Effects of thyroid hormone at the neuromuscular junction

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Hofmann, William W., and Eric H. Denys. Effects of thyroid hormone at the neuromuscular junction. Am. J. Physiol. 223(2): 283–297, 1972.—Neuromuscular transmission has been studied in vitro in phrenic nerve-diaphragm preparations from rats made hyper- or hypothyroid. In preparations from hyperthyroid animals, the amplitude of miniature end-plate potentials (MEPPs) was significantly reduced, as was the muscle-membrane potential. Some muscle fibers from hyperthyroid animals were inexcitable, when tested either directly or indirectly, despite relatively mild depolarization. Membrane potentials were normal in preparations from hypothyroid animals as were the frequency and amplitude of the MEPPs. In muscle fibers from hypo- and hyperthyroid animals, there were no changes in time course or shape of the action potential and no sign of repetitive unit firing. Thus, the prolonged twitches observed in the hypothyroid animals could not be accounted for on this basis. No histological abnormalities were seen in any of the tissues. These findings are discussed in the light of known clinical associations between thyroid diseases and certain neuromuscular diseases in man.

hypothyroidism; hypothyroidism in vitro; neuromuscular transmission; miniature end plate amplitude; transmitter release in hyper- and hypothyroidism; muscle-membrane potential; hyperthyroid muscle contraction; twitch times; T₄ on muscle.

These are a number of muscular and neuromuscular disorders associated in man with disturbances of the thyroid gland (4, 8, 9, 15, 18, 21, 23, 24, 27–29), but very little is known about the direct effects of the hormone on the electrophysiological properties of the mammalian neuromuscular junction. In fact, data available do not indicate that synaptic function has been closely examined in either hyper- or hypothyroidism. In these experiments we have studied isolated neuromuscular junctions in vitro from rats made hyper- or hypothyroid and have compared the findings with those in control animals of similar age and weight at the same temperature. In addition to the microphysiological studies on synaptic events, we have also examined the mechanical properties of the three groups of muscles under a number of different experimental conditions. The latter investigations have been reported separately (3).

MATERIAL AND METHODS

The experimental animals were Sprague-Dawley rats of either sex and initial weight of between 250 and 370 g. Hyperthyroidism was induced by five to eight daily intraperitoneal injections of 500 μg of L-3,3′,5-triiodothyronine (T₃) dissolved in 0.005 N NaOH (10, 34). T₃ was selected because it produces all the known effects of the thyroid hormone in man (2) and is about 7 times as active as tetraiodothyronine (T₄) (33). Hypothyroidism was produced by feeding a low-iodine diet (General Biochemicals Inc., Chagrin Falls, Ohio) (26) and the addition of 2% sodium perchlorate to the drinking water (11, 12, 30) for 14–20 days. The animals were weighed, and weight gains or losses were compared with the averages obtained over the same period of time in control animals. The blood of the test animals was removed by cardiac puncture at the time of thoracotomy, and the serum level of tetraiodothyronine (T₄) was measured. The value obtained was taken as a direct reflection of the animal’s metabolic state, although the injected T₃ may have biased the T₄ determinations to some extent. Control animals were fed a standard diet ad libitum. In all, 12 control, 8 hyperthyroid, and 10 hypothyroid rats were studied.

Electrophysiological measurements were made in vitro on the excised phrenic-nerve diaphragm preparation in all animals and in one or more of the hindleg muscles in animals from each experimental group in vivo. For the in vitro experiments the nerve-muscle preparation was kept in vigorously oxygenated nutrient solution of the composition described by Liley and North (16). If the limiting factor in O₂ availability for the muscles was the replenishment of dissolved gas, the oxygenation technic should have been more than adequate for both control and hyperthyroid preparations. The organ bath temperature was continuously monitored and was kept within 0.5°C of the various desired values by means of a water jacket. Mechanical responses in vitro were recorded with a small transducer (RCA 3734) to the pin of which was fixed the apical portion of the central diaphragmatic tendon.

The muscle was stretched slightly until maximum twitch tension was obtained and was stimulated through the phrenic nerve by supramaximal shocks of .06 msec duration. For direct stimulation the muscle was mounted across two large metal strips, or silver wires, the tendon was fixed as before, and shocks of 1.5–3 msec duration were applied. When the hindlimb muscles were also studied (in vivo), the anesthetized animal’s gastrocnemius-soleus tendon was tied to a larger transducer (Grass FTO3, Grass Instrument Co., Quincy, Mass.), while the sciatic nerve was stimulated in the thigh. The standard tetanus frequency for the in
vitro (diaphragm) preparations was 20 Hz and for the gastrocnemius-soleus (in vivo) 40 Hz. The twitch in both types of preparation were evoked at 1/sec.

Intracellular records of miniature end-plate potentials (MEPPs), end-plate potentials (EPPs), and action potentials were made in vitro with conventional glass micro-electrodes having resistances of from 2 to 10 megohms. These rather large micro-electrodes caused rapid deterioration of some hyperthyroid fibers, and these fibers were not further studied or used in calculations, but electronic noise was reduced to a level permitting measurements of very small transients. Frequent calibration pulses were applied to test the condition of the micro-electrode (Bioelectric Instruments Co., N. Y.). For comparing MEPP amplitudes among the three groups of animals, only depolarizations of 1.5 msec or less rise time were used. Estimates of EPP quantum content were made from 50 to 100 impulse trains of EPPs in partly curarized fibers by calculating the ratio: EPP variance/EPP mean (7, 20). Artifactual differences in quantal depolarization and estimated EPP quantum content could be reduced by applying the usual correction factors for membrane potential and for nonlinear summation to raw data in all three groups (3, 7, 14, 19, 20, 31). However, these corrections assume a standard equilibrium potential for all the fibers and, since we have no information on this point, we have presented the MEPP data with and without correction. The mean EPP amplitude for the entire train (exclusive of the first 10) was used for calculations after it was found that means obtained from successive groups of 5 EPPs were not significantly different and that initial rundown of EPP amplitude has ceased by the 5th or 6th EPP. The variance/mean ratio gave a rough estimate of $q$, the depolarization produced by a single quantum. Dividing mean EPP amplitude by $q$ gave an estimate of $m$, the quantum content. The depletion and mobilization rates of transmitter units were estimated from the rate of rundown of the first 10 EPPs of a train, and the absolute number of quanta released in the first impulse at any junction was calculated from the ratio 1st EPP amplitude/mean $q$, the denominator obtained from the 50- to 100-impulse train following the first 10. A few records were made of action potentials and mechanical responses simultaneously, and from these the latency of neuromuscular conduction (indirect stimulation) or the latency of excitation-contraction coupling (direct stimulation) could be estimated.

RESULTS

A) Evaluation of metabolic status. Table 1 gives the serum $T_4$ values and weight changes in the various test groups. Clinically, the hyperthyroid rats were excited, agitated, and subject to piloerection at any stimulus. They also were intolerant of heat (applied by a lamp), tended to become immobilized by a threatening gesture or noise, and suffered diarrhea, but their gait and posture were normal. Hypothyroid rats, by contrast, were slow in their movements, walked with some ataxia of the hindlimbs, and generally tended to sit quietly huddled together in a corner of their cage. All hypothyroid animals also lost much of the hair on their tails.

B) Membrane responses. Membrane potential values were compared in the three test groups, and after addition of $T_3$ to the bath, as shown in Table 2. The bath-applied compound had no effect on membrane potential. Muscles from the hyperthyroid animals (in vivo injections), however, showed mean values significantly lower than the others in a total of 88 fibers. Fibers which deteriorated rapidly after puncture were not used in calculations for any group, but the total population includes 13 hyperthyroid cells, the initial membrane potentials of which were stable at from 44 to 50 mv. Except for fibers visibly cut or damaged by the isolation procedure, no such depolarized hyperthyroid units were found in the normal or hypothyroid groups. Most of the depolarized, hyperthyroid fibers were excitable on direct and indirect stimulation. Action potentials were not measurably different in the normal and hypothyroid groups, but tended to be somewhat slurred and of lower amplitude in the hypopolarized, hyperthyroid fibers. No sign of repetitive muscle firing or afterdepolarization was seen in any preparation in vitro at the various temperatures selected, despite the fact that hypothyroid muscles contracted more slowly than others in vitro and were apparently more “irritable” in vivo at the time of dissection.

C) Transmitter stores and release. These experiments were all carried out in vitro on partly curarized (2-8 $\times 10^{-7}$ g $d$-tubocurarine/ml nutrient solution) phrenic nerve-diaphragm preparations at 30.0 ± 0.5 C. At this temperature fractional release and early mobilization were investigated by plotting the amplitude of the initial 10 EPPs as a percent of the first response in a series of fibers. The decay of the amplitudes of the first few EPP's was taken as an index of the fraction by which any stimulus depleted the immediately releasable store before any mobilization had occurred. This sort of fractional depletion curve takes no account of possible changes in the initial size of this store under experimental manipulation or the actual numbers of transmitter units ejected per EPP. The slope of the depletion curve, when plotted as a fraction of the first response,

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**TABLE 1. Metabolic effects on body weight**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Serum $T_4$ µg/100 ml</th>
<th>Weight Change, g/day</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
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<tr>
<td>Normal</td>
<td>3.5</td>
<td>3.2-3.6</td>
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<tr>
<td>Hyperthyroid</td>
<td>8.8</td>
<td>5.5-19.0</td>
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<tr>
<td>Hypothyroid</td>
<td>1.0</td>
<td>0.6-1.3</td>
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**TABLE 2. Resting-membrane potentials at 30 C**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>$n$</th>
<th>KMP, mv</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>90</td>
<td>68.9</td>
<td>1.3</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>88</td>
<td>52.6</td>
<td>1.8</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>35</td>
<td>71.2</td>
<td>3.0</td>
<td>&gt;.10</td>
</tr>
</tbody>
</table>

$T_3$ in vitro, 1.6 µg/g diaphragm

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Normal before | 16 | 74.0 | 9.2 | >.10 |
30-60 min after | 19 | 71.0 | 3.5 | >.10 |
could be decreased as much by an inhibition of release as by
more rapid replenishment. Likewise, the effects of very
rapid release from a small pool might look the same, in
terms of fractional depletion, as a total failure of mobiliza-
tion or refilling, and to understand the events behind a given
change it is therefore necessary to make more quantitative
estimates of transmitter release. This has been done by
plotting the actual number of transmitter units released in
the initial impulse and the next nine from junctions in the
three animal groups. The early run-down process, and the
level from which it started then can be used to evaluate
such parameters as the probability of release, the size of the
immediately releasable store, and the rate of replenishment
(7). The two types of depletion curves are shown in Fig. 1.
In A are the values of EPP amplitude, plotted as a percent
of the first, in 10-impulse trains in the three groups of
animals. Fig. 1B illustrates the absolute values of quantum
content (m) per EPP in the first 10 responses, along with the
average number of units ejected by each nerve impulse
during the following sustained tetanus at 20 Hz. Under
these conditions the three fractional depletion curves were
indistinguishable, as were the rundowns of actual quantum
number in the first 10 EPPs from their respective beginning
levels. The mean quantum content of the first EPP, as
shown at the left in Fig. 1B, is not significantly different
from normal in either of the other two groups of animals.
The hyperthyroid nerve terminals did not eject significantly
more than normal amounts of transmitter per EPP during a
50- to 100-impulse steady tetanus after initial rundown.
Surprisingly, however, the hypothyroid junctions were able
to maintain a slightly higher output during tetanization at
30 C (5 animals). The change is barely significant at 30 C
(0.02 > P > 0.01) but is confirmed at 22 C, where seven
junctions from two hypothyroid preparations were able to
maintain a mean "plateau" output of 54.4 ± 12.1 quanta
per EPP, a value higher than that for normal animals at
37 C. It was interest in this seemingly paradoxical plateau
response that led to the study of nearly twice as many
hypothyroid fibers during rundown (31 hypo, 18 normal,
18 hyper). It should be noted in this connection that the
hypothyroid preparations showed this relative potentiation
of transmitter release only after repetitive stimulation and
not at the beginning. In fact, from the curves of Fig. 1, it
appears that the resting hypothyroid nerve terminal has
neither an unusually large store of immediately releasable
quanta nor a higher probability of release. After continued
stimulation, however, the mobilization and replenishment
process seems to be even slightly more active than normal.
From these findings it was concluded that no functional
abnormalities in neuromuscular transmission would be
expected in vivo as a result of change in the immediately
releasable transmitter pool or the kinetics of the early
replenishment process produced by hypo- or hyperthy-
roidism.

1) Spontaneous transmitter release. The amplitude and fre-
cquency of spontaneous quanta1 discharges (MEPPs) were
recorded in a total of 191 satisfactory fibers, 75 normal, 78
hyperthyroid, and 38 hypothyroid at 30 ± 0.5 C. Table 3
shows the results of these studies, and it can be seen that,
while the mean frequency of the spontaneous depolariza-

FIG. 1. Transmitter release and replenishment in the 3 test groups.
A: fractional depletion of acetylcholine in first 10 EPPs at 20 Hz.
Eighteen fibers from 3 normals ( ), 18 fibers from 4 hyperthyroid
animals ( ), and 31 fibers from 5 hypothyroid animals ( ). B: initial
depletion curves plotted from estimates of actual EPP quantum con-
tent. First 10 EPP responses at left, mean ± SE of sustained quanta
per EPP compared with normal animals in plateau values at right.
Plateau data obtained from 50 to 100 EPP trains and pooled from
numbers of fibers shown (n). P values given are for significance of
differences from normal of first EPP quantum output (above run-
down curve) and of plateau values (to right of bar), respectively.
tion was essentially the same in all groups at the test temperature of 30.0 ± 0.5°C, the amplitude at hyperthyroid junctions was less than normal. On the other hand, MEPP amplitude in hypothyroid preparations was not significantly different from normal (0.05 > P > 0.02). The time course of the MEPPs was the same in all preparations. In two experiments the MEPPs were recorded before and for up to 1 hr after addition to the bath of T₃. The final bath concentration of T₃ was approximately the same as would have been established per gram of tissue in vivo after full absorption of the administered dose. There was no change in either the frequency or amplitude of the MEPPs or of the average membrane potential.

**DISCUSSION**

These results suggest that at least two parameters important in neuromuscular transmission may be altered by the hypermetabolic state, while the effects of hypothyroidism appear to be limited to changes within the muscle fibers themselves (3). The first electrophysiological change observed was the relative depolarization of the hyperthyroid fibers, which in many instances was associated with inexcitability on both direct and indirect stimulation. Secondly, there was a significant reduction in MEPP amplitude, even after the lower resting potential was taken into account. Apparently, the conditions of isolation severely tax the hyperthyroid muscle so that it is not able to maintain normal excitability or electrical polarization in vitro. We have made no measurements of membrane potential in vivo in these animals and can offer no evidence about conditions when blood supply is intact. We have no good reason, however, to believe that the muscles are depolarized in the living animal or that such in vitro depolarization as we have recorded would, by itself, produce any clinical change in neuromuscular function. On the other hand, previous investigators have shown increased brain (32) and muscle (17, 25) sodium concentrations in fresh material from hyperthyroid animals and have postulated failure of energy-dependent sodium extrusion in vivo. Furthermore, Zaimis et al. (35) have found that hyperthyroid muscles exposed to decamethonium are not able to repolarize without a prolonged delay, and the sum of evidence suggests that the hyperthyroid state could easily lower the threshold for expression of other neuromuscular and muscular diseases.

The well known clinical association between thyrotoxicosis and myasthenia gravis is perhaps a result of the reduced effectiveness of transmitter quanta in both disorders (6). With hypokalemic periodic paralysis, any "leakiness" of the muscle membrane to Na ions (13) would be exaggerated by concurrent hypermetabolism, and it is clinically obvious that the muscle cells cannot tolerate both disturbances together.

Without tests of membrane resistance, it is not possible to say whether depolarization and increased intrafiber sodium are related to structural surface changes induced in muscle cells by thyroid hormone, or whether the findings indicate failure of metabolic "pumping." The time courses of MEPPs and action potentials were not shortened significantly, as would be expected with major increases in membrane conductance, and we agree with previous investigators who have suggested that the primary factor is some type of inefficiency of the Na extrusion mechanism (17, 25, 32). As judged by the frequency of MEPPs, the nerve terminals do not seem to be depolarized in any of the preparations.

Bath-applied T₃ produced no electrophysiological changes in previously normal muscles, though, after 30 min to 1 hr the twitches became somewhat faster (decreased total twitch time). It is thus suggested that the oxidative metabolism of the tissues must be increased before the typical intracellular effects of thyroid hormone are seen, and it seems reasonable to infer that, after a lengthy exposure to the hormone in vitro, the isolated muscles are twitching faster because of a truly metabolic effect. To establish this point it would be necessary to record oxygen consumption of the test preparations.

Though hyperthyroidism can accentuate neuromuscular disorders, there is no reason to blame the weakness of Graves' disease on any disturbance of synaptic conduction. In fact, if florid thyrotoxicosis in man produces the same electrophysiological changes as are seen in the present experiments, it is important to emphasize that muscle strength would not be at all decreased on the basis of impaired neuromuscular transmission alone. Progressive failure of neuromuscular transmission was never found in any of the preparations exposed to repeated tetanization. Furthermore, even if the smaller MEPPs in hyperthyroidism may indicate a minor junctional abnormality, such a disturbance certainly could not explain the weakened contraction during direct stimulation which we have observed in tetanic activation of histologically normal hyperthyroid fibers in vitro. The muscle weakness of thyrotoxicosis thus appears to involve a biochemical disturbance within the contractile elements themselves, and the most parsimonious explanation of the associated electrophysiological abnormalities is that they, too, are of metabolic origin.

The hypothyroid muscles appeared quite normal histologically and did not even show any convincing electrophysiological defect, despite the striking changes observed in their contraction (3). There was no measurable difference in the latency between nerve stimulation and the muscle excitation at hypothyroid neuromuscular junctions.

A question remains about the slightly higher average voltage (0.05 > P > 0.02) of MEPPs in hypothyroid prep-

<table>
<thead>
<tr>
<th>TABLE 3. Spontaneous discharge of ACh quanta</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Hypothyroid</td>
</tr>
</tbody>
</table>

* Means ± se. † See text.
suggestions that a sampling error has not been completely eliminated, and we cannot make any statement about the activity of acetylcholinesterase in hypothyroid animals. It is worth emphasis at this point that any temperature difference that may exist in vivo between hypothyroid and other groups of animals could not influence results in these experiments in vitro, where the temperature was always controlled.

The slowing of reflex (15) and voluntary muscle responses in hypothyroidism is thus without obvious electrophysiological abnormality, even at unit level (see, however, ref. 22), and there is no good evidence in these experiments of any tendency for myxedema and defective neuromuscular transmission to sum.

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