**In vitro** test system for the screening of histamine H\(_2\) agonists and antagonists

This protocol describes a standard in vitro test on guinea pig gallbladder based on the relaxant response of histamine on isolated, precontracted guinea pig gallbladder.

1. **Tissue preparation**
   - Male Dunkin-Hartley guinea pig (300-400 g), killed by cervical dislocation.
   - Remove and clean the gallbladder in a dissection disc with Krebs-Henseleit solution.
   - Cut longitudinally the gallbladder to obtain two muscle strips (approximately 1 cm by 0.5 cm).
   - Set up the strips in 10 ml organ bath at 37°C containing Krebs-Henseleit solution gassed with 95% O\(_2\) and 5% CO\(_2\) (pH 7.4) under a constant load of \(\pm 0.2\) g.
   - Equilibration period: 60 min. Wash the preparation 4 x 15 min with fresh Krebs-Henseleit solution.

2a. **Histamine H\(_2\) agonists screening**
   - Add 30 µl of cholecystokinin-octapeptide (CCK-8; 1 µM solution) in order to obtain a stable contraction. This tonic contraction reaches a peak within \(~30\) min and remains stable for 60-90 min. Discard preparation showing a spontaneous fade of plateau response.
   - At the top of contraction administer histamine (or the H\(_2\) agonist, amthamine, impromidine and dimaprit) in a cumulative fashion at 0.5 log unit increments. Construct the concentration-response curve (CRC) of agonist (histamine: 1 - 300 µM; amthamine: 0.1 - 100 µM; impromidine: 0.001 - 10 µM; dimaprit: 0.01 - 10 mM). Perform experiments with histamine in presence of mepyramine (1 µM: add 10 µl of mepyramine 1 mM 30 min before histamine administration) to minimize possible H\(_1\)-mediated contraction. Histamine-induced maximal relaxant effect: \(~85\)%.
   - Since the relaxant effect of H\(_2\) agonists is not reproducible, register only one agonist CRC for each preparation.
   - Express the inhibitory effect of H\(_2\) agonists on CCK-8-induced contraction as percentage of the maximal inhibition obtained with each agonist, considering the plateau response to CCK-8 as 100%.
   - From the individual log-concentration-response curve of each agonist calculate the EC\(_{50}\) by the use of a non-linear regression analysis program (Graph Pad Prism 5.0, San Diego, CA, USA). Express the agonist potency as pD\(_{2}\) value (±SEM), which is defined as –Log(FC\(_{50}\)). pD\(_{2}\) histamine: 4.64 ± 0.04.

2b. **Histamine H\(_2\) antagonists screening**
   - At the end of the equilibration period, administer the H\(_2\) antagonist at the desired concentration.
   - Test the antagonistic activity against the relaxation induced by amthamine, that is considered the most selective H\(_2\) agonist among the compounds available.
   - Administer the antagonist 30 min before the construction of CRC to amthamine.
   - Use two gallbladder strips from the same animal for constructing the CRCs to the agonist alone or to the agonist in presence of the antagonist.
   - When testing competitive antagonists, parallel rightward displacement of the agonist CRC with no change of the maximal response is obtained. Then, use linear regression to calculate pA\(_{2}\) value and the slope of the Schild plot. Use at least three antagonist concentrations.
   - In presence of antagonist causing surmountable antagonism only at lower concentrations, calculate pA\(_{2}\) values from single CRC which produces surmountable antagonism, using the Gaddum’s equation:
     \[
     pA_2 = -\log[B] + \log[CR-1]
     \]
     where [B] represents the concentration of antagonist and CR is the concentration-ratio at the EC\(_{50}\) level.

3. **Materials and reagents**
   - Male Dunkin-Hartley guinea pig (300-400 g)
   - Krebs-Henseleit solution: NaCl 113.0 mM; KCl 4.7 mM; MgSO\(_4\)/7 H\(_2\)O 1.2 mM; CaCl\(_2\)/2 H\(_2\)O 1.8 mM; KH\(_2\)PO\(_4\) 1.2 mM; NaHCO\(_3\) 25.0 mM; dextrose 11.2 mM.
   - Cholecystokinin-octapeptide (CCK-8): prepare stock solution, 0.1 mM in absolute ethanol, store at 4°C and dilute in distilled water immediately before the use.
   - Amthamine dihydrobromide solutions: 100 mM - 10 µM in distilled water.
   - Histamine dihydrochloride solutions: 100 mM - 1 µM in distilled water.
   - Dimaprit dihydrobromide solutions: 100 mM - 10 µM.
Dilute all available H$_2$ antagonists (10 mM - 10 µM) in distilled water or in the appropriate solvent as indicated by suppliers. Dissolve famotidine 10 mM in HCl 0.1 M and make further dilutions in distilled water. If you use DMSO, prepare 10 mM stock solution, in 100% DMSO. Dilute 1 mM in 50% DMSO and further dilutions in distilled water.

**References**


CONTRIBUTED BY: Cristina Pozzoli (cristina.pozzoli@unipr.it)
LAST MODIFIED: 2013-02-11