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rapid communication

Thyroid hormone-induced upregulation of Na^+ channels and Na^+-K^+ pumps: implications for contractility

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Harrison, A. P., and T. Clausen. Thyroid hormone-induced upregulation of Na^+ channels and Na^+-K^+ pumps: implications for contractility. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R864–R867, 1998.—We have previously observed in rat soleus muscle that endurance is a function of the ratio between the concentration of Na^+ channels and Na^+-K^+ pumps [Harrison, A. P., O. B. Nielsen, and T. Clausen. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1402–R1408, 1997]. In this study we explore this relationship further by comparing the changes in Na^+ channel and Na^+-K^+ pump concentrations induced by injections of 3,5,3′-triiodothyronine (T_3) with endurance. T_3 induced upregulation of the concentration of Na^+ channels and Na^+-K^+ pumps, which was associated with a progressive loss of contractile endurance. The increase in Na^+ channels preceded that of the Na^+-K^+ pumps and amounted to 49 and 52% (both $P < 0.01$) after 48 and 72 h of T_3 treatment, respectively. Concomitantly, during 90-Hz stimulation, the initial rate of force decline increased by 42 and 45% after 48 and 72 h of T_3 treatment, respectively (both $P < 0.001$). These observations are important for an understanding of the fatigue associated with hyperthyroidism and add further support to the hypothesis that muscle endurance depends on the leak-to-pump ratio for Na^+.

[^{3}H]ouabain binding; [^{3}H]saxitoxin binding

SKELETAL MUSCLE IS ONE of the major target tissues for the action of thyroid hormones, of which most of the effects can be ascribed to the action of 3,5,3′-triiodothyronine (T_3). Hyperthyroid patients complain of muscle wasting, cramps, muscle weakness, and fatigue, symptoms that disappear when individuals are returned to a euthyroid state (16). It is well established that hyperthyroidism leads to marked upregulation of the concentration of Na^+-K^+ pumps in skeletal muscle of animals (1, 2, 6) and humans (12). This appears to run contrary to the finding that hyperthyroidism is associated with muscle weakness and fatigue. Rather it should be expected that muscles from hyperthyroid individuals exhibit an enhanced contractile performance associated with improved maintenance of membrane excitability resulting from upregulation of Na^+-K^+ pump concentration. Evidence that the solution to this problem may lie in another direction was suggested by the findings that *1*) within 48 h of T_3 treatment Na^+ influx at rest is increased in rat skeletal muscle (6), *2*) administration of thyroid hormone leads to upregulation of voltage-dependent Na^+ channels in cultured myotubes (3), and *3*) increased Na^+ influx via Na^+ channels relative to the capacity for Na^+-K^+ pumping induces a considerable decrease in the contractile performance of rat soleus muscle (10). This study has therefore sought to address *1*) whether administration of T_3 induces upregulation of Na^+-channel concentration in skeletal muscles in vivo, *2*) whether such effects of T_3 can be related to an upregulation of Na^+-K^+ pumps, and *3*) whether changes in the concentration of Na^+ channels induced by T_3 can be related to the impairment of contractile performance associated with hyperthyroidism. Part of these results have been presented as a preliminary communication (9).

METHODS

Animals and Muscle Preparation

Wistar rats, 4 (60–70 g) or 10 wk old (200 g), were used. Animals of similar body weight from within the same litter were randomly assigned to either a control or T_3 group. They were fed a standard rat feed (Altromin Nr. 1314; Spezialfutterwerke, Lage, Germany) ad libitum, and water was freely available. Environmental temperature was maintained at 21°C, and lighting was on a 12:12-h light-dark cycle. Rats were killed by decapitation, and intact soleus muscles were excised as described previously (13). Muscles were placed in a standard incubation medium, Krebs-Ringer bicarbonate buffer, containing (in mM) 120.1 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5.0 D-glucose. The buffer was kept at 30°C and equilibrated continuously with a mixture of 95% O_2 and 5% CO_2 (pH 7.3).

Thyroid Hormones

A T_3 solution (40 mM) was prepared in a solvent of 154 mM NaCl containing 1% wt/vol bovine serum albumin, titrated with 2 N NaOH to a final pH of 10–11, as described previously (6). Injections were given once daily at 0900 subcutaneously at a dose rate of 0.2 μg/g body wt, and control rats received an equal volume of the solvent. As described in detail elsewhere, this treatment leads to hyperthyroidism, as evident from an increased heart-to-body weight ratio and a rise in body temperature (6). In the 4-wk-old rats, an initial booster dose of T_3 (0.4 μg/g body wt) was administered on the first day to induce rapid onset of hyperthyroidism.

[^{3}H]Saxitoxin Binding

The concentration of Na^+ channels was determined as the specific displaceable [^{3}H]saxitoxin binding capacity (8). As previously described in detail (10), intact muscles or strips of muscles weighing 20–38 mg were incubated for 2 h at 4°C in

Krebs-Ringer bicarbonate buffer containing [^{3}H]saxitoxin (0.05 μCi/ml) and unlabeled saxitoxin at a final concentration of 2.5×10^{-8} M. After incubation, the muscles were blotted, weighed, and soaked overnight in 2 ml of 300 mM trichloroacetic acid (TCA). The content of [^{3}H]saxitoxin in the TCA extracts was determined by scintillation counting after addition of 2.5 ml of optifluor. For each muscle or strip of muscle, specific uptake of [^{3}H]saxitoxin was measured by incubation in 2.5×10^{-8} M saxitoxin. The unspecific uptake of [^{3}H]saxitoxin was determined after incubation at a final supersaturating concentration of 2.0×10^{-6} M unlabeled saxitoxin. Use of higher concentrations of unlabeled saxitoxin or 2.0×10^{-6} M unlabeled tetrodotoxin for displacement of [^{3}H]saxitoxin gave comparable results for unspecific uptake. Specific displaceable [^{3}H]saxitoxin binding was calculated as the difference between total [^{3}H]saxitoxin uptake and the unspecific uptake of [^{3}H]saxitoxin using the specific activity of [^{3}H]saxitoxin in the buffer and expressed as picomoles per gram wet weight.

[^{3}H]Ouabain Binding

The concentration of Na^+-K^+ pumps was determined as the specific displaceable [^{3}H]ouabain binding capacity (15). After testing for contractile performance, muscles were frozen in liquid N_2 and cut into segments weighing 2–8 mg, washed, and incubated in a buffer of the following composition (in mM): 10 tris(hydroxymethyl)aminomethane (Tris), 3 $MgSO_4$, 1 Tris-vanadate, and 250 sucrose, pH 7.2–7.4. Muscle samples were equilibrated for 2 h at 37°C in buffer containing [^{3}H]ouabain (0.6 μCi/ml) and unlabeled ouabain at a final concentration of 10^{-6} M. This was followed by four 30-min washes in ice-cold ouabain-free buffer to remove [^{3}H]ouabain not bound to the receptors. The muscle samples were then blotted, weighed, and soaked overnight in minivials containing 0.5 ml 300 mM TCA with 0.1 mM ouabain, added to serve as a carrier, before addition of 2.5 ml optifluor and liquid scintillation counting of the [^{3}H]activity. On the basis of the specific activity of [^{3}H]ouabain in the incubation medium, the amount of [^{3}H]ouabain taken up and retained in the samples was calculated and corrected for nonspecific uptake, loss during washout at 0°C, isotopic purity, and incomplete saturation by multiplying by a comprehensive correction factor of 1.334 (for details see Ref. 11). Measurement of [^{3}H]ouabain binding capacity was done in quadruplicate.

Force Development

As previously described (4, 10), muscles were mounted vertically with their tendons intact in thermostatically controlled chambers and were stimulated directly by supramaximal pulses (10 V, 1-ms duration) through two platinum electrodes. Isometric force development was measured using force displacement transducers (Grass Instruments, Quincy, MA), and recordings were made with a calibrated chart recorder and computer-based recording system (ADInstruments, Hastings, UK). Adjustments to muscle length were made to ensure that maximal isometric force was achieved on stimulation, after which muscles were tested by stimulation at 2, 30, and 90 Hz for 1.5-s duration before being left to equilibrate for 30 min without stimulation. Before the start of the experiment, muscles were stimulated once at 90 Hz for 1.5 s to check contractile force and left to rest for 10 min. The ratio between twitch and tetanic force was 1:5. In all experiments, endurance was examined by recording the decline in contractile force during a period of constant stimulation at 90 Hz until ~80% of initial force had been lost. This parameter, which is effectively the inverse of what is often referred to as fatigue, avoids confusion associated with the precise definition of fatigue, which can occur at the level of the central nervous system, peripheral nervous system, and neuromuscular junction, as well as at the level of the muscle fiber. Endurance was measured as the time taken for peak isometric force to decline by 75%. Contractile force was expressed in Newtons per gram wet weight, and the relative decline of force was calculated as a percentage of the maximum isometric force, which was reached ~1 s after the onset of stimulation. The initial rate of force decline was determined as the relative decrease over the following 30 s.

Chemicals and Isotopes

All chemicals used were of analytic grade. [^{3}H]ouabain (15 Ci/mmol) and [^{3}H]saxitoxin (39 Ci/mmol) were obtained from Amersham International, Buckinghamshire, UK. All other chemicals were obtained from Sigma Chemical.

Statistical Analysis

Differences between means were tested for statistical significance using an unpaired *t*-test. Differences showing a P value >0.05 were considered nonsignificant (NS). Data are means ± SE.

RESULTS

Effects of T_3 Over 24, 48, or 72 h on 4-Wk-Old Rats

Body weight, muscle weight, and maximum isometric force. Body weights of 4-wk-old rats injected with T_3 for 24, 48, and 72 h were reduced by 4–5% in comparison with those of sham-injected controls ($P < 0.05$). Soleus muscles taken from 4-wk-old control rats weighed 20.5 ± 0.9 mg wet wt ($n = 13$), a value that was not significantly affected by T_3 injection over 24, 48, or 72 h. Moreover, T_3 injection over 24, 48, or 72 h did not significantly affect maximum isometric tetanic force (16.2 ± 0.5 N/g wet wt; $n = 22$) compared with controls (16.5 ± 1.1 N/g wet wt; $n = 13$).

Muscle endurance. Figure 1 shows the effect of T_3 treatment for 48 h on contractile performance of soleus muscles from 4-wk-old rats. During sustained 90-Hz stimulation, the time taken for peak isometric force to decline by 75% was reduced from 3.27 to 2.20 min, a 33% reduction in endurance ($P < 0.001$). Furthermore, after 72 h of T_3 treatment, a similar reduction in endurance (30%; $P < 0.01$) was found, whereas 24 h of T_3 treatment produced a smaller (19%; $P = 0.02$) decrease in endurance.

[^{3}H]saxitoxin, [^{3}H]ouabain binding, and initial rate of force decline. Figure 2 shows the time course of the effects of T_3 on Na^+ channels, Na^+-K^+ pumps, and the initial rate of force decline. Twenty-four hours after the onset of T_3 treatment, there was no change in Na^+ channel or Na^+-K^+ pump concentration. After 48 and 72 h of T_3 treatment, Na^+-channel concentration increased by 49% ($n = 9$; $P < 0.01$) and 52% ($n = 8$–9; $P < 0.01$), respectively. Over the first 48 h of T_3 treatment, Na^+-K^+ pump concentration increased by only 8% and did not become significantly different from control levels until after 72 h (28% increase, $n = 6$–8; $P < 0.001$). In muscles from T_3-treated rats, the initial rate of force decline over the first 30 s of 90-Hz stimulation was 0.88 ± 0.04 ($n = 9$), 1.11 ± 0.06 ($n = 8$), and 1.13 ±

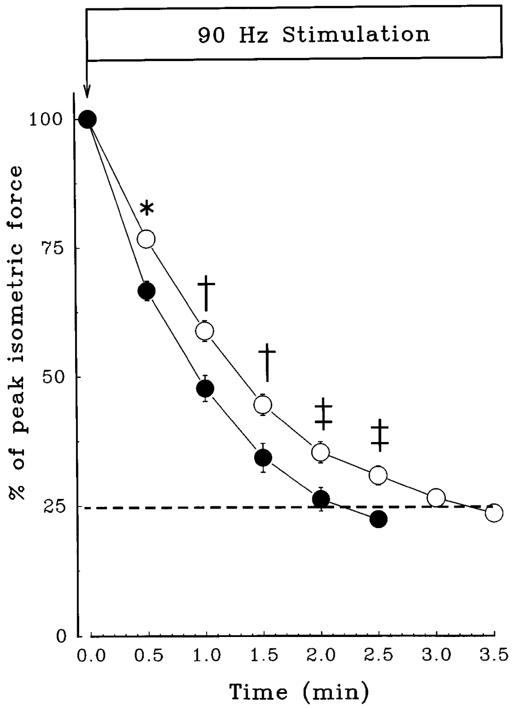

Fig. 1. Time course of decline in isometric force in soleus muscles from control (○) and 48-h 3,5,3′-triiodothyronine (T_3)-treated (●) 4-wk-old rats during continuous electrical stimulation at 90 Hz, applied until ~80% of maximum force was lost. T_3 was administered subcutaneously (0.4 μg·day^{-1}·g body wt^{-1} on *day 1* and 0.2 μg·day^{-1}·g body wt^{-1} on *day 2*) while controls were sham injected with an equal volume of the solvent. Endurance, defined as the time taken for peak isometric force to decline by 75%, was measured by reading the intercept of the dashed line with the curve showing force decline off the *x*-axis. Force decline curves for soleus muscles from control and 48-h T_3-treated rats can be fitted by single-exponential equations: $y = exp(-0.42x + 4.51)$, $r = 0.98$, and $y = exp(-0.60x + 4.52)$, $r = 0.99$, respectively. Each point represents mean ± SE (vertical lines) of observations on 8–18 muscles. $^*P < 0.001$; $^†P < 0.01$; $^‡P < 0.05$ compared with mean of controls (unpaired *t*-test).

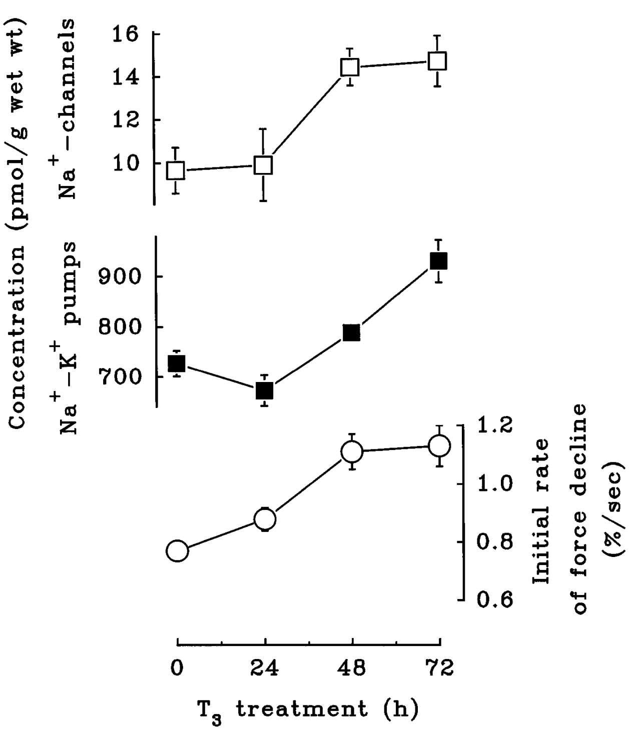

Fig. 2. Time course of the effects of T_3 on Na$^+$ channels (□), Na$^+$-K$^+$ pumps (■), and initial rate of force decline (○) during the first 30 s of 90-Hz stimulation in soleus muscles of 4-wk-old rats. Na$^+$-channel and Na$^+$-K$^+$ pump concentrations were determined using [^{3}H]saxitoxin and vanadate-facilitated [^{3}H]ouabain binding, respectively. T_3 was administered subcutaneously (0.4 μg·day^{-1}·g body wt^{-1} on *day 1* and 0.2 μg·day^{-1}·g body wt^{-1} thereafter) for either 24, 48, or 72 h. Controls were sham injected with an equal volume of the solvent. Each point represents mean ± SE (vertical lines) of observations on 5–9, 4–8, and 8–18 muscles for the concentration of Na$^+$ channels, Na$^+$-K$^+$ pumps, and initial rate of force decline, respectively. For details concerning the significance of differences with duration of treatment see METHODS.

0.07 %/s ($n = 8$) after 24, 48, and 72 h of T_3 treatment, respectively, compared with 0.78 ± 0.03 %/s ($n = 18$) for controls. Thus, after 48 and 72 h of T_3 treatment, the rate of force decline in soleus was already significantly faster than that of control muscles after only 30 s of stimulation (42 and 45%, respectively; both $P < 0.001$). No further significant increase was seen between 48 and 72 h of T_3 treatment.

Effects of T_3 Over 8 Days on 10-Wk-Old Rats

T_3 injections were given over 8 days to adult rats (10 wk old) to assess the effects on Na$^+$ channels, Na$^+$-K$^+$ pumps, and muscle performance of a stable period of hyperthyroidism. Body weights of controls and rats injected with T_3 for 8 days were not significantly different. However, in soleus muscle significant increases in Na$^+$-channel and Na$^+$-K$^+$ pump concentration, greater than those seen in 4-wk-old rats, were observed. As shown in Table 1, T_3 treatment for 8 days produced almost the same relative increase in Na$^+$-channel concentration (154%) and Na$^+$-K$^+$ pump concentration (144%). These changes were associated with a 36% reduction in endurance and a 53% increase in the initial rate of force decline.

Table 1. *Effects of 8 days of T_3 treatment on the concentration of Na$^+$ channels, Na$^+$-K$^+$ pumps, contractile endurance, and initial rate of force decline in soleus muscles from 10-wk-old rats*

Parameters	Control Muscles	Muscles From T_3-Treated Rats
Na$^+$-channel concn, pmol/g wet wt	7.2 ± 0.7 (5)	18.3 ± 1.1 (4)*
Na$^+$-K$^+$ pump concn, pmol/g wet wt	256 ± 11 (4)	625 ± 18 (6)*
Endurance at 90-Hz stimulation until 75% decline in peak force, min	1.1 ± 0.1 (7)	0.7 ± 0.1 (8)†
Initial rate of force decline (0–30 s), %/s	1.5 ± 0.1 (7)	2.3 ± 0.1 (8)*

Values are means ± SE; no. of observations in parentheses. 3,5,3′-Triiodothyronine (T_3) was administered subcutaneously (0.2 μg·day^{-1}·g body wt^{-1}) at the same time each day over an 8-day period. Controls were sham injected with an equal volume of the solvent. Continuous electrical stimulation was at 90 Hz. $^*P < 0.001$; $^†P < 0.01$ compared with mean of controls (unpaired *t*-test).

DISCUSSION

Thyroid Hormone Effects on [^{3}H]Saxitoxin and [^{3}H]Ouabain Binding

In rat myotubes in culture, thyroid hormones have been found to induce a 95% increase in Na$^+$-channel concentration within 24 h of treatment (3). The present results extend these observations by showing that T$_3$ in vivo also leads to upregulation of Na$^+$-channel concentration in skeletal muscle. It is interesting that this upregulation of Na$^+$ channels reaches statistical significance before that of the Na$^+$-K$^+$ pumps. This lends further support to the idea that the effect of T$_3$ on the concentration of Na$^+$-K$^+$ pumps should be regarded as an adaptation to the enhanced passive ion leaks (17), where demand for ion transport provides the primary drive for Na$^+$-K$^+$ pump synthesis. We therefore suggest that the upregulation of Na$^+$-K$^+$ pumps associated with hyperthyroidism is the result of the increased resting Na$^+$ influx induced by T$_3$ treatment (6), as well as an increased excitation-induced Na$^+$ influx in working muscles via newly synthesized Na$^+$ channels.

Muscle Contractile Performance and Hyperthyroidism

For over 160 years muscle weakness and fatigue have been identified as symptoms of hyperthyroidism (16), along with muscle wasting and an elevated metabolic rate (5, 14). T$_3$ injection either over a period of only 48–72 h or for a longer period of 8 days led to a loss of endurance of soleus muscles in 4-wk-old rats or mature 10-wk-old rats, respectively, and a faster initial rate of force decline during periods of sustained high-frequency stimulation (Fig. 1 and Table 1). These findings are in agreement with those of others who have shown that in rats T$_3$ treatment increases fatigue (7), defined as a greater decay in force during contraction in hyperthyroid rats compared with euthyroid controls. Furthermore, this study shows that the initial rate of force decline with the onset of hyperthyroidism (Fig. 2) is correlated with the increase in Na$^+$-channel concentration. This observation lends further support to our earlier results (10) indicating that Na$^+$ influx via Na$^+$ channels relative to the capacity for Na$^+$-K$^+$ pumping is an important determinant of the contractile performance of skeletal muscles. The results strongly suggest that the reduced endurance and increased initial rate of force decline induced by T$_3$ are due to a faster decline in the gradient for Na$^+$ arising from increased Na$^+$ influx via an increased concentration of Na$^+$ channels.

Physiological Perspectives

During periods of hyperthyroidism, upregulation of Na$^+$-channel concentration precedes that of Na$^+$-K$^+$ pumps. Furthermore, upregulation of Na$^+$-channel concentration with hyperthyroidism is involved in the faster rate of force decline and loss of endurance that is symptomatic of the disease. These results are important for the understanding of muscle contractile performance experienced by hyperthyroid patients and, in a more general fashion, indicate that the excitation-induced decline in the transmembrane gradient for Na$^+$ during exercise contributes significantly to muscle fatigue.

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