IN VITRO CONTRACTILE STUDY FROM EXCISED HUMAN GASTROINTESTINAL SPECIMENS: AN IMPORTANT TOOL FOR UNDERSTANDING MECHANISMS OF MOTILITY DISORDERS

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ABSTRACT:

It is well known fact that enteric nervous system plays a major role in regulation of contractile functions of intestinal smooth muscle. A number of neurotransmitters (excitatory/inhibitory) including Acetylcholine (ACh), Histamine, Serotonin, NO, Substance P, Bombesin, Motilin, ATP, VIP, Met-encephalin and Leu-encephalin, Polypeptide Y, Somatostatin are involved in gastrointestinal (GIT) motility mechanism. They are secreted by different neurons of enteric nervous system to modulate contractile and secretory functions of GIT. Dysregulation of some of these transmitters or their receptors have been already implicated in pathophysiology of certain GIT motility disorders. The present paper discusses how an in vitro study on diseased excised specimens may be useful in understanding the pathophysiology of various motility related GIT problems and thereby may be helpful for better medical and surgical management.

Keywords: *In vitro study, Gastrointestinal motility, Smooth muscle contraction, Neurotransmitters, Enteric nervous system*

INTRODUCTION

The regulation of contractile mechanisms of intestinal smooth muscle involves neural elements through extrinsic (sympathetic and parasympathetic) and intrinsic systems, in addition to myogenic mechanisms. The Intrinsic innervation is also known as enteric nervous system, consists of myenteric plexus (Auerbach's plexus) between outer longitudinal and middle circular muscle layers and submucous plexus (Meissner's plexus) lies in the submucosa, near its junction with the circular muscle layer. The intrinsic system contains about 100 millions of neurons in human – as many as are found in whole spinal cord (1). The major regulatory mechanisms of intestinal smooth muscle contractility involve cholinergic, adrenergic and nonadrenergic-noncholinergic systems. The spontaneous contractions involve pacemaker activity and the complex neuromuscular coordination in the intestinal tissue. Although many studies are available on intestinal motility in animals but very few found on human intestine. There are many neurotransmitters (excitatory/inhibitory) involved in GIT motility mechanism, which are secreted by different neurons of enteric nervous system eg. Acetylcholine (ACh), Histamine, Serotonin, NO, Substance P, Bombesin, Motilin, ATP, VIP (Vasoactive intestinal peptide), Met and leu encephalin, Polypeptide Y, Somatostatin etc.(2).

The basic tools that may help in evaluating the functional status of smooth muscle in gastrointestinal tract (GIT) is recording of spontaneous and chemically evoked contractions in *in vitro* preparations (3-5). The contractions evoked by agonist of any above neurotransmitters and their blockers may help in assessment of the status of cholinergic contractile mechanisms which is known as major and important regulating mechanism or other mechanisms of gastrointestinal motility in any GIT disorders where surgical excision of a part of intestine is needed. There are reports showing altered transmitter or/and receptor mechanisms in a number of GIT motility disorders (4). Thus, an *in vitro* study on diseased excised specimens is likely to be useful for not only understanding the basic pathophysiology of the ailment but also may be very helpful for better medical and surgical management.

MATERIAL & METHODS

The studies on GIT motility can be done on freshly excised gastrointestinal tissue from the surgery/pediatric surgery operation theater. Longitudinal or circular strips (2 to 3 mm wide and 15 to 20 mm long) can be prepared from freshly excised specimens operated for intestinal diseases like Hirschsprung Disease, Anorectal malformations, Diverticular disesease or Ulcerative colitis etc (figure 1).

One end of the strip needs to be hooked on lower end of a glass tube and other end with the force transducer in an organ bath filled with Krebs Ringer solution. The composition of Krebs Ringer solution in mM is – NaCl-119, KCl-4.7, $CaCl_2.2H_2O$-2.5, KH_2PO_4-1.2, $MgSO_4.7H_2O$-1.2, $NaHCO_3$-5.0 and

Glucose-11.0. Continuous 100% O_2 supply and temperature at 30 ± 2 C° should be maintained throughout the experiments. It is seen by our previous experiments that the intestinal strips show better response at 30 ± 2 °C temperature (4). Initial tension of 0.5 g should apply on the muscle strip. After stabilization for 30 min, isometric muscle contractions can be recorded by using isometric force transducer bridge amplifier and display onto personal computer (figure 2). Aternatively the same can be also recorded with the help of a ink based chart recorder in place of computer. Spontaneous contractions as well as chemically induced contractions can be recorded by using different chemical agonists and their antagonists. At the end, weight of the strip should be recorded to express the contractions in g/g of wet tissue.

Fig. 1: Photograph showing a single intestinal strip ready for mounting and recording of the contractions

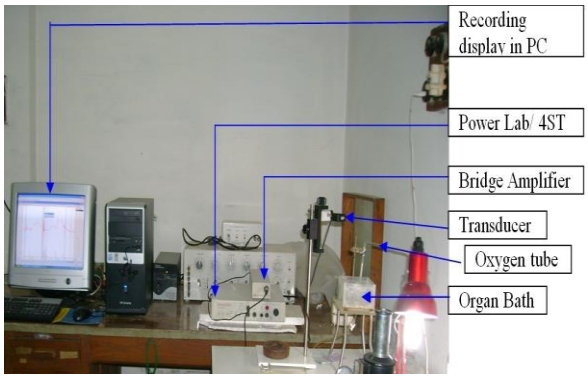

Fig. 2: Photograph showing experimental set-up.

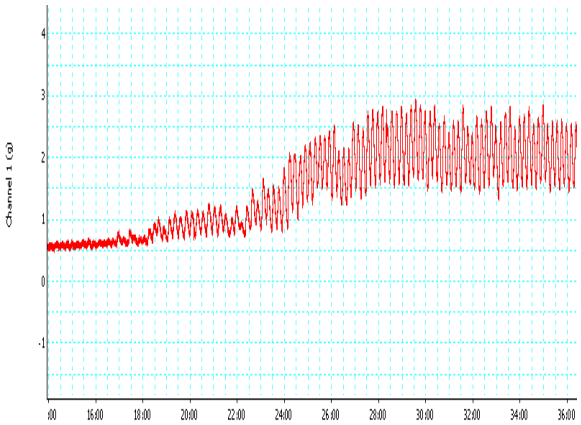

Fig. 3: A saved sample of actual contractions which can be used later for analysis purpose. X-axis shows the time in minutes. Y-axis indicates tension in grams.

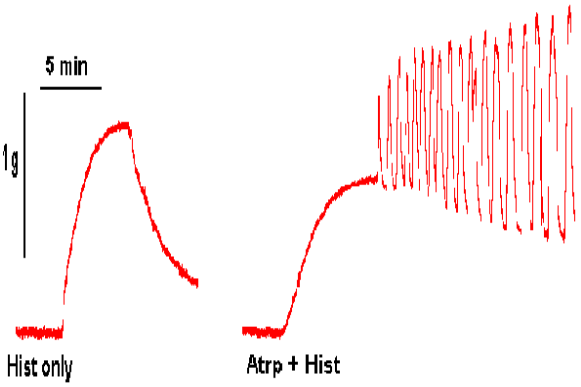

Fig. 4: Actual recordings showing the response of histamine (10 µM) with and without application of atropine (10µM) obtained from a colonic strip prepared from normal looking part of excised specimen of anorectal malformation. Please note that histamine response was increased and become phasic when pre-treated with atropine. This is an example for assessment of functional status of histaminergic contractions and also shows that cholinergic mechanisms inhibit histaminergic contractions.

DISCUSSION

Spontaneous contractions and cholinergic mechanisms play a major role in the mechanisms of the gastrointestinal (GIT) motility. Spontaneous contraction is one of the important parameters to understand the functional status of intestine. Out of a very few available *in vitro* studies on normal human GIT tissue, a normal rhythmic spontaneous contractions from colonic strips have been recorded earlier (6). The origin of spontaneous rhythmic contractions is known to be due to activity of interstitial cells of Cajal (ICC) (7). Ramon Y Cajal described nerve-like cells at the ends of motor neurons in organs innervated by peripheral nerves (8). These cells, which have been best described in the gastrointestinal tract, has provided promising explanations for motor physiology and pathophysiology of the hollow organs. The

spontaneous contractions in the intestinal smooth muscle are elicited by slow waves which are rhythmic oscillations in membrane potential of ICC (9). A fluctuation in intracellular Ca^{++} concentration has a major role in production of slow waves. The spikes on the peak of depolarization slow waves are translated into mechanical contractions. Excitatory neurotransmitters including ACh causes increase in number of spikes and thus amplitude of contractions while inhibitory neurotransmitters like epinephrine decreases the number of spikes and the tension of smooth muscle. The observation by Cao (2006) demonstrated reduced spontaneous contractions of colon in cases of ulcerative colitis (10). Decreased/absent spontaneous contractions were also observed in our lab in pouch colon associated with anorectal malformations (ARM) by using similar *in vitro* method. Such reduction has been attributed to the alteration of neurohumoral status or intracellular signaling pathways in the smooth muscle (10-11).

A number of studies provided the evidences for acetylcholine being the major excitatory neurotransmitter of GIT (12). Histamine is also proved to be involved in the gastrointestinal motility (3,4). Serotonin is also a very important mediator of gastrointestinal transmission (13). Serotonin mediates many processes in GIT eg. motility, epithelial secretion, emesis etc (14). Therefore, the abnormalities in the receptor mechanisms of different neurotransmitter in different diseases can be delineated by using different agents and their receptor blockers specific for various receptor subtypes by conducting *in vitro* study on excised human intestine in organ bath. An altered cholinergic and histaminergic mechanisms in pouch colon with anorectal malformations and Hirschsprung diseases have been documented in our laboratory (3,4) as well as in others (15) for diverticular disease. Further, it may be noted that, our previous study demonstrated cholinergic system could inhibit the histamine mediated large intestinal contraction as we observed potentiating effect of atropine on histamine induced contraction (4)(fig 4). Thus, *in vitro* study appered to be useful to understand changed contractile function of human intestinal smooth muscle.

CONCLUSION

It may be concluded that, *in vitro* study on excised intestinal specimen from operation theatre, may be important tool for understanding of pathophysiology of motility disorders in human and such kowledege may further used for improved medical and surgical management.

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