untitled protocol

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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.

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Materials

Sodium phosphate dibasic 7558-79-4 by Sigma Aldrich
NaHCO3 by Contributed by users
KCl by Contributed by users
CaCl2 by Contributed by users
NaCl 53014 by Sigma Aldrich
MgCl6.H2O by Contributed by users
d-glucose by Contributed by users

Protocol

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Step 1.

The animal was euthanized by cervical dislocation

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

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Step 3.

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

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Step 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.

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Step 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;

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Step 6.

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

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Step 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.

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Step 8.

Tyrode solution

Step 9.

mix in distilled water

CONCENTRATION
11 Mass Percent Additional info: D-glucose
CONCENTRATION
135 Mass Percent Additional info: NaCl
CONCENTRATION
5 Mass Percent Additional info: KCl
CONCENTRATION
0 Mass Percent Additional info: MgCl6.H2O
CONCENTRATION
2 Mass Percent Additional info: CaCl2
CONCENTRATION
0 Mass Percent Additional info: Na2HPO4. H2O
CONCENTRATION
1 Mass Percent Additional info: NaHCO3

Tyrode solution

Step 10.