THE EFFECT OF SODIUM L-THYROXINE ON THE SLOW MUSCLE FIBER

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Many reports have shown since 1900 that there might be some relations between thyrotoxicosis and functional disturbances of muscle contraction in the thyrotoxic form of periodic paralysis, and that on the contrary thyroid hormone had a beneficial action on the paralytic attack in the familiar form of this disease. Taking this point of view some electrophysiological analyses on the skeletal muscle membrane have been reported in recent years. LAPLAUD & GARGOUIL (1960) showed that with triiodothyroacetic acid, one of the thyroid hormones, the negative after-potential of the action potential in the sartorius muscle of the frog was augmented in its height. Further, they observed in vivo that the action potential of the rats pectoralis muscle was increased in its duration by previously injected thyroxine (LAPLAUD et al. 1961). Although it has been known that thyroid hormones affect the basic metabolism in tissues, the mechanisms of this effect on the muscle membrane are not yet too clear in detail.

In this experiment attempts were made to investigate and to discuss the actions of sodium thyroxine on the membrane characteristics of the bull-frog’s slow muscle fibers, which are innervated by small motor nerve fibers, and on neuromuscular transmission.

METHOD

Biceps muscles of Rana catesbiana were dissected with their innervating nerves intact. This nerve muscle preparation was mounted in a perspex chamber of about 4 cc in volume. Ringer solution composed of 111.2 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 2.4 mM NaHCO₃ was perfused during observations at a rate of 5 cc per minute. Two glasscapillary microelectrodes filled with 3 M KCl of the Ling & Gerard's type were inserted into the same slow muscle fiber at the neuromuscular junctional region, one for recording the membrane potential and the other for passing current. The resistance of employed microelectrodes was between 15 to 30 MΩ. The recording microelectrode was connected to a low gain and high input impedance preamplifier. Passing currents were recorded as IR drops across a monitoring resistor (10 KΩ) inserted between an indifferent electrode and the ground. For the voltage-clamp experiment (OOMURA &

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319
A negative feedback amplifier system (a differential amplifier (Tektronix type 122) with a gain of 2000X, and time constant of 4 sec) was connected between the two electrodes, as schematically shown in Fig. 1. The nerve was suspended on a pair of platinum electrodes in a small moist chamber adjacent to the recording electrodes.

**Fig. 1.** A: a schematic diagram of experimental arrangement. Recording electrode is connected to the preamplifier and polarizing electrode is connected to selector switch through high resistance (20 MΩ). 5 MΩ and 20 pF are inserted in the circuit for compensation of high frequency loss of the current pulse. Indifferent electrode is grounded through current monitoring resistor (10 KΩ). For polarizing the membrane the switch is set to pulse generator, and for voltage clamp experiment to output of feedback amplifier. Indirect stimulation is applied with a pair of silver wire electrodes. B: a scheme of the ionophoretic microapplication technique of Ach to the junctional region. 1 and 2 are the recording and polarizing electrodes inserted into the slow fiber respectively. 3 is Ach-pipette filled with 1 M Ach.
to the main chamber for indirect stimulation. No spike action potential was evoked by either direct or indirect stimulations in the slow fibers, but the small nerve junctional potential (s.j.p.) was recorded by indirect stimulation. For selective stimulation of the small motor nerve which innervated the slow muscle fibers, Burke & Ginsborg's method (1956a) was employed. The stimulating current rose to a maximum in a few msec and decayed slowly with a time constant of about 30 msec and to avoid the anode break excitation, was applied to the nerve through two platinum electrodes about 3 mm apart; the electrode closer to the muscle was made positive with respect to the other electrode in order to produce an anodal block of the large motor nerve fibers.

Acetylcholine-potentials (Ach-potential) in the slow muscle fibers were recorded in the junctional region by applying the so-called ionophoretic microapplication technique (Nastuk, 1953; Castillo & Katz, 1955). Acetylcholine-micropipette (Ach-pipette) with 15 to 25 MΩ resistance which was filled with 1 M acetylcholine chloride (Merck) was brought to the surface close to the recording electrode already inserted in the neuromuscular junctional region of the slow muscle fiber. The steady diffusion of Ach was withheld by a small steady negative bias applied to a silver wire dipped in the Ach-pipette. A transient output of Ach was brought about by a brief outward current pulse which was monitored by the resistor between the indifferent electrode and the ground.

Most experiments were carried out in an air conditioned room (temperature 16°C) in summer. Sodium thyroxine used in the experiments was supplied by the British Drug Houses Ltd.

RESULT

The properties of the slow muscle fiber in Ringer solution

The resting potential: Negative potentials within the range of 40 to 55 mV appeared suddenly when the recording microelectrode was inserted into the slow muscle fibers. The mean value of the initial resting potentials of the slow fibers in the present experiments was $-51 \pm 1.0$ mV. The membrane potential tended to decrease gradually while the electrode tip stayed in the fiber. It might be due to the small dimensions, about 60 μ in diameter, 10 mm in length and the appreciable spontaneous movement of the slow fibers. The injury around the inserted electrode might develop and the depolarization due to it might be remarkable.

Membrane properties: The current electrode was inserted into the same slow fiber as close to the recording electrode as possible (within about 50 μ). The changes in the membrane potential caused by square current pulses were recorded (upper traces in records 2, and 3 in Fig. 2). The relations between the currents and the membrane potentials were plotted in Fig. 3. The slope of this curve indicates the effective resistance of the muscle membrane. As seen in the figure, the muscle membrane had a remarkable rectifying property, i.e. a larger resistance to an anodic (inward) current than to a cathodic (outward) one (Burke & Ginsborg, 1956a, b). The mean value of the effective membrane resistance was 0.6 ± 0.06 MΩ. This value was small in comparison with that previously reported by us (Oomura et al. 1963), probably due to
Fig. 2. Effects of thyroxine-Na on the membrane of slow muscle fiber and s.j.p. A: control. B: in $10^{-4}$ thyroxine-Ringer solution. 1: s.j.p.'s, upward deflexion shows depolarization. 2: current voltage relations showing membrane resistance. Upper traces show current and lower ones voltage. Note remarkable increase in value of membrane resistance by thyroxine. 3: relation between amplitude of s.j.p. and shift of membrane potential (toward hyperpolarization). 4: s.j.c.'s (downward deflexion in upper traces) recorded by voltage clamp method keeping membrane potential at resting potential level (shown by lower traces). Calibrations: $5 \times 10^{-8}$ A for current (upper bar), 10 mV for voltage (lower bar). Time scale: 50 msec.
seasonal changes in the muscle membrane. Consequently, the time constant of the muscle membrane measured on the decay of the electrotonic potential at the termination of the inward current pulse was also small, about 76 msec (28-164 msec).

The s.j.p.'s: The s.j.p. was easily recorded by nerve stimulation when the electrode was inserted randomly into the muscle fiber, because of the diffuse innervation along its whole length. Heights of the s.j.p.'s ranged from 1.5 mV to 6.7 mV, and the mean value was 3.7 ± 0.41 mV. In the present experiments, relatively marked hyperpolarization followed main depolarization in most of the s.j.p.'s. Amplitudes of the s.j.p. became larger by a membrane hyperpolarization as shown in record 3 of Fig. 2, and smaller by a depolarization, then the relationship between the amplitudes of the s.j.p.'s and the membrane potentials was approximately linear. In some cases the amplitude of the s.j.p. was not altered when the membrane potential was changed without any appreciable signs of membrane deterioration, such as a reduction in membrane resistance or in the resting potential. This indicated that the electrodes inserted were not close enough to the neuromuscular junctional region. In these cases, as will be mentioned later, the correct s.j.c.'s could not be recorded due to an incomplete voltage clamp. It may be safe to assume that
each of the junctional regions are separated by some distance, even though the slow muscle was thought to be innervated diffusely. Until finding a successful relationship between the amplitude of the s.j.p. and the membrane potential, repeated insertions of the microelectrode for searching the junctional region were carried out.

The small nerve junctional current (s.j.c.): Under the voltage-clamp condition, the membrane potential was kept at the resting level, and in response to stimulations of the small motor nerve the current which flowed across the muscle membrane in the neuromuscular junctional region was recorded. The peak of the s.j.c. was attained much faster than that of the s.j.p., and its falling phase was a simple exponential decay, and terminated much faster than that of the s.j.p. The time course of this current indicates the reaction between the transmitter substance liberated from the nerve terminals and the specific receptor membrane (Oomura & Tomita, 1960a,b). The mean amplitude of the s.j.c. in the resting potential level was $2.1 \pm 0.14 \times 10^{-9}$ A. When both the recording and current electrodes were far from the junctional region as referred to before, the s.j.c. was not the simple monophasic form but a diphasic one just like a mirror image of the s.j.p., indicating a simple feedback of the voltages spread electrotonically under the recording electrode and an insufficient amount of current for the clamp of the membrane potential in the junctional region.

The acetylcholine potential (Ach-potential): The Ach-potential of the slow muscle fiber was shown previously in detail by Oomura et al. (1963). In the present experiments the Ach-potential of about 4.0 mV in amplitude was recorded by a current pulse of $1.37 \times 10^{-8}$ A with 50 msec duration (0.67 $\times 10^{-9}$ Coulomb). The quantity of Ach-output from the Ach-pipette is roughly proportional to the strength, multiplied by the duration of the current, i.e. the charge passed through the pipette. It was conceivable that the amplitude of the Ach-potential was influenced by the sensitivity in the junctional region to Ach and the membrane constant (membrane resistance and time constant), when the quantity of the Ach-output and the magnitude of the membrane potential was constant.

Effect of sodium thyroxine

After measuring the electrical properties of the slow muscle membrane and the transmission in normal Ringer, thyroxin-Ringer solution which contained $10^{-5}$ or $10^{-4}$ g/ml sodium thyroxine were perfused. Ten minutes later the first recordings were carried out.

In $10^{-5}$ thyroxine, the muscle fibers were not affected remarkably. The membrane potential did not change, whereas the amplitudes of the s.j.p. and the s.j.c. increased by about 40% in accompany with an increase in the membrane resistance of about the same degree (Table I).

In $10^{-4}$ thyroxine the membrane characters were markedly changed as illus-
Table I.  
Effect of Thyroxine-Na.

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<tr>
<th></th>
<th>Rp (mV)</th>
<th>N</th>
<th>s. j. p. (mV)</th>
<th>N</th>
<th>s. j. c. (10^{-8}A)</th>
<th>N</th>
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<td>Control</td>
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<td>51±1. 0 (8)</td>
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<tr>
<td>10^{-5} gm/cc</td>
<td>50±3.5 (4)</td>
<td>5.3±0. 95 (4)</td>
<td>2.7±0. 92 (129%)</td>
<td>2.78±0. 05 (4)</td>
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<td></td>
<td>(98%)</td>
<td>(134%)</td>
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<td>(135%)</td>
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<tr>
<td>10^{-4} gm/cc</td>
<td>53±0. 7 (18)</td>
<td>6.7±0. 53 (31)</td>
<td>5.1±0. 37 (243%)</td>
<td>2.02±0. 36 (5)</td>
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<td>(104%)</td>
<td>(181%)</td>
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<td>(348%)</td>
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Rp: resting potential. s. j. c: small nerve junctional current under the clamp condition where the membrane potential was kept at the resting level. Re: effective membrane resistance. N: number of observation. S. E.: standard error. Figures in parenthesis: changes in per cent by thyroxine.

The resting potential hyperpolarized slightly, but statistically insignificantly (after t-test, 0.5<P<0.6). The voltage-current relations, however, indicates an increase in the membrane resistance by about 3.5 times on the average (Fig. 3). The amplitudes of both the s. j. p. and the s. j. c. were strikingly augmented to about 180% and 240% respectively, as compared with those in the control solution. Table I shows the membrane properties measured in the control and test solutions. Although the amplitude of the s. j. c. was augmented by thyroxine, the time course was rather shortened, i.e. the mean value of the time constant of the falling phase was 41.4 msec in 10^{-4} thyroxine, while that in the control was 55.9 msec (Fig. 4).

The relationship between the magnitudes of the membrane potential and the amplitudes of the s. j. c.'s was nearly linear when the latter was plotted on an ordinate and the former on an abscissa. The potential where the membrane potential-s. j. c. curve crossed the abscissa indicated the equilibrium po-

![Fig. 4. Effect of thyroxine on s. j. c. A: control. B: 10^{-4} thyroxine. Note a remarkable increase in amplitude with no significant increase in half decay time. Calibrations: 10^{-8} A for current, 100 msec for time.](image)
potential for the s. j. c. No appreciable change in the equilibrium potential could be detected in the control and test solutions. Furthermore, the shunting resistance in the junctional region which could be estimated from the gradient of this curve was about 2.0 and 0.8 MΩ in the control and test solution respectively.

The Ach-potential was increased significantly by thyroxine. One of typical examples is shown in Fig. 5. In this case in the control solution, the Ach-potential of 2.5 mV required a current of $2.5 \times 10^{-8} \text{A}$ and 50 msec duration

![Figure 5](image_url)

Fig. 5. Effects of thyroxine-Na on s. j. p.'s and Acetylcholine-potentials (Ach-potentials). A: control. B: in $10^{-4}$ thyroxine-Ringer solution. 1: s. j. p.'s. 2: s. j. p.'s (the first deflexion on lower traces) and Ach-potentials (the second one), upward deflexion on upper traces show the currents required for the ionophoretic application of Ach. Note remarkable increase in amplitudes of s. j. p. and Ach-potential in thyroxine-Ringer. Calibrations: $5 \times 10^{-8} \text{A}$ for current (upper), 10 mV for voltage (lower). Time scales: 500 msec for 1, and 1 sec for 2.

(1.25 $\times 10^{-9}$ Coulomb was applied to the Ach-pipette) and in thyroxine only $0.69 \times 10^{-8} \text{A}$ with the same duration ($0.35 \times 10^{-9}$ Coulomb) produced that of 12.1 mV.

DISCUSSION

In the present experiments the most remarkable effects of sodium thyroxine on the neuromuscular junctional region of the slow muscle membrane were the increases in the resting membrane resistance, up to more than three folds of that in the control solution, and in the amplitudes of both the s. j. p.'s and s. j. c.'s. These three facts evidently show that thyroxine may facilitate neuro-
muscular transmission. Actions of many agents which influence the neuromuscular transmission may be classified in three categories from the view of their acting sites, i.e. the presynaptic terminals, the postsynaptic membrane or a combination of both. On the other hand, by an electrically equivalent circuit, the muscle membrane is represented approximately as a condenser (membrane capacity) in parallel with a battery (membrane potential) with a series resistance (membrane resistance). In response to the transmitter substance the muscle membrane in the junctional region is shorted by the shunting resistance (FATT & KATZ, 1951). (In a more exact expression the shunting resistance has a series battery representing the equilibrium potential). According to the ionic theory the shunting resistance was explained by a sudden increase in the ionic fluxes across the membrane, e.g. possible ions are sodium and potassium or chloride in the slow muscle (BURKE & GINSBORG, 1956b). In this regard the augmentation of the s.j.p.’s may be caused by the following factors (OOMURA & TOMITA, 1961): (1) increase in the resting potential (2) increase in the membrane resistance (3) decrease in the membrane capacitance (4) decrease in the minimum shunting resistance or increase in the maximum ions permeability in the membrane in the junctional region: (i) increase in the released amount of transmitter substance; (ii) increase in the sensitivity of the receptor membrane to the transmitter substance; and (iii) inactivation of the cholinesterase at the junctional region (5) prolongation of the whole time course of the temporal change in the shunting resistance.

In these experiments the membrane resting potential was not affected by thyroxine, therefore factor (1) could be excluded. The value of the shunting resistance measured from the gradient of the relationship between the s.j.c. and the membrane potential significantly decreased to below half in the presence of thyroxine. Therefore, one of the reasons influencing the increase of the s.j.p. should be considered factor (4). However, the equilibrium potential of the s.j.c. obtained from the same relationship mentioned above, was not appreciably shifted by thyroxine. In frog’s fast muscles, TAKEUCHI & TAKEUCHI (1960) showed that the transmitter made the end-plate more permeable to sodium and potassium ions but not to chloride ions. They further indicated that the ratio of $g_{Na}$ to $g_{K}$ was 1.29 in the equilibrium potential. Although the relationship between the slow muscle fiber and the ion species is not yet confirmed, if the above is assumed to be adopted in the case of the slow muscle, the decrease in the value of the shunting resistance and no change in the equilibrium potential mean that the values of both $g_{Na}$ and $g_{K}$ increased, but the ratio of them remained constant. On the other hand, the Ach-potentials were significantly augmented by thyroxine. If thyroxine increased mainly the transmitter substance liberated from the presynaptic terminals and did not affect the postsynaptic membrane, the Ach-potentials would not be augmented. Furthermore, in thyroxine the strength of the Ach-driving current needed to produce the
Ach-potential of the same amplitude as in the control was only one seventeenth. If the increase in the sensitivity to the transmitter substance in the postsynaptic membrane is supposed to be seventeen folds, the amplitude of the s.j.c. would be about the same in relation. But the amplitude of the s.j.c. during the thyroxine treatment was only doubled. Consequently, it is more likely to assume that by thyroxine the amount of the released transmitter substance in response to the nerve stimulation may not be increased but rather reduced. These influences will be discussed later.

Under the voltage-clamp condition the effect of the membrane capacitance can be ruled out from the electric phenomena. Further, as shown in Fig. 4 the time constant of the falling phase of the s.j.c. did not increase, but rather decreased in the test solution, though the amplitude of the s.j.c. increased. Therefore, the action of cholinesterase in the neuromuscular junctional region seems not to be depressed by thyroxine. Concerning this evidence, it may be unconceivable that the membrane capacity was an attributable factor for the increase in the amplitude of the s.j.p.

From these points it may be concluded that the facilitation of the neuromuscular transmission was caused by a marked increase in the membrane resistance at rest and in the sensitivity in the receptor membrane to the transmitter substance.

One of the effects of thyroxine on the slow muscle membrane, is to increase the resting membrane resistance. This is similar to those of Ca or Ba ions reported previously by OOMURA et al. (1961b). Thyroxine is easily combined with the cations such as Ca, Ba or Mg (GEMMILL & PLUNKETT, 1952). Using I\(^{131}\)-labelled thyroxine and the rat diaphragm muscle HOGNESS et al. (1957) clearly showed not only its special bindings on the surface membrane of the muscle fibers, but also its significant entrance into the same. In this regard, thyroxine was supposed to combine first with the muscle membrane in some unknown (probably physicochemical) fashion and secondarily absorb Ca ions and manifest the effect of such ions as an increase in the membrane resistance. On the other hand, regarding the importance of Ca ions on the excitable membrane TOBIAS (1958) presented a scheme of the molecular structure of the membrane in which the skeleton of the membrane was composed of lipoprotein molecules combined with Ca ions. Then an alternative explanation may be presented for the increase in the resting membrane resistance, that thyroxine may combine with the muscle membrane at the sites where the Ca ions are combined, then quantitatively alter the fashion of the ions permeability in the membrane. Another conceivable explanation for that may be an acceleration in the active transport of such as sodium, potassium and other ions in the muscle membrane caused by incorporating thyroxine into the muscle fiber, since the effect of thyroxine on the muscle fiber was not too rapid. LAPLAUD & GARGOUIL (1960) postulated an activation of the potassium pump in the muscle membrane, after
recognition of an augmentation and prolongation of the negative after potential in the frog's sartorius muscle by triiodothyroacetate. This may not be conceivable, however, because the resting potential in thyroxine was not altered significantly.

Many reports have shown that thyroid hormones affect the function of the tonic as well as phasic muscle activities. Wolf (1943) found in the familial form of periodic paralysis, that thyroid medication benefited the paralytic attacks, although an adverse effect in the thyrotoxic group of this disease (Shinosaki, 1926; Robertson, 1954). Shy et al. (1961) reported that an increased weakness following withdrawal of 1-triiodothyronine on familial periodic paralysis was promptly reversed by its re-administration. From these points there were differences between the thyrotoxic and familial forms of periodic paralysis. Our analyses of the effects of thyroxine on the electric phenomena in the neuromuscular junctional region of the slow muscle, show possible mechanisms toward the favorable action of thyroid hormones on the familial form of periodic paralysis.

On the contrary, the adverse effect of an administration of the thyroid hormone in the thyrotoxic form of periodic paralysis might be explained as follows. It has not yet been proved, but it may be likely that the sensitivity to the transmitter substance in the neuromuscular region may not be increased but rather decreased, for example the 'desensitization', by continual applications of thyroid hormone due to thyrotoxicosis (Katz & Thesleff, 1957). Moreover, the amount of transmitter substance liberated from the nerve terminals that had been previously reduced by this hormone as discussed before, might be further reduced by a hormone treatment. These two may act together as the functional disturbances of neuromuscular transmission. However, further investigations to clear up this problem will be necessary in the future.

SUMMARY

The effects of $10^{-5}$ and $10^{-4}$ gm/ml sodium l-thyroxine on the bullfrog's slow muscle fibers innervated by the small motor nerve and on the neuromuscular transmission were investigated by means of the intracellular microelectrodes technique.

1. The slow muscle fibers were affected by more than $10^{-4}$ thyroxine-Ringer solution. The resting potential was slightly hyperpolarized but insignificantly. The effective membrane resistance, however, considerably increased to three folds of that in the control solution. The neuromuscular transmission was facilitated, i.e. amplitudes of the s. j. p.'s were augmented by 180%. The s. j. c. under the voltage clamp condition was also augmented by 240%, while their durations somewhat decreased.

2. Ach-potentials produced by the ionophoretic microapplications of acetyl-
choline are remarkably increased by $10^{-4}$ thyroxine.

3. From these evidences, it was concluded that thyroxine accelerated the neuromuscular transmission by both increasing the resting muscle membrane resistance and enhancing the sensitivity of the receptor membrane for the released transmitter substance from the nerve endings. It was also discussed that other factors such as the anticholinesterase-like action of thyroxine and an increase in the amount of the transmitter substance from the nerve endings were not conceivable.

4. The relationships between the thyroid hormones and the neuromuscular disorders in the cases of either the thyrotoxic or the familial form of periodic paralysis were discussed.

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REFERENCES


OOMURA, Y. AND TOMITA, T. (1961a). Some observations concerning the end-plate


