PROTOCOLS FOR ELECTROPHYSIOLOGY STUDIES OF MUSCLES EX VIVO – IN GENERAL

Dystrophic/mechanome affected muscles (5 minutes between every protocol/TREAT-NMD)

- Twitch (base tension)
- 5x ISO (to determine the specific muscle force)
- 5x ECC (loss of force under stress)
- 5x ISO (ability to recover after stress, reversible/irreversible loss of force)

Metabolic affected muscles – Glycolysis /glycogen decomposition (can be blocked in muscles)

- Twitch (base tension)
- 5x ISO (to determine the specific muscle force)
- Fatigue (ability to resist fatigue)

Metabolic affected muscles – Fatty acid conversion (cannot be less than 0, = fatal)

- Twitch (base tension)
- 5x ISO (to determine the specific muscle force)
- Fatigue – long protocol to glycogen depletion until the curve flattens out
- 5x ISO (short stimulation 200ms)

Ion channel defects – force affected

- Twitch (base tension)
- 5x ISO (to determine a specific muscle force)
    - Force-velocity determination (myofibrillary force fusion)
    - Hill-coefficient (gradient – flatter top = decreased recruitment of myofibrils)
- Fatigue (can be varied but indicates if the fiber repolarizes correct and in-time for a new depolarizing).

Ion channel defects – indirect force affected

- Twitch (base tension)
- 5x ISO (to determine a specific muscle force)
- Fatigue (can be varied however the idea is to look into if calcium-homeostasis or other ion-homeostasis is affected). Therefore select/design the protocol in relation to calcium-kinetics in muscles – release of Ca^{2+} through RYR1 and ATP-demanding uptake through SERCA into SR.
- The literature is expansive on this topic.

Myofibrillary myopathies

- Twitch (base tension)
- 5x ISO (to determine a specific muscle force)
    - Force-velocity determination
- 5x ECC (loss of force under stress)
- 5x ISO (ability to recovery, reversible/irreversible loss of force)
- Passive shortening is made separate and not in connection with the above. Deterimines the impact of mechanical changes of the sarcomere structure. May be combined with passive lengthening for a loop. Active /stimulated shortening can also be made but might be more difficult to interpret.

As the motor and stimulation are not required in all protocols, it is possible to examine ultrastructural damage in a reproducible way to use the chamber to make the damage with the motor, without making a force measurement, and then measuring the uptake of e.g. Procion orange, which is a membrane exclusion dye, i.e. it cannot penetrate healthy fibers.

This can be varied with stimulation, again without doing force measurement, but afterwards one looks at which cellular systems are affected by intense work (8 stimulations) or ultrastructural damage.

The major advantage of the 840MD is that non-stimulating protocols can be programmed into the 840MD as easy as protocols for force measurements that involves a stimulus to achieve reproducible results.

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