ACTION OF THYROXINE ON FUNCTIONS OF THE SARCOPLASMIC RETICULUM OF RABBIT SKELETAL MUSCLES

A. I. Marzoev, B. V. Rubtsov, G. I. Klebanov, and Yu. A. Vladimirov

UDC 612.74.014.21-06:612.441.018

KEY WORDS: Sarcoplasmic reticulum; thyroxine; Ca-ATPase; accumulation of Ca^{++}.

The body level of thyroid hormones largely determines the functional state of the myofibers. For example, an increase in ATPase activity has been found in myofibrils isolated from the heart muscles of hyperthyroid muscles [3, 8, 9], and, conversely, a decrease in its activity has been found when thyroid hormone production *in vivo* is deficient [6]. It has also been shown that myofibrils from heart muscles of hyperthyroid animals can contract faster, develop a higher amplitude of force of contraction, and relax faster than the corresponding myofibrils obtained from normal animals [2, 3]. There have been isolated reports that thyroid hormones affect the functions of the sarcoplasmic reticulum (SR) and that in hyperthyroidism they cause an increase in the rate of Ca^{++} uptake by SR preparations from heart muscles. Meanwhile, an increase in the Ca-ATPase activity of these preparations also has been observed [8, 10]. Workers cited above [2, 3, 9] found a tendency toward positive correlation between the thyroid hormone level and muscle function. At the same time it is known that in developed hypothyroid states (thyrotoxicosis) muscular weakness is observed, sometimes to a considerable degree [5].

The object of this investigation was to study the functional state of SR of skeletal muscles in thyrotoxic states.

EXPERIMENTAL METHOD

Rabbits weighing from 2 to 2.6 kg were used. Experimental thyrotoxicosis was induced by intraperitoneal injection of a solution of L-thyroxine in 0.05 M KOH in a dose of 120-150 mg/kg body weight daily for 25-28 days. Rabbits kept under the same conditions as the experimental animals served as the control.

SR was isolated from white muscles of the hind limbs and spinal region [7]. The rate of accumulation of Ca^{++} ions (V_{Ca}) and activity of Ca-dependent ATPase (V_{ATP}) were measured by a pH-metric method [1]. In some experiments the kinetics of accumulation of Ca^{++} ions by SR preparation was recorded by measuring the scattering of light by a suspension of SR fragments at 546 nm.

The incubation medium in which the functional parameters of the SR fragments were measured contained 100 mM NaCl, 4 mM $MgCl_2$, 2 mM ATP, 5 mM sodium oxalate, and 1.5 mM imidazole, pH 7.0. Temperature conditions are given below. The protein concentration in the samples was determined by the biuret reaction [4].

EXPERIMENTAL RESULTS

By the time of isolation of SR the rabbits receiving thyroxine had lost on average 30-35% of their body weight compared with the control, reflecting the development of severe thyrotoxicosis.

Graphs showing the ATPase activity and rate of accumulation of Ca^{++} ions by SR fragments from rabbits with thyrotoxicosis and from normal rabbits are shown in Fig. 1. Clearly the rate of accumulation of CA^{++} ions by SR fragments was 60% slower in thyrotoxicosis than normally (data obtained at 32°C). The difference between the experiment and control was more

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 5, pp. 541-543, May, 1980. Original artical submitted May 15, 1979.

 0007-4888/80/8905-0586$07.50

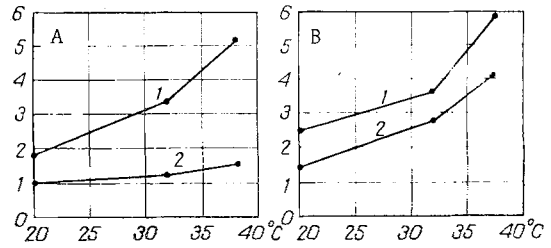

Fig. 1

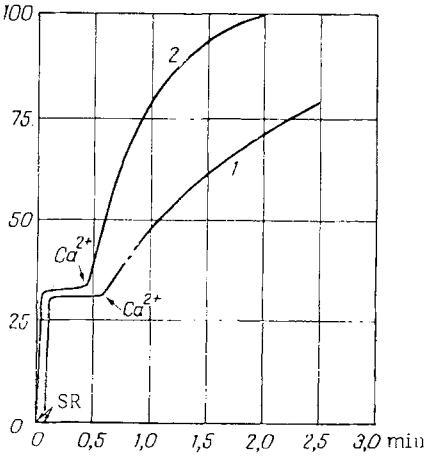

Fig. 2

Fig. 1. Rate of uptake of Ca^{++} and activity of Ca-dependent ATPase of SR preparations as a function of temperature. A) Rate of uptake of Ca^{++} (in μmoles Ca^{++} /min/mg protein) by SR fragments obtained from normal rabbits (1) and from animals with thyrotoxicosis (2); B) activity of Ca-ATPase (in μmoles ATP hydrolyzed per minute per milligram protein) of SR fragments obtained from normal rabbits (1) and from animals with thyrotoxicosis (2).

Fig. 2. Kinetics of changes in intensity of scattering of light (in relative units) by SR suspension (32°C) during accumulation of Ca^{++} ions as a function of time. 1) Change in intensity of scattering of light by SR preparations preincubated for 1 min with L-thyroxine (10^{-5}M) before addition of Ca^{++} (3×10^{-5}M); 2) change in intensity of scattering of light by SR preparation incubated in medium without thyroxine. Protein concentration in sample 0.1 mg/ml. Arrows indicate times of addition of SR fragments and Ca^{++} to incubation medium.

TABLE 1. Action of Thyroxine on Functional Parameters of SR Fragments (M ± m)

Concn. of hormone in sample	V_{Ca}, μmoles Ca^{2+}/min/mg protein	V_{ATP}, μmoles ATP/min/mg protein	Ca/ATP
0	2,86±0,16	3,40±0,26	0,84
10^{-5} M	1,53±0,16	2,20±0,07	0,69

Legend. The SR preparations were incubated with the hormone for 1.5-2 min at 32°C. Protein concentration in sample 0.1 mg/ml.

marked at higher temperatures (Fig. 1A). Activity of Ca-dependent ATPase of the SR preparations also was 25% lower (at 32°C) from rabbits with thyrotoxicosis, although the decrease was less than that of the rate of Ca^{++} accumulation (Fig. 1B). The Ca/ATP ratio, reflecting the efficiency of operation of the calcium pump, was reduced in thyrotoxicosis (at 32°C) to 0.42 compared with the normal value of 0.92.

Investigation of the functional parameters of SR fragments from the heart muscle of hyperthyroid rabbits likewise revealed a decrease in efficiency of the Ca-accumulating system [10]. However, the absolute values of the rate of Ca^{++} uptake and of Ca-ATPase activity under these circumstances were higher than normally. The differences between data cited in the literature and the results of the present experiments can perhaps be attributed to the fact that the doses of thyroxine which we used led to severe thyrotoxicosis, which was evidently accompanied by a fall in CA-ATPase activity.

When the molecular mechanisms of action of biologically active substances such as hormones are studied it is important to compare effects as a result of direct interaction between hormone and object with changes that develop after administration of the hormone *in vivo*. The results of a series of experiments to study the effect of thyroxine on functional parameters of SR fragments *in vitro* are given in Table 1.

As this table shows, direct inhibition of Ca^{++} uptake by thyroxine takes place against a background of lowered Ca-ATPase activity of the preparation. The efficiency of Ca^{++} accumulation is reduced, just as after administration of the hormone to animals.

Inhibition of Ca^{++} accumulation by SR fragments incubated in medium with thyroxine also was observed in analogous experiments in which the dynamics of uptake of Ca^{++} ions was recorded as a change in scattering of light. The results of these experiments show (Fig. 2) that the rate of Ca^{++} accumulation falls after incubation of SR fragments for a short time with the hormone.

A marked increase in the thyroid hormone level in the body can thus lead to a decrease in the levels of functional parameters of SR of the skeletal muscles. High correlation was observed between the effects of thyroxine *in vivo* and *in vitro*. It cannot, of course, be concluded from this that the mechanisms of action of thyroxine are identical in both cases, but it does allow the possibility of direct inhibition of the Ca-ATPase of SR by thyroid hormones *in vivo*. The second probable cause of the change in SR function in thyrotoxic states may be changes induced in the membranes of these structures by thyroxine through metabolic pathways. The efficiency of Ca^{++} transport in SR in fact depends on many factors: the mobility and orientation of the Ca-ATPase molecules, interaction between Ca-ATPase and lipids, and also with other SR proteins, and so on. The factors listed above determine on the whole the concrete protein–lipid interactions in the structure of the membranes. Thyroid hormones are powerful regulators of both protein and lipid metabolism. It can therefore be suggested that when their body levels are abnormally high, structural changes take place in the membranes of SR which lead to a fall in the levels of functional parameters.

LITERATURE CITED

1. V. B. Ritov, Biokhimiya, 36, 393 (1971).
2. R. A. Buccino, J. F. Spann, P. E. Pool, et al., J. Clin. Invest., 46, 1669 (1967).
3. M. J. Goodkind, G. E. Dambach, P. T. Thyrum, et al., Am. J. Physiol., 226, 66 (1974).
4. A. G. Gornal, C. Bardawill, and M. M. David, J. Biol. Chem., 177, 751 (1949).
5. F. L. Hoch, Physiol. Rev., 42, 605 (1962).
6. A. L. Jacobson, B. Humphrey, and V. Grey, Int. J. Biochem., 3, 518 (1972).
7. A. Martonosi, J. Donley, and R. A. Halpin, J. Biol. Chem., 243, 61 (1968).
8. W. G. Nayler, N. C. R. Merrillees, D. Chipperfield, et al., Cardiovasc. Res., 5, 469 (1971).
9. S. C. Seagren, C. L. Skelton, and P. E. Pool, Am. J. Physiol., 220, 847 (1971).
10. J. Suco, Biochim. Biophys. Acta, 252, 324 (1971).