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SHORTENING INDUCED DEACTIVATION IN PIG URINARY BLADDER: CHAOS AND CALCIUM

Aims of Study

When a muscle is allowed to shorten during an active contraction, the maximum force attained after shortening is smaller than the isometric force at the same muscle length without prior shortening. This shortening induced deactivation was found to depend both on shortening amplitude and shortening velocity [1]. Furthermore it was shown that the intracellular calcium concentration after shortening is positively related to the shortening amplitude [2] and that a sudden change in muscle cell length may lead to disorganization of actin and myosin filaments [3]. In a previous study [4] we found that the shortening induced deactivation increased with increasing shortening amplitude. We explained the deactivation by a combination of the rearrangement of actin and myosin and the intracellular concentration of calcium. We hypothesized that after shortening, the increase of the intracellular calcium concentration quickly freezes the existing disorganized actin-myosin cross-bridges thereby preventing the formation of new ones. In this study we investigated the relationship of force amplitude (number of cross-bridges) during shortening and shortening induced deactivation.

Method

Fourteen muscle fibers (3x 0.7 mm) from 4 pig urinary bladders were subjected to a stop test. Each fiber was clamped between two pairs of tweezers: one linked to a force transducer and the other to a length controller. The initial length was the length at which a slight stretch resulted in a passive force of 150 μN. The muscle fibers were fixed at a stop length (l_{stop}) defined as 200% of the initial length. The fibers were electrically stimulated at a pre-set start length (l_{start}) for 30 s. Shortening started 2, 3, 5, 8, 11 or 14 s after the stimulation. The fibers were shortened from l_{start} to l_{stop} with a shortening velocity of 50 or 100 μm/s during 4 seconds. L_{start} was defined as l_{stop} + 4x shortening velocity. The maximum force attained during stimulation before shortening was called F_{max}. The force attained at the end of shortening was called F_{short}. The force attained during ongoing stimulation after shortening was called the redevelopment force F_{red} (Fig. 1). After 5 min the fibers were stimulated again for 14 s at l_{stop} to establish the isometric force (F_{iso}). F_{max}-F_{short} was considered as a measure for the number of detached cross-bridges as a result of shortening and F_{red}/F_{iso} was considered as a measure for shortening induced deactivation. A bi-exponential curve was fitted to F_{red}. This resulted in two force redevelopment amplitudes F_{red1} and F_{red2} and corresponding time constants τ_1 and τ_2.

Results

With increasing delay between stimulation and shortening ($\Delta t_{stim-short}$), F_{max}-F_{short} increased and F_{red}/F_{iso} decreased (Fig. 2). The value of τ_1 (mean $\pm$ SD: 2.4 $\pm$ 0.7s) is similar to the time constant of an isometric contraction. Presumably F_{red1} describes the development of force related to the influx of extracellular calcium [5]. Since τ_2 is smaller (0.5 $\pm$ 0.2s), we assume that F_{red2} describes the force development related to calcium already present in the cytoplasm. The contribution of the two force components to the redevelopment force was calculated as a percentage of the total. %F_{red1} remained larger than %F_{red2} at all $\Delta t_{stim-short}$ but decreased with increasing $\Delta t_{stim-short}$ (%F_{red1}(2s): 91 $\pm$ 8, (14s): 84 $\pm$ 13, %F_{red2}(2s): 15 $\pm$ 5, (14s): 21 $\pm$ 13).

Conclusion

Presently we find that shortening induced deactivation increases with increasing $\Delta t_{stim-short}$. This is explained by the fact that with increasing $\Delta t_{stim-short}$ the difference between the maximum and the shortening force increases. This force difference represents the number of cross-bridges that is detached. Presumably, more detached cross-bridges lead to more free intracellular calcium. We hypothesize that the chaos due to the disorganization of actin and myosin is 'frozen' by the high calcium concentration. Cross-bridges remain attached which

prevents other cross-bridges from being formed. This hypothesis is supported by the results of the bi-exponential fit. With increasing $\Delta t_{stim-short}$ the slow force component $\%F_{red1}$ decreased whereas the fast component $\%F_{red2}$ increased. In other words, the role of extracellular calcium decreased and that of intracellular calcium increased.

Obviously, chaos and calcium are intimately related. In future experiments we will vary the calcium concentration to further unravel the processes involved in shortening induced deactivation.
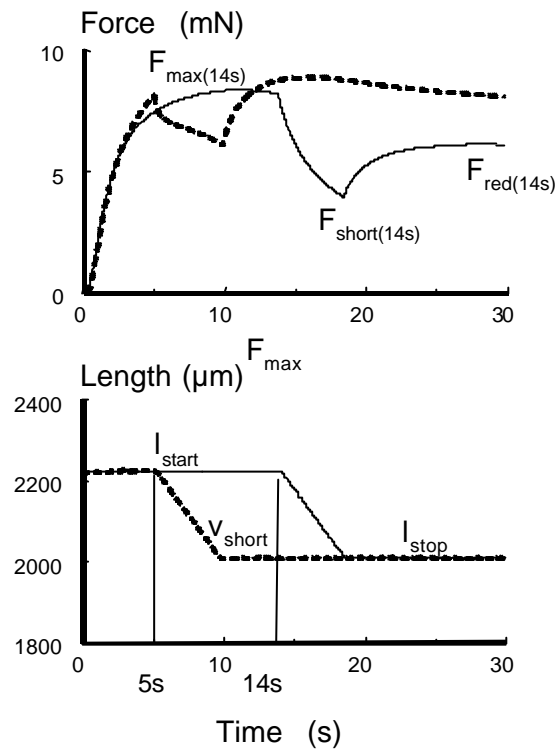
Fig. 1   **Fig. 2**
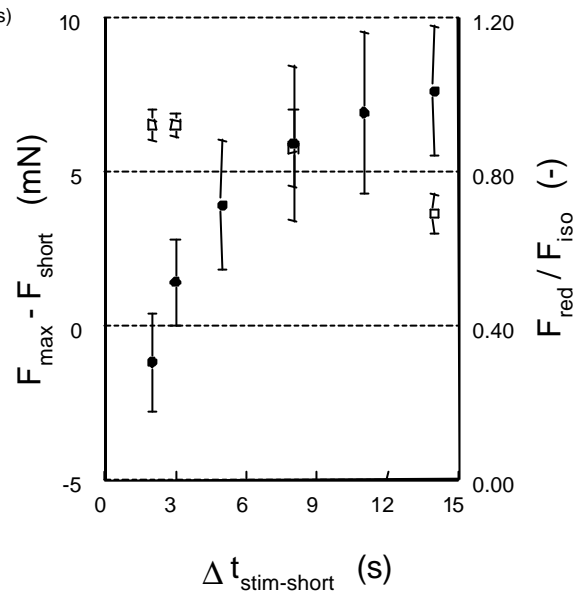
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