EFFECT OF THYMUS EXTRACT UPON ELECTROMYOGRAPHIC RESPONSES OF SKELETAL MUSCLE IN THE LIVING FROG

MARC L. LEAVITT, ALFRED W. RICHARDSON AND HAROLD M. KAPLAN
Department of Physiology, Southern Illinois University, Carbondale, Illinois 62901

ABSTRACT.—Electromyographic responses of the gastrocnemius muscle to injection of acetone-soluble extract of thymus glands extracted from calves were recorded on the intact living frog with bipolar electrodes. Eleven of 13 frogs injected with the extract showed a significant, although transient depression in their electromyographic responses. The thymus secretes some substance that depresses the activity of skeletal muscles. These responses, which help assay muscle activity, indicate the influence of the thymus upon muscle contraction and may ultimately be of practical importance in conditions such as myasthenia gravis.

Various attempts have been made to demonstrate the relation of thymus gland activity to skeletal muscle function. Thus, Adler (1938) induced a myasthenic reaction in the dog by injecting calf thymus extracts. Zacks and Cohen (1955) observed a decrease in muscle tension in the isolated frog sartorius muscle after injecting thymus extracts from myasthenia gravis patients. Wilson and Wilson (1955) produced paralysis of limb muscles in the mouse by injecting acetone extracts from fetal whale thymus. Nowell *et. al.* (1959) found that fetal whale thymus extracts depressed contraction of the isolated frog rectus muscle. Parkes and McKinna (1966) described a depression of contraction and of the action potentials of the isolated rat tibialis anterior muscle after injecting acetone extracts of thymus from myasthenia gravis patients. The present investigation examined the effects of injecting extracts of calf thymus on the electromyographic response (EMG) of the gastrocnemius muscle in the living intact frog.

METHODS AND MATERIAL

Electromyographic Technic

Frogs were anesthetized by intraperitoneal injection of 5.8 ± 1.2 mg/kg of sodium pentobarbital. The intact sciatic nerve of the continually moistened frog was stimulated tetanically with square-wave pulses of 2 msec duration from a Physiograph stimulator. A resistance of 800 ohms was placed in series with the electrodes so that the current remained relatively constant. The EMG was recorded from the gastrocnemius muscle and its tendon with a Hewlett Packard 141A storage oscilloscope with a 1422A time base and a 1401A amplifier.

Stimuli of increasing voltage (0.6 to 3 volts) were given at intervals of 5 minutes with about 20 to 30 stimuli until a maximal response resulted. The signal was stored on the oscilloscope and measurements were made of the first, tenth, and twentieth EMGs.

Method of Extraction

Thymus from calves was emulsified in a 1:5 w/v dilution in double distilled acetone, at 0°C (see Zacks and Cohen, 1955). All glassware used was packed with dry ice. The total volume of the filtrate was about 1 liter for every 200 gm of frozen thymus.

Liter aliquots of the filtrate were evaporated in vacuo at 37°C until the volume was 100-200 ml. The extract was transferred to a retort and acetone washings of the distillation flask were added. Continued evaporation at temperatures up to 47°C yielded a residue and vis-

cous fluid. The active thymus fraction was present in very small amounts. The 642 gm (frozen weight) of thymus yielded only 4 gm of residue and viscous fluid. The residue and fluid in 0.6% Ringer's solution will be called the acetone-soluble extract (ASE).

Biological Tests

Individual readings were taken and the values for all frogs averaged to obtain the untreated normal EMG. Additional sham controls were injected with 1 ml of Ringer's solution to find if the solvent had any effect on th EMG. Then, 13 untreated controls were given dorsal lymph sac injections of 1 ml of ASE and an average of 10 readings were obtained for each injected frog. The time during which the response to ASE was fully expressed is shown within a circle in Fig. 1.

Results

The difference in EMG between untreated frogs and frogs injected with Ringer's solution was not significant ($P > .01$).

There was no change from tetanic stimulation following solvent injection for periods up to 7 hours (Fig. 1). Thus, any weakening of response in the ASE treated frogs is not due to electrical stimulation.

The mean EMG of 13 untreated frogs was 4.57 mV. This value was significantly decreased to 2.30 mV ($P < .001$) after the injection of ASE. The average percentage drop in mV in the muscles was 52%.

With the exception of 2 frogs, Fig. 1 characterizes the response of ASE-injected frogs. The EMGs of 2 atypical frogs did not significantly change.

One frog of the 13 treated displayed an almost immediate response to ASE, a drop from 3.3 to 1.2 mV within 2 minutes after injection. The range of response in all other frogs was between 17 and 45 minutes, the period of maximum response being 20 to 40 minutes.

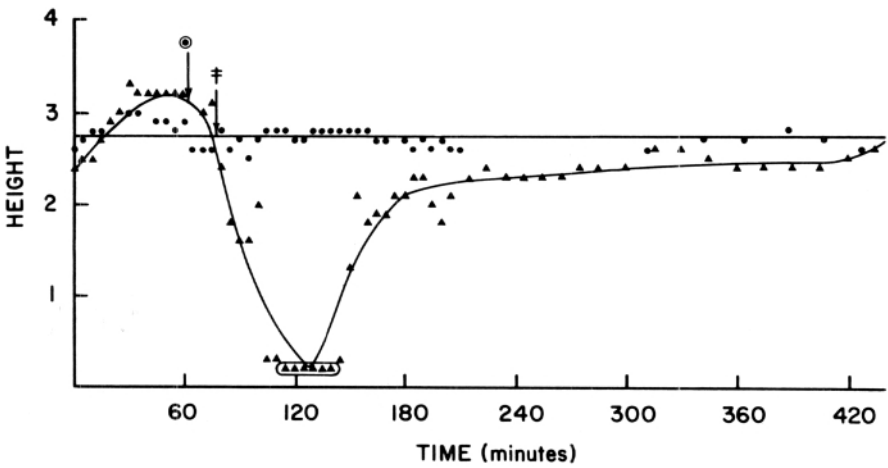

Figure 1.—Electromyographic response of a frog to thymus extract.
• = Frog injected with 0.6% Ringer's solution
△ = ASE injected frog
Arrows show time of injection; ‡ = Ringer's solution; ⊙ = ASE
Height = Electromyographic response in millivolts

Recovery, as seen in the EMG, then progressed to normal (Fig. 1).

Discussion

The significant transient depression in the EMG observed in living frogs following injections of acetone extracts from calf thymus correlates well with the findings of prior investigators (see Introduction). It is becoming increasingly evident that the thymus gland influences muscle contraction. The relationship has especial applicability to the understanding of myasthenia gravis.

Literature Cited

Adler, H. 1938. Physiologie und Pathologie des Thymus. Deutsch Ztschr. f. Chir., 250:614-649.

Nowell, P. T., A. Wilson, and H. Wilson. 1959. Some effects of foetal whale thymus glands. Quart. J. Exp. Physiol., 44:229-238.

Parkes, J. D. and J. A. McKinna. 1966. Effects of thymic extract on the neuromuscular junction. Nature, 214:1116-1117.

Wilson, A. and H. Wilson. 1955. The thymus and myasthenia gravis. Amer. J. Med., 19:697-700.

Zacks, S. and R. Cohen. 1955. Myasthenia gravis. I. Importance of potassium in inhibitory action of normal and myasthenic thymus extracts. Proc. Soc. Exp. Biol. Med., 90:601-606.

Manuscript received July 8, 1971.