van Asselt E\textsuperscript{1}, Pel J \textsuperscript{1}, van Mastrigt R \textsuperscript{1}
1. Erasmus MC

SHORTENING INDUCED DEACTIVATION IN URINARY BLADDER SMOOTH MUSCLE

Hypothesis / aims of study
When 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 phenomenon has been described in striated, heart and smooth muscle and is called force depression, force deficit or shortening induced deactivation. Several hypotheses have been presented to explain shortening induced deactivation \cite{1,2}. They involve the attachment/detachment of cross bridges, the reorganization of myofilaments and the availability of calcium. We studied deactivation in pig urinary bladder muscle using the stop test and focussed on the effects of shortening amplitude and the interruption of stimulation immediately after shortening.

Study design, materials and methods
Small muscle bundles (~ 0.7 x 3 mm) were dissected from the anterolateral wall of the pig urinary bladder. Bundles were clamped between two tweezers, one connected to a force transducer and one to a length controller. The bundles were stimulated electrically using two platinum electrodes. The stop test (Fig.1) was performed with the stimulation turned off and on, to measure passive and total force (the difference was called active force).

Bundles were slowly stretched from stop ($l_{\text{stop}}$) to a start length ($l_{\text{start}}$). $l_{\text{stop}}$ was defined at 200% of slack length.

At stimulation, the active force increased to a maximum $F_{\text{max}}$. After 14 s, the bundle was shortened for 4 s with velocity $v_{\text{short}}$ from $l_{\text{start}}$ to $l_{\text{stop}}$ and force decreased to $F_{\text{min}}$. Then force redeveloped to $F_{\text{red}}$ and stimulation was stopped. After 5 min, the bundle was stimulated again to measure the maximum force $F_{\text{iso}}$ at $l_{\text{stop}}$.

Deactivation $F_{\text{sid}}$ was defined as $F_{\text{red}} / F_{\text{iso}}$.

In series 1, 20 bundles were subjected to a shortening amplitude ($l_{\text{short}}$) of 100, 200, 300, 400 and 700 µm. In series 2, 19 bundles were shortened for 4 s at 100 µm/s and the stimulus was interrupted immediately after shortening for 2, 3, 4, 6, 12 or 22 s ($t_{\text{int}}$). Contraction at $l_{\text{start}}$ and $l_{\text{stop}}$ and redevelopment contractions were fitted with multi-exponential functions to study the rate of force development (Fig. 2).

Results
In series 1, $F_{\text{sid}}$ decreased from 0.86 ± 0.10 (mean ± SD) to 0.69 ± 0.08 with $l_{\text{short}}$ ranging from 100 to 700 µm (ANOVA p<0.001). In series 2, $F_{\text{sid}}$ increased from 0.79 ± 0.08 to 1.13 ± 0.15 with $t_{\text{int}}$ ranging from 0 to 22 s (ANOVA p<0.001) (Fig. 3). The contractions at $l_{\text{start}}$, after interruption of the stimulus and 5 min after the stop test were best fitted with a mono-exponential function with time constants $\tau_{\text{max}} = 3.3 \pm 0.7$ s, $\tau_{\text{redint}} = 2.7 \pm 1.2$ s and $\tau_{\text{iso}} = 3.1 \pm 0.7$ s. Redevelopment contractions without interruption were best fitted with a bi-exponential function.
function with $\tau_{\text{red}1} = 3.7 \pm 1.7 \text{s}$ and $\tau_{\text{red}2} = 1.2 \pm 1.9 \text{ s}$. When fitted with a mono-exponential function, $\tau_{\text{red mono}}$ was $3.0 \pm 1.7 \text{ s}$. There was no significant correlation between $\tau_{\text{red mono}}$, $\tau_{\text{red}1}$, $\tau_{\text{red}2}$ and $t_{\text{short}}$ or between $\tau_{\text{red mono}}$, $\tau_{\text{red int}}$ and $t_{\text{int}}$ (Pearson’s correlation test).

**Interpretation of results**

Presumably, due to the relatively fast active shortening, the contractile filaments become disorganized, and the larger the shortening amplitude, the larger the disorganization, the larger the deactivation. When the stimulus is interrupted, (all) cross bridges detach enabling filaments to reorganize, resulting in better redevelopment. The two time constants during redevelopment without interruption suggest two different processes: relatively slow recruitment of calcium released from intracellular and/or extracellular sources and the immediate availability of cytoplasmic calcium released during active shortening [3]. When the stimulus is interrupted, the calcium released into the cytoplasm may be taken up into the sarcoplasmatic reticulum, which explains the finding of only one slow time constant during the following force redevelopment.

**Concluding message**

Shortening induced deactivation also occurs in the pig urinary smooth bladder muscle. It increases significantly with the shortening amplitude and decreases significantly with stimulus interruption time after shortening. During an isometric contraction, force development can be described by one time constant. After active shortening, a second process becomes involved in the redevelopment of force. We presume that this (faster) process is driven by the immediate availability of cytoplasmic calcium. Future experiments will focus on the effect of the calcium concentration (extracellular and intracellular) on deactivation using the stop test.

**References**