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Russell R. Rulon
Luther College

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RUSSELL R. RULON [1]

Abstract. Rats made hypothyroid by surgical thyroidectomy were compared with controls relative to the shortening velocity and maximum shortening ability of the intestinal smooth muscle and the gastrocnemius. Hypothyroidism showed some potentiation of maximum shortening, but no effect on the shortening velocity of the gastrocnemius. Intestinal smooth muscle maximum shortening as well as shortening velocity tend to be depressed. There is some evidence for a differential effect of thyroid hormone lack in the two muscle types.

This study has derived out of a desire to ascertain the effect of hypothyroidism on shortening velocity and maximum shortening of two contractile tissues, skeletal muscle and intestinal smooth muscle. It is often stated that thyroxin stimulates motor activity in the intestine and promotes vigorous skeletal muscle contraction (Gorbman, 1962). Lambert *et al.* (1951) studied the Achilles tendon reflex and have confirmed the impression that contraction is slow in most persons who are definitely hypothyroid. Because of a decreased tetanus fusion frequency, Schwartz and Lein (1955) found that in muscle from hypothyroid rats compared with euthyroid and hyperthyroid rats, the steady state tensions tend to be greater when stimulation frequencies are low.

MATERIALS AND METHODS

Male, Holtzman laboratory rats were used in this study. Seven thyroidectomized animals were prepared by standard procedures (Farris and Griffith, 1949). Six control animals were used. Approximately eight weeks elapsed between thyroidectomy and the measurement of the contractile parameters. Metabolic rates on both control and thyroidectomized animals were obtained by indirect calorimetry just prior to examining the contractile activity using a technique described by Watts and Gourley (1953). One modification of the technique which reduced exploratory activity and movement of the rat involved placing the rat in a light cotton sock prior to inserting the animal into the respirometer. This modification improved the precision of the five serial determinations of O_2 consumption which were used to compute the metabolic rate of each animal.

Measurement of contractile velocity and maximum shortening of the rat gastrocnemius was determined using standard, student

[1] Luther College, Biology Dept.

grade, isotonic muscle lever, kymograph, inductorium etc. The rats were anesthetized with sodium pentobarbital using a dosage of 40 mg./kg., given subcutaneously. The gastrocnemius was exposed and the tendon of Achilles was cut to include a portion of the calcaneium. The rat was fastened to a supporting board and the gastrocnemius was allowed to project down through a small opening. The femur was clamped and the muscle afterloaded with 50 grams. Force-velocity data was obtained on the muscles through a range of 50-550 grams. The velocity of shortening was determined by taking the maximum velocity indicated on the record for each weight.

The rat abdominal wall was then opened exposing the intestinal tract. A string was tied around the intestine next to the stomach and a second string was tied 2 inches down the duodenum. The segment was removed from the animal and immediately placed in aerated Locke's solution. The segment was placed in a tissue cup containing 37° C Locke's solution and its activity recorded using a heart lever and kymograph. The segment was stimulated with three serial additions of acetylcholine to the tissue cup, with the second and third additions of acetylcholine stimulating the segments in the presence of concentrations remaining from previous addition(s). Shortening velocities were obtained from the record. The serial additions of acetylcholine resulted in tissue cup concentrations of 3.5, 10.6 and 21.1 x 10^{-5} mg./ml. The segment was then placed in fresh Locke's solution and subjected to an acetylcholine stimulus of 14.3 x 10^{-5} mg./ml. The record of shortening in this case was used to compute the maximum shortening of the muscle.

Statistical tests were run by testing the significance of the difference of two means, comparing the control values with those from the thyroidectomized animals. An analysis of variance was done on the gastrocnemius, maximum shortening data.

Results

Metabolic rate data indicated that the control and thyroidectomized animals were significantly different ($P < 0.01$) relative to their metabolic rates. The six control animals had an average metabolic rate of 59.3 C/M²/Hour with a standard error of 4.3; whereas the seven thyroidectomized animals had a mean value of 45.6 C/M²/Hour and a standard error of 5.3.

Gastrocnemius, Force-Velocity Curves for control and thyroidectomized animals show no differences ($P = 0.7 - 0.9$) relative to shortening velocity at the eleven different loads tested. Table 1 shows the maximum shortening at loads tested. The thyroidectomized animals show greater overall shortening averages at all but the 550 gram load. An analysis of variance shows the

Table 1. Maximum shortening Averages Of The Gastrocnemius Subjected To Tetanic Stimulation Under Various Loads

Load (gms.)	Maximum Shortening (cm.)	
	Thyroidectomized (N = 7)	Controls (N = 6)
50	1.26	1.15
100	1.09	1.01
150	0.97	0.83
200	0.87	0.74
250	0.76	0.65
300	0.70	0.61
350	0.64	0.54
400	0.56	0.51
450	0.51	0.48
500	0.46	0.41
550	0.41	0.43

values are approaching significance, with the computed level of significance falling between 5 and 10 percent.

Shortening velocities of intestinal segments subjected to serial stimulation with acetylcholine are shown in Table 2. Although the numerical averages tend to indicate a slower contraction velocity in the thyroidectomized animals, the values were not shown to be significantly different.

Table 2. Average Shortening Velocities Of Two Inch Intestinal Segments Stimulated With Serial Applications of Acetylcholine.

Acetylcholine Concentration	Shortening Velocity ± S.E. (cm./sec.) Thyroidectomized (N = 7)	Control (N = 6)	P Value
3.5×10^{-5} mg./ml.	0.060 ± 0.007	0.075 ± 0.016	0.2
10.6×10^{-5} mg./ml.	0.041 ± 0.005	0.043 ± 0.012	0.9
21.1×10^{-5} mg./ml.	0.033 ± 0.002	0.050 ± 0.009	0.4

Table 3 indicates the average maximum shortening of the two inch intestinal segments when subjected to the 14.5×10^{-5} mg./ml. acetylcholine concentration. The average shortening of the segments from the thyroidectomized animals showed nearly a centimeter less contraction.

Table 3. Average Maximum Shortening Of Intestinal Smooth Muscle Segments Stimulated With Acetylcholine

Acetylcholine Concentration	Shortening ± S.E. (cm.) Thyroidectomized (N = 7)	Control (N = 6)	P Value
14.3×10^{-5} mg./ml.	1.59 ± 0.7	2.53 ± 0.6	< 0.3

Discussion

A technique for investigating the comparative mechanics of skeletal and intestinal smooth muscle of the rat utilizing standard student laboratory equipment has been described. Using the

techniques described, the intestinal smooth muscle seems to be somewhat more sensitive to the thyroid state than skeletal muscle. The data suggests that the hypothyroid state decreases the overall shortening capability of intestinal muscle, but potentiates the amount of shortening in skeletal muscle. This apparent potentiating effect might be an artifact associated with a decreased fusion frequency in the hypothyroid animal's muscles (Schwartz and Lein, 1955). Although there was no significant effect of the hypothyroid state on skeletal muscle shortening velocity, the intestinal smooth muscle did seem to be somewhat slower. This information may suggest a difference in the hormone-muscle interaction in the two tissues.

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