Effect of Alterations in the Thyroid State on the Intrinsic Contractile Properties of Isolated Rat Skeletal Muscle

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ABSTRACT Contractile properties of soleus muscles isolated from 31 euthyroid (EU), 20 hyperthyroid (HT), and 18 myxedematous (MY) rats were studied in a myograph. At 100 stimuli/sec maximum isometric tension was essentially identical in EU (17.2 ±0.5 g/mm²) and HT (17.7 ±0.5 g/mm²) muscles, but was significantly depressed in MY muscles (11.5 ±0.7 g/mm²). The rate of tension development was increased in HT (103 ±4.5 g/sec per mm²) as compared to both EU (86.2 ±4.6 g/sec per mm²) and MY (38.4 ±2.2 g/sec per mm²) muscles, while the duration of the active state was shortened in HT (77.1 ±2.3 msec) as compared to EU (105.1 ±1.1 msec) muscles and was prolonged in MY muscles (153.3 ±6.0 msec). The mean rate of isometric relaxation was 26.5 ±4.9 g/mm² per sec in EU muscles, more rapid in HT muscles (33.1 ±1.3 g/sec per mm²), and slower in MY muscles (16.0 ± g/mm² per sec). The fusion frequency was greater in HT muscles, averaging 68.5 ±3.6 stimuli/sec compared to EU muscles (38.1 ±1.2 stimuli/sec) and to MY muscles (33.3 ±4.0 stimuli/sec). At 40 stimuli/sec tension averaged 16.4 ±0.8 g/mm² in EU muscles while at the same frequency tension was reduced in HT muscle, averaging 14.2 ±0.5 g/mm². All differences were significant ($P < 0.01$). In conclusion, HT and MY result in profound alterations in the intrinsic contractile properties of skeletal muscle. While tension in HT muscles is maintained in vitro at a stimulus frequency of 100 stimuli/sec, the reduction in duration of active state may lower tension in vivo by preventing complete fusion of contractile events. In MY tension is reduced as a consequence of the lowered intensity of the active state. These changes explain, at least in part, the weakness of muscle activity in both HT and MY.

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INTRODUCTION

It is well known that alteration in the level of thyroid activity result in alterations in neuromuscular function. Skeletal muscle weakness, tremor, and a shortened duration of the tendon jerk are frequently seen in hyperthyroidism, while prolongation of the tendon jerk and slowness of muscle contraction are among the characteristic manifestations of myxedema (1–4). Studies by Lambert, Underdahl, Beckett, and Mederos confirmed the clinical impression that muscle contraction time is shortened in patients with hyperthyroidism and prolonged in patients with myxedema. These investigators also demonstrated that the velocity of conduction of nervous impulses through the reflex arc is identical in hyperthyroid, euthyroid, and myxedematous patients (5) and suggested that the neuromuscular abnormalities in hyperthyroid and myxedematous patients are due to alterations in the contractile mechanism of the muscle rather than to abnormalities of motor nerve transmission.

The fundamental effects of an excess or deficiency of thyroid hormone on the contractile properties of skeletal muscle have not been defined. The aims of this investigation were to determine the effects of experimentally produced hyperthyroidism and hypothyroidism on the intrinsic contractile properties and fusion frequencies of the isolated rat soleus muscle.

METHODS

Sprague Dawley male rats weighing 40–60 g were employed. Hyperthyroidism was produced by the intraperitoneal injection of 1 mg/kg per day of l-thyroxine for 14–17 days. A second group of animals was rendered myxedematous by a single injection of radioactive ^{131}I (1 μCi/kg) and studied 4–6 wk later (6). The third group of animals was designated as normal controls. All animals were studied between 20–45 days of age (7). At the time of study, animals from each group were decapitated and their left soleus muscle dissected free and placed in a muscle bath

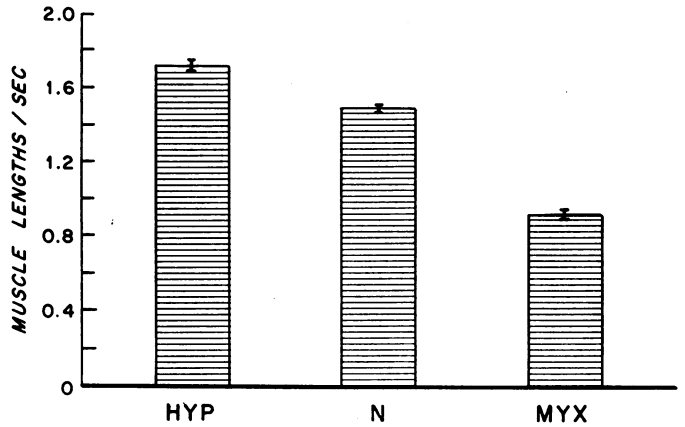

FIGURE 1 The average maximum velocity of shortening of isolated soleus muscles, expressed in units of muscle lengths per second, from hyperthyroid (HYP), euthyroid (N), and hypothyroid (MYX) rats at a load of 0.5 g/mm². Horizontal bars represent ±SEM.

within 2.5 min of the time of sacrifice. The muscle bath was filled with Krebs-Ringer solution (Na 148, K 4.0, Ca 5.0, Mg 2.5 Cl 123, HCO_3 25 mEq/liter, phosphate 1.2 mM, and glucose 5.6 mM at pH 7.4) and aerated with 95% O_2 and 5% CO_2 at a constant temperature of 20°C.

The distal tendon of the soleus was inserted into a spring-loaded lucite clip which formed the rigid extension of a force transducer.[1] The proximal end was tied with a short length of wetted Ethicon 4-0 braided silk (Ethicon, Inc., Somerville, N. J.) to a lever mounted on a rigid Palmer stand arranged so that both isotonic and isometric contractions could be studied.

The muscles were stimulated[2] with a square wave DC impulse delivered through field electrodes placed parallel to the long axis of the muscle. After allowing the muscle to stabilize for several minutes after placement in the bath, muscle length was adjusted to L_{max}, the length at which tetanic tension was maximal. Tetanic stimulation (100 stimuli/sec, each 5 msec in duration with a voltage 80% above threshold) was delivered for 1-sec periods at intervals of 1 min. Length and tension changes, along with the stimulus artifact, were recorded on a multichannel oscillographic recorder. Maximum isotonic velocity of shortening, maximum rate of tension development, and the rate of relaxation were measured from these recordings. Maximum isotonic velocity of shortening was measured as the maximum slope of the shortening curve; the maximum rate of tension development was measured as the maximum slope of the isometric tension curve; and the rate of relaxation was obtained by measuring the maximum slope of the descending limb of the tension curve. The duration of the maximum active state was measured as the interval from cessation of stimulation to the beginning of the decline in tetanic tension (8). As the active state declines the tension departs from the course of the tetanic contraction and relaxation ensues (9). Fusion frequency was determined by varying the frequency of individual stimuli between 15 and 120 stimuli/sec and determining the frequency at which complete fusion of mechanical events occurred (10, 11).

In order to compare the mechanical function of soleus muscles of different sizes, tension was corrected for cross-sectional area and expressed in grams per square millimeter; and velocity of shortening was expressed in terms of muscle lengths per second (8, 12). Statistical analyses were performed utilizing Student's unpaired t test to compare the muscles from the three groups (13). Differences between groups were considered to be statistically significant at $P < 0.01$. 10 animals from each group were sacrificed and the thyroid state documented by measurement of serum thyroxine by column levels (14).

RESULTS

Characterization of the thyroid state

In each of the hyperthyroid animals the serum thyroxine level exceeded 20 μg/100 ml, a value significantly above the normal range of 2.2–2.8 μg/100 ml; in each of the hypothyroid animals this level was less than 1.0 μg/100 ml, a value significantly below the normal range.

Mechanics

Analysis of isotonic contractions. Maximum velocity of isotonic shortening was found to vary directly with the level of the thyroid state (Fig. 1); at the lightest load studied (0.5 g/mm²), velocity averaged 1.51 ±0.10 muscle lengths/sec in muscles from nine euthyroid animals, was significantly lower in muscles obtained from nine hypothyroid animals (0.93 ±0.13 muscle lengths/sec), and significantly higher in 15 hyperthyroid animals (1.77 ±0.12 muscle lengths/sec) (Fig. 1).

Analysis of isometric contractions. When muscles were stimulated at the tetanic stimulation frequency of 100 stimuli/sec, active tension at the peak of the length-active tension curve averaged 17.7 ±0.5 g/mm² in mus-

[1] Statham G1-4-250, Statham Instruments, Inc., Los Angeles, Calif.

[2] AEL Laboratory Stimulator 104A.

cles from 20 hyperthyroid rats and was similar, 17.2 ±0.5 g/mm², in muscles from 31 euthyroid rats. It was significantly lower, 11.5 ±0.7 g/mm², in muscles from 18 hypothyroid rats (Fig. 2). The rate of tension development and of tension decline varied directly with the level of thyroid activity, the former averaging 38.4 ±2.2 g/sec per mm², 86.2 ±4.6 g/sec per mm², and 103 ±4.5 g/sec per mm² in muscles from the hypothyroid, euthyroid, and hyperthyroid groups respectively (Fig. 3), while the latter averaged 16.2 ±1.0 g/sec per mm², 26.5 ±4.9 g/mm² per sec, and 33.1 ±1.3 g/sec per mm² in these three groups respectively (Fig. 4). The duration of the active state was significantly shortened in muscles from hyperthyroid animals, averaging 77.1 ±2.3 msec compared to that in muscles from euthyroid animals (105.1 ±1.1 msec) and was significantly prolonged in muscles from hypothyroid animals, in which it averaged 153.3 ±6.0 msec (Fig. 5).

Analysis of fusion frequency. The frequency of stimulation at which complete fusion of individual contractions became manifest was significantly greater in muscles from hyperthyroid rats in which it averaged 68.5 ±3.6 stimuli/sec when compared both to muscles from euthyroid rats in which it averaged 38.3 ±1.2 stimuli/sec and in muscles from hypothyroid rats in which it averaged 33.3 ±4.0 stimuli/sec. The fusion frequencies of muscles from euthyroid and hypothyroid rats were not significantly different but it must be pointed out that a relatively small number of muscles was studied in each group (Fig. 6). At a stimulation frequency of 40 stimuli/sec, mechanical fusion was complete in the muscles obtained from the euthyroid group and tension development averaged 16.4 ±0.8 g/mm² at this stimulation frequency. At this stimulation frequency, mechanical fusion was incomplete in the muscles obtained from the hyperthyroid group and tension development was significantly lower, averaging 14.2 ±0.5 g/mm².

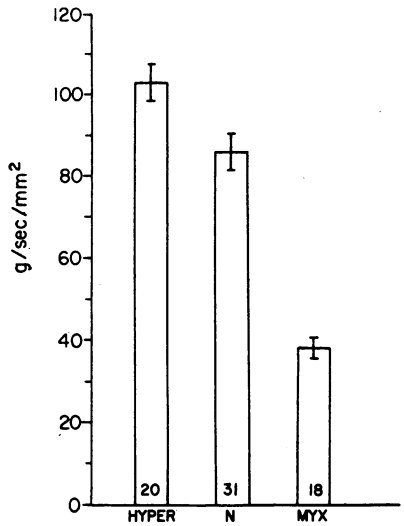

FIGURE 3 The rate of rise of tension as a function of level of thyroid state. The rate of rise of tension was measured as the maximum slope of the tension curve corrected for cross sectional area.

DISCUSSION

The activity of a muscle at any given instant after stimulation can be characterized in terms of the active state of the contractile component (15, 16). The latter is a measure of the mechanical energy derived from chemical reactions in the contractile element and is

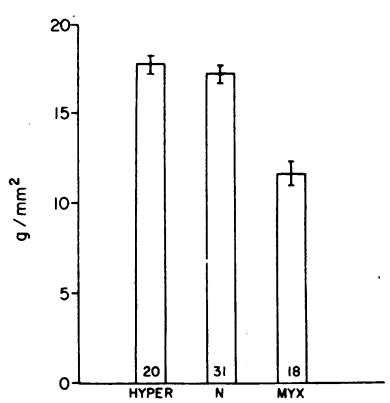

FIGURE 2 Isometric tension developed as a function of level of thyroid state. Tension represents tetanic tension measured at the peak of the length-tension curve corrected for cross sectional area (g/mm²). In this and subsequent figures, the number of muscles in each group is shown with results expressed as the mean ±SEM. HYPER = hyperthyroid, N = euthyroid, MYX = myxedema.

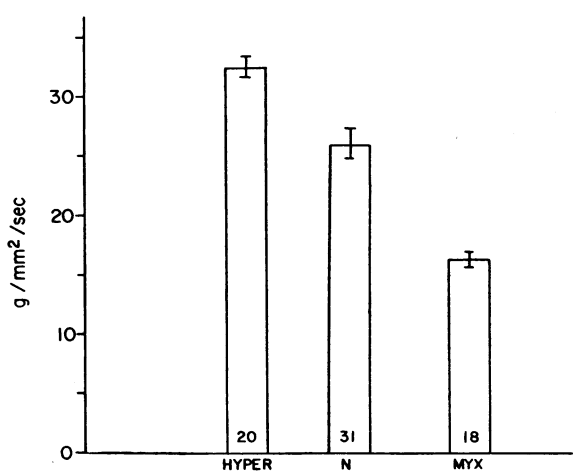

FIGURE 4 Rate of muscle relaxation as a function of the level of thyroid activity. The rate of relaxation was measured as the maximum slope of the decline of tension of the tension curve and corrected for cross sectional area.

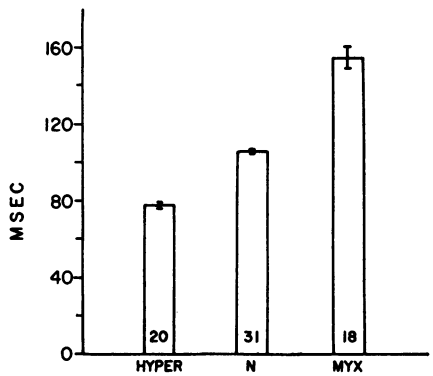

FIGURE 5 Duration of active state as a function of level of thyroid activity. The duration of maximum active state was measured as the interval between the end of the stimulus train and the first perceptible fall in tetanic tension.

reflected either in the force developed during isometric contraction or the velocity of shortening if the muscle is unloaded (17). Thus, the contractile state of skeletal muscle can be described by the force-velocity relation in which the force of contraction is inversely related to load (15). In the absence of any load, i.e. when force development is zero, the velocity of contraction is maximal and this maximal velocity of shortening, V_{max}, serves as a useful means of characterizing the contractile state (17). In cardiac muscle, V_{max} can be altered acutely by many influences such as adrenergic stimuli, cardiac glycosides, barbiturates, etc. Experiments carried out in isolated cat papillary muscle have demonstrated that chronic alterations in the level of thyroid activity also alter the speed of shortening of the contractile element, as reflected by V_{max} (18). In contrast to cardiac muscle, the V_{max} in skeletal muscle is usually considered to be essentially constant, except with changing temperature (15, 19–21).

A major finding of the present study is that variations in thyroid activity influence the active state of skeletal muscle by altering the maximal velocity of shortening and the rate of tension development. This observation is consistent with the view that thyroid activity influences the rate of force generating processes at contractile sites in skeletal muscle thus affecting the conversion of chemical energy into mechanical work. It is of interest in this connection that there is an alteration in the efficiency of energy utilization in cardiac muscle in hyperthyroidism leading to a reduced efficiency in the conversion of chemical energy to mechanical work (22).

The intensity of the active state can also be assessed by measuring the maximum force which the muscle is capable of developing during a tetanic contraction, i.e., when the contractile component is neither lengthening or shortening (23, 24). Although the time course of the active state cannot be measured directly, the mechanical correlates can be determined under isometric conditions by measurement of the maximum rate of tension development and the time interval from the last stimulus in a tetanus to the first perceptible fall in tension (9, 11, 23, 25, 26).

Variations in the level of thyroid activity were found to produce significant alterations in these measurements; the maximum rate of tension development varied directly while the duration of active state varied inversely with the level of thyroid activity. Although the tetanic tension observed in muscles from hypothyroid animals was significantly lower than in those from euthyroid rats, this variable was not significantly different in muscles isolated from normal and hyperthyroid animals.

As might be anticipated, muscle fusion frequency is inversely proportional to the duration of muscle contraction (27). As a result of the abbreviation of the active state in muscles from hyperthyroid animals, it would be anticipated that fusion of contractions might be incomplete at frequencies of stimulation which produce fusion in muscles from the euthyroid group. This was indeed observed in the present investigation (Fig. 6), a finding consistent with that of earlier investigators of this problem (28, 29). If these considerations can be extended to intact man, then the observed shortening of the duration of the active state and the more rapid rate of tension development and decline of hyperthyroid muscles may explain the shortened muscle contraction time which has been observed in hyperthyroid patients (5).

Although the present studies are in agreement with observations (29) that tetanic tension development in muscles obtained from hyperthyroid animals does not

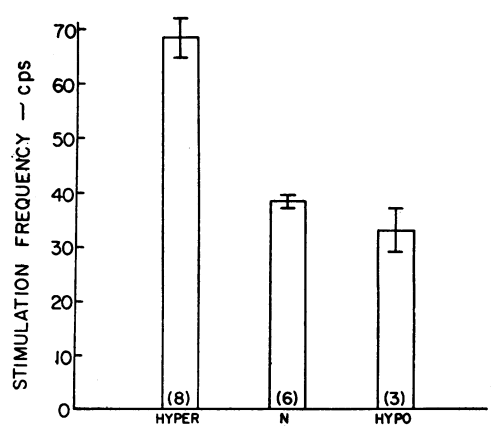

FIGURE 6 Stimulation frequency at which complete fusion of mechanical events occurred as a function of the level of thyroid state. HYPO = hypothyroid.

significantly differ from tetanic tension development in normal muscle, it is important to emphasize that tetanic contraction of individual muscle fibers may not occur in intact animals (30). Tetanic activation of the individual fibers of a muscle is unlikely because direct observation has shown the discharge frequencies of motor neurons to be inadequate to secure tetanic contraction. Since Denny-Brown's original observation, a number of investigators have shown that the discharge frequency of individual motor neurons in man rarely exceeds 30 impulses/sec for normal submaximal activity (31, 32). Bigland and Lippold, investigating the human gastrocnemius by electromyographic methods, observe maximum frequencies ranging between 25 and 35/sec during strong contractions of this muscle (33). These frequencies are well below those required to produce typical tetanic fusion of muscle preparations. Thus, at physiological ranges of stimulation, fusion of contraction might be incomplete in hyperthyroid muscle and more complete in normal muscle. Such incomplete fusion of contraction would, in turn, lead to reduced tension development. It was observed in this study that the tension developed in muscle from hyperthyroid animals was indeed reduced when compared to that developed by muscle from euthyroid animals when stimulation was carried out at a near maximal physiological frequency of stimulation (40 stimuli/sec).

If these observations of a reduced maximal tension development in muscle obtained from hyperthyroid rats at 40 stimuli/sec can be extended to intact man and if the frequency of motor nerve impulses is not altered by changes in thyroid status, then one might expect that muscle weakness would occur. At present, however, there is no information on the frequency of motor nerve impulse traffic in hyper- or hypothyroidism.

In skeletal muscle obtained from myxedematous rats, a significant reduction in peak tension was observed at all frequencies of stimulation. This reduction was associated with a decrease in the rate of tension development, a lower velocity of contraction of the minimally loaded muscle despite a prolongation of the duration of the active state, and reflects a reduction in the intensity of active state produced by the low level of thyroid activity. These findings would explain, at least in part, the muscular weakness observed in patients with myxedema.

In the present investigation the rate of tension decline was observed to be prolonged significantly in muscles from hypothyroid rats and to be significantly abbreviated in muscles from hyperthyroid animals. Thus, the well-known alterations in the duration of the tendon jerk which have been shown to occur in patients with hyper- or hypothyroidism may be a consequence of these intrinsic alterations in the muscle's relaxation process produced by variations in thyroid state.

In conclusion, hyperthyroidism and myxedema result in profound alterations in the intrinsic contractile properties of isolated skeletal muscle with the maximal velocity of contraction varying with the level of the thyroid state. In hyperthyroidism, the observed reduction in the duration of the active state in vitro may be expected to reduce tension in vivo by preventing complete fusion of contractile events; in myxedema tension is reduced as a consequence of the lowered intensity of active state. The slow rate of muscle relaxation in myxedema and the rapid rate in hyperthyroidism are intrinsic properties of these muscles. These changes explain, at least in part, the weakness of muscle activity in both hyper- and hypothyroidism.

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