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CONTRACTILE DEACTIVATION BY RAPID, MICROWAVE-INDUCED TEMPERATURE JUMPS

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Activation of muscle is controlled by Ca^{++} (released by changes in membrane potential), myofilament overlap, and muscle load. Understanding has been hindered by inability in many cases to achieve rapid changes in potential or intracellular concentration of agents. Biochemical approaches are hindered by the loss of the physiological ordered lattice and by loss of the coupling to external load.

A microwave temperature-jump system has been developed for the study of rapid-heating relaxation of single frog striated muscle cells, using a modified magnetron conditioner (4J50, 9.4 GHz, 200 kW peak power, 0.002 duty cycle) (Fig. 1). The isolated muscle cell is bathed in saline contained in an acrylic chamber (150 μl volume) passing through a waveguide (RG51) one quarter wavelength from the shorted end. Slits are provided to allow continuous measurement of sarcomere spacing by means of the diffraction of a HeNe laser beam, and isometric force is recorded using an AME silicon transducer element. Temperature increase was linear with duration of the heating train 0.2°K/ms, and cooling of the ambient temperature had a half-time exceeding 10 s. No cellular damage is apparent even with scores of T-jumps over some hours, if excessive heating (T <28°C) is avoided. Heating for 5–10 ms preceding a twitch altered the twitch in the same fashion as altered steady T; heating during the rising phase caused an increase in the rate of tension development, followed by the more rapid relaxation

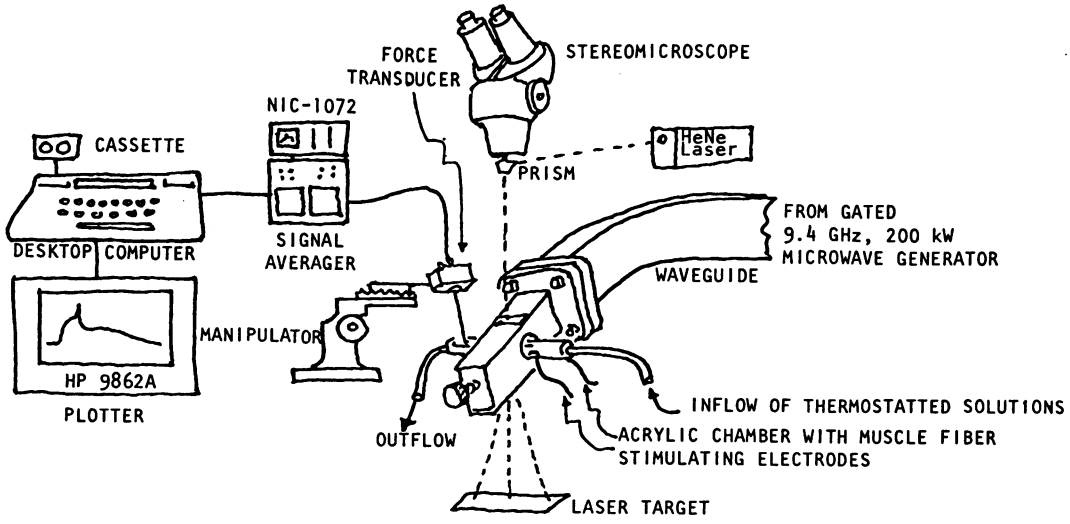

FIGURE 1

characteristic of twitches in warmer muscle; heating during relaxation resulted simply in more rapid relaxation. During the plateau of a tetanus, brief heating caused a two-phase rise to greater force. The initial phase of about 2% per degree, lagging slightly behind the heating (time constant ~5 ms at 10°C), varied with sarcomere spacing in the same way as tetanic force; the slower phase had a time constant of about 20 ms. Muscle fibers depolarized with elevated potassium concentration were briefly heated (10–20 ms) at the peak of force development. At maximal depolarization (100 mM K^+), there was a rapid rise to greater force, followed by the relatively slow spontaneous relaxation characteristic of the new T. At submaximal depolarization (25 mM K^+), there was a rapid activation beginning with the onset of heating and peaking in 50–80 ms, followed by a deactivation relaxation to the (lower) peak force characteristic of the new T at that depolarization. Both activation (order of 0.03/ms) and deactivation (order of 0.005/ms) rate constants decreased as the membrane potential (or preheating level of activation) became less negative. Speed of deactivation increased with increasing sarcomere spacing, in contrast to the rate of relaxation from tetanic contractions.

There are thus three major processes to be studied by this method, empirically identified by the time constants at 10°C—5 ms, 30 ms (activation), and 100–200 ms (deactivation). The practical limit of resolution with single-sweep operation is presently processes with time constants of 5 ms or greater. With signal averaging the present limit is 1 ms, a time scale at which the frequency response of the present transducer is itself limiting.

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