BIOASSAY OF HISTAMINE BY USING ISOLATED CHICKEN ILEUM

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ABSTRACT

Study of isolated organ/tissue preparation study has to sacrifice laboratory animals just for teaching purpose. The objective of present investigation was to study effect of histamine on dose response curve (DRC) and to find out unknown concentration of histamine by interpolation and three-point bioassay using isolated chicken ileum. The results suggested that histamine produced a dose dependent increase in response of contraction using tyrode physiological salt solution (PSS). Unknown concentration matches nearby with actual concentration by interpolation and three-point bioassay. The result shows that the chicken ileum is suitable for performing bioassay of histamine, an alternative to isolated ileum preparation from laboratory animal (guinea pig) without sacrificing the experimental animals.

KEYWORDS: chicken ileum, histamine, interpolation, three-point bioassay.

INTRODUCTION

The usually accepted methods for bioassay of histamine are cat's blood pressure, guinea pigs uterus and the guinea pigs ileum. The most widely used tissue for the bioassay of histamine is guinea pigs terminal portion of the ileum. (Chowdhury et.al., 1956).Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), a statutory body framed under the Prevention of Cruelty to Animals Act, 1960, which is duty bound to take all such measures as may be necessary to ensure that animals are not subjected to unnecessary pain or suffering before, during or after the performance of experiments on them. CPCSEA has instructed Universities and College to avoid experiments...
on animals wherever it is possible to do so for teaching purpose and introduce use of alternative to animal experimentation like CDs, computer simulations, models, in-vitro methods etc. Substitute to in vivo animal experiments are in vitro cell culture technique and in silico computer simulation. Still there is need to develop alternative to animal testing. (Undale et.al., 2013). Also restriction to experiments using laboratory animals for bioassay has provoked researcher to search for alternate tissues. (Parthasarathy, 2014). Guinea pig ileum has been used to study various receptors present in ileum. It responds to most of the common spasmogen and particularly suitable for study of histaminergic receptors. For these study laboratory animals has to be sacrificed. Chicken ileum is simply obtainable from the slaughter house and animal need not to be killed just for teaching and demonstration purpose. (Bhutada et.al., 2006). Use of chicken ileum might reduce, refine and replace the number of animals for laboratory experiment just for teaching purpose. No attempt has been made to study effect of histamine on isolated chicken ileum. The objective of present investigation was to study effect of histamine on isolated chicken ileum is easily available from slaughter houses as an alternative for dose response curve (DRC) and estimation of unknown concentration of histamine by interpolation and three-point bioassay. Histamine (stock solution: 1mg/ml) used in the study was purchased from S.D.Fine Chem. Mumbai. Initially different physiological salt solutions were used. But tissue responses to histamine were not uniform and taking long time for relaxation. Therefore tyrode solution was used for experimentation. Composition of tyrode physiological solution were (in g/liter) NaCl 8.0; KCl 0.2; CaCl₂ 0.18; MgCl₂ 0.1; NaHCO₃ 1.0; NaH₂PO₄ 0.05 and glucose 2.0 weighed accurately and dissolved in distilled water maintained at 32-35˚C.

Fresh intestine of Chicken were obtained from a government registered slaughter house. The tissue was immediately transported to the laboratory under ice and kept for aeration. The intestinal content were removed by washing with PSS. With gentle care, the mesentery and adhering tissues were removed. The ileum was cut into small segments of 2-3 cm long and mounted the tissue in the organ bath containing PSS maintained at 32-35˚C, bubbled with air and allowed to equilibrate for 30 min. Mounting was similar to guinea pig ileal preparation mounting.(Kulkarni, 2009) Tension adjustments were made and tissue were washed after every 15 min. The histamine induces the contraction were recorded on kymograph by using frontal writing lever. Contact time of 30 sec, and 5 min time cycle was kept constant throughout the experiment which was followed by 3 times washing with PSS at an interval of 1 min. Concentration of histamine were added in geometric manners. DRC was recorded till
maximum response (E max or ceiling effect) to histamine was obtained and height of response were measured in terms of mms. Interpolation bioassay was carried out in six set of experiments with standard concentration 5, 10, 15, 20 & 30ug/ml. Record the contraction due to graded doses of test histamine. Measure various responses to calculate the mean of each test response. Plot the graph with log dose on X-axis and % response on Y-axis and interpolate the response of test on to the DRC so as to find the standard dose that gives an equivalent response of that of the test. Calculate the potency of the test by converting the log of the standard dose that has produced an equivalent response as that of test in to antilog and determine potency of test (ug/ml). To measure the potency of the test solution, four different set of experiment were carried out by three point bioassay method. Two doses of standard (S1 and S2) from the DRC of standard drug and one dose of test (T) which lies between S1 and S2 were selected. Three sets of responses using S1, S2, and T were recorded for histamine in randomized Latin square fashion and mean heights of S1, S2, and T responses were measured.

Potency= n1/t x antilog {T-S1/S2-S1 x log n2/n1 }x C
Where n1 = lower standard dose; n2 = higher standard dose; t = test dose; S1 = response of n1; S2 = response of n2; T = response of test (t) and C=concentration of standard. (Vrushabendra et.al., 2014)

Interpolation bioassay, Histamine (1 ug to 30 ug) produced a concentration dependent increase in concentration of isolated chicken ileum preparation. These results show that histamine can induce contractions in isolated chicken ileum. Concentration of histamine used were 5, 10, 15, 20 & 30 ug/ml and concentration obtained after performing bioassay nearly match with the actual concentrations. Table no. 1 show the concentration obtained after performing bioassay.

Table 1. Concentration of unknown sample of histamine by interpolation bioassay

<table>
<thead>
<tr>
<th>Concentration of histamine used (ug/ml)</th>
<th>Concentration of histamine obtained (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>05</td>
<td>5.37 ± 0.1739</td>
</tr>
<tr>
<td>10</td>
<td>9.275 ± 0.2056</td>
</tr>
<tr>
<td>15</td>
<td>14.08 ± 0.2501</td>
</tr>
<tr>
<td>20</td>
<td>19.12 ± 0.2394</td>
</tr>
<tr>
<td>30</td>
<td>26.39 ± 0.6397</td>
</tr>
</tbody>
</table>

(*Values are expressed as Mean ±SEM from 6 preparations for individual test samples of Histamine.)
Concentration of the test was determined using S1 (2 ug) and S2 (4 ug) by three point bioassay (Table 2). The potency of unknown sample of histamine was found to be 14.03 ± 0.1707. The actual value of T was 15ug and by bioassay, it was 14.00ug that is 93.34% of the actual strength.

**Table 2. Three point bioassay of histamine using isolated chicken ileum preparation**

<table>
<thead>
<tr>
<th>Height of response in mm</th>
<th>S1 (2 ug)</th>
<th>S2 (4 ug)</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26</td>
<td>50</td>
<td>38</td>
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<td></td>
<td>26</td>
<td>51</td>
<td>39</td>
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(* Three point bioassay with Histamine was carried out in four experiments)

Experiment was design to study effect of histamine on DRC and estimate unknown concentration using isolated chicken ileum which will mimic the actual laboratory condition without sacrificing the experimental animals. Histamine produced a dose dependent increase in response of contraction using tyrode physiological salt solution (PSS). Unknown concentration matches nearby with actual concentration by interpolation and three-point bioassay. By interpolation bioassay, potency of test concentration was found nearly match with actual concentration. Three point bioassay results have given good response for about 4 to 6 hrs. The result shows that chicken ileum is suitable for performing bioassay of histamine. Ileum preparation is most widely used for the study of drugs on isolated muscle preparation. It consists receptors like muscarinic, histaminic, GABAergic, serotonergic, and adrenoreceptors. (Ghosh, 2005 and Jain et.al., 2012) The advantages of using chicken ileum preparation are that it is cheap, easy to mount in organ bath, stable and reproducible for about 4- to 6- hours and no need to sacrificed the experimental animals.(Jain et.al.,2012). Furthermore this is needed to perform antagonistic study. However, the model has certain limitation, i.e., ileum has to be brought from slaughter house for study and the characteristic and nature of receptor present are not fully understood. They use to be studied by radioligand binding assay. (Bhutada et.al., 2006). Bioassay is used to identify the nature of receptors and to find out the unknown substance. M3 muscarinic receptors were reported in chick ileum. Chick ileum gives significant stability and reproducibility. (Parthasarathy et.al., 2013)

Chick ileum can be used as an alternative for tissues from guinea pigs for bioassay of histamine. Chicken ileum preparation can be used to demonstrate anti-Histaminic and anti-
spasmogenic activity. It is also suitable for carrying out different experiments like pA₂ value; multiple point bioassay, drug antagonism and comparison of different agonists.

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REFERENCES