In Vitro test system for the screening of histamine H₃ antagonists

This protocol describes a standard in vitro test on guinea pig jejunum based on the inhibitory effect of (R)-α-methylhistamine (RAMH) on the electrically-evoked contractile response of isolated guinea pig jejunum.

1. Tissue preparation

- Male Hartley Dunkin guinea-pig – kill by a blow on the head.
- Prepare small intestine – 20-50 cm proximal to the ileocaecal valve place in Dulbecco’s Phosphate-Buffered Saline (PBS) with 1·10⁻⁶ M Indomethacin.
- Rinse the lumen of the gut carefully with PBS with 1·10⁻⁶ M Indomethacin and place in fresh portion of PBS with 1·10⁻⁶ M Indomethacin.
- Cut ±2cm preparations of jejunum that lies 10-15cm from ileocaecal valve and mount them between two platinum electrodes (4mm apart) under constant load of 1g in Krebs-buffer at 37°C gassed with Carbogen.
- Equilibration period – wash preparations 4x10 min in fresh portions of 20ml Krebs-buffer (without any stimulation).

2. Histamine H₃ antagonists screening

- Place preparations in 20ml Krebs-buffer and begin electrical stimulation – frequency 0.1Hz, duration 0.1 msec, voltage 10/30volts. After 20 minutes twitches should be regular. Add 100µL Pyrilamine solution. After 5 minutes twitches should be regular.
- Add further concentrations of (R)-α-methylhistamine dihydrogenmaleate solutions – start with 2·10⁻⁶ M - and record cumulative concentration-response curves (CRC) of (R)-α-methylhistamine until no changes in response are found.
- Record 3-5 CRC of tested H₃ antagonists. Use different molar concentrations of tested compounds (start with the lowest concentration). Add the tested H₃ antagonist 20 min before generation of CRC with (R)-α-methylhistamine.
- Wash the preparations (Vide supra – Equilibration period) between two succeeding measurements.
- Analyze statistical concentration-response curves with the Student’ t-test. Calculate pA₂ value according to Schild regression analysis.

3. Materials and reagents

- Male Hartley Dunkin guinea-pigs (300-400g)
- Dulbecco’s Phosphate-Buffered Saline (PBS). Composition: KCl 2.68 mM, KH₂PO₄ 1.47 mM, NaCl 136.89 mM, Na₂HPO₄ 8.1 mM (pH 7.4 at room temperature)
- Krebs-buffer. Composition: NaCl 118 mM, KCl 5.6 mM, MgSO₄ 1.18 mM, CaCl₂ 2.5 mM, NaH₂PO₄ 1.28 mM, NaHCO₃ 25 mM, Glucose 5.5 mM (pH 7.4 at 37°C).
- (R)-α-Methylhistamine dihydrochloride (Sigma-Aldrich) solutions: 2·10⁻⁶ M - 2·10⁻² M (in 0.9% NaCl).
- Pyrilamine solution: 2·10⁻³ M (in H₂O).
- Indomethacin solution: 1·10⁻² M (in DMSO).
- Thioperamide maleate (Sigma-Aldrich) solutions: 2·10⁻⁶ M - 2·10⁻² M (in H₂O).
- Carbogen (O₂/CO₂ – 95%/5%) (Linde Gas).
- Tested compounds solutions: 2·10⁻⁶ M- 2·10⁻³ M (in H₂O or DMSO).

References


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