**In vitro test system for the screening of histamine H\textsubscript{2} agonists and antagonists**

This protocol describes a standard *in vitro* test on guinea pig heart, based on the changes in force of contraction induced by histamine \( H\textsubscript{2} \) receptor agonists on isolated, electrically driven left papillary muscle.

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 papillary muscle (3-4 mm in length and 0.5-0.8 mm in diameter) with ventricular base.
- Suspend the preparation in 10 ml organ bath containing Ringer solution gassed with 95% \( O\textsubscript{2} \) and 5% \( CO\textsubscript{2} \) and kept at 37°C.
- Drive electrically the tissue by means of two platinum electrodes implanted into the ventricular basis and connected to an electronic stimulator delivering single square wave pulse at 2 Hz frequency, 1 ms duration. Adjust the current strength at a level 20% above threshold.
- By the use of an isometric transducer connected with a pen-writing polygraph, record changes in force of contraction.
- Equilibration period: 60 min. During this period, repeatedly expose the tissues to a threshold concentration of histamine (0.1 \( \mu \text{M} \): add 10 \( \mu \text{l} \) of 0.1 mM histamine), at 15 min intervals, until a stable response is obtained. After each administration, wash the preparation until the value of basal contractile activity returns to baseline.

2a. **Histamine H\textsubscript{2} agonist screening**

- After the stabilization of \( H\textsubscript{2} \)-agonist responses, construct a cumulative concentration-response curve (CRC) to histamine or to other \( H\textsubscript{2} \) agonists by increasing concentrations in the organ bath at 0.5 log unit intervals. Leave each concentration to act until the response had reached a plateau (usually 1-2 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. The second CRC is usually superimposable to the first in terms of agonist potency (EC\(_{50}\)) and maximal response (E\(_{\text{max}}\)), provided that a 60 min interval is allowed between curves and frequent washouts (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: 5.92 ± 0.18.

2b. **Histamine H\textsubscript{2} antagonist screening**

- To study the activity of \( H\textsubscript{2} \)-antagonists, construct the first CRC to \( H\textsubscript{2} \) agonist (control condition), wash the preparation (as above described) and add increasing concentrations of the \( H\textsubscript{2} \) antagonist, 30 min before constructing the further CRCs to the agonist.
- Express the response to the \( H\textsubscript{2} \) agonist in antagonist-treated preparations as a percentage of the maximal response to \( H\textsubscript{2} \) agonist obtained under control condition.
- When testing competitive antagonists that induce a surmountable effect, 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 \( H\textsubscript{2} \)-antagonist causing surmountable antagonism only at lower concentrations, calculate pA\(_{2}\) values from single CRC which produces surmountable antagonism, using the Gaddum’s equation:

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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 \( H\textsubscript{2} \) agonists (for histamine: 100 mM - 1 \( \mu \text{M} \)) in distilled water or in the appropriate solvent as indicated by the suppliers.
- Dilute all available \( H\textsubscript{2} \) antagonists (10 mM - 10 \( \mu \text{M} \)) 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. If you use 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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