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HISTAMINE RECEPTORS;
RESPONSES AND MODULATIONS TOWARDS AGONIST AND ANTAGONIST ON THE RABBIT ILEUM, IN VITRO STUDY

DR. AFTAB TURABI
MBBS, M. Phil, Ph.D., Post doc. (USA)
Professor and Head
Department of Pharmacology & Therapeutics
Islam Medical College, Pasroor Road, Sialkot

DR. GHULAM ALI ASGHAR QURESHI
MBBS, MPH (UK), MSPH (USA)
Associate Professor and Head
Department of Community Medicine
Islam Medical College, Pasroor Road, Sialkot

DR. ZIAULLAH
MBBS, FCPS
Associate Professor
Department of Pathology
Rehman Medical Institute, Hayattabad, Peshawar

Dr. S. Saud Hasan
MBBS, M. Phil
Associate Professor and Head
Department of Pharmacology & Therapeutics
Dow Medical College,
Dow University of Health Sciences, Karachi

ABSTRACT...Objective: This research work deals with the mechanism of action involved in determining the therapeutic potential of histamine and its blockers in gastrointestinal motility. **Study Design:** Rabbits of equal weights were used in this study. They were brought from the animal house of BMSI, sacrificed in the Pharmacology Research laboratory. Ileum strip were isolated and with special recommended methodology, longitudinal and circular muscles were separated. Individual muscle strip were then exposed separately to the desired drugs in the organ bath and reading were recorded on the polygraph machine. **Setting:** Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. **Period:** 1996 to 1998. **Results:** Histamine increases the contractile effects of longitudinal and circular muscle. H_1 and H_2 blockers potentiate its effects on longitudinal muscle while in circular muscle no change was observed with H_1 blocker whereas H_2 blocker antagonized the histaminic effects. However when H_1 blocker applied directly it increases the amplitude of contraction in longitudinal and circular muscle whereas H_2 blocker decreases the height of contractions. Histamine in the presence of H_1 and H_2 blocker augmented their effects in longitudinal muscle and antagonizes in circular layer. **Conclusion:** Gastrointestinal motility can be controlled through histamine and its antagonist. New drugs can be formulated on the basis of this study for the regulation of intestinal motility.

Key words: H (histamine), G.I. (gastrointestinal), IP^3 (inositol tri phosphate), 5-HT (5-hydroxytryptamine), $_cAMP$ (cyclic amino mono phosphate), Ca^{++} (calcium)

INTRODUCTION

The smooth muscle of Gastrointestinal tract undergoes almost continuous but slow electrical activity. Slow electrical waves are generated in the longitudinal muscle layer of small intestine and are not found in the circular muscle in the absence of longitudinal muscle.

Thus, if the longitudinal muscle layer is removed, the circular layer no longer exhibits spontaneous, rhythmical activity. However, they do control the appearance of intermittent spike potentials[1]. In the Gastrointestinal smooth muscle, the channels responsible for the action potential are called calcium and sodium channels. The calcium channels are much slower to open and also to close, in comparison to the rapid sodium channels, which accounts for longer duration of action potentials[2].

The advent, in 1972, of selective antagonists of the H2-receptors responsible for the acid secretory properties of histamine confirmed a physiological role for this amine in the control of parietal cell function. Initial indirect studies on guinea pig ileum indicated that histamine could stimulate the breakdown of membrane inositol phospholipids. This was later confirmed using the more measure of inositol phosphate accumulation in the presence of lithium ions[3]. Histamine affects on the membrane potential and conductance of the longitudinal smooth muscle of the taenia of the guinea pig caecum by using sucrose in the solution, causing decrease in the depolarization significantly[4].

Histamine receptors have been divided into three major subtypes, H_1, H_2, and H_3 in vitro studies. Classic H-receptor-mediated responses include contraction of many visceral smooth muscles including guinea pig trachea, uterus, and longitudinal smooth muscle of the ileum[3]. Histamine contracts both ileal longitudinal and circular smooth muscle layer via activation of H_1

receptors, but can probably also relax circular muscle via an H_2 receptor induced hyperpolarisation of the membrane[5]. It has been reported that histamine inhibits the release of 5-HT from enterochromaffin cells of the porcine small intestine and pharmacological experiments indicated that H_3 receptors mediate the effect of histamine on the enterochromaffin cells[6]. Histamine is normally present in typical and atypical mast cells found in the bowel and is thought to participate in acute inflammatory reactions. It can act at H_1 and H_2 receptors present on mucosal cells, myenteric plexus inter neurons of smooth muscle to produce actions that might contribute to the symptoms of inflammatory bowel disease[7]. A third receptor type, H_3, first identified as an inhibitory presynaptic autoreceptor in the cerebral cortex, is now thought to exist also in peripheral neurons and to subserve presynaptic inhibition of transmitter release[6,8,9]. Cimetidine is a H_2 receptor antagonist markedly inhibits the basal and meal stimulated gastric secretion in normal and in peptic ulcer patients.

MATERIALS AND METHODS

Animals

Adult rabbits of either sex were obtained from animal house of BMSI and were killed by blow on the head and sacrificed by cutting the neck with a sharp surgical knife. Segments of intestine were dissected out and placed in a petri-dish containing kreb's[10] nutrient solution with 95% oxygen.

Instruments and Chemicals

- Transducers FT03C (USA) – Pressure and force displacement type
- Grass Polygraph machine (model 7B USA)
- Kreb's nutrient solution[10]
- Drugs: Histamine, Cimetidine, Chlorpheniramine

Longitudinal and Circular Muscle Preparation

In this model experiment, the method used was that adopted by Craig and Clark[11]. The intestinal segment was slipped over a glass rod and with the help of scalpel blade a longitudinal superficial incision was given along the mesenteric border through out the entire length of the segment. The superficial muscle layer was then stripped off, in a circular manner starting from the site of incision with a piece of moist cotton wool. The separated muscle layer was the outer longitudinal muscle and the remaining underlying circular muscle was then taken out from the glass rod. Both muscle layer strips were placed immediately in the petri dish containing Krebs's nutrient solution bubbled with oxygen. The longitudinal and circular muscle strips were then mounted in separate organ baths connected to the force and pressure displacement transducers respectively. The organ baths had a continuous supply of oxygen and nutrient solution.

METHODOLOGY

Histamine, Cimetidine and Chlorphinaramine were diluted in the concentrations of 10^{-3} to 10^{-9}. Longitudinal and circular muscle strips were exposed to each dilution and the response was recorded on the polygraph. Each dilution used in a quantity of 0.2ml and was left in contact with the tissue for a period for 60 seconds. The response was calculated from the amplitude of force of contraction observed before and after the drug administration and the values were taken in mm as well as in percentage. Before each reading the resting period of 45 minutes was given for equilibration, which was checked by recording base line muscle contractions.

The percentage values of various dilutions were arranged in descending orders and the median value were taken. This procedure was repeated five times for each drug. The determined EC-50 of histamine was then added to both organ baths containing longitudinal and circular muscle and the responses conducted through transducers were recorded on the polygraph machine. Cimetidine was then added in the tissue chambers without washing and the responses were recorded. After washing and resting phase of 45 minutes the same procedure was performed with histamine and chlorpheniramine. In the next step the procedure was repeated in vice versa manner, in which the tissues were initially exposed to antagonists (cimetidine and

chlorpheniramine) and after observing their responses, the tissues were exposed to agonist (histamine) without washing and results were recorded.

RESULTS

When longitudinal muscle was treated with histamine there was an increase in amplitude to 5 mm of base line. Addition of chlorpheniramine in the presence of histamine causes further increase of amplitude to 1 mm and the percentage of difference between agonist vs antagonist comes to 54.5%. In the next step, the chlorpheniramine was first applied to longitudinal muscle strip and there was an increase in amplitude form 3.8 mm (base line amplitude) to 4.6 mm, followed by addition of histamine in the presence of chlorpheniramine, the amplitude further increases to 15.5 mm. The difference of percentage between antagonist vs agonist comes to 70.3% vide table I, Fig 1.

Table-I. Contraction Effects of Drugs on Longitudinal Muscle of Rabbit ileum

Before drug	Histamine	Histamine + Chlorpheniramine
3.4mm	5.0mm	11.0mm
Before drug	Chlorpheniramine	Chlorpheniramine + Histamine
3.8mm	4.6mm	15.5mm
Before drug	Histamine	Histamine + Cimetidine
3.4mm	13.3mm	15.6mm
Before drug	Cimetidine	Cimetidine + Histamine
8.6mm	8.4mm	7.6mm

Each reading represents mean value of 5 observations.

muscle strip the amplitude was further increased to 2.3 mm. The percentage of difference was 14.7% in agonist vs antagonist. In the second step, when the cimetidine was applied first, the amplitude of contraction was reduced to 0.2 mm from the base line amplitude of 8.6 mm. On the addition of histamine in the presence of cimetidine there was further decrease in amplitude to 7.6 mm. So the difference in percentage comes to -9.5% vide table I, Fig 3.

Table-II. Contraction Effects of Drugs on Circular Muscle of rabbit ileum

Before drug	Histamine	Histamine + Chlorpheniramine
4.3mm	4.0mm	12.0mm
Before drug	Chlorpheniramine	Chlorpheniramine + Histamine
5.6mm	6.2mm	5.0mm
Before drug	Histamine	Histamine + Cimetidine
5.2mm	5.6mm	5.5mm
Before drug	Cimetidine	Cimetidine + Histamine
6.4mm	5.6mm	6.0mm

Each reading represents mean value of 5 observations.

The circular muscle showed the different result. When histamine was applied the base line amplitude was decreased from 4.3 mm to 4.0 mm and on application of chlorpheniramine there was no further change in amplitude, hence the percentage of difference comes to zero. In next step the chloropheniramine was applied first that resulted in increase in amplitude to 6.2 mm. Histamine was then added and the amplitude was decreased to 5 mm. The percentage of difference in this case was 19.3% Vide table II, Fig 2.

In case of cimetidine circular muscle showed decrease in amplitude to 5.5mm and the percentage of difference between agonist v/s antagonist was -1.7%. On reversing the step, cimetidine was applied first to circular muscle strip; the amplitude was reduced from 6.4 mm to 5.6 mm. Subsequently the histamine was added and the amplitude was increased to 6 mm. The percentage of

The Effect of Histamine and Chlorpheniramine on Longitudinal and Circular Muscles of Rabbit Ileum

Fig-1. Longitudinal muscle

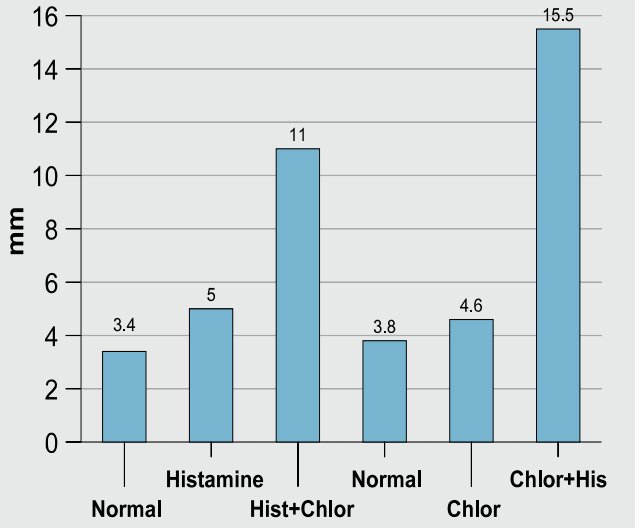
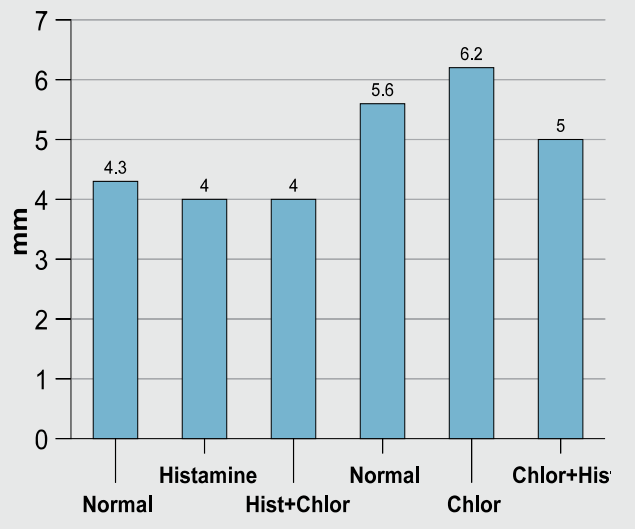


Fig-2. Circular muscle



difference between antagonist v/s agonist was 6.6% vide table II, Fig-4.

DISCUSSION

This study was carried out with the aim to explore the action and effects of histamine on the smooth muscle of small intestine and to see whether effects are mediated through histaminergic receptors or via different mediators and also see that it has any direct effect. Several researchers have already been carried out on different animals. We had selected rabbit's intestine for model experiment, as it is similar in action and physiology to human. No such research has been carried out previously on longitudinal and circular muscle separately simultaneously. Our observation in this study is that

The Effect of Histamine and Cimetidine on Longitudinal and Circular Muscles of rabbit Ileum

Fig-3. Longitudinal muscle

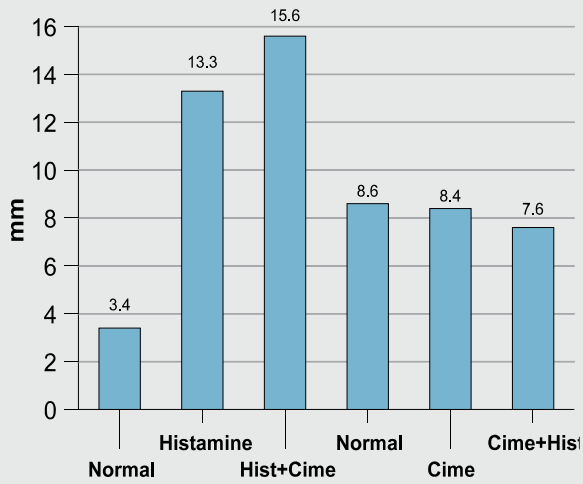
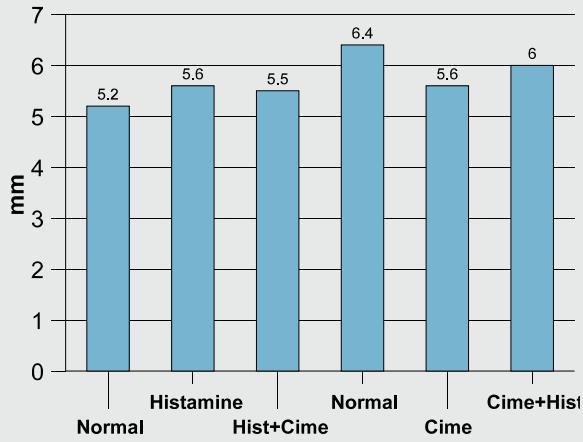


Fig-4. Circular muscle



histamine increases the contractile effects of longitudinal and circular muscle. H1 and H2 blockers chlorpheniramine and cimetidine potentiates its effects on longitudinal muscle while in circular muscle no change was observed with H1 blocker whereas cimetidine antagonized the histamine effects vide table I and II. In next step chlorpheniramine when applied directly it increases the amplitude of contraction in longitudinal and circular muscle whereas cimetidine decreases the height of contractions. Histamine in he presence of H1 and H2 blocker augmented their effects in longitudinal muscle and antagonizes in circular layer as shown in table I and II.

It has been suggested that histamine, an endogenous amine and a putative neurotransmitter at aminergic synapses in the brain, may have physiological functions as a neuromodulator at other types of synapses like cholingeric. Activation of presynaptic histamine receptors (both H1 and H2 types) can either increase or decrease the release of acetylcholine from pregangilionic nerve terminals in frog sympathetic ganglia and motor nerve terminals at frog's neuromuscular junctions[12,13]. It was concluded that histamine, like acetylcholine, act primarily on the smooth muscle directly14. Histamine, contracts smooth muscle of guinea pig intestine in a concentration dependent way acted via at least two distinct receptors H1 and H2. Histamine contracts both ileal longitudinal and circular smooth muscle layer via activation of H1 receptors15, but can probably also relax circular muscle via an H2 receptor induced hyperpolarization of the membrane. Moreover, other H2 receptor antagonists also proved to be able to relax intestinal smooth muscle at high concentrations. Involvement of the H3 receptor in the inhibitory response of histamine was studied. It was shown that under conditions of electrical stimulation, that release acetylcholine, the histamine induced inhibition could be denoted as an H3 receptor effect. The intestinal smooth muscle might therefore be a suitable preparation for the evaluation of either H1 receptor or H3 receptor effects[16].

Two histamine receptors types coupled to distinct signal transduction pathway coexist in intestinal muscle of guinea pig intestine; a histamine H1 receptor sensitive to mepyramine that indicates Ca++ dependent contraction and a histamine H2 receptor sensitive to cimetidine that mediates the cAMP dependent relaxation. Although the contractile effects mediated by H1 receptors on muscle cells was predominant, the effect mediated by H2 receptors was evident in several features of the response and could be fully unmasked when H1 receptors were blocked with selective antagonist. Though when the effects mediated by H2 receptors were blocked by cimetidine.

1) Contraction induced by histamine was augmented significantly 2) decline in contraction at supra maximal concentrations were eliminated and 3) the competitive pattern of inhibition by mepyramine was restored, similarly reported by[3]. The relaxant effect mediated by H2 receptors is most evident in cells maximally pre contracted with a non histamine agonist: in these cells, histamine in the presence of an H1 receptor antagonist caused relaxation that could be reversed by cimetidine. Histamine caused an increase in Ca++ that was inhibited by mepyramine and an increase in cAMP that was inhibited by cimetidine. The results implied that the two signal transduction pathways were functionally linked converging to regulate the level of Ca ++ as the main determinant of response[6]. Several sources and routes of Ca++ mobilization in response to receptor activation may be identified in smooth muscle. There are two major classes of Ca++ channels; PDCs and ROCs. The dependence of response, or a component there of, on the availability of extracellular Ca++ has long been regarded as indicative of a role for extracellular Ca++[17].

It was reported that histamine via H3 receptor can also inhibit the release of 5-HT from the porcine small intestine. Moreover, experiments carried out in the presence of neurotoxin tetrodotoxin suggested that the inhibitory H3 receptors localized directly on the enterochromaffin cells are the only described non neuronal (although neuroendocrine) cells which appear to be reduced with inhibitory H3 receptors[18].

The results of most studies suggest that the responses to histamine are mediated only by H1 receptors and its

direct action on the ileum and colon were blocked by H1 receptors. However, there were marked differences amongst the regions in their sensitivity to histamine, receptor subtypes or regional differences in receptor density. The basis for the variation in the sensitivity to the contractile actions of histamine is best explained to regional differences in the density of a homogenous population of H1 receptors15.

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Article received on: 11/08/2010 Accepted for Publication: 11/10/2010 Received after proof reading: 04/11/2010

Correspondence Address:
Dr. Aftab Turabi
Professor & Head
Department of Pharmacology & Therapeutics
Islam Medical College, Pasroor Road, Sialkot
aftabturabi@hotmail.com
aftabturabi@gmail.com

Article Citation:
Turabi A, Qureshi GAA, Ziaullah, Hasan SS. Histamine receptors; responses and modulations towards agonist and antagonist on the rabbit ileum, in vitro study. Professional Med J Dec 2010;17(4):691-697.

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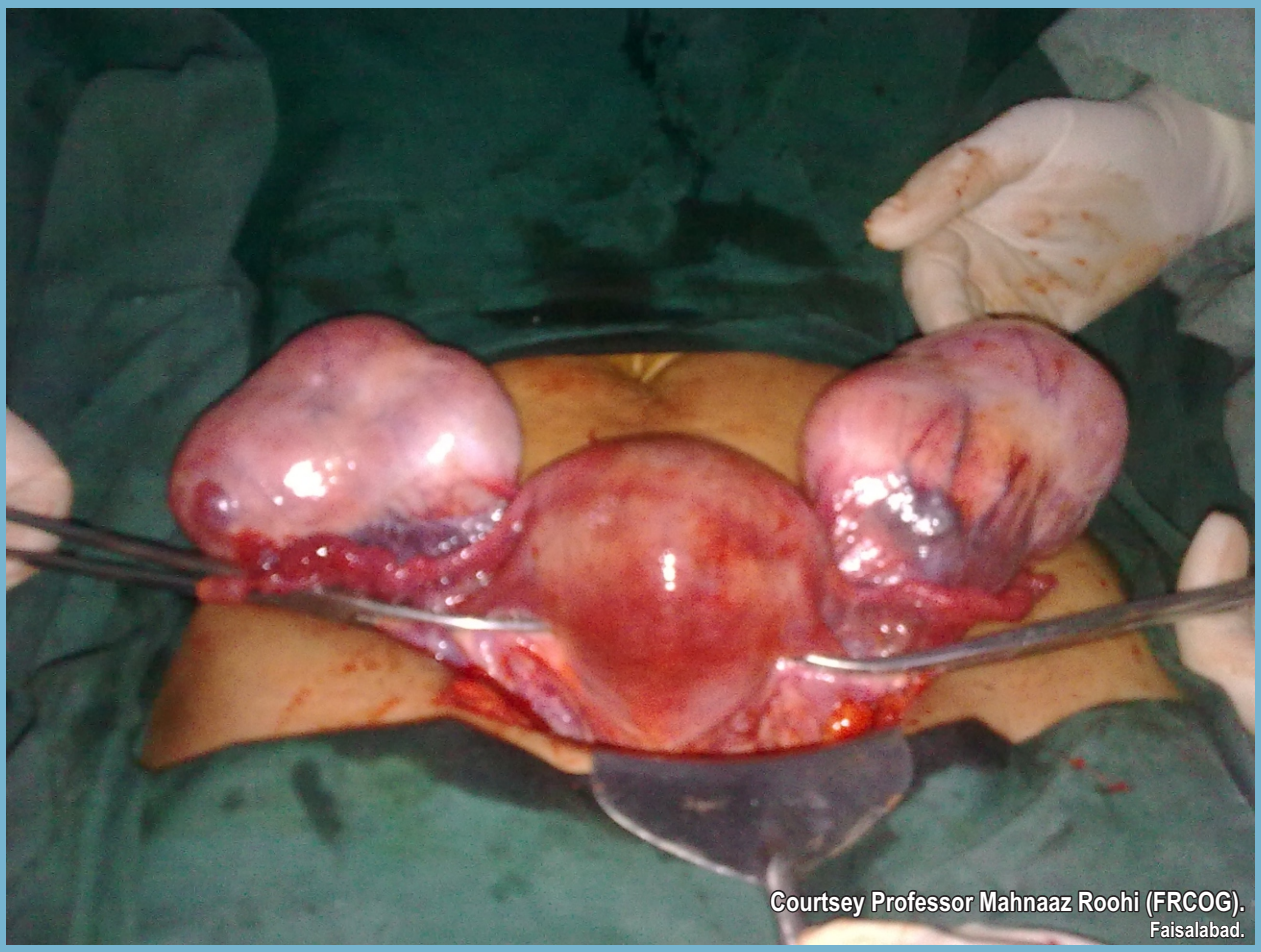

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