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In vitro Evaluation Techniques for Gastrointestinal Motility

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ABSTRACT

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In vitro techniques involve isolation of pieces of tissue taken from a recently killed animal and are kept alive in a suitable salt solution for biological evaluation of investigational drugs. Different parts of the small intestine are used in the pharmacological experiments. Various types of patterns of intestinal movements are involved in the physiological progression of chyme along the gastrointestinal tract (GIT). Peristaltic movements are myogenic and mainly initiated by local reflexes. Any substance affecting the motility can disturb functionality of the GIT. The study of gastrointestinal (GI) motility may be helpful in (1) determining alteration in motility (2) evaluating effect of pathological condition on GI transit (3) determining the therapeutic potential of drugs in motility disorders. Pieces of intestines can be easily obtained from smaller animals. For assay purposes, guinea pig ileum is chosen because it yields steady base-line, for studying the effects of drugs on pendular movements (Finkleman method), rabbit jejunum is used. Effect of drugs on peristalsis can be studied by setting up guinea pig ileum preparation by the method of Trendelenburg. The functional components of the isolated intestines are terminal sympathetic and parasympathetic synapses as well as parasympathetic ganglionic synapse. Stimulation of sympathetic nerves inhibits peristaltic movements, while parasympathetic stimulation increases movement. Most experiments involve the investigation of drug action on the contractions of the longitudinal muscle. A simple method was developed to generate spatio-temporal maps of patterns of movement in the isolated guinea-pig ileum. A new approach was developed for a quantitative and comprehensive evaluation of the force-time curve of guinea pig gut contractions. Isolated colon smooth muscle cells can be used to assess the receptor binding studies.

KEYWORDS: Jejunum, Ileum, Intestinal motility, Longitudinal muscle

.INTRODUCTION

The controlled progression of ingested food or chyme along the gastrointestinal tract (GIT) is an essential part of digestion process which results in supply of nutrients, water and electrolytes to various parts of the body. Different patterns of intestinal movements are involved in the physiological progression of chyme along the GIT and are the result of the interplay between spontaneous activity of intestinal smooth muscle, enteric or intrinsic and extrinsic neural circuits.[1-3] These functions are influenced by the motility patterns of the gut and any substance affecting the motility can disturb functionality of the GIT. A decrease in motility can lead to the stasis of chyme in the intestine which increase the quantum of bacterial growth, which may cause breakdown of the barrier, leading to a bacterial translocation to other organs of the body. On the other hand, increased motility interferes with the digestion and absorption processes and can lead to diarrhea and the malabsorption syndrome. [4]

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The study of gastrointestinal (GI) motility by *in vitro* techniques may be helpful in determining (1) the therapeutic potential of newer drugs in motility disorders (2) the alterations in motility secondary to physiological or pharmacological stimuli (3) the effect of pathological condition on GI motility.

***In vitro* techniques:** These techniques involve isolation of one or more pieces of tissue which have been taken from a recently killed animal and are kept alive in a suitable salt solution, temperature, pH etc., for biological evaluation of investigational drugs. The advantages of *in vitro* techniques over *in vivo* experiments are that (1) several new drugs can be tested on tissues obtained from a single animal (2) relatively small amount of the test material is required and (3) the drug effect is tested directly and perceived its action as the drug is free from the factors of absorption, metabolism, excretion or interference due to nerve reflexes. [5,6]

Suitability of isolated small intestine

Generally, the small intestine is used in the pharmacological experiments because it is long and slender can be cut into smaller pieces. In man, the small intestine is subdivided into a duodenum, which is short and devoid of mesentery, a jejunum which accounts for the proximal two-fifths of the remainder

of the intestine, and an ileum which accounts for some three-fifths of the organ. These portions show small variations from species to species. The duodenum of the mouse and rat is only a few millimeters long.[6-8]

Many *in vitro* preparations have been used to study motility patterns and responses of GI smooth muscle, these include- (1) Guinea pig ileum, (2) Rabbit ileum, duodenum and jejunum, (3) Stomach of mice, rat and hamster . Guinea pig and rabbit intestines are routinely used in preference to other species.[5] Pieces of intestine from any animal will continue to give responses for many hours if kept in a suitable salt solution. Usually they are placed in Tyrode's solution through which air is blown. With pieces of guinea pig ileum, there is relatively little spontaneous activity. Whereas, with rabbit intestine there is regular pendular movement, i.e., continuous contraction and relaxation. For assay purposes, guinea pig ileum is chosen, because of need of steady base-line for studying the effects of drugs on movements. For the effects on pendular movements (Finkleman method), it is necessary to use rabbit intestine, usually pieces of jejunum. Effect of drugs on peristalsis can be studied by setting up guinea pig ileum preparation by the method of Trendelenburg.[6-8]

Intestinal muscle is innervated by both parasympathetic (PS) and sympathetic fibres of the autonomic nervous system (ANS). Parasympathetic system supplies preganglionic fibres to enteric nervous system of (ENS) which synapse with myenteric plexus (Auerbach's) and submucosal plexus (Meissner's). Fibres from the cell bodies in these plexuses travel to the smooth muscle of the gut to control motility and to secretory cells in the mucosa. The parasympathetic system is responsible for maintaining normal intestinal motility through releasing acetylcholine (ACh) and it is the major one. Ascending reflex contractions of the small intestine involves predominantly cholinergic neurotransmission.[1,9]

Sympathetic system is usually run periarterially through the mesentery to the muscle. It supplies postganglionic nerve fibres to ENS, which synapse at submucosal plexus and to the cells of mucosal layer, releases noradrenaline (NA) as neurotransmitter. Fibres from the cell bodies in this plexus travel to the smooth muscle of the gut to control motility and to secretary cells in the mucosa. Stimulation of sympathetic or adrenergic nervous system produces relaxation GI smooth muscle, reduction in GI motility and secretions.[1,10]

Peristaltic movements are myogenic and mainly initiated by local reflexes. This reflex can occur without any neural connections to the brain or spinal cord, and the extrinsic nerves to the intestine appear to have only a minor role in modulating the activity of the organ. Stimulation of sympathetic nerves inhibits peristaltic movements, while parasympathetic stimulation increases peristaltic activity. The PS action is mediated via muscarinic receptors, while the inhibitory effects of sympathetic nerve stimulation are mediated via α- and β-adrenoceptors. Thus, the intestine is unusual in that both α- and β-receptor types mediate a similar biological response, that is inhibitory. The β-receptors are located on smooth muscle fibres, whilst the α- receptors are located presynaptically on PS ganglion cells of the myenteric plexus.[10]

The myogenic spontaneous activity of the intestine varies along its length and from species to species. Spontaneous activity is often artificially reduced by running the experiment at a temperature 5-7 ºC below normal body temperature. Rat, mouse and rabbit intestine exhibit spontaneous activity. This strong and consistent response of rabbit intestine is used for the study of relaxation produced by sympathetic nerve stimulation or sympathomimetic drugs.[6]

Stimulation of sympathetic nerves to rabbit jejunum may be achieved by periarterial stimulation; a method described by Finkleman. If the preparation is very fresh, and taken from a young rabbit it is sometimes possible to observe PS effects by using a low stimulation rates. Higher rates of stimulation produces exclusively sympathetic effects, and the effects of drugs on adrenergic transmission can accordingly be studied.[6-8]

The longitudinal smooth muscle fibers are present on the outer surface of the intestine. Most experiments involve the investigation of drug action on the contractions of the longitudinal muscle. The caecal end of the guinea pig ileum gives a steady baseline which is important for studying drug-induced contractions.[6-8]

Stimulation of the intramural nerves of the guinea pig ileum can be achieved by either using transmural or field stimulation. The myenteric plexus-longitudinal muscle strip is a very useful alternative preparation when field stimulation is used. The main benefits of this preparation are that the tissue is much less complex and useful in the studies of cholinergic system. In addition, muscle spasm is reduced as circular fibres are absent, and the onset and offset of drug action is more rapid. The nerve plexus in the wall of the gut is complex and has been described as providing a paradigm of mechanisms at synapses in the CNS.[6]

Guinea pig ileum: Isolated ileum being relatively more resistant to trauma, easier to set up and produces larger contractions than jejunum or duodenum.[5] A guinea pig is killed by a blow on the head and cutting the throat. The abdomen is opened through a midline incision and when the caecum is lifted forward, the ileum will be found joined on the

back of it. A suitable length of ileum is removed and placed in a dish containing Tyrode's solution (NaCl 137 mM; KCl 2.7 mM; $CaCl_2$ 1.8 mM; $MgCl_2$ 0.1mM; $NaHCO_3$ 11.9 mM; NaH_2PO4 0.4mM; Glucose 5.55 mM). A thread is tied at the top, i.e., jejunal end to serve as a marker. Care should be taken to avoid damaging the gut muscle. It should be handled with the fingers rather than gripped with forceps. The mesentery is trimmed away and pieces are cut from the length of ileum as required, starting above Peyer's patch. The tissue should give responses to drugs even if 2 or 3 h have passed since the animal was killed. If the animal has not been fed recently, the gut may be clean enough to use directly. Otherwise, it may be necessary to wash out the contents by forcing Tyrode's solution with little pressure. It is also important to wash the gut through when it has had time to relax fully in Tyrode's solution at about 37 ºC. Pieces of ileum will keep for several hours in clean Tyrode's solution at room temperature (below 20 ºC). A section of splanchnic nerve (mesenteric pedicle) may be left attached to the muscle strip for stimulation. This method is most useful in pharmacological studies of peripheral acting drugs.[6-8]

Rabbit jejunum: In a similar way, jejunum is obtained from a freshly killed rabbit. The abdomen is exposed and opened. The jejunum can be identified by following it back to the stomach. The intestine is cut at a point 5 to 10 cm below the stomach and a length taken from here downwards towards the caecum. It does not usually require to be washed through, partly because it is wider than guinea pig intestine and partly because its spontaneous activity causes it to clear itself. A suitable length free from mesenteric attachments is transferred to a dish containing Tyrode's solution. It is then cut into 2-3 cm in length several pieces. A thread is tied at each end, taking care to see that the jejunum is left open and the threads do not close the lumen.[6-8]

(1) Setting-up the preparation by the method of Magnus

The isolated ileum, as first described by Magnus (1904), is probably the most widely used model in experimental pharmacology.[11] This method has been used for many purposes, such as the study on the effects of adrenaline on the lower segments causing contraction and on the segments of the upper end causing relaxation by Munro (1951) or the study on the origin of ACh release from guinea pig intestine and longitudinal muscle strips by Paton and Zar (1968) either retaining or being denervated from Auerbach's plexus.[12,13] This model is used as a basic screening procedure for spasmolytic activity, whereby an anti-ACh or anticarbachol effect indicates antimuscarinic activity and an anti-barium chloride effects indicates a musculotropic, papaverine-like effect.[14]

Procedure: A piece of small intestine is from guinea pig or rabbit as stated previously. The mesenteric membrane is trimmed from a length of intestine so that the muscle is quite free, and a thread is attached at each end by inserting a needle from the inside of the gut outwards. In no circumstances should be lumen of the gut be closed off. One thread is tied to a fixed pin in the organ bath and the other to a lever which has a frontal – writing point.[6-8]

I. For the guinea pig ileum, the load should be about 0.5 g and the magnification from 5 to 7-fold; for a bath whose length is 5-8 cm., and volume about 10 ml., the length of the piece of ileum should be 2 to 3 cm when relaxed.

II. For rabbit intestine, a slightly wider bath is necessary and the volume is usually about 20 ml. The magnification should be smaller, usually not more than 5-fold, and the load should be bigger usually about 1 g, but should not be so big that it causes the muscle to relax fully.

Guinea pig ileum: The preparation should contract in response to drugs acting on parasympathetic ganglia, at postganglionic cholinergic receptors, or at certain other receptors on smooth muscle. Because the guinea pig ileum is fully relaxed under its load, it should not be seen to relax any further in response to drugs affecting sympathetic postganglionic receptors; likewise, as there should not be any spontaneous activity of the ileum, it will not be possible to see that these drugs inhibit it. If the lever is very lightly loaded or if the ileum is spontaneously active, a relaxation and inhibition can be seen but drugs which produce these effects are better tested on rabbit jejunum.[6-8] Experiments with this preparation are usually performed at 37 ºC in Tyrode's solution through which air is blown. Drugs such as ACh or histamine produce a very rapid response and can be washed out after 30 seconds or less. The following time cycle is convenient:

0 min- To start the kymograph.

1 min- To add the drug.

1.5 min- To stop the kymograph and wash the preparation

3 min- To start the kymograph

Occasionally, it may be necessary to wash the preparation a second time and to lengthen the cycle to 4 min, but often it is possible to work with a shorter cycle, though there is a risk of the tissue not having had enough time to recover completely, especially if large effects were produced with the previous dose. Although the guinea pig ileum is chosen because it may produce a steady base-line, there is often some spontaneous activity; this is more likely to subside if the tissue is given with drugs at regular intervals than if the experimenter waits

in the hope that it will stop of it own accord. This preparation should give reasonably steady responses to drugs within about 30 min of being set up, particularly if it is being given doses at regular intervals.[6-8]

Use of the guinea pig ileum as an assay preparation: Although it is not as sensitive as the eserinized leech muscle, the guinea pig ileum is a very useful preparation for the assay of solutions of cholinergic drugs. It is also convenient for the assay of solutions of histamine, though it is not quite as sensitive to histamine as to acetylcholine. If a biological extract, e.g., a perfusion fluid is being assayed, it is necessary to perform a histamine assay with atropine (2×10^{-6} M to 10^{-7} M) in the Tyrode's solution, because ACh may also be present. Similarly it is advisable to perform cholinergic assays with mepyramine (5×10^{-6} M or 2×10^{-7} M) present in Tyrode's solution, because histamine may also be present.[6-8]

Use of the Rabbit jejunum: This preparation is treated in the same manner as the guinea pig ileum but as it is a thicker piece of muscle it is probably better to aerate it with a mixture of oxygen (95%) and carbon dioxide (5%) rather than air. It is also likely that it may be necessary to allow longer for drugs to be washed out of the tissue. For this reason, it may not be possible to work with a cycle shorter than 3 min and it may be necessary to lengthen it. This is particularly likely to be necessary if advantage is being taken of the spontaneous activity of this tissue to study the actions of adrenergic drugs, which inhibit movement. There is a possibility that some of the compounds are producing effects by actions on parasympathetic ganglia and for which it is required to repeat the experiment with Tyrode's solution containing hexamethonium (10^{-4} M).[6-8]

Many modifications of the Magnus technique have been described in the literature, mainly with the isolated ileum. Okwuasaba and Cook (1980) dissected the myenteric plexus and longitudinal muscle free of the underlying circular muscle according to the method of Paton (1957), Paton and Zar (1968) and stimulated the preparation with trains of supramaximal rectangular pulses of 1.0 m sec duration at a frequency of 0.2 Hz.[15,16,13] De Graaf et al (1983) described a fully automated system for in vitro experiments with isolated tissues.[17] The apparatus consists of an organ bath equipped with (1) a gradient pump supplying a logarithmic concentration / time gradient of agonists; (2) pumps and valves for dispensing bath fluid, antagonist solution, and an oxygenation gas mixture; and (3) a transducer with automatic baseline adjustment. The information coming from the preparation is fed into a minicomputer. The data of various experiments can be accumulated and Schild-plots obtained. Barnette et al (1990) used electrically stimulated strips of circular smooth muscle from the lower oesophageal sphincter of dogs to study the inhibition of neuronally-induced relaxation by opioid peptides.[18] Bradykinin antagonism can be studied in the isolated guinea pig ileum bathed in a solution containing atropine, diphenylhydramine, indomethacin and captopri.[19]

Cascade superfusion technique: The technique of isolated organ superfusion was developed by Gaddum (1953) for the assay of biologically active substances.[20] Extension of the technique for multiple tissue superfusion with particular reference to the identification and the assay of prostaglandin-like activity was used by various authors. *Procedure*: The apparatus consist of a double-wall glass container (height 20-25 cm, inner diameter 7-8 cm) with an outlet at the bottom. A constant temperature of 38 ºC is maintained by circulation of warm water through the outer jacket. Inside the glass container can be suspended up to 5 pieces of tissue of various origins. The multiple preparation tissue holder consists of a vertical rectangular rod and plastic platforms for attachment of the tissues and for accurate deflection of the superfusate on to the lower tissue. The rod is grooved at 10 mm intervals with 1 mm deep slots set at an angle of 20º to the horizontal. To the upper surface of the nonwettable platform a small plastic hook is cemented at such a distance from the rod that when the tissue is in position its attachment thread passes between the V-shaped notch cut into the margin of the upper tissue platform. The individual platforms are inserted on to the vertical rod by slotting into the requisite grooves appropriate to the tissue length. Thus, the superperfusate passes at a uniform flow rate down the tissues of the cascade and the tension recording threads are separated from each other by about 5 mm gap so that the responses can be conveniently recorded. The threads from the organs are connected over isotonic levers to isometric tension transducers. The lever is used for preloads according to the individual origin. Tension exerted by each tissue is recorded on a polygraph. Various media can be used for superfusion. Many tissue preparations can be used for the cascade, such as (1) Rat fundic strip, duodenum, colon (2) Guinea pig proximal colon (3) Rabbit stomach.[14]

(2) **Setting-up the preparation by the method of Trendelenburg**

A piece of guinea pig intestine is placed in the organ bath containing Tyrode's solution, with the caecal end mounted over a tube connected to a reservoir also containing Tyrode's solution. The intestine is carefully washed through in order to expel any air and the other end is then tied off with a thread which is attached to a lever. Although both the inside and the outside of the gut are therefore in Tyrode's solution, there is no

connection between the two and the pressure inside the gut may be raised or lowered by altering the height of the fluid in the reservoir relative to the level of fluid in the bath. The temperature is usually 37 ºC and air is blown through the Tyrode's solution in the organ bath, but it is not possible to aerate the Tyrode's solution in contact with the inside of the intestine. The piece of intestine should be about 6 cm when relaxed. The organ bath also should be longer and wider, to accommodate the tube over which the preparation is mounted; its capacity should be between 30 and 50 ml.[6-8, 21]

A rise in internal pressure will set off the peristaltic reflex and the fluid inside the gut will be driven back into the reservoir. The fluid cannot actually be propelled along the gut but will be driven to and fro, and the changes in volumes will be registered by the volume recorder or pressure transducer attached to the reservoir. These changes in volume are produced by the contractions of both the longitudinal and the circular muscle. However, the lever attached to the gut will record only the movements of the longitudinal muscle.[6-8]

Drugs can be added to the organ bath, but with this simple arrangement it is not possible to add drugs to the fluid inside the gut. Even washing drugs out of the organ bath raises difficulties because emptying the bath will disturb the gut greatly. The preparation should therefore be washed by overflow. If the gut is really active, it is best to leave it with the pressure inside rather below that outside when it is desired to rest the preparation. It is important, however, to check that the negative pressure does not cause the muscle to be sucked back into the tube on which it is mounted. A modification of this method, in which drugs can be applied to the inside of the gut as well as to the outside, is described by Bulbring, Crema and Saxby.[22]

Drugs and the peristaltic reflex: First, it is investigated to find out the effect of different heads of pressure on the peristaltic reflex. With an increased pressure in the gut of 1 or 2 cm of Tyrode's solution, it should be possible to produce peristalsis which lasts for 30 sec. With pressures greater than this, the preparation will tend to become fatigued. The reflex is tested once in every two min to find what pressure regularly produces peristalsis; this will be used as the standard stimulus for testing the effects of drugs on the reflex. 0.1ml of 2×10^{-3} M cocaine is added (or 0.1 ml of lignocaine, 5×10^{-3} M) to the bath, allowed it to act for 2 min and then tested the reflex. If this is unaltered by the drug, higher doses are tried. When an effect has been obtained, preparation is washed and waited until the reflex returns to normal. [6-8]

The effects are observed on the reflex by adding 0.1 ml of hexamethonium, 3×10^{-3} M, to the bath, again allowing the drug 2 min in which to produce an effect and increasing the dose if the expected result is not obtained. The preparation is washed and waited until the peristaltic reflex returns to normal.

Similarly, the effects on the reflex are observed by adding 0.1 ml of atropine, 10^{-5} M, to the bath; the reflex is tested after the atropine has been in contact with the tissue for 2 min and again at intervals, for up to 10 min. If it is still unaltered, the dose of atropine is increased.

With some preparations the peristaltic reflex can regularly be maintained for 60 sec but with others it may become fatigued even in 30 sec. The experiment can be performed with a shorter length of stimulus than the 30 sec period suggested.

This experiment shows that the peristaltic reflex involves nervous pathways, i.e., that it is a local reflex which does not involve the spinal cord, and it is not, merely a property of the muscle itself as is the pendular movement of rabbit intestine. The preparation could be used for testing local anaesthetic, ganglion-blocking agents, or atropine-like compounds, but it is difficult to use it for quantitative work. The sensitivity of the tissue fluctuates very considerably during the experiment and it is really only suitable for qualitative work.[6-8]

(3) Setting up the preparation by the method of Finkelman

A piece of rabbit jejunum is taken, together with the mesentery. The nerve supplying the intestine lies in the mesentery along with the arterial blood supply and can easily be dissected out. The intestine is mounted but the mesentery is threaded through electrodes, connected to a stimulator which will deliver rectangular-wave pulses of 0.5 m sec duration. If the preparation is very fresh, stimulation at a slow rate, 2-4 pulses/sec, may produce parasympathetic effects. With higher rates 30-50 pulses / sec, only sympathetic effects are seen, pendular movement ceases and the intestine relaxes. It is usually necessary to use rather a large stimulus, about 10 volts, because of the electrical insulation provided by the fat in the mesentery. The stimulus should be applied for not more than 30 sec, to avoid fatigue and 1.5 min should be allowed for recovery or longer if the pendular movement has not returned to normal. The bath must be slightly wider than that normally used for rabbit intestine, to allow space for the electrodes; its volume is usually at least 25 ml.[6-8, 23]

(4) Setting-up the rat fundus strip

At rat is killed by a blow on the head and cutting the throat and the abdomen opened. The fundal part of the stomach can easily be identified because it is grey, whereas the pyloric part is pink. The stomach is dissected out and the pink pyloric end cut away from the grey fundal end. The fundal end is split open so as to form a sheet, the contents are washed away and

preparation transferred to a dish containing Krebs' solution. Cuts are made in this sheet of muscle so as to produce a strip, to which a thread is attached at each end and the preparation mounted in the organ-bath.[7,8,24]

A thread is attached at each end and the preparation is mounted in Krebs' solution at 37 ºC, aerated with carbogen. The organ bath should be relatively long (8-10 cm) but can be narrow, so its volume can still be quite small (5-10 ml). One end of the strip is attached to a fixed pin in the bath and the other end to a lever writing frontally on a smoked drum. It is better to use an auxotonic lever, with which the load increases as the muscle shortens. The load in the horizontal position is usually about 1 g and although a magnification as high as 16 may be used, it is usual only to work with magnification of between 5 and 7. The muscle does not relax spontaneously after it has been caused to contract so it must be stretched, to assist its recovery, by adding an extra 1 g to the load. The preparation should be left for 30 min to stretch before use. Because the tissue only contracts slowly, a long time-cycle is necessary,

0 min: To start the kymograph

1 min: To add the drug

2.5 min: To stop the kymograph, wash the preparation and add the extra load.

6 min: To remove the extra load and start the kymograph.

The interval between doses is therefore 6 min. Sometimes the tissue only recovers slowly after stretching making it very difficult to obtain to steady base-line. If this happens, do not stretch the preparation for as long as 3.5 min but allow a longer period for recovery without stretching e.g., stretch the preparation for 2 min and then allow 5.5 min for recovery, so doses are added once every 10 min. [7,8,24] The sensitivity of the preparation is investigated by the doses of 0.1 or 0.2 ml of the following; 5-HT (10^{-7} M); ACh (10^{-6} M); nicotine 10^{-3} M; Histamine 10^{-3} M.

(5) Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps.

Hennig et al (1999) developed a simple method to generate spatio-temporal maps of patterns of movement in the isolated guinea-pig ileum.[3] They evoked peristalsis in small intestine by slow fluid infusion and recorded onto video and digitized. Spatio-temporal maps of diameter and longitudinal movement were constructed and parameters of motion were calculated. During the filling of the isolated segments of intestine, rhythmic local longitudinal movements were observed at several points along the preparation. In addition, occasional synchronized longitudinal muscle contractions caused net shortening of the preparation and always preceded the onset of peristaltic emptying. Peristaltic emptying was characterized by a contraction of the circular muscle which usually started at the oral end of the preparation, that propagated aborally, propelling the contents. However, in 19 % of trials, the first circular muscle contraction occurred in the aboral half of the preparation. The propagation of peristalsis consisted of separate sequential circular muscle contractions several centimeters long, particularly in the oral half of the preparation, giving a 'step-like' appearance to the spatio-temporal map. The gut was transiently distended aboral to the propagating circular muscle contraction due to the propulsion of contents. At each point in the preparation, the longitudinal muscle remained contracted during the propulsive part of the circular muscle contraction.

The maps generated can be used to quantify changes in the length and diameter of the intestine during peristalsis evoked by slow fluid infusion resolution. From such detailed analysis, organized patterns of longitudinal muscle contractility became apparent during the preparatory or filling phase. It was also clear that the longitudinal muscle contracts in synchrony with the circular muscle during the propagating wave of circular muscle contraction, as suggested by some authors rather than relaxing as others have suggested.[25-27] In addition, the emptying phase of peristalsis was shown to consist, in many preparations, of a 'step-wise' sequential activation of separate circular muscle contractions at different points along the intestine, rather than as a smooth propagating wave of contraction.

(6) New method for evaluating intestinal contractions in guinea pig by curve fitting

Sugimori et al (2000) using a "hybrid logistic function," attempted to develop a new approach for a quantitative and comprehensive evaluation of the force-time curve of guinea pig gut contractions.[28] They recorded ileum twitch and proximal colon spontaneous isometric longitudinal contractions because of their high regularities. They digitized the force-time curves of both contractions and performed curve fitting to them by hybrid logistic functions with a personal computer. They found that the fitness of these functions to both contractions was excellent. The respective best-fit parameters of these functions were closely correlated with the observed mechanical indexes, all of which are physiologically meaningful. The result suggests the possibility that these parameters can characterize the magnitudes and time courses of F(t) curves of the intestinal contractions. Furthermore, it might be able to show an effect of a pharmacological agent specifically either on the contraction phase, the relaxation phase, or other parameters of each. Therefore, their study emphasized its use for evaluating gut motility is promising for clinical application.

(7) Preparation of colon longitudinal smooth muscle cells

Kobayashi et al (2001) developed this technique using the colon of guinea pig.[29] The distal colon of guinea pig, measuring approximately 25 cm in length from the beginning of the rectum forward, is excised. The longitudinal muscle tissue should be obtained by gently pulling off the muscle strips from the colon with serrated tweezers. The isolated longitudinal muscle is minced into small pieces with iris scissors and then enzymatically dispersed by shaking gently for 30 min at 37 ºC in 8 ml of Ca^{2+} -free Hanks' balanced salt solution(HBSS, pH 7.4) containing 20 mM 2-[4(2-hydroxyethyl)-1- piperazinyl] ethanesulfonic acid (HEPES), 0.1% bovine serum albumin (BSA), 15 mg papain (from Papaya Latex, 15 units/mg), 4 mg hyaluronidase type I-S (from bovine testes, 440 units/mg), and 3 mg dithiothreitol. The pieces are washed 3 times in phosphate-buffered saline(PBS) and again dispersed in 8 ml of Ca^{2+} -free HBSS containing 20 mM HEPES (pH 7.4), 0.1% BSA, and 10 mg collagenase S-I (from Streptomyces parvulus subsp. citrinus 260 units/mg; Nitta Gelatin, Osaka, Japan) at 37 ºC for an additional 30 min. The digestion is terminated by adding 80 ml of 250 mM ethyleneglycol-bis-(b-aminoethylether) N,N, N0, N0-tetraacetic acid (EGTA) solution. The dissociated cells were washed 3 times in PBS, filtered through a 250 mm nylon mesh, and then resuspended in phenol red free HBSS (pH7.4) containing 20 mM HEPES and 0.1% BSA (HBSS/BSA). The prepared cells were stored at 4 ºC until testing. The cells can be used for evaluation receptor-agonist-antagonist binding potencies.

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