In vitro test system for the study of histamine H₂ agonists and antagonists

This protocol describes an in vitro test on human atrium based on the positive inotropic activity induced by histamine H₂ receptor agonists on isolated, electrically stimulated, pectinate muscle.

1. Tissue preparation

- Obtain some specimens from atrial appendages of patients undergoing corrective heart surgery.
- Place the tissue at 4°C in oxygenated (95% O₂ and 5% CO₂) Krebs solution.
- Isolate and clean from the surrounded tissues thin pectinate muscle segments (0.6-1 mm width, 6-10 mm long).
- Suspend the tissue, under a tension of 0.8-1.5 g, in 10 ml organ bath filled with a modified Krebs-Heinsleit solution gassed with 95% O₂ and 5% CO₂ and kept at 37 °C.
- Apply electrical pacing by means of two ring platinum electrodes positioned 0.5 cm apart and connected to an electronic stimulator, delivering single square wave pulse at 1 Hz, 0.2 ms, 20% above threshold stimulation (70-140 mA).
- By the use of an isometric transducer connected with a pen-writing polygraph, record the electrically-evoked contraction.
- Equilibration period: 60 - 90 min. Set the duration of single pulse current at 1-2 ms for the first 90 min, then change it as above described. During this period wash the preparation (4 for 15 min) until the inotropic activity evoked by electrical pacing became constant.

2a. Histamine H₂ agonists screening

- After the stabilization period, construct a cumulative concentration-response curve (CRC) to the H₂ agonist by increasing concentrations in the organ bath by 0.5 log unit progression, leaving each response to reach a plateau (3-4 min).
- When the maximal effect is obtained, repeatedly wash the tissue until the pre-drug level of contraction is restored.
- You can construct two or more agonist CRCs for each preparation. Second CRC is usually superimposable to the first in terms of agonist potency (EC₅₀) and maximal response (Eₘₐₓ) 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 EC₅₀ by the use of a non-linear regression analysis program (Graph Pad Prism 5.0, San Diego, CA, USA). Express the agonist potency as pD₂ value (±SEM), which is defined as –Log EC₅₀.
- Compare two series of data by means of Student’s t-test for paired or unpaired data. Fix at P<0.5 the level of statistical significance.

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<thead>
<tr>
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<th>% of max histamine effect</th>
<th>pD2</th>
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<tbody>
<tr>
<td>Histamine</td>
<td>100 (0.69±0.8 g)</td>
<td>5.19±0.30</td>
</tr>
<tr>
<td>Amthamine</td>
<td>103±7.5</td>
<td>5.38±0.36</td>
</tr>
<tr>
<td>Impromidine</td>
<td>79±8.9*</td>
<td>6.59±0.35*</td>
</tr>
<tr>
<td>Dimaprit</td>
<td>106±8.7</td>
<td>4.37±0.59*</td>
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Each datum is the mean±SEM of 5-8 observations. * Significantly different (P<0.5) from Histamine.

2b. Histamine H₂ antagonists screening

- To study the activity of H₂-antagonists, construct the first CRC to the H₂ agonist (control condition), wash the preparation (as above described) then add increasing concentrations of the H₂ antagonist, 30 min before construct the further CRCs to the agonist.
- Perform pilot experiments to check the reproducibility of the H₂ agonist response by repeating (three times) the cumulative administration 60-90 min after washing.
- Express the response to the H₂ agonist in antagonist-treated preparations as a percentage of the maximal response to the H₂ agonist obtained under control condition.
- The concentration of agonist required to elicit half-maximum response (EC₅₀) is calculated from individual log-CRCs by non-linear regression analysis.
- 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 pA₂ 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 pA₂ 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

- Human right atria specimens immediately placed in Krebs solution.
- Krebs-Henseleit solution: NaCl 113.0 mM; KCl 4.7 mM; MgSO_{4} \cdot 7 \text{H}_{2}\text{O} 1.2 mM; CaCl_{2} \cdot 2 \text{H}_{2}\text{O} 1.8 mM; KH_{2}PO_{4} 1.2 mM; NaHCO_{3} 25.0 mM; dextrose 11.2 mM.
- Dilute all available H_{2} agonists (for histamine: 100 mM - 1 \mu M) in distilled water or in the appropriate solvent as indicated by the suppliers.
- Dilute all available H_{2} antagonists (10 mM - 10 \mu M) 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. When using DMSO, prepare 10 mM stock solution, in 100\% DMSO. Dilute 1 mM solution in 50\% DMSO and make further dilutions in distilled water.

References


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LAST MODIFIED: 2013-02-11