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Mouse Heart Perfusion - Ultrastructural Analysis (Using Karnovsky's Fixative)

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Protoplasmic Astrocytes in CA1 Stratum Radiatum Occupy Separate Anatomical Domains

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Protocol status: Working

We use this protocol and it's working

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Abstract

Optimal fixation for mouse tissue analysis.

Materials

- 1) (Cacodylic acid, sodium salt trihydrate: Ted Pella, cat # 18851)
- 2) (Paraformaldehyde, Prills: EMS cat. #19202)
- 3) 25% glutaraldehyde (EM grade). (Ted Pella, Cat. # 18426)
- 4) Detrose
- 5) Drug: Nembutal "Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml).
- 6) 2% Lidocaine hydrochloride
- 7) 1 N NaOH
- 8) 95% O₂/5% CO₂ gas tank
- 9) Ringer's solution
- 10) NaCl (79.8 g/L stock, 4 °C fridge)
- 11) Na₂HPO₄ (18 g/L stock, room temperature)
- 12) KCl (37.5 g/L stock, 4 °C fridge)
- 13) MgCl₂ . 6 H₂O (20.0 g/L stock, 4 °C fridge)
- 14) CaCl₂ X 2H₂O (30.0 g/L stock 4 °C fridge)
- 15) NaHCO₃ (50.0 g/L stock, 4 °C)
- 16) 25 gauge needle
- 17) 3X125ml Erlenmeyer flask
- 18) 100ml Erlenmeyer flask
- 19) water bath
- 20) #1 Whatman filter
- 21) Funnel
- 22) Parafilm
- 23) Heating Plate
- 24) Heprin (1,000 use units per mL)
- 25) Perfusion pump
- 26) Surgical instruments: large and small scissors, hemostat, tweezers, and iris scissors.
- 27) Stir bar

Safety warnings

 Make sure perfusion is done in fume hood. Wear PPE.

Ethics statement

Protocol Number: S06211

Nembutal "Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml).

Preparing Karnovsky's Modified Fixative Solution

- 1 For 100 ml of **2.0% paraformaldehyde - 2.5% glutaraldehyde** in 0.15M sodium cacodylate buffer, pH 7.4 with 2mM CaCl_2 :
- 2 Add 2 g prills paraformaldehyde into 125ml Erlenmeyer flask. (**Paraformaldehyde, Prills: EMS cat. #19202**)
- 3 Add 30 ml of ddH₂O to flask.
- 4 Add about 3-5 drops of 1 N NaOH and allow to mix with stir bar on heating plate at 60-65°C until solution clears.
- 5 Filter cleared solution through a #1 Whatman filter (using funnel) into a 100 ml graduated cylinder.
- 6 Add 50 ml 0.3M cacodylate stock solution (pH 7.4) (**Cacodylic acid, sodium salt trihydrate: Ted Pella, cat # 18851**)
- 7 Add 10 ml of **25% glutaraldehyde (EM grade)**. (**Ted Pella, Cat. # 18426**).
- 8 Add 1 mL of 0.204 M $\text{CaCl}_2 \times 2\text{H}_2\text{O}$.
- 9 Bring total volume to 100 ml with ddH₂O and check pH is 7.4.
- 10
 1. Pour into 100ml Erlenmeyer flask, cover with Parafilm and keep at 35°C in a water bath for perfusion.

Preparing Ringer's Solution

- 11 For 100 ml:

Add the following components:
 - 9.9 ml NaCl stock solution (79.8 g/L; 1.365M)
 - 1.0 ml Na_2HPO_4 stock solution (18 g/L; 0.127M)

1.0 ml KCl stock solution (37.5 g/L; 0.503M)
1.0 ml MgCl₂ · 6 H₂O stock solution (20.0 g/L; 0.098M)
2.5 ml NaHCO₃ stock solution (50.0 g/L; 0.595M)

- 12 Bring total volume to 95 ml.
- 13 Add 0.2 grams dextrose.
- 14 Bubble 95% air/5% CO₂ through the solution @ 35°C for at least 5 min.
- 15 After bubbling with CO₂, add 1.0 ml CaCl₂ · 2 H₂O stock solution (30.0 g/L; 0.204M).
- 16 Add 1.0 mL of 2% Lidocaine (anesthetizes smooth muscle) and 2.5 mL of 1000U/mL heparin (prevents blood clotting).

Perfusion

- 17 Turn on pump and allow fix solution to reach valve. Turn valve to Ringer's and set pump rate to proper setting depending on size of animal. Watch and make sure that all air is removed from the line.
- 18 For mice, use 25 gauge needle.
- 19 Set out the necessary surgical instruments: large and small scissors, hemostat, tweezers, and iris scissors.
- 20 Anesthetize animal by giving an intraperitoneal injection of 0.05ml "Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml). Ensure the needle is in the peritoneal cavity by drawing back needle and checking for air (no blood).
- 21 Allow several minutes for anesthetic to take effect and test depth of anesthesia using tail pinch and paw prick. Ensure that breathing does not stop.
- 22 Secure limbs of animal. Using small scissors and tweezers, lift up skin below rib cage and cut until liver is visible. Cut upward along sides of body cavity until diaphragm is visible. Try to avoid injuring organs. Cut diaphragm and then *quickly* cut up along sides



of body, being very careful not to damage heart and trying to avoid lungs. Once heart is sufficiently exposed, use hemostat to pull skin up over rat's head. Place needle into left ventricle and then cut right atrium with iris scissors. Turn on flow of Ringer's solution. The **heart should continue to beat and liver should quickly turn pale in color.** Perfuse with Ringer's for about 1 minute at a rate of approximately 5 mL / minute. Switch valve on pump to fix solution. The heart should stop beating and the body should begin to stiffen. Perfuse with fix solution for 10 minutes. Take needle out of heart.

- 23 Dissect the brain without any artificial damage and place in vial containing same fixative and keep at 4°C for 2 hours.

Protocol references

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