Original Article

Role of 5HT in the modification of intestinal motility, *in vitro* study
Aftab Turabi, Syed Saud Hasan, Ahmed Danyal, Naseer Baluch

From Departments of Pharmacology, Islamic International Medical College Rawalpindi, Dow University of Health Sciences Karachi, Frontier Medical College Abbottabad and Bolan Medical College Quetta

Correspondence: Dr Aftab Turabi, House # 356, Street # 72 G 11/ 2, Islamabad. Email: aftabturabi@hotmail.com

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ABSTRACT

**Objective:** To determine with the mechanism of action involved in the therapeutic potential of serotonin and its blocker on gastrointestinal motility.

**Method:** The standard method was used for obtaining the longitudinal and circular muscles strip of rabbit ileum for *in vitro* studies. Each muscle strip was exposed to serotonin and its blocker and the result obtained was recorded on polygraph apparatus. The effects were recorded in vice versa fashion i.e. agonist v/s antagonist and antagonist v/s agonist on longitudinal and circular muscle strip separately.

**Results:** Serotonin had depressant effect on the force of contraction. On addition of antagonist in the presence of agonist, the effects were increased. Longitudinal muscle showed more pronounced effect i.e. 52.7% with methysergide in comparison to circular muscle, which was 15.6%. Circular muscle showed reduction in the force of contraction with serotonin, which was increased on addition of antagonist, but still below the level of base line contraction.

**Conclusion:** Serotonin when given from external source *in vitro*, decreased the force, however, there was minimal increase in the rate of contraction. Hence, serotonin decreases the intestinal motility giving an impression of having antispasmodic effect. The results of this study can be utilized in the development of new drug related to G.I. motility mediated through 5HT receptors.
KeyWords: Serotonin, 5HT receptors, Irritable bowel syndrome

INTRODUCTION

Serotonin is widely distributed in nature, being found in plant and animal tissues, venoms and stings. The actions of 5HT are mediated through variety of cell membrane receptors. 5 HT receptors are classified as 5 HT₁, 5HT₂, 5HT₃, 5HT₄, 5HT₅, 5HT₆ & 5HT₇. The 5HT₄ receptor is involved in the intestinal contraction by activation of cholinergic nerves of guinea pig ileum, and ascending colon. It is observed that the stimulatory effects on EC cells are mediated by nicotinic and muscarinic receptors of β-adrenoceptors whereas inhibitory effects are mediated by somatostatin, α adrenoceptors, vasoactive intestinal polypeptide, γ aminobutyric acid and benzodiazepine receptors.

Response to serotonin in the ileum is in part due to the interaction of serotonin with presynaptic neuronal receptors that activate acetylcholine release. 5HT causes contraction of resting ileum partly by a direct action on the longitudinal muscle and partly by causing tetrodotoxin-sensitive release of acetylcholine from myenteric nerves. In contrast, 5HT inhibits the peristaltic reflex evoked in isolated ileum. There seems to be ample opportunity for an interaction between 5HT and adrenaline. Two different actions of 5HT on the peristaltic reflex were observed on isolated intestine of guinea pig that if applied on serosal surface it inhibits peristalsis and when applied to the mucosal surface it facilitates the peristaltic reflex. Methysergide used as an antagonist as it inhibits the vasoconstrictor and pressor effects of 5HT as well as the action of amine on a variety of extra vascular smooth muscle cells.

Several studies have already been carried out on different animals; however, no such research has been carried out previously on longitudinal and circular muscle separately and simultaneously. We selected rabbit’s intestine for model experiment, as it is similar in action and physiology to human. This study was carried out with the aim to explore the action and effects of 5HT on the smooth muscle of small intestine and to see whether effects are mediated through 5HT receptors or via different mediators and also to see that it has any direct effect.
MATERIALS AND METHODS

Adult rabbits of either sex 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 nutrient solution with 95% oxygen. The longitudinal and circular muscle strips were then mounted in separate organ baths connected to the force and pressure displacement transducers (FT 03C, USA) respectively. The organ baths had a continuous supply of oxygen and nutrient solution.

Serotonin and its antagonist, methysergide 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 (Model 7B, USA). 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 height (amplitude) of contraction observed before and after the drug administration and the values were taken in mm and 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 ED50 of serotonin was then added to both organ baths containing longitudinal and circular muscle and the responses conducted through transducers were recorded on the polygraph machine. Methysergide was then added in the tissue chambers without washing and the response was recorded. In the next step, the tissues were initially exposed to methysergide and after observing the response, the serotonin was added in to the tissue bath without washing and results were recorded.

RESULTS

The amplitude of contraction decreased to 3.2 mm from the base line amplitude of 5.0 mm on addition of serotonin in the tissue bath containing longitudinal muscle strip. When methysergide was added in the presence of serotonin, the height of contraction increased to 7.0 mm. The change in amplitude of contraction in case of agonist v/s antagonist was 52.7%. With
physiological base line of 9.5 mm, when methysergide was added in the tissue bath containing longitudinal muscle strip, there was reduction in amplitude of contraction to 7.8 mm. With serotonin added in the same tissue bath, in the presence of methysergide; there was further decrease in the amplitude of contraction to 5.8 mm. The percentage of reduction in amplitude of contraction in antagonist v/s agonist experiment was 25.6% (Fig.1). The circular muscle was then treated in the same fashion. The percentage of increase in amplitude of contraction in antagonist v/s agonist was 26.6% (Fig. 2).

**DISCUSSION**

This study shows that in longitudinal muscle 5HT reduces the amplitude of contraction while methysergide potentiate it. Reversal by methysergide initially after the recording of base line contraction shows decrease in the amplitude of contraction, which is further reduced by the application of 5HT. Circular muscle shows the same effect with 5HT and methysergide.
Although when methysergide given initially, there was a mild increase in amplitude, which was further increased by the addition of 5HT.

Intestinal motility can both be inhibited and stimulated by exogenous 5HT by exerting dual effect on the cholinergic neurons of the guinea pig ileum for the release of acetylcholine. Increase in muscle tension is mainly due to the release of acetylcholine. Regarding 5HT the excitatory receptors on sympathetic nerve terminals are pharmacologically different from those on myenteric cholinergic neurons. Methysergide antagonized the inhibitory action of 5HT but did not affect the 5HT induced increase in acetylcholine outflow. This suggests that the inhibitory 5HT receptor may roughly corresponds to the 5HT\textsubscript{1} subtype, characteristic for an interaction between an agonist and a competitive antagonist.

**Fig. 2. Effects of Serotonin & Methysergide on Circular Muscle of Rabbit Ileum**

5HT receptors are at present classified as 5HT\textsubscript{1}, 5HT\textsubscript{2}, 5HT\textsubscript{3}, 5HT\textsubscript{4}, 5HT\textsubscript{5}, 5HT\textsubscript{6} and 5HT\textsubscript{7}. The 5HT\textsubscript{4} receptor is involved in contraction by activation of cholinergic verves of guinea pig ileum.\textsuperscript{2} 5HT receptor subtypes can be categorized into three major families. Each family consists of multiple receptor subtypes that share similarities in their molecular biological, pharmacological, biochemical and / or physiological properties. The 5HT\textsubscript{1} family includes at least three distinct subtype 5HT\textsubscript{1A}, 5HT\textsubscript{1B}, and 5HT\textsubscript{1D} receptors. The 5HT\textsubscript{1c} receptor should now be more
appropriately placed in the 5HT\textsubscript{2} family of receptors. 5HT\textsubscript{2} include 5HT\textsubscript{1c}, 5HT\textsubscript{2A} and 5HT\textsubscript{2B}. Regarding receptors, data suggest that they are coupled to an ion channel, probably a calcium channel. The 5HT\textsubscript{3} receptor therefore shares more similarities with the nicotinic receptor than with 5HT\textsubscript{1} or 5HT\textsubscript{2} receptors.\textsuperscript{9} Next member of this family is 5HT\textsubscript{4} receptor, which has been discovered and shown to have wide tissue distribution and to mediate an impressive array of functional responses.

The gut is the only organ that manifests complex integrative behaviors and reflexes in the absence of input from the CNS.\textsuperscript{10} This ability is conferred on the bowel by its intrinsic innervations, the enteric nervous system, which contains intrinsic primary afferent neurons and the inter-neurons.\textsuperscript{11} EC cells thus signal by secreting massive amounts of 5-HT, which is efficiently inactivated within the bowel mediated by a plasmalemmal 5-HT transporter or serotonin reuptake transporter (SERT).\textsuperscript{12} Inactivation of 5-HT is critical to prevent toxic effects on distant targets and from desensitizing its receptors. Inhibition of mucosal SERT potentiates responses of intrinsic primary afferent neurons to the 5-HT and, if severe and prolonged, causes neuronal 5-HT receptors to desensitize.\textsuperscript{12}

MAO (MAO-A, MAO-B) and all of the other enzymes that catabolize 5-HT are intracellular.\textsuperscript{13} Uptake into cells is therefore essential for 5-HT to be inactivated. However, 5-HT is highly charged and hydrophilic at a physiological pH and thus does not readily permeate plasma membranes. A mechanism to transport 5-HT into cells is thus required to metabolize it after it has been secreted, as well as to reduce its concentration in the vicinity of 5-HT receptors. SERT is the only transporter expressed in the bowel with a high affinity for 5-HT.\textsuperscript{12}

Catecholamines strongly stimulate 5HT release from EC cells\textsuperscript{13}, and sympathetic nerves innervate enteric serotonergic neurons. Infections or fright, which causes the release of catecholamines from sympathetic nerves or the adrenal medulla, release enough 5HT leads to alternate diarrhea and constipation i.e. irritable bowel syndrome.\textsuperscript{14} Alosetron, a 5-HT\textsubscript{3} antagonist, is strikingly effective in treating diarrhea-predominant IBS.\textsuperscript{14} Conceivably, a defect resulting in an excessive concentration of 5-HT reaching enteric receptors, caused by either over secretion or by inadequate inactivation of SERT, might contribute to the pathogenesis of IBS. In conclusion, this study suggests that 5HT has a definite role in the intestinal peristalsis but other still unknown mechanisms that involve other neurotransmitters like acetylcholine, may also play a part.
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