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PHARMACOLOGICAL CHARACTERIZATION OF THE RECEPTORS OF THE GUINEA-PIG TERMINAL ILBUM, CARDIAC AND ANAL SPHINCTERS

BY

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PHARMACOLOGICAL CHARACTERIZATION OF THE RECEPTORS OF THE GUINEA-PIG TERMINAL ILEUM, CARDIAC AND ANAL SPHINCTERS

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY FACULTY OF PHARMACEUTICAL SCIENCES UNIVERSITY OF NIGERIA, NSUKKA

BY

OFFIAH, VERONICA NKECHI B.Sc; M.Sc (IBADAN)

IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DOCTOR OF PHILOSOPHY (PH.D) DEGREE IN PHARMACOLOGY

SUPERVISOR: PROFESSOR F. IWE AKUKUE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY FACULTY OF PHARMACEUTICAL SCIENCES UNIVERSITY OF NIGERIA.
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OF THE DOCTOR OF PHILOSOPHY (PH.D.) DEGREE
IN PHARMACOLOGY OF THE
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DEAN, SCHOOL OF POSTGRADUATE STUDIES
DEDICATION

This work is affectionately dedicated to my son, Aniwetalu, for his understanding and love.
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Naschi.

NSUKKA, 1957.
The effects of cholinergic, adrenergic and histamine receptor agonists and antagonists were investigated on the guinea-pig isolated terminal ileum, cardiac and anal sphincters, with the aim of characterizing the receptors present on these tissues. The receptors of the terminal ileum were compared with those of the proximal ileum.

Cholinergic stimulants: acetylcholine, carbachol and methacholine caused dose-dependent contractions of the guinea-pig terminal ileum; cardiac and anal sphincters. The mean $P_{D_2}$ values for acetylcholine on the terminal ileum; proximal ileum; cardiac and anal sphincters were $7.7 \pm 0.03; 7.85 \pm 0.14; 5.68 \pm 0.1$ and $5.4 \pm 0.23$ respectively.

Atropine caused dose-dependent right shifts in the log-dose response curves of acetylcholine. There was depression of maximal response to acetylcholine in the presence of atropine on the cardiac sphincter while elevation of maximal response was observed for the anal sphincter. The mean $P_{A_2}$ values for atropine against acetylcholine on these tissues were $9.60; 9.74; 8.77$ and $11.57$ for the terminal ileum, proximal ileum; cardiac and anal sphincters respectively.

On all these tissues Pirenzepine ($<2.55 \times 10^{-7}M$) caused potentiation of acetylcholine-induced contractions while pirenzepine ($>3 \times 10^{-7}M$) inhibited acetylcholine-induced contractions competitively.
The F42 values for h-DAMP on the terminal and proximal ileum were 9.51 ± 0.14 and 9.67 ± 0.12 respectively. These results suggested the presence of inhibitory muscarinic M1 and excitatory muscarinic M2-receptors in all these tissues.

Histamine caused dose-dependent contractions of the guinea-pig terminal and proximal ileum, but had no marked effect on the cardiac and anal sphincters. Mepyramine caused dose-dependent right shifts while cimetidine caused left shifts (which were dose-dependent in the terminal ileum) in the log-dose response curves for histamine showing the presence of six population of histamine H1- and H2-receptors in the guinea-pig ileum.

Alpha-adrenoceptor agonists contracted the terminal ileum and anal sphincter, but had no effect on the cardiac sphincter. This α-excitatory effect was blocked by phentolamine and prazosin suggesting the involvement of α1-adrenoceptor. Yohimbine potentiated these α-excitatory effects which show the presence of inhibitory α2-adrenoceptors. The excitatory effects of NA on the proximal ileum were converted to relaxations by yohimbine. This suggested that α2-receptor activation on this tissue is excitatory.

Both α - and β-adrenoceptor agonists inhibited twitch responses of the guinea-pig ileum. They also relaxed the cardiac and anal sphincters when their tone was raised with
carbachol. These relaxant effects of $\alpha$-adrenoceptors were blocked by prazosin and yohimbine while those caused by $\beta$-adrenoceptors were blocked by propranolol.
CHAPTER ONE

INTRODUCTION
INTRODUCTION

THE DIGESTIVE SYSTEM

The digestive system or tract, also known as the gastrointestinal tract (GIT) is composed of a series of organs through which the body is provided with continual supply of water, electrolytes, and nutrients. The mechanisms by which this vital objective is achieved are complex:-(a) food substances taken into the system are reduced to simpler forms, which can be absorbed by the intestinal mucosa. This action is achieved through the actions of enzymes and other digestive adjuncts such as bile, that are secreted by the digestive system. (b) Food substances must be mixed and transported to various segments of the system and retained for a sufficient interval to accomplish both digestion and absorption.

The digestive system, is a hollow tube which consists of the following: mouth, oesophagus, stomach, small intestine, large intestine, rectum and anal canal. Sphincters occur at some points along the digestive system. There are sphincters between (a) the oesophagus and the stomach, (b) the stomach and the small intestine (c) the bile duct and the small intestine, (d) the small intestine and the large intestine and (e) at the end of the GIT (Figure 1.1).

The wall of the digestive tract shows a basic structural pattern. Figure 1.2 illustrates a typical section of the digestive system wall (Cut wall), showing the following layers
FIGURE 1.1


The digestive system of human is illustrated instead of that of guinea-pig because of the clinical importance of the sphincters in human.

1 = Mucous Layer or Mucosa
2 = Submucous layer or submucosa
3 = Muscular Layer
4 = Serous layer or serosa
from inner surface outwards (Guyton, 1981).

(1) The mucous layer or mucosa. This consists of the surface epithelium with its glands, loose fibrous tissue with capillaries and lymphatic vessels. The type of epithelial tissue varies with site and function. The mucous layer is in close contact with gut contents and is specialized for secretion and absorption.

(2) Submucous layer or submucosa.

This layer is made up of dense fibrous tissue in which lies blood vessels, lymphatic vessels, and tissues and Meissner’s nerve plexus. There are glands in the submucosa of the oesophagus and the first part of the duodenum (McNaught and Callander, 1975).

(3) Muscular coat or muscularis externa.

This layer consists of smooth muscle layers except in the upper two third of the oesophagus where the muscle is either skeletal or mixed skeletal and visceral (McNaught and Callander, 1975; Counsell et al, 1977; Guyton, 1981). The smooth muscle layers are made up of inner circular arrangement and outer longitudinal arrangement. In the stomach there is a third muscle layer which is obliquely arranged. Between these muscle layers are blood and lymphatic vessels and Auerbach’s nerve plexus. The muscular coat is for movement. It controls the diameter of the tube and mixes contents
along the tube.

(4) Serous coat or serosa.

This layer has fibrous tissue with fat, blood and lymphatic vessels. Where the tube is suspended by a mesentery, the serosa is formed by visceral layer of peritoneum, but where there is no mesentery, the visceral layer of peritoneum is replaced by fibrous tissue, which merges with surrounding fibrous tissue.

**Sphincters of the Digestive System**

The sphincters of the digestive system are the regions whose specialized function is to control the time materials move from one region of the digestive tract to the other, that is, they control the gastrointestinal emptying time.

Morphologically, the term "sphincter" implies a thickening of the circular muscle fibre in a hollow viscus (Botha, 1962). Sphincter muscle has a high tonus and may be considered to be in a state of continuous contraction. The contraction of circular muscle fibres brings about closure of sphincter, while contraction of longitudinal fibres brings about opening of the sphincter. This means that both opening and closing of sphincters are active phenomena which occur at various levels along the digestive tract as well as in other systems.

The clearly identifiable sphincters of the digestive tract are:— the lower oesophageal (cardiac) sphincter; the pyloric sphincter; the sphincter of Oddi (Choledochoduodenal);
the ileo-colic sphincter or in some animals that have cecum (e.g. guinea-pig), it is known as the ileo-cecal sphincter; and the anal sphincters.

**THE LOWER OESOPHAGEAL (CARDIAC) SPHINCTER**

The cardiac sphincter occurs at the junction between the oesophagus and the stomach. It is believed to be a physiological and not an anatomical sphincter. This is because histological evidence for a sphincteric structure at this lower end of the oesophagus in some animals like cat is absent (Clark and Vane, 1961). On the other hand, Mann and Shorter (1964), observed an anatomical gastroesophageal sphincter in the last 1 to 2 cm of the oesophageal tube of canine which is composed of circularly disposed smooth muscle. However, whether physiological or anatomical, this area is always associated with increased pressure in man (McCallum et al, 1983).

**THE PYLORIC SPHINCTER**

The pyloric sphincter occurs at the junction of the stomach and the duodenum. It is a muscular ring formed by marked thickening of the circular layer of the muscular coat. Dindo and Anderson (1970), observed in man, that some of the longitudinal fibres turn in and interlace with the fibres of the sphincter.

In man the pyloric sphincter contracts momentarily in a rhythmic manner 4 to 6 times a minute in response to each propagated atrial peristaltic wave (Loukes et al, 1960).
It has been suggested by Edward and Rowland, (1968), that the gastroduodenal junction (pylorus) cannot be regarded as a true sphincter. This is because its functional activity cannot be separated from the rest of the stomach, and it is not associated with a region of raised intraluminal pressure.

**THE SPHINCTER OF ODDI**

The circular muscle around the lower part of the bile duct, including the ampulla and the terminal part of the main pancreatic duct is thickened and is called the sphincter of the hepatopancreatic ampulla or the sphincter of Oddi (William and Warwick, 1980).

It has been shown in man that there is no sphincteric arrangement of the musculature around the opening of the bile and pancreatic ducts into the duodenum (Kirk, 1944). Kirk (1944) also observed that the sphincter of Oddi surrounds the bile duct as it passes through the submucosal zone of the duodenal wall, and that the sphincter is continuous with the circular muscle coat of the duodenum, which is thickened at this site. However subsequent studies by Boyd (1957) suggest strongly that in man and other primates there is a common sphincteric apparatus surrounding both ducts, and that the common bile duct has a second sphincter of the type described above.

**THE ILEOCecal SPHINCTER**

In man, the lower end of the ileum opens into the medial
and posterior aspect of the large intestine, at the point of junction of the caecum with the colon. The ileocaecal orifice is represented on the surface at the point of intersection of the right lateral and transstubiccular planes. About 2 cm below this point is the Vermiform appendix which opens into the caecum. The opening is provided with a "valve", consisting of two segments or flaps which projects into the lumen of the large intestine (William and Warwick, 1960; Guyton, 1981). In the guinea-pig, the last part of the ileum (terminal ileum) opens into the caecum directly. In man the circular and longitudinal muscle coats of the terminal part of the ileum are continued into the valve and form a sphincter.

The main function of this sphincter is to prevent the contents of the ileum from passing too rapidly into the caecum (Guyton, 1981). This promotes more complete digestion and absorption of the intestinal contents.

**THE ANAL SPHINCTER**

The anal sphincter consists of two parts: the external and the internal anal sphincters. The external anal sphincter surrounds the lowest part of the anal canal. It is intimately adherent to the skin, and it overlaps the internal anal sphincter. The external anal sphincter is composed of striated muscle (Duthie and Watt, 1965), and because of this it is not regarded as a true sphincter. The internal anal
sphincter results from a thickening of the circular muscle of the anal canal (Furness, 1969; Furness and Costa, 1974; Lim and Mair, 1983; 1985). The anal sphincters reduce the lumen of the terminal portion of the intestine.

The tone of both internal and external anal sphincters keep the anal canal and the anus closed (Mayner, 1979). During defecation these muscles are relaxed and the lower part of the anal canal is opened out and flattened, so that the mucous membrane of the upper part of the canal appears at the surface (William and Warwick, 1960; Guyton, 1981). The external anal sphincter can be voluntarily contracted because it is composed of skeletal muscle.

**CLINICAL IMPORTANCE OF SPHINCTERS**

Generally disorders of sphincter function may result from overactivity or incompetent functions of the sphincter. Since both opening and closing of sphincters are active phenomena, obstruction may result from a spasm of circular muscles or a lack of contraction of longitudinal fibres which dilate the segment of bowel, or both.

Some of the common disorders of the sphincters are: Achalasia of the cardiac; Pyloric stenosis; and Incompetent anal sphincter.

Disorders of sphincters are of clinical importance because sphincters control the time substances move from one part of the gut to another, thereby controlling the rate of digestion and absorption of substances.

**SMOOTH MUSCLE**

A true sphincter is that which is formed by the thickening of the circular smooth muscle layer, (Botha, 1962). Smooth muscle generally occurs as part of a complex tissue. It is usually associated with secretory epithelium, often with nerve cells, nerve plexuses and nerve endings. Smooth muscle cells
are fusiform or spindle-shaped with centrally placed nuclei. Their dimensions vary considerably. In the intestine they are 5 - 6 \( \text{mm} \) in diameter and 30 - 40 \( \text{mm} \) long (Bowman and Rang, 1960). The activity of smooth muscles is sensitive to small changes in the environment and its response to stimuli is often variable. Many smooth muscles, such as the intestine of rabbit and the taenia of guinea-pig cecum are spontaneously active (Bulbring and Tomita, 1960; Bolton, 1971; 1972; Suzuki and Kuriyama, 1975; Bulbring and Tomita, 1987), and their tonus (expressed as basal length or as tension) fluctuates from time to time. The sphincters of the digestive tract have tone (Gonella et al., 1979; Bouvier and Gonella, 1981; Brock-Uttre and Downing, 1983; Dent et al., 1983). The physiological and pharmacological properties of smooth muscle vary greatly depending on the type of organ from which it is taken, the age, condition, and sex of the animal and the species. No two smooth muscles from different organs are identical in their electrical and mechanical properties (Burnstock et al., 1963).

In spite of the diversity of their physiological properties, Bozler (1948) divided vertebrate smooth muscles into two groups according to their degree of dependence on an extrinsic nerve supply and their ability to respond in an all - or - none manner to various stimuli. Muscles of the first group include the straining membrane and iris, ciliary muscle, pilomotor and some blood vessels. Under normal conditions they contract only in response to excitation of their extrinsic motor nerves. Muscles of the second group include most visceral smooth muscles, i.e., those of the gastrointestinal tract, ureter, and uterus. These muscles frequently show continuous rhythmic activity and although this may be modified by extrinsic nerves, the rhythmic activity does not appear to depend on them, (extrinsic nerves),
for rhythmic activity continues after the removal of nerves (Bozler, 1948). The spontaneous activity of this second group of muscle is more readily stimulated by stretch. This diversity of properties probably represents their adaptation to their particular function.

Smooth muscle cells are arranged in bundles and layers in organs. These bundles and layers always contain numerous elastic fibres which are continuous with those in surrounding connective tissue. This arrangement gives uniform transmission of tension throughout the tissue. In the gastrointestinal tract like in most tubular organs, the smooth muscle cells are arranged in layers with the fibres orientated parallel to one another, so that the cells form a spiral around the tube. In the intestine and in certain other organs (e.g. ureter and vas deferens) in which the function of the smooth muscle is to promote movement of the contents, there is an inner layer orientated in a close spiral and an outer layer forming a long spiral. These layers are usually described as circularly arranged and longitudinally arranged smooth muscle.

INNERVATION OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract is innervated by both intrinsic and extrinsic (autonomic) nervous systems.
The intrinsic nervous system has an intrinsic nervous system of its own that occurs from the oesophagus to the anus. This system controls most gastrointestinal functions, especially gastrointestinal movements and secretion. On the other hand, both parasympathetic and sympathetic nervous signals to the gastrointestinal tract from the brain can strongly alter the degree of activity of this intrinsic nervous system. Parasympathetic activity in general increases the activity of the intrinsic nervous system while sympathetic activity decreases it.

The intrinsic nervous system is composed principally of two layers of neurons and appropriate connecting fibres:

- The outer layer is called the myenteric plexus or Auerbach’s plexus. It lies between the longitudinal and circular muscular layers. The inner layer is called the submucosal plexus or Meissner’s plexus. It lies in the submucosa.

The myenteric plexus controls mainly the gastrointestinal movements (Wood, 1970; Furness, 1969) while the submucosal plexus is important in controlling secretion and also subserves many sensory functions, receiving signals principally from the gut epithelium and from stretch receptors in the gut wall (Guyton, 1981).

In general, stimulation of the myenteric plexus increases the activity of the gastrointestinal tract, causing the following effects:

1. Increased tonic contraction, or
"tone" of the gut wall, (2) increased intensity of the rhythmic contractions, (3) increased rate of rhythmic contraction, and (4) increased velocity of conduction of excitatory waves along the gut wall (Paton and Zar, 1968; Wood, 1970; 1973; 1975; Bolton, 1971; Hirst and McKirdy, 1974). On the other hand some myenteric plexus fibres are inhibitory rather than excitatory (Burnstock et al, 1966; Wood, 1970; Biber and Fara, 1973). These inhibitory fibres are believed to be purinergic - that is, they secrete adenosine triphosphate (ATP) or some similar purine-based transmitter substance (Burnstock et al, 1966). The excitatory fibres are mainly cholinergic - that is, they secrete acetylcholine (Paton and Zar, 1968; Bolton, 1971).

The intrinsic nervous system, including both the submucosal sensory plexus and the myenteric motor plexus, is especially responsible for many neurogenic reflexes that occur locally in the gut, such as reflexes from the mucosal epithelium to increase the activity of the gut muscle or to cause localized secretion of digestive juices by the submucosal glands. 

AUTONOMIC NERVOUS SYSTEM

The gastrointestinal tract receives extensive extrinsic (parasympathetic and sympathetic) innervation that is capable of altering the overall activity of the entire gut or specific parts of it.
Parasympathetic innervation

The parasympathetic supply to the gut is divided into cranial and sacral divisions. Except for a few parasympathetic fibres to the mouth and pharyngeal regions of the gastrointestinal tract, the cranial parasympathetics are transmitted almost entirely in the vagus nerves. The vagus fibres provide extensive innervation to the oesophagus, stomach, small intestine, and first half of the large intestine (Johnson, 1977; Bolton, 1979; Bowman and Rand, 1980). The sacral parasympathetics originate in the 2nd, 3rd and 4th sacral segments of the spinal cord. The sacral fibres supply the distal half of the large intestine (Guyton, 1981). For the parasympathetic nerves both the pre- and postganglionic fibres are cholinergic.

Sympathetic innervation

The sympathetic fibres to the gastrointestinal tract originate in the spinal cord between the segments of 8th thoracic and 3rd lumbar vertebrae. The preganglionic fibres, after leaving the cord, enter the sympathetic chains and pass through the chains to outlying ganglia, such as the coeliac ganglion and various mesenteric ganglia (Elliott, 1904; M'Fadden et al., 1935; Furness and Costa, 1974). The postganglionic neuron cell bodies are located in these ganglia, and postganglionic fibres spread from them along with blood vessels to all parts of the gut (Kuntz and Jacob, 1955; Norberg, 1964);
Jacobowitz, 1965).

Only a few adrenergic fibres supply the muscle of the non-sphincter region (Furness and Costa, 1971; 1974), but most sphincter muscle is supplied by a dense plexus of adrenergic nerves (Baumgarten and Lange, 1969). In the lower oesophageal (cardiac) sphincter of the guinea-pig there is a dense network of adrenergic fibres (Costa and Gabella, 1971). In the monkey, cat and rat there are few adrenergic fibres in the musculature of the cardiac sphincter. The amount of adrenergic fibres in these animals is not more than the amount in adjacent non-sphincter area (Gillespie and Maxwell, 1971). The circular muscle of the pyloric region of the rat and guinea-pig is densely supplied by adrenergic fibres (Gillespie and Maxwell, 1971; Costa and Gabella, 1971). The common bile duct and the sphincter of Oddi of the cat, guinea-pig, monkey and rabbit have intramuscular adrenergic fibres (Baumgarten and Lange, 1969; Mori et al, 1971; Persson, 1971). There is no significant increase in the density of the adrenergic innervation in the region of the ileo-caecal sphincter of the guinea-pig, whereas the ileo-caecal sphincter in rat and rabbit is more densely innervated than in the adjacent non-sphincter muscle (Furness and Costa, 1975). The internal anal sphincter is richly supplied by adrenergic nerves in guinea-pig, cat and

The origins of the adrenergic fibres supplying the sphinctera have not been extensively investigated. They seem to correspond with the sources of adrenergic fibres to adjacent non-sphincter muscle. It has been shown by Gonella et al, (1979), that the sympathetic fibres to the cardiac sphincter come from the stellate ganglion while some run along the splanchnic nerve. The fibres arising from the stellate ganglion were found to join the vagus nerve at the thoracic level while the fibres running along the splanchnic nerve pass through the coeliac ganglion without synapsing. The cell bodies of the adrenergic nerves to the sphincter of Oddi are located in the coeliac plexus (Baumgarten and Lange, 1969). Adrenergic nerve innervating the ileocaecal sphincter are located in the superior mesenteric ganglion (Furness and Costa, 1974). The internal anal sphinctera of cat and dog receive sympathetic nerves running in the colonic and hypogastric nerves (Furness and Costa, 1974, Rayner, 1979; Bouvier and Gonella, 1981). In the guinea-pig it was observed by Furness and Costa, (1973b), that little or no adrenergic fibres to the internal anal sphincter originate in the inferior mesenteric ganglion, but that adrenergic
axons run from the sacral sympathetic chains and from the posterior pelvic plexuses to the sphincter.

Usually stimulation of the sympathetic nerves leads to relaxation of the non-sphincteric area and contraction of the sphincter except the rabbit's internal anal sphincter that is relaxed by sympathetic nerve stimulation and adrenaline (Langley, 1901; Rayner, 1979; Bouvier and Gonella, 1981a). Todorov and Papasova (1984) also showed that noradrenaline induces contraction of the internal anal sphincter of the cat. Generally parasympathetic stimulation causes contraction of non-sphincteric area and relaxation of the sphincter (McNaught and Callander, 1975; Rayner, 1979; Guyton, 1981). Stimulation of the parasympathetic outflow to the internal anal sphincter through the second ventral sacral root, inhibited spontaneous electrical activity of the circular muscle (Bouvier and Gonella, 1981b). Acetylcholine and carbachol caused relaxation of the cat's internal anal sphincter in vitro, which is only blocked by simultaneous administration of atropine and hexamethonium (Todorov and Papasova, 1984). In the cardiac sphincter of the cat, Gonella et al (1977) observed that the vagus nerve has a dual control:— that is vagal excitatory and inhibitory effects. They also showed that preganglionic vagal fibres are cholinergic and they activate
(a) intramural excitatory cholinergic neurones, 
(b) intramural nonadrenergic inhibitory neurones (purinergic neurones). Gorella et al (1977) also found that the preganglionic fibres leading to inhibition have a higher threshold than those leading to excitation, and that both excitatory and inhibitory pathways are interconnected inside the intramural network. They also observed that activation of intramural inhibitory neurones, by relaxing the oesophagus inhibits intramural excitatory neurones and subsequently blocks vagal excitatory responses. The same workers in 1979, observed that the sympathetic control of the cardiac sphincter of cat is mainly exerted through cholinergic myenteric neurones which could be excited directly or indirectly by inhibition of inhibitory intrinsic neurones. In 1985, Coruzzi and co-workers suggested from results of experiments they did with different agonists and antagonists that contraction of cardiac sphincter from the cat was associated mainly with the stimulation of postsynaptic muscarinic receptors.

Receptors are those specialized sites in the tissue on which substances act to produce their individual effect on the tissue. This site (receptor) is likely to be either an enzyme or a site on a cell membrane (Waud, 1968). Substances which produce an observable response from a tissue are classified as agonists while antagonists are those that do not themselves produce an observable response but prevent the
response to agonists. Drug substances have their different receptors on which they act, for example:

**ACETYLCOLINE RECEPTORS**

Acetylcholine is the neurotransmitter at all peripheral autonomic ganglia as well as at the postganglionic parasympathetic nerve endings. The pharmacological actions of acetylcholine are classified into two groups: those that are mimicked by the naturally occurring alkaloid, muscarine, (hence referred to as - muscarinic actions), and those that are mimicked by nicotine (nicotinic actions).

Nicotinic actions of acetylcholine are produced by its action on autonomic ganglia and skeletal muscle, while muscarinic actions are produced on effector cells. The acetylcholine receptors whose stimulation leads to nicotinic and muscarinic effects/actions are known as nicotinic and muscarinic receptors respectively.

**MUSCARINIC RECEPTORS**

The nature of muscarinic receptors has been studied mainly by investigating the chemical and physical characteristics of the drugs with which they interact (Barlow, 1964; Bebbington and Brimblecombe, 1965). Such studies have revealed that the muscarinic receptors consist of negatively charged particles that form an integral part of the lipoprotein matrix of the sub-neural portion of the cell membrane.
Muscarnic receptors are found on many peripheral tissues and organs such as the guinea-pig ileum. Most of the organs and tissues in the body contain differing quantities of muscarinic receptors (Heilbronn and Bartfai, 1976; Kuhar and Yamamura, 1976; Aronston et al, 1977). Muscarinic receptors also exist presynaptically, where they regulate the release of acetylcholine. Muscarinic agonists inhibited while atropine stimulated acetylcholine release from cerebral cortex and cortical-slices (Polak and Bertel-Meeuws, 1966; Bertel-Meeuws and Polak, 1968; Szerb and Somogyi, 1973) and from hippocampal slices (Hahazvy and Szerb, 1977) of the rat. Presynaptic muscarinic receptors have also been demonstrated on smooth muscles like guinea-pig ileum (Kilbinger and Wagner, 1975).

Muscarnic receptors are currently classified with respect to their affinity for pirenzepine (Hammer and Giachetti, 1982). Those muscarinic receptors exhibiting a high affinity towards pirenzepine are \( M_1 \); while those exhibiting a low affinity towards pirenzepine are \( M_2 \) (Hirschowitz et al, 1984). \( M_1 \)-receptors are considered to be located almost exclusively on neural tissue, while \( M_2 \)-receptors exist on both neural tissues, such as the cerebellum, and peripheral effector organs (Hammer et al, 1980). However, it has also been proposed that muscarinic
receptors present in the periphery do not form a heterogeneous population.

Barlow et al (1976) have proposed that ileal and atrial muscarinic receptors differ, since antagonists such as 4-diphenylacetoxycarbamoylmethyl piperidine methiodine (4-DAMPA) are more selective for ileal muscarinic receptors. Mitschler and Lambrecht (1984) have also identified a series of antagonists derived from procyclidine and difenidol which are illeoselective. Antagonists such as gallamine, pancuronium and stercuronium exhibit the converse selectivity in that they are more selective for atrial muscarinic receptors (Mitchelson, 1984). However, the antagonist properties of these compounds are complicated by allosteric interactions and are therefore, non-competitive in nature (Birdsall et al, 1984).

The muscarinic receptors on the gastrointestinal tract of many animal species have been studied by many workers. Results of such studies revealed that the muscarinic receptors on these G.I.T. smooth muscles are the $M_2$ subclass. Such smooth muscles are the guinea-pig ileum (Barlow et al, 1972; 1976; 1980; Beddington and Brimblecombe, 1965; Brown et al, 1980, Hammer et al, 1980), guinea-pig stomach (Hammer, 1980), rat anococcygeus muscle (Tayo, 1982; Oriowo, 1983). Using the rat, Doss and Van Zweiten (1972) observed that the muscarinic receptors involved in gastric acid
secretion were different from muscarinic receptors on the GIT smooth muscles. Later, Hammar (1980) showed that the muscarinic receptors on the fundic mucosa from dog's stomach were different from those on the smooth muscle of the same stomach. These receptors were classified $M_1$ and $M_2$ respectively.

**ADRENERGIC RECEPTORS**

Adrenergic receptors, also known as adrenoceptors are those receptors that are stimulated by noradrenaline (sympathetic neurotransmitter) and similar drugs known as sympathomimetics. Adrenergic receptors have been classified into alpha ($\alpha$)- and beta ($\beta$)- receptors (Ahliquist, 1968). The $\beta$-adrenoceptors which mediate bronchodilatation were shown to differ pharmacologically from those which mediate cardiac actions, and because of this, they were classified as $\beta_1$- and $\beta_2$-receptors respectively (Lands et al, 1967a; 1967b). Subsequently $\alpha$-adrenoceptors were also subdivided since they were shown to be present not only on smooth muscle cells ("postjunctional" receptors) but also on nerve terminal ("prejunctional" receptors) which innervates the smooth muscle. From the effects of various agonist and antagonists, it was found that post- and prejunctional receptors have different properties, and thus they were subdivided into $\alpha_1$- and $\alpha_2$-receptors respectively (Langer, 1974).
It is clear, however, that both $\chi_1$- and $\chi_2$-receptors as well as $\rho_1$- and $\rho_2$-receptors, are present in the post-junctional smooth muscle membrane (Timmermans and Van Zwieten, 1981). Therefore, the different types of receptors cannot be subdivided by their location and they are now classified according to their relative responsiveness to agonists and antagonists. For example, $\chi_1$-receptors are more powerfully activated by phenylephrine than by clonidine, and the reverse is true for $\chi_2$-receptors. The responses mediated by $\chi_1$-receptors are antagonised by prazosin or phenoxybenzamine, while those mediated by $\chi_2$-receptors are antagonised by yohimbine or rauwolscine. Similarly, examples of agonists and antagonists for $\rho_1$-receptors are trezolol and practolol respectively, while those for $\rho_2$-receptors are fenoterol and ICI 118,551 (erythro-4R-1-(7-methylindan-4-yl)-3-isopropylaminobutan-2-ol), respectively (Bulbring and Tomita, 1987).

**Histamine Receptors**

Histamine stimulates the contraction of smooth muscle from various organs, such as the gut and bronchi, and this effect can be suppressed by low concentrations of mepyramine—a typical antihistaminic drug. The pharmacological receptors involved in these mepyramine-sensitive, histamine responses have been defined as $H_1$-receptors (Ash and Schild, 1966). Histamine also stimulates the secretion of acid by the
Loew and Chickering, 1941), increases the heart rate (Trendelenburg, 1960), and inhibits contractions in the rat uterus (Dews and Graham, 1946). These actions cannot be antagonised by mepyramine and related drugs. These mepyramine-insensitive histamine receptors were blocked by burimamide and hence classified as $H_2$-receptors (Black et al, 1972). Other drugs that can block histamine $H_2$-receptors include metiamide, cimetidine, famotidine and ranitidine.

**AIM OF THE PRESENT STUDY**

Although the receptors of many parts of the gastrointestinal tract have been the subject of extensive pharmacological investigations, not much is known about the receptors present in the sphincters of the gastrointestinal tract. It was therefore decided to study the response to drugs and to characterize the receptors on the guinea-pig gastrointestinal sphincters. The cardiac and anal sphincter were used. The terminal ileum which extends into the ileocaecal junction and is usually discarded during pharmacological experiments was used to represent the ileocaecal sphincter. Since the pyloric sphincter is not regarded as a true sphincter (Edwards and Howland, 1968), it was not used in the study.

The present study therefore was aimed at the following:

1. To investigate the effects of cholinergic, adrenergic and histaminergic receptor agonists on the terminal ileum,
cardiac and anal sphincters of the guinea-pig in vitro.

(2) To characterize the receptors in these tissues using specific antagonists.

(3) To study the nature of the intrinsic innervation of the sphincter and the transmitters involved by means of electrical stimulation.

(4) To compare the receptors on the terminal ileum with those on the proximal ileum.
CHAPTER TWO

MATERIALS AND METHODS.
MATERIALS AND METHODS

ANIMALS

Guinea-pigs of either sex weighing between 300 and 500g were used throughout this study. The animals were bred locally in animal houses belonging to the Faculties of Biological and Veterinary Sciences, University of Nigeria, Nsukka. They were fed on standard livestock pellets (Pfizer) supplemented with green grass and with free access to water.

PREPARATION OF TISSUES

Animals that were fasted of food but not water for 16 hours were killed by cervical dislocation followed by severing the carotid arteries. The abdomen was opened and the appropriate tissues were treated as follows.

ILEUM

The ileum was removed from the ileo-caecal junction and placed in a dish containing Tyrode solution aerated with air. The mesentery was trimmed off. Pieces of about 2cm in length were routinely used. The last 2cm of the ileum from the ileo-caecal junction was used as the terminal ileum, while any 2cm after the first 10cm from the ileo-caecal junction was regarded as the proximal ileum. The part of the ileum between the terminal and the proximal ileum were also used for the experiments. This area was described in the study by its
distance from the ileo-cecal junction, that is 2 - 4 cm, 4 - 6 cm, 6 - 8 cm, and 8 - 10 cm.

**CARDIAC SPHINCTER**

Some part of the stomach together with 3 cm of oesophagus was removed and immersed in Tyrode solution aerated with air. The stomach contents were removed by washing with Tyrode solution. The remaining part of the stomach was trimmed off, leaving the gastro-oesophageal junction. Since anatomical gastro-oesophageal sphincter was observed in the last 1 to 2 cm of the oesophagus (Kann and Shorter, 1964), the last 1 cm from the gastro-oesophageal junction was used in the study. This 1 cm tube was cut vertically to get a sheet. Longitudinal incisions were made at opposite sides of the sheet to get the sphincter strip used for the experiment as shown in Figure 2.1.

**INTERNAL ANAL SPHINCTER**

The visceras was removed and the pubic symphysis split. A segment of the rectum, including the anal region was removed and transferred to a dish containing Tyrode solution which was aerated with air. The content of the rectum was removed gently without applying much pressure. The rectum was trimmed out and the skeletal muscle and connective tissue forming the external anal sphincter removed. The internal anal sphincter which corresponds to a region of swelling (Lim and Muir, 1985)
FIGURE 2.1

Sketch of method of preparation of cardiac sphincter strip.

(a) = Cardiac Sphincter i.e. the 1cm tube.
(b) = The sheet obtained after the vertical cut.
(c) = The sphincter sheet after longitudinal incisions at opposite sides are made to produce a strip.
(*) = Shows where the tissue was tied.
was then dissected out from the anal margin. A longitudinal incision was made to get a flat sheet of tissue, then the horizontal strip was obtained by cutting horizontally. The sketch of this preparation is shown in Figure 2.2.

MOUNTING OF THE TISSUES

The tissues were suspended by means of threads attached to both ends of the tissues. One end of the tissue was tied to a tissue holder which was lowered to the bottom of the organ bath containing Tyrode solution. A 10ml organ bath was used except for transmural electrical stimulation when a 60ml organ bath was used in order to make room for the electrodes. The other end of the tissue was tied to an isotonic transducer (Ugo basile, 7006). The Tyrode solution was gassed with air and maintained at a temperature of 36 ± 1°C thermostatically. The muscle preparations were allowed to equilibrate for 30min under a resting tension of 1g for the sphincter strips and 0.5g for the ileum before they were exposed to drugs or to electrical stimulation. During the equilibration period, the tissues were washed with fresh Tyrode solution every 10min to prevent the accumulation of metabolic end products.

Non-cumulative concentration responses to agonists were established. For the ileum the agonists were allowed to act for 30s in a 2min time cycle while 60s contact time with 5min time cycle was used for the sphincter strips. After addition
FIGURE 2.2

Sketch of method of preparation of internal anal sphincter strip.

(a) = The internal anal sphincter
(b) = The sheet obtained after the longitudinal incision,
(c) = The horizontal strip obtained after making the horizontal cut.
(*) = Indicates where the tissue was tied.
(Merck); (-)-adrenaline (Richter); (l)-isoprenaline hydrochloride (Sigma); phen tolamine hydrochloride (Ciba); DL-propranolol hydrochloride (ICl); Yohimbine hydrochloride (Sigma); prazosin hydrochloride (Sigma); 1-phenylephrine hydrochloride (Sigma); Dopamine hydrochloride (Fluka AG), and others are the market type or racemic. The concentrations of the drugs were calculated as their salts.

**DRUG SOLUTIONS**

Stock solution of prazosin (1mg/ml) was made in 50% methanol with 0.01N hydrochloric (HCl) acid constituting the other 50%. Physostigmine was dissolved in 0.01N HCl and stabilized with equal amount of sodium meta-bismuthite.

The rest of the drugs were dissolved in freshly distilled water. All drugs were freshly prepared before use with the exception of prazosin, physostigmine and 4-DAMP, whose stock solutions were stored at a temperature of -20°C and diluted fresh in distilled water before use.

**PHYSIOLOGICAL SOLUTION**

Throughout this study, Tyrode solution was used as the physiological salt solution. The composition was as follows in mmol per litre:

- Sodium Chloride: 136.8
- Potassium Chloride: 2.7
- Calcium Chloride: 1.8
- Sodium dihydrogen phosphate: 0.3
- Sodium hydrogen carbonate: 11.9
Magnesium sulphate, 1.05 and
Glucose, 5.6.

**ANALYSIS OF RESULTS**

Results are expressed as mean ± standard error of the mean of 'n' observations, where 'n' represents the number of observations. Data were represented as percentage of the absolute maximum contractile response. The maximum response obtained was determined on each piece of tissue.

Agonist potency \((-\log EC_{50})\) was determined on each piece of tissue. Antagonist affinities \((PA_2)\) were determined by constructing concentration-response curves to the agonists at 3 concentrations of antagonist allowing 30min equilibration time at each concentration and calculated according to the method of Arunlakshena and Schild (1959). Three concentrations of antagonist were tested on each piece of tissue. The dose-ratios were calculated for each concentration and for each tissue. All the data were then pooled for each antagonist. The intercept on the abscissae and slope of the Arunlakshena and Schild plots were determined using linear regression by the method of least squares.

**STATISTICAL ANALYSIS**

Where appropriate, comparison between paired group was made using student "t" test. The difference between the groups is taken to be significant when \(P<0.05\).
CHAPTER THREE
CHARACTERIZATION OF RECEPTORS ON THE GUINEA-PIG TERMINAL ILEUM

INTRODUCTION

METHODOLOGY

RESULTS

DISCUSSION
INTRODUCTION

The ileum is the last part of the small intestine before the large intestine (colon). In the guinea-pig and in other animals that have caecum, the ileum joins the caecum, while in those animals that do not have caecum, the ileum joins the colon.

A lot of work has been done on the ileum and small intestine revealing how they behave to drugs and the receptors present on them.

Cholinergic drugs contract the intestinal smooth muscle (Paton and Rang, 1965; Delatteca et al., 1968; Barlow et al., 1976; Clague et al., 1985). These contractions were antagonised by atropine (Arunlakshana and Schild, 1959) and were therefore assumed to involve muscarinic receptors. Muscarinic receptors are currently classified into \( M_1 \) and \( M_2 \) subtypes, with respect to their affinity for pirenzepine (Hammer et al., 1980; Hammer and Giachetti, 1982). The \( M_1 \) muscarinic receptors are those that exhibit high affinity towards pirenzepine and are located almost exclusively on neural tissue (Hirschowitz et al., 1984; Hammer et al., 1980). Muscarinic receptors exhibiting a low affinity towards pirenzepine and high affinity to \( L \)-DAMP are the \( M_2 \) subtype and are located on peripheral effector organs such as the guinea-pig ileum (Hirschowitz et al., 1984; Hammer et al., 1980; Barlow et al., 1976).
The effects of catecholamines on the gastrointestinal tract of several mammalian species are mediated through activation of $\alpha$- and $\beta$-adrenoceptors. Stimulation of these receptors produces mainly inhibitory responses (Ahlquist and Levy, 1953; Puschgott, 1960; Furness and Burnstock, 1974; Fontaine et al, 1984). The relative importance of the two receptor populations varies in different parts of the alimentary tract; $\beta$-adrenoceptors on the muscle are nearly always inhibitory and $\alpha$-adrenoceptors may mediate excitation (Munro, 1951; Lands, 1952; Regoli and Vane, 1964; Innes and Kohli, 1969; Gagnon, 1970, Furness and Costa, 1974; Minker et al, 1977; Bauer, 1981). These $\alpha$-adrenoceptor mediated contractions of the gut smooth muscle were observed on the guinea-pig terminal ileum (Munro, 1951; Lands, 1952; Innes and Kohli, 1969; Minker et al 1977; Bauer, 1981; Gaion and Trento, 1983) and rat isolated colon (Regoli and Vane, 1964; Gagnon, 1970). There is considerable evidence that prejunctional $\alpha$-adrenoceptors distributed on the cholinergic nerve terminals play an inhibitory role on the acetylcholine release from the intramural myenteric plexus (Kosterlitz et al, 1970; Paton et al, 1971; Drew, 1977), and that the postjunctival $\alpha$-adrenoceptors present in the smooth muscle membrane mediate the relaxation of smooth muscle of the ileum (McDougall and West, 1952; Kosterlitz et al, 1970).

However, there is also evidence for the existence of inhibitory
and excitatory postjunctional $\alpha$-adrenoceptors, which participate in the contraction or relaxation of the guinea-pig ileum by catecholamine (Anderson and Lees, 1976; Baer, 1976; Wikberg, 1978).

It has been recently found that the presynaptic inhibitory and postjunctional inhibitory alpha adrenoceptors are homogeneously distributed in all regions of the guinea-pig ileum, whereas the postjunctional excitatory alpha adrenoceptors have different densities of distribution in terminal and proximal regions of the ileum (Baer, 1980, 1981).

Histamine is known to produce contraction of various smooth muscles, such as ileum, bronchial and spleen (Dale and Dubrey, 1921; Guggenheim, 1912). The receptors involved in the contractile effects of histamine were classified as $H_1$-receptors (Ash and Schild, 1966) whereas those involved in relaxation of rat uterus were classified as $H_2$-receptors (Black et al, 1972).

Generally, it has been the practice to discard the first 10cm of the ileum from the ileocaecal junction whenever isolated tissue experiments are carried out on the ileum (Dale and Laidlaw, 1910; Verma and McNeill, 1979; Fagboomi and Salako, 1982; Galon and Trento, 1983). This stems from the general belief that this part behaves differently from the rest of the ileum. In order to substantiate this claim, this work was designed to re-examine this issue and if it behaves...
differently from the rest of the ileum to determine the point on the ileum where the change occurs.

**Methodology**

The experiment was set up as described in chapter two. The last 2cm from the ileo-caecal junction was used as the terminal ileum while any 2cm after the first 10cm from the ileo-caecal junction was used as the proximal ileum. The segment between the terminal and proximal ileum was used and was described by its distance from the ileo-caecal junction, that is 2 - 4cm; 4 - 6cm, 6 - 8cm and 8 - 10cm.

**Results**

The guinea-pig terminal ileal preparations often (63.4% of cases) \( n=13 \) developed spontaneous rhythmic contractions within 10min after they were set up. The proximal ileum did not possess any spontaneous activity. The terminal ileal spontaneous contractions were not readily blocked by replacing the bathing fluid with that containing quarter calcium ion concentration \( \frac{1}{4}(Ca^{2+}) \) or that containing double magnesium ion concentration. However, the spontaneous rhythmic contractions were completely abolished when the bathing fluid was replaced with calcium-free Tyrode solution. These spontaneous contractions were also not abolished by atropine \( (7.24 \times 10^{-5}M) \); meprobamate \( (2.4 \times 10^{-5} \text{ to } 1.2 \times 10^{-5}M) \) pirenzepine \( (2.4 \times 10^{-8} \text{ to } 1.2 \times 10^{-8}M) \). On the other hand, the contractions were blocked by higher concentrations of atropine \( (> 3.60 \times 10^{-8}M) \).
The contractions declined steadily during regular dosing of the preparation with agonists.

**EFFECTS OF CHOLINOMIMETICS**

Acetylcholine (Ach) \((2.02 \times 10^{-10} \text{ to } 1.56 \times 10^{-7} \text{M})\); methacholine \((2.53 \times 10^{-10} \text{ to } 2.82 \times 10^{-7} \text{M})\) and carbachol \((1.75 \times 10^{-10} \text{ to } 7.08 \times 10^{-7} \text{M})\) produced concentration-related contractions of all the segments of the ileum, that is, the terminal \((0-2cm)\); 2-4cm; 4-6cm; 6-8cm; 8-10cm and proximal \((\text{any } 2cm \text{ after the first } 10cm)\) ileal segments. All measurements were made from the ileocecal junction. The potency of acetylcholine on these ileal segments measured as the PD₂ values is shown in Table 3.1.

From the PD₂ values for acetylcholine on these ileal segments, there is slight increase in the sensitivity to Ach as the distance from the ileocecal junction increases, though not statistically significant \((P = 0.05)\).

**EFFECTS OF CHOLINOMIMETIC IN THE PRESENCE OF TETRAMISOLE**

Because of the difference in the sensitivity to acetylcholine at different ileal segments, the effects of acetylcholine, methacholine and carbachol were determined for both segments the terminal and proximal ileum in the presence of phystostigmine \(2.42 \times 10^{-7} \text{M}\). The values of the PD₂ obtained for the terminal ileum, the PD₂ values were \(7.7 \pm 0.03\);
**TABLE 3.1**

FD$_2$ values of acetylcholine at different segments of the ileum.

<table>
<thead>
<tr>
<th>PART OF ILEUM FROM THE ILEOCOCCecal JUNCTION</th>
<th>FD$_2$ VALUES * For ACH:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal (0 - 2cm)</td>
<td>7.53 ± 0.27</td>
</tr>
<tr>
<td>2 - 4cm</td>
<td>8.09 ± 0.05</td>
</tr>
<tr>
<td>4 - 6cm</td>
<td>8.19 ± 0.18</td>
</tr>
<tr>
<td>6 - 8cm</td>
<td>8.19 ± 0.22</td>
</tr>
<tr>
<td>8 - 10cm</td>
<td>8.35 ± 0.40</td>
</tr>
<tr>
<td>Proximal (Any 2cm after 1st 10cm)</td>
<td>8.7 ± 0.26</td>
</tr>
</tbody>
</table>

Each value is a mean ± SEM of 4 observations.
7.2 \pm 0.06 and 7.18 \pm 0.2 for acetylcholine, carbachol and methacholine respectively; while values obtained for the proximal ileum were 7.85 \pm 0.14; 7.45 \pm 0.1 and 7.3 \pm 0.4 for acetylcholine, carbachol and methacholine respectively.

EFFECTS OF MUSCARINIC RECEPTOR ANTAGONISTS

Atropine (7.24 \times 10^{-9} to 1.82 \times 10^{-7} M) competitively antagonised acetylcholine induced contractions of all segments of the guinea-pig ileum, that is the terminal; 2 - 4 cm; 4 - 6 cm; 6 - 8 cm; 8 - 10 cm and proximal ileal segments. Figures 3.1 and 3.2, show the effects of increasing concentrations of atropine on acetylcholine induced contractions of the terminal and proximal ileal segments respectively. The pA2 values and the slopes of A - S plots for the different ileal segments are shown in Table 3.2.

N-1-ethyl-1-aziridino-N\textsubscript{2} \textsubscript{2} methyl piperidine meth bromide (4-DMNP) (1.24 \times 10^{-8} to 1.24 \times 10^{-6} M) reduced competitively the effects of acetylcholine at both the terminal and proximal ileum (Figures 3.3 and 3.4). Using pirenzepine which is an M1 muscarinic receptor antagonist, it was observed that lower concentrations (2.36 \times 10^{-8} to 2.36 \times 10^{-7} M), potentiated acetylcholine-induced contractions thereby shifting the log-dose-response curves of acetylcholine to the left (Figures 3.5a and 3.5b). Higher concentrations of pirenzepine (2.36 \times 10^{-6} to 5.89 \times 10^{-5} M), however, reduced competitively the effects of acetylcholine (Figures 3.6a and b). The antagonist
FIGURE 3.1
Acetylcholine-concentration response curves of the guinea-pig terminal ileum in the absence and presence of atropine. Each point is a mean of 4 experiments and the vertical bars represent standard error of the mean.

\[ \rightarrow \rightarrow \] = Control
\[ \circ - \circ \] = Atropine $7.24 \times 10^{-9} \text{M}$
\[ \square - \square \] = Atropine $3.63 \times 10^{-8} \text{M}$
\[ \Delta - \Delta \] = Atropine $1.82 \times 10^{-7} \text{M}$
Fig. 3.1  ACH conc. (log molar)
FIGURE 3.2

Acetylcholine-concentration response curves of the guinea-pig proximal ileum in the absence and presence of atropine. Each point is a mean of 4 experiments and the vertical bars represent standard error of the mean.

\[ \text{Control} \]
\[ \text{Atropine } 7.2 \times 10^{-9} \text{M} \]
\[ \text{Atropine } 3.63 \times 10^{-8} \text{M} \]
\[ \text{Atropine } 1.82 \times 10^{-7} \text{M} \]
<table>
<thead>
<tr>
<th>Part of ileum from the ileocecal junction</th>
<th>( \text{PA}_2 \pm \text{SEM} )</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal (0 - 2cm)</td>
<td>9.74 ± 0.11</td>
<td>0.85 ± 0.17</td>
</tr>
<tr>
<td>2 - 4cm</td>
<td>9.68 ± 0.15</td>
<td>0.91 ± 0.15</td>
</tr>
<tr>
<td>4 - 6cm</td>
<td>9.72 ± 0.28</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>6 - 8cm</td>
<td>9.57 ± 0.18</td>
<td>0.96 ± 0.14</td>
</tr>
<tr>
<td>8 - 10cm</td>
<td>9.80 ± 0.45</td>
<td>0.97 ± 0.18</td>
</tr>
<tr>
<td>Proximal (after the 1st 10cm)</td>
<td>9.60 ± 0.23</td>
<td>1.10 ± 0.04</td>
</tr>
</tbody>
</table>

Each value is a mean ± SEM of 4 observations.
Acetylcholine-concentration response curves of the guinea-pig terminal ileum in the absence and presence of l-DAMP. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

\[\begin{align*}
\n\n\n\triangle & = \text{l-DAMP } 1.23 \times 10^{-6}\text{M} \\
\n\n\n\n\n\end{align*}\]
**FIGURE 3.4**

Acetylcholine concentration response curves of the guinea-pig proximal ileum in the absence and presence of L-DAMP. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

- ▽ ▽ = Control
- ○ ○ = L-DAMP $1.23 \times 10^{-8}$ M
- □ □ = L-DAMP $1.23 \times 10^{-7}$ M
- △ △ = L-DAMP $1.23 \times 10^{-6}$ M
FIGURES 3, 5 a and b

Acetylcholine concentration response curves of the guinea-pig terminal (a) and proximal (b) ileum in the absence and presence of low concentrations of pirenzepine.

Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

\[ \text{\textbullet} \rightarrow \text{\textbullet} = \text{Control} \\
\circ \rightarrow \circ = \text{Pirenzepine } 2.36 \times 10^{-8}M \\
\square \rightarrow \square = \text{Pirenzepine } 2.36 \times 10^{-7}M \]
FIGURES 3,6 a and b

Inhibitory effects of higher concentrations of pirenzepine on the concentration response curves produced by acetylcholine at the guinea-pig terminal (a) and proximal (b) ileum. Each point is a mean of 4 experiments and the vertical bars represent standard error of the mean.

\[ \nabla \quad \nabla = \text{Control} \\
\circ \quad \circ = \text{Pirenzepine } 2.36 \times 10^{-6} \text{M} \\
\square \quad \square = \text{Pirenzepine } 1.18 \times 10^{-5} \text{M} \\
\Delta \quad \Delta = \text{Pirenzepine } 5.89 \times 10^{-5} \text{M} 
\]
affinity (PA₂) values for 4-DAMP and pirenzepine at the muscarinic receptors using acetylcholine as agonist are shown in Table 3.3.

EFFECT OF HISTAMINE

Histamine (3.98 x 10⁻⁹ to 1 x 10⁻⁵M) produced concentration-related contractions of all the ileal segments, that is, the terminal; 2-4cm; 4-6cm; 6-8cm; 8-10cm, and proximal ileal segments. The sensitivity of histamine on these ileal segments measured as the PA₂ values are 6.45 ± 0.1; 7.4 ± 0.09; 6.0 ± 0.12; 6.57 ± 0.05; 6.93 ± 0.21 and 7.6 ± 0.22 for the terminal (0-2cm); 2-4cm; 4-6cm; 6-8cm; 8-10cm and proximal ileal segments respectively.

EFFECT OF Mepyramine ON HISTAMINE-INDUCED RESPONSES OF THE GUINEA-PIG ILEAL SEGMENTS

Mepyramine (2.49 x 10⁻⁸ to 1 x 10⁻⁷M) antagonised competitively the effects of histamine on the guinea-pig terminal (0-2cm); 2-4cm; 4-6cm; 6-8cm; 8-10cm and proximal ileal segments. Figures 3.7 and 3.8 show the effect of mepyramine on the log dose response curves to histamine on the guinea-pig terminal and proximal ileal segments respectively.

The antagonist affinities measured as the PA₂ values for the different ileal segments used and the slope of the A-S plot for the ileal segments are shown in Table 3.4.
Table 3.3

PA$_2$ values of l-DAMP and Pirenzepine at both the terminal and proximal ileal segments, using acetylcholine as agonist.

<table>
<thead>
<tr>
<th>Part of ileum</th>
<th>PA$_2$ values ± SEM</th>
<th>l-DAMP</th>
<th>Pirenzepine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal</td>
<td>9.51 ± 0.14</td>
<td>5.86 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>9.67 ± 0.12</td>
<td>5.91 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Each value is a mean ± SEM of 4 observations.
FIGURE 3.7

Histamine-concentration response curves of the guinea-pig terminal ileum in the absence and presence of mepyramine. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

\[
\begin{align*}
\n\text{□□□□} & = \text{Mepyramine } 2.49 \times 10^{-6} \text{M} \\
\n\text{△△△△} & = \text{Mepyramine } 4.98 \times 10^{-6} \text{M} \\
\n\text{△△△△} & = \text{Mepyramine } 9.96 \times 10^{-6} \text{M}
\end{align*}
\]
Fig. 3.7  Histamine conc. (log molar)

Response (% max.)

-7  -6  -5  -4
FIGURE 3.8

Histamine concentration response curves of the guinea-pig proximal ileum in the absence and presence of mepyramine. Each point is a mean of 4 experiments and the vertical bars represent standard error of the mean.

▽ — ▽ = Control
○ — □ = Mepyramine 2.49 x 10^{-6} M
□ — △ = Mepyramine 9.96 x 10^{-6} M
△ — △ = Mepyramine 3.99 x 10^{-7} M
Fig. 3.8  Histamine conc. (log molar)
TABLE 3.4

Sensitivities of histamine and antagonist affinities of methapyrone (measured as PD$_2$ and PA$_2$ values respectively) at H$_1$-receptors on different ileal segments from guineapig.

<table>
<thead>
<tr>
<th>Part of ileum</th>
<th>PD$_2$ Values ± SEM</th>
<th>PA$_2$ Values ± SEM</th>
<th>Slope (A - S plot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal</td>
<td>6.45 ± 0.1</td>
<td>9.25 ± 0.5</td>
<td>1.08</td>
</tr>
<tr>
<td>2 - 4cm</td>
<td>7.4 ± 0.09</td>
<td>8.95 ± 0.34</td>
<td>0.99</td>
</tr>
<tr>
<td>4 - 6cm</td>
<td>6.8 ± 0.12</td>
<td>8.54 ± 0.1</td>
<td>0.98</td>
</tr>
<tr>
<td>6 - 8cm</td>
<td>6.57 ± 0.05</td>
<td>8.06 ± 0.2</td>
<td>0.91</td>
</tr>
<tr>
<td>8 - 10cm</td>
<td>6.93 ± 0.21</td>
<td>8.54 ± 0.2</td>
<td>0.97</td>
</tr>
<tr>
<td>Proximal</td>
<td>7.6 ± 0.22</td>
<td>8.67 ± 0.18</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 4 observations.
EFFECT OF CIMETIDINE ON HISTAMINE-INDUCED CONTRACTIONS OF THE GUINEA-PIG ILEUM

Histamine $H_2$-receptor antagonist, cimetidine ($3.66 \times 10^{-7}$ to $9.16 \times 10^{-6}$M) potentiated the contractions produced by histamine on the guinea-pig terminal ileum (Figure 3.9). On the proximal ileum, cimetidine ($3.66 \times 10^{-7}$ to $9.16 \times 10^{-6}$M) potentiated the contractions produced by histamine but the effects were not dose-related (Figure 3.10).

EFFECTS OF SYMPATHOMIMETICS ON THE GUINEA-PIG ILEUM

Noradrenaline ($2.4 \times 10^{-7}$ to $3.98 \times 10^{-5}$M) contracted the guinea-pig terminal ileum, but had no observable effect on the proximal ileum (Figures 11a and b). In some of the proximal ileal preparations there was slight depression of baseline (Fig. 3.12). Higher doses ($>6.31 \times 10^{-5}$M) of noradrenaline produced dose-related contractions of both the terminal and proximal ileal segments (Figures 3.11a, 3.11b and 3.12).

Adrenaline ($2.16 \times 10^{-7}$ to $2.81 \times 10^{-5}$M) produced dose-related contraction of the terminal ileum while on the proximal ileum, adrenaline in concentration less than $2.84 \times 10^{-5}$M, did not produce any effect. However, higher doses ($>2.84 \times 10^{-5}$M) produced contractions which were not dose related (Figure 3.13).

From the result it was observed that there are differences between the effects of noradrenaline and
FIGURE 3.9

Histamine concentration response curves of the guinea-pig terminal ileum in the absence and presence of cimetidine. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

- Control
- Cimetidine $3.66 \times 10^{-7}$M
- Cimetidine $1.83 \times 10^{-6}$M
- Cimetidine $9.16 \times 10^{-6}$M
Fig. 3.9  Histamine conc. (log molar)
Figure 3.10

Histamine concentration response curves of the guinea-pig proximal ileum in the absence and presence of cimetidine. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

- ▼▼ ▼▼ Control
- O--O Cimetidine 3.66 x 10^-7M
- □--□ Cimetidine 1.83 x 10^-6M
- △--△ Cimetidine 9.16 x 10^-6M
Fig. 3-10 Histamine conc. (log molar)
FIGURE 3.11a and b
Noradrenaline-concentration response (contraction) curves of the guinea-pig terminal (a) and proximal (b) ileal segments. Each point is a mean of 4 experiments and the vertical bars represent standard error of the mean.
Effects of noradrenaline on the guinea-pig proximal ileum; (a) showing the slight depression of baseline. This figure also shows the elevation of baseline caused by introduction of prazosin.
FIGURE 3.13

Effects of adrenaline on the guinea-pig terminal and proximal ileal segment showing that adrenaline (2.10 x 10^-7 to 2.83 x 10^-5g) contracted the terminal ileum but had no effect on the proximal ileum.
adrenaline on the terminal and proximal ileum. This observation led to investigating the effects of these agonists (adrenaline and noradrenaline) on all the ileal segments, that is, terminal; 2 - 4 cm; 4 - 6 cm; 6 - 8 cm; 8 - 10 cm and proximal ileal segments. Noradrenaline \(<3.0 \times 10^{-6} \text{M}\) caused contraction of the terminal; 2 - 4 cm and 4 - 6 cm, ileal segments. These contractions due to noradrenaline decreased as the distance from the ileocecal junction increases and became zero after the first 10 cm (proximal ileum) (Figure 3.14). Adrenaline contracted the terminal ileum and the second segment (that is 2 - 4 cm from the ileocecal junction), and produced mixed contraction and relaxation on the other segments after 2 - 4 cm (Figure 3.15).

Isoprenaline did not produce any observable effect on all the ileal segments.

Phenylephrine \((3.29 \times 10^{-6} \text{ to } 1.32 \times 10^{-4} \text{M})\) caused dose dependent contraction of the terminal ileum but had no effect on the proximal ileum (Figure 3.16).

**EFFECT OF ALPHA ADRENOCEPTOR ANTAGONISTS ON CONTRACTIONS PRODUCED BY ADRENOCEPTOR AGONISTS**

The alpha adrenoceptor antagonists phentolamine, prazosin and yohimbine caused increase in the tone of the ileal preparations. This was observed as elevation of
FIGURE 3.1b

Effects of noradrenaline on the different ileal segments, showing the contractions produced by noradrenaline ($4.302 \times 10^{-5} M$) on 2 - 4 cm and 4 - 6 cm ileal segments. The ileal segments were obtained from the same animal.
FIGURE 3.15

Shows the contractions produced by adrenaline on the terminal and 2 - 4 cm ileal segments. It also shows the mixed contraction and relaxation produced by adrenaline on the other segments after 2 - 4 cm from the ileocaecal junction. The segments were obtained from the guinea-pig.
baseline plus increased rhythmic contractions (Figure 3.17).

Phentolamine (1 x 10^{-7} to 2.5 x 10^{-6}M) produced dose-related inhibition of the contractile effects produced by noradrenaline on the terminal ileum. On the proximal ileum, there was no noticable effect. Prazosin (1.1 x 10^{-8}M; 5.5 x 10^{-8}M; and 2.75 x 10^{-7}M) converted noradrenaline-induced contractions into relaxations (Figures 3.12 and 3.17). However higher doses of noradrenaline (> 7.76 x 10^{-6}M) were still able to overcome the blockade produced by prazosin and contracted the tissue. In the presence of 2.75 x 10^{-7}M prazosin, the maximal response to noradrenaline was not always obtained even with as high as 1.91 x 10^{-3}M noradrenaline (Figure 3.18a). Prazosin, however did not produce any signifiant effect on the contractile responses produced by noradrenaline on the proximal ileum (Figure 3.18b).

Yohimbine (0.25 to 5 x 10^{-6}M) potentiated noradrenaline induced contraction of the terminal ileum (Figure 3.19) while it converted the contractions produced by noradrenaline into relaxation on the proximal ileum (Figure 3.20).

**RESPONSE OF THE GUINEA-PIG ILEUM TO ELECTRICAL STIMULATION**

Electrically-induced responses of the guinea-pig ileum were elicited by field stimulation with 1ms; 100V, 0.2Hz. In the presence of atropine or hyoscine (2 x 10^{-5}M) the
FIGURE 3.16 a and b

Effects of phenylephrine on the guinea-pig terminal (a) and proximal (b) ileal segments. (a) also shows the effect of phenylephrine (3.29 x 10^{-5}M) in the absence and presence of prazosin (6.46 x 10^{-7}M).
FIGURE 1.17

This illustrates the increase in tone developed by the terminal ileal preparation in the presence of alpha-adrenoceptor antagonist (prazosin). It also shows the relaxation caused by noradrenaline ($6.03 \times 10^{-5}$M) in the presence of prazosin.
FIGURES 3.18 (a) and (b)

Noradrenaline concentration response curves of the guinea-pig terminal (a) and proximal (b) ileum in the absence and presence of prazosin. Each point is a mean of 4 observations and the vertical bars represent standard error of the mean.

- ▼▼ Control
- ○○ Prazosin $1.4 \times 10^{-6}$M
- □□ Prazosin $5.5 \times 10^{-6}$M
- △△ Prazosin $2.75 \times 10^{-7}$M.
FIGURE 3.10

Histogram showing the effects of different concentrations of yohimbine on the contractile response produced by $2.4 \times 10^{-4}$M noradrenaline on the guinea-pig terminal ileum.

- Control
- Yohimbine $2.5 \times 10^{-7}$M
- Yohimbine $1.0 \times 10^{-6}$M
- Yohimbine $5.0 \times 10^{-6}$M
FIGURE 3.20

Shows the contraction and relaxation produced by noradrenaline ($2.4 \times 10^{-4}$ M) on the guinea-pig proximal ileum in the absence and presence of yohimbine respectively.
twitch responses to field electrical stimulation were abolished and transformed into a rapid and sustained relaxation (Figure 3.21).

**EFFECTS OF SYMPATHOMIMETIC AGONISTS ON TWITCH RESPONSES DUE TO FIELD STIMULATION**

Noradrenaline (1.94 x 10^{-9} to 6.4 x 10^{-6}M) inhibited the twitch responses produced by field stimulation of both the terminal and proximal ileum. The inhibition was dose-related (Figures 3.22 and 3.23). It was observed that the height of the twitch responses increased after each inhibition caused by the drug (NA) (Figure 3.22). The inhibition was more on the proximal ileum than on the terminal ileum, hence lower dose of noradrenaline was needed to completely inhibit the twitch responses on the proximal ileum. On the terminal ileum, noradrenaline caused brief potentiation of twitch responses before the inhibition (Fig. 3.22).

Adrenaline, clonidine, phenylephrine and isoprenaline (all in doses of 10^{-9} to 10^{-4}M) inhibited twitch responses on both the terminal and proximal ileum in a dose related manner.

Using equal doses (6 x 10^{-6}M) of these adrenoceptor agonists, it was observed that adrenaline was the most potent in inhibiting twitch responses. This was followed by noradrenaline. Isoprenaline and clonidine were the least
FIGURE 3.21

Effect of hyoscine \(2 \times 10^{-6} \text{M}\), on the twitch response produced by field electrical stimulation of the guinea-pig terminal ileum. Stimulation parameters were 1 ms; 100V and 0.2 Hz.
FIGURE 3.22
Shows the inhibition of twitch responses due to field electrical stimulation produced by noradrenaline on the guinea-pig terminal ileum. It also shows the slight potentiation of twitch responses before inhibition.
FIGURE 3.23
The inhibition of twitch responses produced by noradrenaline on the guinea-pig proximal ileum.
potent in inhibiting twitch responses to field electrical stimulation. The adrenoceptors depressed the baseline in addition to inhibition of twitch responses.

**EFFECTS OF ADRENOCEPTOR ANTAGONISTS ON TWITCH INHIBITION CAUSED BY ADRENOCEPTOR AGONISTS**

The concentration-dependent inhibition of twitch responses caused by norepinephrine, adrenaline, clonidine and phenylephrine was antagonised by alpha adrenoceptor antagonists, phentolamine, prazosin and yohimbine in dose-dependent manner. The inhibition of twitch responses caused by noradrenaline at both the terminal and proximal ileum was antagonised by prazosin and yohimbine. On the terminal ileum, yohimbine was more potent in antagonizing NA-induced inhibition than prazosin. In the presence of yohimbine \((2 \times 10^{-7} M)\), noradrenaline \((5 \times 10^{-5} M)\), caused only 13.18 percent inhibition of twitch response whereas in the presence of prazosin \((2 \times 10^{-7} M)\), the same \(5 \times 10^{-6} M\) noradrenaline caused 3.67 percent inhibition of the twitch response.

Noradrenaline induced inhibition of twitch responses were completely abolished when prazosin and yohimbine were introduced together.

Prazosin, yohimbine and propranolol inhibited the inhibition of twitch responses caused by adrenaline.

The inhibition of twitch responses caused by isoprenaline was only blocked by propranolol.
DISCUSSION

This study has investigated the actions of muscarinic, histaminergic and adrenergic receptor agonists and antagonists at their receptors present in the terminal ileum and compared them with those present in the proximal ileum.

The guinea-pig ileal segments (used in the study) were contracted by muscarinic receptor agonists such as acetylcholine, methacholine and carbachol. The sensitivity of acetylcholine (PD₂) on the terminal ileum in the absence of anticholinesterase was less than that for the same receptor on the proximal ileum. However, the difference was not statistically significant (P<0.05). When the sensitivity (PD₂) of acetylcholine was determined for all the segments, (that is terminal; 2 - 4cm; 4 - 6cm; 6 - 8cm; 8 - 10cm and proximal ileal segments) in order to establish the point where the change in sensitivity started, it was observed that the sensitivity of acetylcholine on the ileums increased as the distance from the ileo-caecal junction increased. This finding leads to the determination of muscarinic receptor agonists' potencies (PD₂ values) in the presence of an anticholinesterase - physostigmine (1 x 10⁻⁶ M). The results obtained showed that the order of potency for these agonists were similar for both the terminal and proximal ileal segments: (Acetylcholine > Carbachol > Methacholine). The values were