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Thyroid hormone differentially affects mRNA levels of Ca-ATPase isozymes of sarcoplasmic reticulum in fast and slow skeletal muscle

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mRNA levels for the type I and type II isoforms of sarcoplasmic reticulum (SR) Ca-ATPase were determined in soleus (SOL) and extensor digitorum longus (EDL) muscle of euthyroid (normal), hypothyroid, and hyperthyroid rats. Total Ca-ATPase mRNA content of hyperthyroid muscle was 1.5-fold (EDL) and 6-fold (SOL) higher compared to hypothyroid muscle, with corresponding increases in total SR Ca-ATPase activity. EDL contained only type II Ca-ATPase mRNA. In SOL type I mRNA was the major form in hypothyroidism (98%), but the type II mRNA content was stimulated 150-fold by T_3, accounting for 50% of the Ca-ATPase mRNA in hyperthyroidism.

Ca-ATPase isozyme; Ca-ATPase activity; Sarcoplasmic reticulum; Thyroid hormone; Rat skeletal muscle

1. INTRODUCTION

It is now well established that thyroid hormone (T_3) regulates the sarcoplasmic reticulum (SR) Ca-ATPase level in skeletal muscle. This Ca^{2+} pump is responsible for the re-uptake of cytosolic Ca^{2+} released from the SR during a contraction-relaxation cycle, and its increase induced by T_3 is in line with the well-known stimulative effect of this hormone on the relaxation rate. Measurement of SR Ca-ATPase activity, or coupled Ca^{2+}-transport, in muscle homogenates or isolated SR [1-4,17], as well direct determination of enzyme levels [5] and electron microscopy [6] has shown that Ca-ATPase levels are increased by T_3 up to 6-fold in slow muscle and 1.5-fold in fast muscle. Slow muscle fibers (type I) express the cardiac isoform of the Ca-ATPase (designated type I in this paper), and fast muscle fibers (type II) express 5 to 7 times higher levels of the somewhat more active isoform of the Ca^{2+} pump (designated type II) [5,14,28]. Both isoforms are encoded by separate genes [7,8], and it is not known whether T_3 merely stimulates the expression of the isoform already present in a muscle fiber, or that the two isoforms are differently affected. Because the T_3-induced transformation of type I fibers to fibers with fast characteristics includes shifts in isozyme expression, e.g. the replacement of myosin isozymes with low activity with isoforms typical of type II fibers [9-13], it is possible that the expression of Ca-ATPase isozymes is similarly controlled by T_3. To test this possibility, we determined the mRNA levels for each Ca-ATPase isozyme in fast and slow skeletal muscle from normal and hypo- and hyperthyroid rats. Because it is also unclear whether T_3 acts at a pre- or post-translational level in stimulating Ca-ATPase levels, we determined the maximal SR Ca-ATPase activity of these muscles and correlated this with the total levels of Ca-ATPase mRNA.

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Abbreviations: SR, sarcoplasmic reticulum: SOL, soleus; EDL, digitorum longus

2. MATERIALS AND METHODS

2.1. *Animals*

Age-matched male rats of the Wistar strain, weighing 250-300 g at the time of sacrifice, were used. Animals were maintained on a low iodine diet with drinking water supplemented with either 6.5 mg $KI \cdot l^{-1}$ (euthyroid control), or 1% $KClO_4$ for a period of 6 weeks to induce hypothyroidism [15]. Hyperthyroidism was induced by daily treatment of euthyroid animals with subcutaneous injections of 20 μg T_3 per 100 g b.w. for 2 or 4 weeks [16]. Animals were killed with an overdose of Nembutal and the soleus (SOL), a typical slow twitch muscle with 80% type I and 20% type II fibers, and extensor digitorum longus (EDL), a typical fast twitch muscle with 98% type II fibers, were excised and immediately frozen in liquid nitrogen.

2.2. *SR Ca-ATPase activity*

Homogenates of single SOL or EDL muscles were prepared as described before [4] and SR Ca-ATPase activity was determined spectrophotometrically (340 nm) as described in detail elsewhere [17]. Briefly, homogenate samples were incubated in buffer containing 200 mM KCl, 15 mM $MgCl_2$, 10 mM NaN_3, 1 mM EGTA, 0.005% Triton-X-100, 20 mM Hepes, 4 mM MgATP, 10 mM phosphoenolpyruvate, 0.2 mM NADH, 18 $U \cdot ml^{-1}$ each of purified pyruvate kinase and lactate dehydrogenase (Boehringer), pH 7.5, 25°C. The maximal SR Ca-ATPase activity is obtained by recording the ATPase activity in the presence of 10 μM free $[Ca^{2+}]$ (0.5 mM $CaCl_2$), and subtracting the activity in the presence of 20 mM $CaCl_2$, at which concentratrion the SR Ca-ATPase is selectively inhibited.

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2.3. *Isolation of RNA and Northern blot analysis*

Single frozen muscles were pulverized and total RNA was extracted from 100 mg powder by the guanidinium thiocyanate method described in [18]. The final RNA pellet was resuspended in 0.5% sodiumdodecylsulphate and stored at −70°C. RNA was denatured in 1 M glyoxal, 50% dimethylsulphoxide, 10 mM sodium phosphate buffer (pH 7.0). Up to 30 μg total RNA per lane was electrophoresed on 1% agarose gels (10 mM sodium phosphate buffer (pH 7.0)) and subsequently transferred to Hybond-N membrane (Amersham), fixed with UV light and hybridized with ^{32}P-labelled random-primed cDNA or an end-labelled oligonucleotide. A 520 base *Bam*HI(1854)-*Pvu*II(2373) cDNA fragment from the coding region of type II Ca-ATPase (rabbit) was a kind gift from Dr D.H. MacLennan (Banting and Best Inst. Toronto). The sequence of this cDNA fragment is 90% homologous with the corresponding region of type I Ca-ATPase and hybridization and wash conditions were such that the probe did not discriminate between type I and II transcripts and can be used to quantify both. The level of skeletal-muscle α-actin mRNA was determined using a synthetic oligonucleotide (5′-GCAACCATAGCACGATGGTC-3′) described in [19]. Blots were hybridized with the cDNA probe (2×10^8 c.p.m./μg) for 20 h in 5× SSPE (SSPE: 180 mM NaCl, 0.1 mM EDTA, 10 mM sodiumphosphate buffer, pH 7.4), 5× Denhardt's, 50% formamide, 60 μg/ml herring sperm DNA at 42°C and washed to a stringency of 2× SSPE, 0.1% sodiumdodecylsulphate, 42°C. Blots were reprobed with the α-actin oligo (1×10^6 c.p.m./pmol) under similar conditions, but at 55°C and in the absence of formamide. Signals on autoradiograms (exposed for several periods of time) were quantified with an LKB 2202 Ultroscan laser densitometer. Control (euthyroid) samples and samples from treated animals were analyzed on the same gel.

3. RESULTS

Total RNA was isolated from EDL and SOL muscle of rats at various thyroid states. Using the non-discriminating cDNA probe for Ca-ATPase in Northern analysis, two distinct signals just below the 28S band could be identified as shown in Fig. 1. These correspond to messages for the type I (upper signal) and type II Ca-ATPase [7]. Reprobing of the Northern blots with the synthetic oligonucleotide yielded a single strong signal just below 18S corresponding with the message for skeletal muscle α-actin [16]. The latter is not affected by the thyroid status [19] and was used to normalize the Ca-ATPase mRNA levels in the various preparations. Figure 2 summarizes the results from the analyses of SOL and EDL RNA.

3.1. *Ca-ATPase mRNA in EDL*

Only type II Ca-ATPase mRNA was detected when RNA from this muscle was analyzed. Although euthyroid EDL contains about 1–3% slow type fibers (6% in hypothyroidism), as indicated by the presence of the slow myosin isozyme [9,10], no signal corresponding to type I Ca-ATPase was observed in any group, even at prolonged exposure of blots. Treatment with excess T_3 for two or four weeks (hyperthyroidism) had no effect on the total level of Ca-ATPase mRNA in this muscle, but hypothyroidism resulted in a 50% decrease of the Ca-ATPase mRNA level.

3.2. *Ca-ATPase mRNA in SOL*

A dramatically different picture was found for SOL muscle. Rat SOL muscle comprises some 15–20% type II fibers [9–13,21] and the data in Fig. 2 show a similar percentage type II Ca-ATPase mRNA in euthyroid muscle. In hypothyroidism the total level of Ca-ATPase

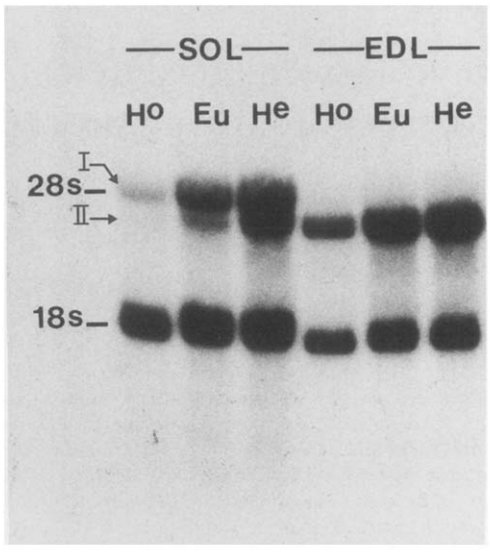

Fig. 1. Northern blot of total RNA from SOL and EDL muscles of hypothyroid (H°), euthyroid (Eu) and euthyroid rats treated for two weeks with T_3 (hyperthyroid, H^e), probed first with Ca-ATPase cDNA and again with a synthetic oligonucleotide for skeletal muscle α-actin (signal at 18S), as described under section 2. Autoradiograms of the consecutive probings were superimposed for this photograph. Position of type I and II Ca-ATPase mRNA is indicated. Longer exposure revealed type II message in hypothyroid SOL, but not type I in EDL. (This blot indicated a 6-fold higher Ca-ATPase mRNA content in EDL than in SOL (euthyroid), assuming the α-actin mRNA content of both muscles to be the same.)

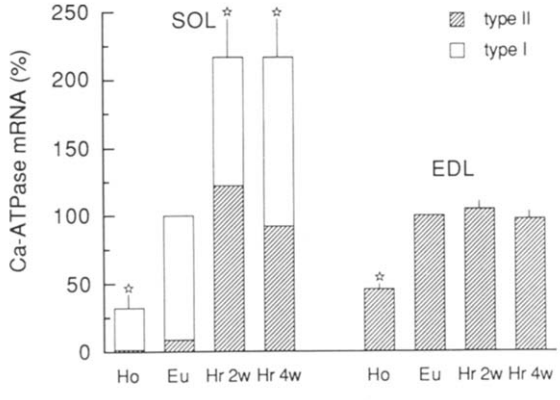

Fig. 2. Average Ca-ATPase mRNA levels of SOL and EDL obtained from Northern blot analyses. Autoradiograph signals of hypothyroid (Ho), hyperthyroid (2 weeks and 4 weeks) (Hr 2w, Hr 4w) were quantified and normalized using the α-actin signal as T_3-independent standard, and are expressed as percentage of total euthyroid levels (Eu) (mean ± SE, number of preparations given below). The distribution of Ca-ATPase mRNA of type I (open column) and type II (hatched column) in SOL is indicated; relative values of type II mRNA in SOL were (%): Ho: 2 ± 1 (6), Eu: 9 ± 2 (6), Hr 2w: 57 ± 9 (3) and Hr 4w: 43 ± 6 (6), mean ± SE of (n) preparations, all differences were significant ($P<0.05$) except the one between 2 and 4 weeks hyperthyroidism. *:$P<0.05$ versus euthyroid, two-sided Student's t-test.

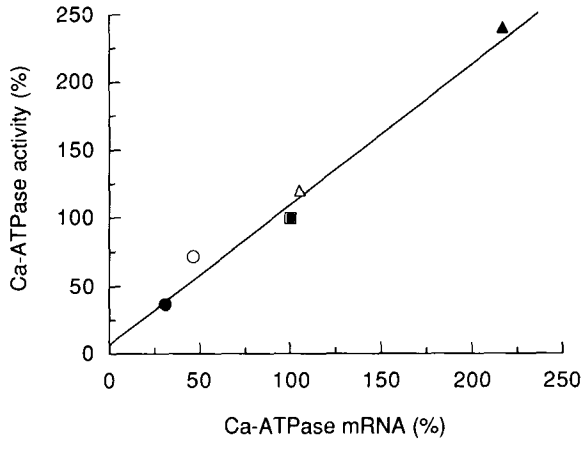

Fig. 3. Total Ca-ATPase mRNA levels and Ca-ATPase activity of SOL (closed symbols) and EDL (open symbols). Maximal Ca-ATPase activities were determined in muscle homogenates of hypothyroid (●○), euthyroid (■ □) and hyperthyroid (two weeks (▲ △) rats, as described under section 2, and relative values (euthyroid = 100%) are plotted against the relative total Ca-ATPase mRNA levels for these muscles (see Fig. 2). Absolute Ca-ATPase activities were (μmol/g wet weight · min):hypothyroid: SOL 4.9±0.4 (5), EDL 57±3 (5), euthyroid SOL 13.5±1.3 (5), EDL 79±3 (5), hyperthyroid (2 weeks): SOL 32.4±1.5 (4), EDL 94±7 (5), means ± SE of (*n*) preparations (effects of hyper- and hypothyroidism were significant ($P<0.05$) except for hyperthyroidism in EDL). Correlation coefficient of the line through the data points was 0.987.

mRNA (type I and type II) dropped by 70%, and the percentage type II mRNA decreased relatively more, reaching a level of about 2% of total. T_3 treatment of euthyroid rats more than doubled the total Ca-ATPase mRNA content of SOL already after two weeks, and prolonged treatment with T_3 (4 weeks) had no further effect. This increase was almost entirely due to a 10-fold stimulation of the level of type II mRNA bringing it to 50% of the total Ca-ATPase mRNA level.

3.3. *mRNA levels versus Ca-ATPase activity*

The effect of T_3 on the level of Ca-ATPase mRNA was then compared to the maximal SR Ca-ATPase activity determined in muscle homogenates. In agreement with the published difference in Ca-ATPase content between EDL and SOL [5,14,28], we found for the euthyroid condition a 7-fold higher Ca-ATPase activity in EDL as compared to SOL, and the maximal T_3-dependent stimulation of this activity (hyper- versus hypothyroid) was 6-fold for SOL and 1.5-fold for EDL (see legend to Fig. 3 for data). Fig. 3 shows the correlation between the relative Ca-ATPase mRNA levels and Ca-ATPase activities of SOL and EDL muscle from the four experimental groups.

4. DISCUSSION

Thyroid hormone has been shown to induce a transformation of phenotypically slow muscle fibers to fibers with fast contraction and relaxation characteristics. The full effect of T_3, i.e. the transition from hypo- to hyperthyroidism, entails in EDL muscle the transformation of almost all type I fibers (6%) to type II, at least with respect to the isozymes of myosin [9]. No such isoform shift, however, appears to apply to the Ca-ATPases, since under all conditions only the type II Ca-ATPase mRNA could be detected. This is in agreement with recent studies on fast muscle in which it was also shown that expression of type I Ca-ATPase can be induced, but only by imposing a level of contractile activity characteristic of slow muscle, which at the same time gave complete repression of type II Ca-ATPase [22,23]. Regulation by T_3 is evidently limited in EDL to a stimulation of mRNA levels of the already expressed type II isozyme of the Ca-ATPase. This increase was quantitatively similar to the observed stimulation of Ca-ATPase activity, which places the site of action of T_3 at a pre-translational level in this fast-type muscle.

The latter conclusion also applies to SOL muscle, where the T_3-induced increase in total Ca-ATPase mRNA level correlated with the 6-fold increase in Ca-ATPase activity. However, the results also suggest a more concerted effect of T_3 on the expression of Ca-ATPase- and myosin isozymes in the transformation of type I fibers into fibers with fast (type II) characteristics in this slow muscle. Previous analyses of fiber composition at the level of myosin have shown an increase in type II fibers from 6% in hypothyroidism, through 20% in euthyroidism, to a maximum of 60% after 4 weeks of hyperthyroidism [9–13], which correlates well with the gradual increase of the percentage type II Ca-ATPase mRNA presented here, i.e. 2%, 10% and 50%, resp. That the maximal effect on Ca-ATPase expression is already reached after two weeks, is in agreement with earlier analyses of myosin- and SR Ca^{2+}-transport activity in SOL [24]. Although stimulation of both Ca-ATPase isozymes contributes to the T_3-induced increase in SR Ca-ATPase activity in SOL muscle, the difference in extent is striking. Whereas the level of type I mRNA was stimulated 3.5-fold, which is about equal to the maximal stimulation of this isoform by T_3 in rat heart [25,26], the type II mRNA level was increased more than 150-fold. We propose that expression of the type II Ca-ATPase gene is repressed in SOL muscle, either due to intrinsic factors or to the activity pattern of slow muscle, as mentioned above, and that this repression can be relieved by T_3. In situ hybridization studies are required to establish whether the isozyme shift observed here, entails a switch from type I to type II Ca-ATPase in specific fibers and stimulation of type I in others, or that both isozymes may be simultaneously expressed in one fiber. Further work will also be needed to determine if T_3, through its receptor, interacts directly with the Ca-ATPase genes, as has now been shown for several other T_3-responsive genes [27].

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