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Diaphragm Skeletal Muscle Preparation

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Manuscript citation:
For isolated diaphragm skeletal muscle preparation, male Swiss mice of 90 to 120 days old were euthanized by cervical dislocation, the hemi-diaphragms were dissected and removed, before the washing with Tyrode nutrient solution (135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2.6\ H_2O$, 15 mM $NaHCO_3$, 2 mM $NaH_2PO_4\ H_2O$, 2 mM $CaCl_2.2\ H_2O$, 11 mM D-glucose in distilled water, pH 7.4), and mounting accordingly to previously described by Duarte et al. [25]. Briefly, each hemi-diaphragm segment was transferred to an organ bath filled with 5 mL of Tyrode's solution at 37ºC, and the dissected tissue was tied up to a holder fixed at the bottom of the glass organ bath, while the upper extremity of the tissue was fixed to a force transducer, adjusted to impose an optimal tension (*i.e.* a condition in which the maximum contraction of each skeletal muscle preparation is observed). The contraction of the diaphragm muscle was induced by transmural electrical stimuli conducted through the platinum electrodes, at a frequency of 0.1 Hz and under supramaximal voltage, with duration of 2 ms each. After 30 min of stabilization of contraction amplitudes, to avoid the interference of acetylcholine released from the presynaptic terminals, the nicotinic acetylcholine receptor antagonist *d*-tubocurarine (1 µM) was added to the preparations. After further 30 min of stabilization of contraction amplitudes now in the presence of *d*-tubocurarine, the tension of the preparation was rearranged. The influence of crotamine on the isometric twitch contraction was evaluated after confirming the stabilization of contraction amplitude for additional one hour and half, followed or not by the addition of K^+ ion channel blockers [26], one hour before the end of experiment, as schematically demonstrated in the graphic below.

Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: skeletal muscle, ex vivo assay, diaphragm skeletal muscle preparation, isolated diaphragm skeletal muscle preparation, diaphragm muscle, contraction of the diaphragm muscle, diaphragm segment, platinum electrode, organ bath, transmural electrical stimuli, tissue, dissected tissue, force transducer, upper extremity of the tissue, glass organ bath

Abstract

For isolated diaphragm skeletal muscle preparation, each hemi-diaphragm segment was transferred to an organ bath, and the dissected tissue was tied up to a holder fixed at the bottom of the glass organ bath, while the upper extremity of the tissue was fixed to a force transducer. The contraction of the diaphragm muscle was induced by transmural electrical stimuli conducted through the platinum electrodes.

Materials

MATERIALS

- Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**7558-79-4
- NaHCO3
- KCl
- CaCl2
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- MgCl6.H2O
- d-glucose

Troubleshooting

1

1 The animal was euthanized by cervical dislocation

2 the diaphragm muscle was removed and transferred to a petri dish containing Tyrode's solution

3 A 1.5 cm segment of muscle diaphragm containing the central tendon and the ribs was used.

4 rib was gently pinned to a holder fixed in the bottom of an organ bath filled with 5 ml of Tyrode's solution, while the central diaphragm tendon portion was attached to a PowerLab force transducer, which was maintained at 30°C and continuously gassed with 95% O2/5% CO2.

5 Isometric twitch contraction was elicited by electrical stimulation of muscle strips through silver electrodes, with 0.1 Hz frequency, 2 ms duration, and supramaximal voltage;

6 Tissue was rinsed four times with Tyrode´s solution and incubated with 1 µM d-tubocurarine

7 After a 20 to 30-min stabilization, muscle length was readjust to give an optimal twitch tension, and 30 min later the effect of drugs was investigate.

8

Tyrode solution

9 mix in distilled water

[M] 11 Mass Percent D-glucose
[M] 135 Mass Percent NaCl
[M] 5 Mass Percent KCl
[M] 0 Mass Percent MgCl6.H2O
[M] 2 Mass Percent CaCl2
[M] 0 Mass Percent Na2HPO4. H2O

[M] 1 Mass Percent NaHCO3

10