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 (Lstop) defined as 200% of the initial length. The fibers were electrically stimulated at a pre-set start length (Lstart) for 30 s. Shortening started 2, 3, 5, 8, 11 or 14 s after the stimulation. The fibers were shortened from Lstart to Lstop with a shortening velocity of 50 or 100 μm/s during 4 seconds. Lstart was defined as Lstop + 4x shortening velocity. The maximum force attained during stimulation before shortening was called Fmax. The force attained at the end of shortening was called Fshort. The force attained during ongoing stimulation after shortening was called redevelopment force Fred (Fig. 1). After 5 min the fibers were stimulated again for 14 s at Lstop to establish the isometric force (Fiso). Fmax/Fshort was considered as a measure for the number of detached cross-bridges as a result of shortening and Fred/Fiso was considered as a measure for shortening induced deactivation. A bi-exponential curve was fitted to Fred. This resulted in two force redevelopment amplitudes Fred1 and Fred2 and corresponding time constants τ1 and τ2.

Results
With increasing delay between stimulation and shortening (Δtstim-short), Fmax-Fshort increased and Fred/Fiso decreased (Fig. 2). The value of τ1 (mean ± SD: 2.4 ± 0.7s) is similar to the time constant of an isometric contraction. Presumably Fred1 describes the development of force related to the influx of extracellular calcium [5]. Since τ2 is smaller (0.5 ± 0.2s), we assume that Fred2 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. %Fred1 remained larger than %Fred2 at all Δtstim-short but decreased with increasing Δtstim-short (%Fred1(2s): 91 ± 8, (14s): 84 ± 13, %Fred2(2s): 15 ± 5, (14s): 21 ± 13).

Conclusion
Presently we find that shortening induced deactivation increases with increasing Δtstim-short. This is explained by the fact that with increasing Δtstim-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 $\Delta t_{\text{stim-short}}$ the slow force component $\%F_{\text{red1}}$ decreased whereas the fast component $\%F_{\text{red2}}$ 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**

![Graph 1](image1)

**Fig. 2**

![Graph 2](image2)

**References**

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