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

This protocols describe a standard in vitro test on guinea pig heart based on the changes in contraction frequency induced by histamine H\(_2\) receptor agonists on isolated spontaneously beating atria.

1. Tissue preparation

- Male Dunkin-Hartley guinea pig (250-300 g), killed by cervical dislocation.
- Quickly remove and clean the heart in a dissection disc with Ringer solution.
- Rapidly dissect left and right atria free from the ventricles.
- Suspend the preparation in 10 ml organ bath containing Ringer solution gassed with 95% O\(_2\) and 5% CO\(_2\) and kept at 30°C.
- By the use of an isometric transducer, connected with a pen-writing polygraph, record changes in contraction frequency.
- Equilibration period: 60 min. During this period, repeatedly expose the tissues to a threshold concentration of histamine (0.1 µM; add 10 µL of 0.1 mM histamine) at 15 min intervals until a stable positive chronotropic response is obtained. After each administration, wash the preparation until the value of contraction frequency returns to baseline.

2a. Histamine H\(_2\) agonists screening

- After the stabilization, construct a cumulative concentration-response curve (CRC) to the H\(_2\) agonist by increasing doses at 0.5 log unit intervals after each response had reached a plateau (3-4 min).
- When the maximal effect is obtained, repeatedly wash the tissue until the pre-drug level of contraction is obtained.
- You can construct two or more agonist CRCs for each preparation. Second CRC is usually equivalent in terms of agonist potency (EC\(_{50}\)) and maximal response (E\(_{max}\)) provided that a 60 min interval is allowed between curves and frequent washout (every 15 min) are made.
- 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 (EC\(_{50}\)). pD\(_2\) histamine: 6.01±0.24.

2b. Histamine H\(_2\) antagonist screening

- To study the activity of H\(_2\)-antagonists, construct the first CRC to H\(_2\) agonist (control condition), wash the preparation (as above described) and add increasing concentrations of the H\(_2\) antagonist, 30 min before constructing the further CRCs to the agonist.
- In antagonist-treated preparations, express the response to the H\(_2\) agonist as a percentage of the maximal response to the H\(_2\) agonist obtained under control condition.
- When testing competitive antagonists, parallel rightward displacement of the agonist CRC with no change of the maximal response is obtained. Use linear regression to calculate pA\(_2\) value and the slope of the Schild plot. Use at least three antagonist concentrations.
- In presence of H\(_2\)-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 (250-300 g)
- Ringer solution: NaCl 154.0 mM; NaHCO\(_3\) 5.9 mM; KCl 5.0 mM; CaCl\(_2\) 2 H\(_2\)O 2.1 mM; glucose 5.6 mM.
- Dilute all available H\(_2\) agonists (for histamine: 100 mM - 1 µM) in distilled water or in the appropriate solvent as indicated by the suppliers.
- Dilute all available H\(_2\) antagonists (10 mM - 10 µM) in distilled water or in the appropriate solvent as indicated by the 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


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