Chicken Intestine an Alternative Isolated Preparation in Experimental Pharmacology

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Research Article

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Abstract

The object of study was to evaluate the use of isolated chicken intestine as an alternative preparation for dose response curve (DRC) and estimation of unknown concentration of acetylcholine (ACh). The DRC was performed and graded doses of ACh have shown dose dependent increase in response of contraction. The obtained responses of ACh on chicken intestine were similar to those where performed in isolated preparations. Also different experiment viz. cumulative DRC, potentiation effect, nature of antagonist, calculation of PA2 value were performed in our pharmacology laboratory. The results suggest that a chicken intestine preparation is suitable for the purpose experiment on isolated preparation and multipoint bioassay. The chicken intestine can be used successfully in routing pharmacology experiment in place of isolated preparation from frog, rodents and other experimental animals to perform or demonstrate the student for action of acetylcholine.

Keywords: acetylcholine, dose response curve, isolated preparation

Introduction

The isolated tissue preparation and method of bioassay may well be considered the trademark of pharmacologist. Isolated tissue preparations have been extensively used for measuring effects due to receptor interaction as well antagonist.^[1] Guinea pigs, rabbit and rats are usually the common sources of isolated tissue. Isolated tissue preparations are commonly used to study the effects of drugs on specific type of receptors. These preparations are also used for bioassay of drugs, characterization of specific receptor or its subtypes, to determine concentration response curve of an agonist, to study antagonism of drug and in new drug discovery.^[2] The advantages of isolated tissue over intact animals are that several preparations can be tested on tissue obtained from a single animal; relatively small amount of test material is required and the drug effect is tested directly and perceived its action as the drug is free from the factor of absorption, metabolism and excretion or interference due to nerve reflexes.^[3]

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is an authority which monitors animal experiments conducted in institutions through ethics committee and is mainly concerned with promoting the humane care of animals used in biomedical and behavioral research. ^[4] For this purpose, the Government has made "Breeding of and Experiment on Animals (Control and supervision) Rule 1998" as amended during 2001 and 2006, to regulate the experimentation on animal. ^[5] So the use of an alternative source definitely serves the purpose of decreasing, if not completely eliminating, the killing of laboratory animals just for a strip of tissue. ^[6]

The isolated strip of intestine are most commonly employed smooth muscle preparation because material are abundant, more resistance to handling, relatively easy to set up. ^[7] It is both ethical and economical in using chicken intestine for experimental purpose as it is waste material in slaughter house. The present investigation sought to examine the sensitivity and suitability of chicken intestine for experiments in pharmacology.

Material and Method

Fresh intestine of health chicken was collected from local chicken center immediately after slaughter in cold (0-4 $^{\circ}$ C) Krebs-Henseleit solution ^[8, 9] and was transported to the laboratory, where it was immediately aerated. With the help of pipette, the lumen of intestine gently rinsed out by Krebs solution; waste material and mesenteric attachment were removed. Segment approximately 2 cm of intestine was cut and suspended in 30 ml isolated organ bath containing Krebs-Henseleit physiological solution of composition (in g/litre) NaCl 6.96; KCl 0.34; CaCl 0.28; KH PO 0.16; NaHCO 2.1; MgSO 0.29; and

glucose 2.00 maintained at 37 ± 1 C and continuously aerated ^[10], and was allowed to equilibrate for at least 1 hr under a resting tension of 3 grams. ^[11]

The responses were recorded on smoked paper with an isotonic frontal writing lever. The drugs used were acetylcholine chloride, physostigmine sulphate, atropine sulphate, noradrenaline bitartrate etc. Concentration response curve was recorded using standard diluted stock concentration of drug and was constructed till ceiling effect. After recording response the kymograph was fixed with fixing solution containing colophony in alcohol. Then the height and percentage response were calculated.

Results

Acetylcholine (1 μ g-16 μ g) produced dose dependent increase in contraction of isolated chicken intestine (Figure 1).

Figure 1 : Concentration response curve of Ach (1µg-16 µg) by using isolated chicken intestine



The contractions were blocked by atropine, selectively antagonized the effect of acetylcholine and were potentiated by physostigmine sulphate. Responses to ACh were recorded as changes in height from baseline and expressed as percent of maximum response of the ACh.





Figure 2 shows the log dose-% response curves in the chicken intestine preparations for the Ach, which is in sigmoid shape. Many intestinal strips (oesophagus, duodenum, jejunum and sometimes ileum) exhibited marked contractility action to acetylcholine in dose dependent manner.

Discussion and Conclusion

Isolated tissue preparations are commonly used to study the effects of drugs on specific type of receptors. These preparations are also used for bioassay of drugs, characterization of specific receptor or its subtypes, to determine concentration response curve of an agonist, to study antagonism of drug and in new drug discovery.^[2] Selection of an experimental animal depends on three factors, viz. sensitivity, reproducibility and availability. Laboratory used frog experiments for demonstrating potentiation and antagonism of drugs on rectus abdominis muscle preparation to pharmacy students. Isolated rectus abdominis of frog and smooth muscle from experimental rodent are the two commonly used preparations to teach undergraduate and postgraduate student of pharmacy.

The Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA) has instructed universities, laboratories and institutions not to use frog for teaching purposes and procure frogs only from licensed breeders, but there is no licensed breeder of frog in India. This has now forced many laboratories to switch from frogs to mammals e.g. use of



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the rat ileum for demonstration of drug actions. ^[12] However the use of mammal tissue in scientific research raises strong emotions to researcher. Pharmacology as a subject is particularly hit badly by CPCSEA and other ethical rules, because the subject is so heavily dependent on animals for research and teaching ^[13] and hence this has driven us to conduct alternative isolated tissue experiments.

Intestinal muscle is innervated by both parasympathetic and sympathetic fibers of ANS. Parasympathetic system supplies preganglionic fibers to enteric nervous system of smooth muscle of gut to control motility. Parasympathetic system is responsible for maintaining intestinal motility through normal releasing acetylcholine. Ascending reflex contractions of small involves intestine predominantly cholinergic transmission. ^[14] Chicken intestine consists of a number of receptors. Both acetylcholine and 5-HT cause contractions of isolated smooth muscle preparations of the chicken rectum. ^[15, 16] The response to acetylcholine was atropine-sensitive, suggesting muscarinic receptors. Adrenaline induces relaxation of the chicken rectum, acting via alpha-adrenoceptors. ^[15] However, chicken intestine preparation is easily available from the slaughter house, and animals need not be killed just for teaching purposes.

The advantages of using this preparation are that it is easy to perform, economical, stability of the preparation is high and importantly, unnecessary killing of laboratory animals for teaching purpose shall be prevented. Thus the isolated chicken intestine was found suitable for isolated experimental pharmacology.

In conclusion, this study has demonstrated the suitability of the isolated chicken intestine preparation for use in performing student routine experiments in pharmacology and for the assay of acetylcholine.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests