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ORIGINAL ARTICLE

EFFECT OF PROSTAGLANDIN $F_2\alpha$ AND INDOMETHACIN ON THE RABBIT ILEUM; *INVITRO* STUDY

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ABSTRACT

Introduction: The smooth muscle of gastrointestinal tract undergoes almost continuous but slow electrical activity. This activity tends to have two basic types of electrical waves. 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. The other waves are peristaltic waves which are a reflex response. The purpose of the study was to deal with the mechanism of action involved in determining the therapeutic potential of $PGF_2\alpha$ and its antagonist in gastrointestinal motility.

Materials and Methods: Rabbits of equal weights were brought from the animal house of BMSI and sacrificed in the Pharmacology Research laboratory. Ileum strips were isolated and with a special recommended methodology, longitudinal and circular muscles were separated. Individual muscle strips were then exposed separately to the desired drugs in the organ bath and readings were recorded on the polygraph machine. The study was performed at Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi from 1996 to 1998.

Results: $PGF_2\alpha$ decreases the contractile effects of longitudinal muscles whether applied before or after the antagonist whereas in circular muscle it increases the amplitude of contraction. Indomethacin antagonizes the effects of $PGF_2\alpha$ in both longitudinal and circular muscle. Secondly when indomethacin applied directly it causes reduction in the amplitude of contraction in longitudinal muscle and increase in the force of contraction in circular muscle.

Conclusion: Prostaglandin has a definite role on the smooth muscle of ileum; hence can be used in the regulation of intestinal motility. New drugs as an analogue or as an antagonist can be developed on the basis of these results.

Keywords: PG (Prostaglandin), BMSI (Basic Medical Sciences Institute), GI (Gastrointestinal).

INTRODUCTION

The smooth muscle of gastrointestinal tract undergoes almost continuous but slow electrical activity. This activity tends to have two basic types of electrical waves. 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. The slow waves pace and coordinate the rhythmic contractions of both muscle layers but significant contractile force is not developed unless action potentials are generated during the crest to the waves. It appears that the slow wave in circular muscle is entirely passive, resulting from outward flowing membrane current produced by active depolarization in longitudinal muscle. Both muscle layers of the small intestine normally exhibit spontaneous rhythmicity. In the intact intestine slow waves are generated by longitudinal muscle and are transmitted, via interconnecting muscle strands, to the underlying circular layer where they serve to coordinate contractions of the latter. Thus, if the longitudinal muscle layer is removed, the circular layer no longer exhibits spontaneous, rhythmical activity[5].The slow waves do not cause calcium ion to enter the smooth muscle fiber but instead sodium ions. Therefore, the slow waves by themselves usually cause no contractions or very little. Instead it is during spike potentials that are generated at the peaks of slow waves that cause large quantities of calcium ions to enter the fibers and cause most contractions.[12] Two major types of innervations have been recognized in much visceral smooth muscle, first is by small bundles of axons in which the apposition between the nerve fiber and the smooth muscle cell is approximately 2000A°. In the second type, the single nerve fibers form close contacts with all or most smooth muscle cells.[22]

The nervous control displays three levels of organization, i.e. the intramural or enteric nervous system, the prevertebral ganglia, and the central nervous system. A general feature of the nervous connections in these different systems is that they are essentially composed of polysynaptic nervous pathways with unmyelinated axons.[19] In response to the above mentioned control system there are two basic types of movements in the intestinal tract, segmentation contractions and peristaltic waves. The segmentation contractions are ring like contractions that appear at fairly regular intervals along the gut, then disappear and replaced by another set of ring contractions in the segments between the previous contractions.Peristaltic waves are a reflex response that is initiated when the gut is stretched by the contents of the lumen, usually initiates a circular contraction behind the stimulus and area of relaxation in front of it. The wave of contraction then moves in an oral to caudal direction; propelling the contents of the lumen forward at rates that vary from 2 to 25 cm/s. The response to stretch is called the myenteric reflex. Peristaltic activity can be increased or decreased by the autonomic input to the gut, but its occurrence is independent of extrinsic innervations.[12]

Autacoids and related locally acting substances are clearly part and parcel of the physiological and pathological phenomena that provide the rationale for drug therapy; their existence provides numerous possibilities for therapeutic interventions by the use of drugs that mimic or antagonise their actions or interfere with their synthesis or metabolism.[11] Autacoid is a group of diverse substances that includes the biogenic amines, histamine and serotonin, small peptides such as kinins and lipids, such as prostaglandins. All these substances are released from tissues during injury or inflammatory reactions. Histamine was the first of these substances to be discovered. Prostaglandins are among the most potent naturally occurring autacoids. Longitudinal muscle is contracted by PGE_2 and $PGF_2\alpha$ whereas circular muscle is relaxed by PGE_2 The contractile effects are mediated by binding to specific cell surface receptors resulted in the release of calcium and the generation of cyclic AMP. Indomethacin, introduced in 1963 for the treatment of rheumatoid arthritis, is a potent inhibitor of the prostaglandin cyclo-oxygenase.

MATERIAL AND METHOD

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[6] 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.[17]

Drugs: Prostaglandin $PGF_2\alpha$: Sigma Chemical Co. St. Louis, USA. Batch No. 96H0133, Indomethacin.

Longitudinal and Circular Muscle Preparation

In this model experiment, the method used was the one adopted by Craig and Clark.[7] 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

Prostaglandin $PGF_2\alpha$ and Indomethacin 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 of 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, that 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 the drug was then added to both organ baths containing longitudinal and circular muscle and the responses conducted through transducers were recorded on the polygraph machine. Prostaglandin $PGF_2\alpha$ was then added in the tissue chambers without washing and the responses were recorded. After the resting phase of 45 minutes the same procedure was performed with Indomethacin.

Table 1: *Effect of PGF2α and Indomethacin on longitudinal smooth muscle of rabbit ileum.*

	B.D (N)	*Agonist*	*Agonist + Antagonist*	*Difference*	*% in DIFF*	*B.D (N*	*Antagonist*	*Antagonist + Agonist*	*Difference*	*% in DIFF*
RATE	12	12	12	00	00	16	12	13	01	7.6
AMPLITUDE	3.9	3.5	5.4	1.9	35.1	12.4	8.9	3.3	-5.6	62.9

Table 2: *Effect of PGF2α and Indomethacin on circular smooth muscle of rabbit ileum.*

	B.D (N)	*Agonist*	*Agonist + Antagonist*	*Difference*	*% in DIFF*	*B.D (N*	*Antagonist*	*Antagonist + Agonist*	*Difference*	*% in DIFF*
RATE	19	18	19	01	5.2	21	22	21	-1	4.5
AMPLITUDE	2.3	3.1	3.0	-0.1	3.2	3.0	3.1	3.6	-0.5	13.8

In the next step the procedure was repeated in a vice versa manner, in which the tissues were initially exposed to antagonists (Indomethacin) and after observing their responses, the tissues were exposed to agonist ($PGF_2\alpha$) without washing and results were recorded.

RESULTS

Longitudinal muscle was treated with prostaglandin $PGF_2\alpha$ after attaining the base line amplitude of 3.9 mm. There was a decrease in amplitude to 3.5 mm in response to $PGF_2\alpha$ while on addition of indomethacin to the organ bath there was an increase in amplitude to 5.4 mm. The difference in percentage was 35.1%. In the next step the indomethacin was applied first, there was decrease in amplitude from the base line of 12.4 mm to 8.9 mm. Subsequently the agonist $PGF_2\alpha$ was applied resulted in decrease in amplitude to 3.3 mm. The percentage in difference of amplitude was 62.9% (table 1).

Circular muscle was then treated with $PGF_2\alpha$ and the result was an increase in amplitude from the base line amplitude of 2.3 mm to 3.1 mm and then the indomethacin was applied this resulted into a decrease in amplitude to 3 mm. The percentage evaluated between agonist v/s antagonists was 3.2%. Whereas on reversing the steps, the indomethacin was applied first resulting in increase of amplitude from the base line of 3 mm to 3.1 mm and on the subsequent addition of $PGF_2\alpha$ there was a further increase in amplitude to 3.6 mm. The percentage in difference of antagonist v/s agonist was 13.8% (table 2).

DISCUSSION

Rabbit model was used in this *invitro* study. Prostaglandin $PGF_2\alpha$, an endogenous mediator and its antagonist indomethacin were used on longitudinal and circular muscles of rabbit ileum to evaluate their role on the regulation of contractions and relaxations. Our object was to propose a hypothesis on the role played by these mediators on two different muscle layers of ileum related to gastrointestinal motility. Several researchers have already been carried out on different animals. We selected rabbits' 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.

Excitatory response in smooth muscle that are dependant upon membrane depolarization are thought to be mediated through the opening of several types of ion channels, e.g. receptor – operated channels, voltage – sensitive channels (potassium, sodium / calcium) and calcium – activated channels.[4] The inhibition of noradrenaline release from several isolated sympathetically innervated organs by prostaglandins of E series is well established. However, their possible role in the release of acetylcholine from cholinergic nerves has been a matter of controversy. It was concluded that PGE_1 did not affect the release of acetylcholine from parasympathetic nerve terminals of guinea pig ileum. Opposite results were reported by Ehrenpreis *et al*[10] where they found that electrically induced neurogenic contractions of isolated guinea pig ileum were inhibited by indomethacin and this inhibition was reversed by

PGE_1 or PGE_2. The results clearly suggest that the release of acetylcholine from the isolated guinea pig ileum is affected by smaller doses of PGE_2 than the release of nor adrenaline, thus, it appears likely that prostaglandins play a physiological role in modulation of cholinergic transmission. In the segments of ileum which were pretreated with indomethacin, the addition of $PGF_2\alpha$ was accompanied by a small increase in the output of acetylcholine and output was increased further after the washout of $PGF_2\alpha$. This was reverse in case of PGE_2 in which acetylcholine output was increased considerably and decreased as PGE_2 was washed out. In longitudinal muscle strip preparation pretreated with indomethacin, a large dose of $PGF_2\alpha$, only slightly augmented the output of acetylcholine but the output increased further after the washout of $PGF_2\alpha$.

The $PGF_2\alpha$ depolarization was attenuated much more than the other drug responses in low sodium solution and the same effect was seen to a lesser extent when sucrose was the substitute although the differences were not significant. The inhibitory component of $PGF_2\alpha$ response was also apparent at times in normal solutions. These results could be explained by the action of $PGF_2\alpha$ at both excitatory and inhibitory receptors.[1] Several researchers[3,10,16] reported that neurogenic contractions of the guinea pig ileum could be increased by the administration of prostaglandin synthetase inhibitor, indomethacin. The inhibition was reversible and the contractions were restored by the administration of prostaglandin. Indomethacin significantly decreases the output of acetylcholine. $PGF_2\alpha$ did not increase the output of acetylcholine in a degree comparable to PGE_2. In guinea pig ileum the release of nor – adrenaline is also modulated by the prostaglandins of E series. This effect, however, could be rather of a pharmacological native because of high concentration of PGE_2 and indomethacin required to influence the adrenergic transmission.[15]

$PGF_2\alpha$ had no measureable effect on the segmental pressures in the sigmoid colon after intravenous infusion.[13] In animals PGE_1 and $PGF_2\alpha$ inhibits intestinal sodium transport and produce loss of fluid from intestinal loops.Isolated human intestinal muscle responds to PGs with contractions or relaxations, depending on the site and the PG used.[8] Ions may selectively interfere either with binding of agonists at a particular receptor or with the specific mechanism by which that activated receptor is linked to the ionic channel.[2] Prostaglandin F_2 alpha has been shown to increase contractility in the small intestine and colon in vitro, and increasedmucosal prostaglandin synthesis has been reported in ulcerative colitis. $PGF_2\alpha$ caused an increase in spike potential frequency and contractile activity in the terminal ileum and proximal and distal colon. Indomethacin alone increased spike potential frequency andcontractile activity in the terminal ileum and proximal colon but decreasedmyoelectric activity in the distal colon. It is concluded that PGF2 alpha may play an important role in modulating intestinal motility, especially inthe distal colon but to a lesser extent in the terminal ileum and proximalcolon.[6]

The study conducted on the guinea pig stomach showed that $PGF_{2\alpha}$ produced excitatory responses in circular muscle of fundus and corpus similar to those in the longitudinal layer of the same regions. These effects of PGs appeared in the presence of adrenergic and cholinergic blocking agents as well as of tetrodotoxin and were therefore, direct effects on smooth muscle. Indomethacin (10^{-7}–10^{-6} mol/1) suppressed spontaneous tone of the fundus and corpus and increased phasic activity of inner pylorus. This indicates that endogenous PG synthesis may be involved in the control of spontaneous activity in gastric muscle.[18] Prostaglandin $F_{2\alpha}$ affected some electrical patterns and stimulated the mechanical responses of isolated longitudinal muscle of the guinea pig caecum. $PGF_{2\alpha}$ reduced the value of the membrane depolarization at which the electrical stimulation produced action potentials, decreased both the membrane potential and the membrane resistance. Atropin and indomethacin did not change the character of the $PGF_{2\alpha}$ effects on the membrane depolarization and the contractile responses. It is concluded that the stimulant effects of $PGF_{2\alpha}$ might be related to the sodium and calcium ions increased permeability of the cell membrane.[21] The same results were observed in the study that concluded, contraction of smooth muscle due to $PGF_2\alpha$ is also associated with increased action potential discharge and increase in membrane conductance.[9,14,20,21]

Our results conclude that $PGF_2\alpha$ decreases the contractile effects of longitudinal muscles whether applied before or after the antagonist whereas in circular muscle it increases the amplitude of contraction. Indomethacin antagonizes the effects of $PGF_2\alpha$ in both longitudinal and circular muscle vide table 1 and 2. Secondly when indomethacin applied directly it causes reduction in the amplitude of contraction in longitudinal muscle and increase in the force of contraction in circular muscle as shown in Fig. 1 and 2.

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