantity of the active enzyme in the respective preparations. In agreement with previous results [20, 22] the membranes from slow muscle are characterized by a low phosphoprotein level as compared to fast muscle. In contrast to calcium transport and ATPase activity, thyroxine treatment significantly affects only the phosphoprotein level in the membranes from soleus muscle leading to a twofold enhancement. The true turnover of the enzyme results if the calcium-dependent ATPase activity is related to the respective phosphoprotein levels [27]. Surprisingly, the phosphate turnover of the transport protein is twofold higher in soleus than in psoas preparations and thyroxine treatment reduces the high turnover of the soleus preparations by about 60%, thus reaching a value which is not significantly different from the turnover of the psoas membranes. This indicated that the specific properties of the transport protein in the muscles are different and are differently affected by thyroxine.

The observed significant reduction of the basic ATPase activity of the membranes from soleus muscle after thyroxine treatment reciprocally complements the increase in the activity of the membranes' calcium transport system. The physiological significance of this change is unknown as is the function of the basic ATPase [28–30].

The marked alteration of soleus fibre pattern observed by histochemical study of myofibrillar ATPase is very similar to that found in the rat [2, 31, 32, 33]. While subtype II C fibres can also be found in the soleus of control rat, their presence in the normal

adult rabbit is disputed. The results here presented point out stronger than those obtained in the rat the capability of thyroid hormone of changing the type of skeletal muscle myosin and confirm the importance of subtype II C fibres as an intermediate step for the transformation of slow type I to fast type II fibres.

In the past, possible relations between the muscles' fibre patterns on the one hand and the speed of contraction on the other hand have repeatedly been discussed [1, 34]. These considerations, however, must be regarded with great care since the maximal speed of shortening of the soleus muscle of the rat is not changed during thyroxine treatment, although the fibre pattern is considerably altered [2]. Similarly, the speculations concerning the relation between the activity of the sarcoplasmic reticulum calcium transport system and the onset and decline of contraction are premature as long as the most important features of the membranes, their ability to release calcium, is only poorly understood (*cf.* [35]). Although nothing can be deduced with certainty from altered functions of the sarcoplasmic reticulum for the time course and the intensity of the single muscle contraction, the replacement of red by white fibres must significantly affect total energy expenditure of the animal. That is because maintenance work is much more economically performed by slow than by fast twitch fibres [36]. Since maintenance of tension by fast twitch fibres requires a considerable increase in motoneuron activity, the question arises whether thyroxine reciprocally affects the properties of skeletal muscle and its nerve supply or both simultaneously.

Acknowledgements

We thank Dr. Beny Schmidt from the Department of Clinical Pathology of Escola Paulista Medicina, São Paulo, for carrying out preliminary histochemical studies and Dr. Dagmar Hellmann-Rajanayagam for valuable secretarial assistance.

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