CHANGES OF STIFFNESS OF SKELETAL MUSCLE DURING LATENCY RELAXATION

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Summary: Changes of stiffness of muscle are measured during latency relaxation of frog toe muscle, using a piezo-electric device that imposes sinusoidal length changes of small amplitude and high frequency (kHz) on the muscle. It is shown that a decrease in stiffness is associated with the beginning of latency relaxation and that there is an increase in stiffness before the onset of the contractile force development.

It is suggested that the latency relaxation (LR) of skeletal muscle is due to changes in the elastic properties of intracellular structures which contribute to the resting force of muscle. There are, however, different opinions whether the LR originates in the contractile filaments or in the longitudinal sarcoplasmic reticulum (1, 2, 3). In order to further clarify this phenomenon it is necessary to investigate the stiffness of muscle during LR.

As early as 1950 Hill (4, 5) demonstrated that there is an increase in stiffness of muscle before the onset of force development after stimulation, but up to now there have been no investigations in which the changes of stiffness are correlated to LR. We have, therefore, developed a method, by means of which changes of stiffness of muscle during LR may be measured with great accuracy. This paper deals with the method and our first results.

Method: Generally the stiffness of muscle is measured from the changes of force which result from sinusoidal length changes

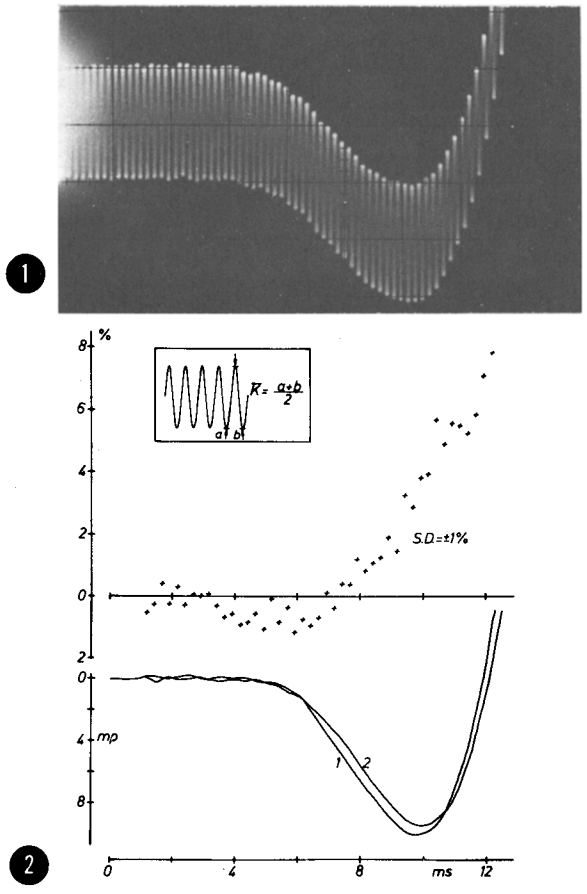

Fig. 1
Original photograph of the latency relaxation curve with the sinusoidal length change superimposed

Temperature: 3.5° C; Calibration box, horizontal: 2 ms
vertical : 2.6 mp (for LR)

Fig. 2
Changes in stiffness during latency relaxation

upper part:
ordinate: Per cent deviation of the mean stiffness of the stimulated muscle from the mean stiffness of the resting muscle. Each cross represents the mean of six experiments on one representative muscle. Crosses below the zero line show negative deviation. S.D.: standard deviation
lower part:
First [1] and last [2] latency relaxation out of a series of a total of 12 experiments (6 with and 6 without sine wave).
ordinate: Change of force during latency relaxation [mp] .
abscissae: Time [ms] . Calibration is identical for the upper part and the lower part of the figure. The origin of the abscissa coincides with the beginning of the stimulus.
Inset:
Scheme for the evaluation of stiffness, see text.
Temperature: 5.5° C

imposed on muscle. Because of the very small decrease in force during LR (a few mp) and its rapid time course (duration: a few ms), common electro-mechanical vibrators cannot be used here. We therefore used a piezoxide which is able to oscillate with very small amplitude in the range of some kilohertz.

The experiments are performed on isolated M. ext. brev. prof. digiti IV, caput. acc. of Rana esculenta (winter frogs). Leaving a small piece of metacarpal bone at its proximal end, the muscle is attached to a small steel hook by means of this. The hook is glued to the centre of a piezoxide device (PXE 5, Valvo). This may be moved along the long axis of the muscle by means of a micrometer screw so that the length of the muscle may be accurately adjusted. The piezoxide is activated by a sinusoidal voltage. The experiments are performed at constant oscillation amplitudes in the range of 2 - 5 μ, the oscillation frequency being held constant at 4 kHz. By means of its distal tendon, the other end of the muscle is attached to the stylus of a RCA 5734 transducer tube. The output of the transducer tube is fed into two amplifiers set to different sensitivity in order to display the LR and the resting force simultaneously on the two beams of an oscilloscope. Responses are then photographed. The whole device is isometric with respect to the muscle. For further details about both the recording of LR and the preparation see (3). Up to now the experiments have been performed at constant temperatures lying between 3.5 and 5.5° C. The muscle is stimulated with supramaximal single rectangular wave pulses (duration: 0.7 ms, frequency: 1 pulse/ 2 min) via massive Pt-electrodes. Composition of the physiological buffer solution (mM): NaCl, 110; KCl, 2.5; $CaCl_2$, 2.5; Na_2HPO_4, 2.9; NaH_2PO_4, 0,85. The solution is saturated with O_2.

After being placed in the small, solution-filled experimental chamber, the isolated muscle is stretched to about 130 % of its in situ-length and allowed to equilibrate for about 1.5 hours at the set temperature. After this the muscle is released and adjusted to a resting force of 1 p which is held constant during the whole experiment.

Evaluation of results: Stiffness of muscle is defined as maximum of force minus minimum of force, divided by the superimposed length change. In order to correct distortions of the sinusoidal force changes which are caused by the decrease and increase of force during LR, it is necessary to calculate a mean difference of force per period of the sine wave ($\overline{K}$, see inset in Fig. 2). The stiffness, so defined, is stated as a per cent deviation from the mean stiffness of the resting muscle (mean calculated from 30 periods, registered a few seconds before the stimulation of the muscle).

Results and discussion: Fig. 1 shows an original tracing of the LR of a muscle with the sinusoidal length change superimposed. Fig. 2 shows the first [1] and the last [2] LR (lower half of fig.) from a series of experiments on one muscle, as well as the corresponding change of stiffness (upper half of fig.) which is plotted in the way described previously. The result is that within a period of 3 - 4 ms at the point where the LR begins, the mean stiffness of the muscle diminishes by a small but significant amount. The stiffness of the muscle increases above the mean resting value at about 7 ms after stimulation. This beginning of the increase in stiffness coincides, at the temperature used here, with the point of the maximum velocity of force decrease of the LR.

The latter result is contradictory to the conclusions drawn

from similar experiments on the M. scapulo antebrachii of Testudo graeca (6). It agrees, however, with the results obtained from quick-release experiments carried out on the M. sartorius of Bufo spec. and Rana spec. (4, 5). There is also good agreement with investigations carried out on single fibres of the M. semitendinosus of Rana temporaria (7) which show that the initial phase of the "active state" begins very soon after stimulation. The results obtained in studies concerning the intracellular kinetics of activator calcium (8, 9, 10, 11) have demonstrated that a rapid Ca^{++} transient occurs in immediate response to the electrical stimulus. These results, too, are in accordance to our findings.

There is a decrease in stiffness which starts even before the first downward deflection of the LR. Thus it may be assumed that there is a close connection between this response and the cause underlying the LR. Our findings are in accordance with the suggestion that LR and contraction originate in different intracellular structures. They agree well with the hypothesis (1) that the LR of skeletal muscle is caused by a loss of tension in the longitudinal sarcoplasmic reticulum.

The experiments are being continued.

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