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INFLUENCE OF HYPERTHYROIDISM ON THE SUPERPRECIPITATION RESPONSE AND Ca^{2+}-SENSITIVITY OF NATURAL ACTOMYOSIN IN CARDIAC AND SKELETAL MUSCLE

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Summary

In cardiac natural actomyosin prepared from hyperthyroid rabbits, the time of onset of the superprecipitation response was shortened by 58% and the rate of response was increased 4-fold compared with euthyroid animals. However, Ca^{2+}-sensitivity of cardiac natural actomyosin prepared from either euthyroid or hyperthyroid rabbits was not changed over the range of 10^{-7} to $6.6 \cdot 10^{-5}$ M free Ca^{2+} concentrations. Skeletal natural actomyosin prepared from either euthyroid or hyperthyroid rabbits showed a far higher Ca^{2+}-sensitivity than cardiac natural actomyosin, but there was no difference in either time of onset or rate of superprecipitation response.

In the hyperthyroid state, a variety of clinical manifestations relate to effects on both cardiac and skeletal muscle [1,2], and cardiac muscle in hyperthyroid state appears to show direct effects on the rate of tension development in the papillary muscle [3] and on contractility in the whole heart [4]. The administration of L-thyroxine to either the guinea pig [5] or rabbit [6] has been shown to increase cardiac myosin Ca^{2+}-activated ATPase activity, without changing myosin EDTA-activated ATPase activity [6—8].

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In order to clarify the physiological significance of increased myosin ATPase activity, it seems to be essential to analyze its effects in the presence of actin and the regulatory proteins, since these myofibrillar proteins are essential to physiological muscle contraction [9]. For this purpose, we employed superprecipitation response of natural actomyosin, a model for muscle contraction in vitro [10,11].

Groups of nine male New Zealand white rabbits weighing 1.0 to 1.5 kg received daily injection of either L-thyroxine (200 μg/kg body weight) or saline intramuscularly for two weeks. Then cardiac and skeletal natural actomyosins were prepared from ventricular muscle and paravertebral muscle, respectively [10].

After the addition of ATP to natural actomyosin, both cardiac and skeletal muscle samples from euthyroid and hyperthyroid rabbits showed clear superprecipitation responses. As demonstrated in Fig. 1, cardiac natural actomyosin from hyperthyroid rabbits revealed a prominent increase in superprecipitation response compared with cardiac natural actomyosin from euthyroid animals, both in high and low concentration of Ca^{2+}. As summarized in Table I, when the time of onset of superprecipitation ($t_{1/10}$) was defined as time to reach one-tenth of the absorbance after the addition of

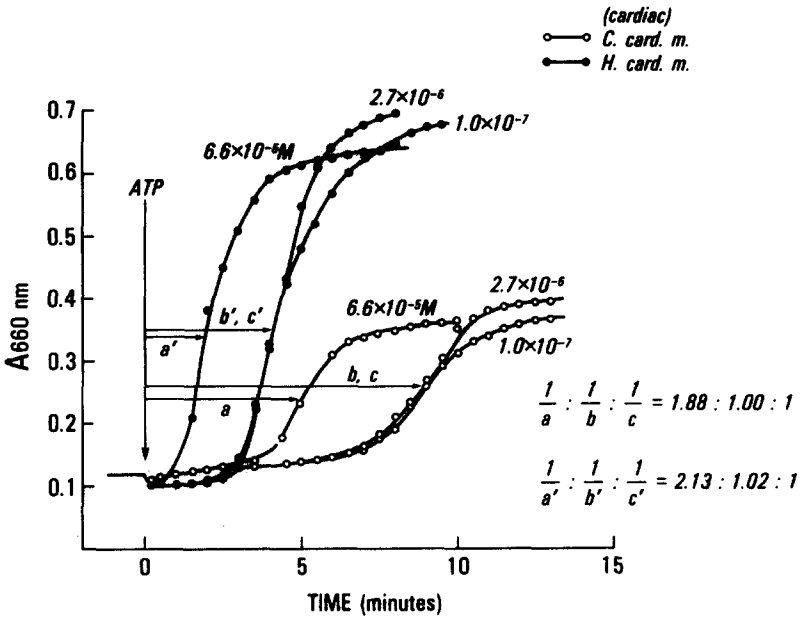

Fig. 1. Superprecipitation response of natural actomyosin from a euthyroid rabbit heart (○—○) or hyperthyroid rabbit heart (●—●). The reaction mixture contained 0.30 mg/ml natural actomyosin, 0.1 M KCl, 1 mM $MgCl_2$, 0.1 mM Ca-EGTA buffer and 20 mM Tris-maleate buffer (pH 6.8) and preincubated at 30° C for 5 min. The reaction was started with the addition of ATP (0.1 mM in final concentration) at zero time, and the absorbance at 660 nm was monitored by Gilford R 2000® spectrophotometer. Numbers at the right of each curve denote the free Ca^{2+} concentration in the reaction mixture [13].

TABLE I

EFFECT OF L-THYROXINE ADMINISTRATION ON SUPERPRECIPITATION RESPONSE OF NATURAL ACTOMYOSIN PREPARED FROM EITHER CARDIAC AND SKELETAL MUSCLE OF RABBITS

Mean ± S.E.M. Compared with euthyroid animals, significantly different at $P < 0.05$ (**) or $P < 0.025$ (*). Compared with skeletal samples, significantly different at $P < 0.001$ (§). Concerning the detailed assay conditions, see the legend to Fig. 1. To measure Ca^{2+}-sensitivity of natural actomyosin, the final concentration of KCl and ATP was changed to 0.03 M and 0.5 mM, because this condition has been shown to be better for the detection of minor change of Ca^{2+}-sensitivity [12]. Time ratio to reach half maximal turbidity after the addition of ATP was compared, changing free Ca^{2+} concentration in the reaction mixture from $1.0 \cdot 10^{-7}$ to $6.6 \cdot 10^{-5}$ M with Ca-EGTA buffer [13].

Muscle	Thyroid state	N	Time of onset ($t_{1/10}$, min)	Maximal rate of rise $(dA/dt)_{max}$, A/min	Ca^{2+}-sensitivity
Cardiac	Euthyroid	9	3.06 ± 0.48	0.0507 ± 0.011	3.05 ± 0.42§
	Hyperthyroid	8	1.27 ± 0.18**	0.212 ± 0.063*	3.08 ± 0.39§
Skeletal	Euthyroid	9	1.88 ± 0.50	0.130 ± 0.046	19.4 ± 1.1
	Hyperthyroid	8	2.17 ± 0.37	0.174 ± 0.080	20.6 ± 1.8

ATP, $t_{1/10}$ in cardiac natural actomyosin from hyperthyroid rabbits was shortened to 42% of that in cardiac natural actomyosin from euthyroid rabbits ($P < 0.005$). When $(dA/dt)_{max}$ was taken as a parameter of the superprecipitation response, cardiac natural actomyosin from hyperthyroid rabbits showed a 4-fold greater value than that from euthyroid rabbits (Table I, $P < 0.025$). However, Ca^{2+}-sensitivity of natural actomyosin was not dependent on thyroid state, and a similar ratio of time to reach half maximal turbidity was obtained (Table I) under this condition which was sensitive to detect Ca^{2+}-sensitivity [12].

When natural actomyosin from skeletal muscle was compared with natural actomyosin from cardiac muscle, the rate of rise in turbidity, $(dA/dt)_{max}$, was similar to that of cardiac natural actomyosin from hyperthyroid rabbits and faster than that of cardiac natural actomyosin from euthyroid rabbits (Table I). The time of onset, $t_{1/10}$, of superprecipitation skeletal natural actomyosin from the hyperthyroid group was not shortened, compared with that from the euthyroid group (Table I). Comparison of skeletal natural actomyosin from hyperthyroid and euthyroid animals revealed no difference in Ca^{2+}-sensitivity (Table I).

Thus, the change in superprecipitation after thyroxine treatment was demonstrated only in cardiac natural actomyosin, and no changes were shown in skeletal muscle natural actomyosin. Banerjee and Morkin reported that the apparent dissociation constant (K_{app}) of actin for heavy meromyosin fragments was smaller in hyperthyroid cardiac myosin [15], indicating an increase in affinity of the fragments for actin; this finding might correspond to the increased $(dA/dt)_{max}$ in hyperthyroid natural actomyosin which we observed, since the rapid phase of superprecipitation has been shown by electron microscopy to result from association of myosin and actin filaments [16]. This phenomenon might also relate to the evidence for decreased time to peak tension and increased speed of muscle contraction in hyperthyroid

states reported in cat papillary muscle and the left ventricle of the intact dog [4], since muscle contraction is considered to be a result of myosin and actin interaction [9,11].

We have previously reported that the free Ca^{2+} concentration required for 50% tension development in glycerin-treated fibers of rabbit papillary muscle is $3 \cdot 10^{-6}$ M [17]. This value was in good agreement with the present results on Ca^{2+}-sensitivity measured by the superprecipitation response (data not shown). Furthermore, we have previously demonstrated that glycerin-treated fiber of cardiac muscle was far less sensitive to changes of Ca^{2+} concentration than those of skeletal muscle [17]. These findings correlate well with the difference of Ca^{2+}-sensitivity between skeletal and cardiac natural actomyosin as measured by the superprecipitation (Table I). The content of the regulatory proteins in natural actomyosin was not changed in cardiac and skeletal natural actomyosin (data not shown), and the decrease of Ca^{2+}-sensitivity was not thought to be due to the decreased amount of the regulatory proteins.

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