EFFECTS OF HYPERGLYCAEMIA ON SMALL INTESTINAL AND ANORECTAL MOTOR FUNCTION IN HUMANS

Antonietta Russo

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Department of Medicine

University of Adelaide

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SUMMARY

This thesis presents studies which relate to the effects of changes in the blood glucose concentration on the organisation and control of motility in the small intestine and anorectum.

The motor mechanisms by which acute hyperglycaemia slows small intestinal transit in healthy humans were investigated. Hyperglycaemia (blood glucose concentration ~15mmol/l) stimulated phase III activity, but there was overall suppression of small intestinal pressure waves, when compared to euglycaemia. These results indicate that marked hyperglycaemia has major effects on small intestinal motility in normal subjects. The observed suppression of small intestinal motility provides a possible mechanism for the slowing of small intestinal transit during hyperglycaemia.

Among the candidate mechanisms mediating the effects of hyperglycaemia on small intestinal motility is modification of tonic inhibition mediated by enteric nerves containing nitric oxide. The effects of a specific inhibitor of nitric oxide (NO) synthase, N^G-monomethyl-L-arginine (L-NMMA), on small intestinal motor activity, were evaluated in healthy human volunteers. Administration of L-NMMA was associated with stimulation of small intestinal phase III activity and a reduction in the duration of phase I activity. These results indicate that NO mechanisms are involved in the initiation of small intestinal phase III activity.

Disordered defaecation occurs commonly in patients with diabetes mellitus and is associated with heterogenous anorectal motor and sensory dysfunctions. These abnormalities may potentially be due to hyperglycaemia, rather than irreversible neural dysfunction. In healthy humans, measurements of anorectal motility and sensation were performed, during euglycaemia (4mmol/l) and hyperglycaemia

(8mmol/l and 12mmol/l). At a blood glucose concentration of 12mmol/l the number of spontaneous internal anal sphincter relaxations was greater and the strength of the external anal sphincter less when compared to euglycaemia. The threshold for perception of rectal balloon distension was lower at a blood glucose of 12mmol/l when compared to 4mmol/l. These observations demonstrate that acute changes in the blood glucose concentration affect both the smooth and striated muscle of the anal sphincter, as well as rectal sensation, and suggest that hyperglycaemia may contribute to incontinence in patients with diabetes mellitus.

Autonomic nervous system dysfunction occurs frequently in patients with diabetes mellitus and is associated with disordered gastrointestinal motor function. The possibility that the blood glucose concentration may affect cardiovascular autonomic (parasympathetic and sympathetic) function was evaluated in healthy human volunteers by performing paired studies during euglycaemia and hyperglycaemia (~15mmol/l). Hyperglycaemia was associated with changes in cardiovascular parasympathetic function. This observation indicates that acute changes in the blood glucose concentration affect cardiovascular autonomic function, which may be important in mediating the effects of hyperglycaemia on gastrointestinal motor function.

The studies reported in this thesis provide new insights into the control of gastrointestinal motility in healthy humans and effects of changes in the blood glucose concentration. The latter observations are likely to be relevant to an understanding of gastrointestinal motor function in patients with diabetes mellitus.

DECLARATION

The work presented in this thesis has been submitted to the University of Adelaide for the degree of Master of Medical Science. The studies reported within are all original and were performed by the author between 1994 and 1996. None of the material contained in this thesis has been submitted for the award of any other degree or diploma at any University. The results of these studies have only been published as scientific papers. The literature review includes figures used from work of other authors and permission has been granted. The material contained within this thesis may be photocopied, and the manuscript may be made available for loan at the discretion of the University of Adelaide.

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CHAPTER 1

ANATOMY OF THE SMALL INTESTINE

1.1 INTRODUCTION

The small intestine is responsible for the digestion and absorption of ingested nutrients, and expulsion of non-digested material into the colon. These functions are carried out by contractions of the small intestinal smooth muscle which mixes chyme with intestinal and pancreatic secretions and optimises the exposure of the intraluminal contents to the absorptive surface of the small intestinal mucosa. Feedback from small intestinal chemical and mechanical receptors regulates gastric emptying and, hence, small intestinal filling. The small intestine exhibits a variety of motor patterns which are necessary for the effective propulsion of chyme. These motor patterns are largely under the control of the intrinsic nervous system of the gut, with modulation by the central neural inputs and circulating hormones. This chapter will discuss the anatomical factors responsible for small intestinal motility with particular emphasis on the roles of the autonomic nervous system and nitrergic innervation.

1.2 GROSS ANATOMY

The small intestine extends from the pyloric sphincter to the ileocaecal valve. At 2-3 m in length, it accounts for 80-90% of the entire length of the gastrointestinal tract and comprises three regions: the duodenum, jejunum and ileum. The duodenum is the shortest and widest part of the small intestine, extending in a curved 25 cm long loop from the pylorus to the ligament of Treitz. The jejunum extends from the ligament of Treitz to the ileum and comprises approximately 40% of the total small intestinal length. The ileum, which extends from the jejunum to the ileocaecal valve, makes up approximately 50%-60% of the small intestine. The jejunum and ileum are attached to the posterior abdominal wall by a fan-like mesentery. This allows for relatively free movement, with each coil adapting to changes in form and position. Although the small

intestine is often considered to have a uniform structure throughout, there is a modest decrease in luminal diameter from the proximal to distal small intestine, so that the diameter of the duodenum (4cm) is greater than the ileum (3.5 cm) (Weisbrodt 1987). Duodenal wall thickness is approximately twice that of the distal ileum predominantly due to an increase in smooth muscle and collagen (Gabella 1987). As a consequence of this, the compliance of the proximal small intestine is less than that of the distal ileum (Storkholm et al 1995).

1.3 HISTOLOGY

The human small intestine comprises four layers: mucosa, submucosa, muscularis externa and the serosa (Farrar & Zfass 1967) (figure 1.1). The mucosa is characterised by numerous circular folds which project into the lumen. These folds are especially prominent in the duodenum, disappearing almost entirely in the distal ileum. As well as increasing the absorptive surface area, these folds may retard passage of luminal contents, and by preventing laminar flow, increase mixing .

The major components of the submucosa are collagen and elastin (Gartner & Hiatt 1990) which provide a strong resistance to distension.

The muscularis externa is the major muscular component of the small intestinal wall and is responsible for propulsion of chyme. Although cells are arranged as a thick inner circular layer and a thinner outer longitudinal layer of tightly packed smooth muscle cells which extend throughout the whole length of the small bowel, bundles of muscle cells often pass from one layer to another (Gabella 1993). The thickness of the circular muscle layer is greater in the proximal intestine. The circular smooth muscle cells are arranged at a more oblique angle in the ileum when compared to the smooth muscle cells in the duodenum which lie approximately at right angles to the longitudinal muscle cells (Daniel et al 1972). These differences are likely to contribute to the contrast in mechanical response to distension between the duodenum and ileum. The propulsive activity of the small intestine is coordinated by so-called pacemaker cells (cells with a

higher intrinsic frequency of electrical discharge than the surrounding tissue) (Rumessen & Thuneberg 1996), which are located at the junction between the longitudinal and circular musculature. The interstitial cells of Cahal, which are histologically distinct cells found in close relationship to both neural and muscular cells, may be the possible source of pacemaker activity (Daniel & Allescher 1990) (section 1.5).

The serosa, the outermost layer, consists of a thin sheet of epithelial cells, and contains a rich vascular neural supply. In addition the extrinsic nerve fibers pass with the blood vessels to synapse on the enteric ganglia.

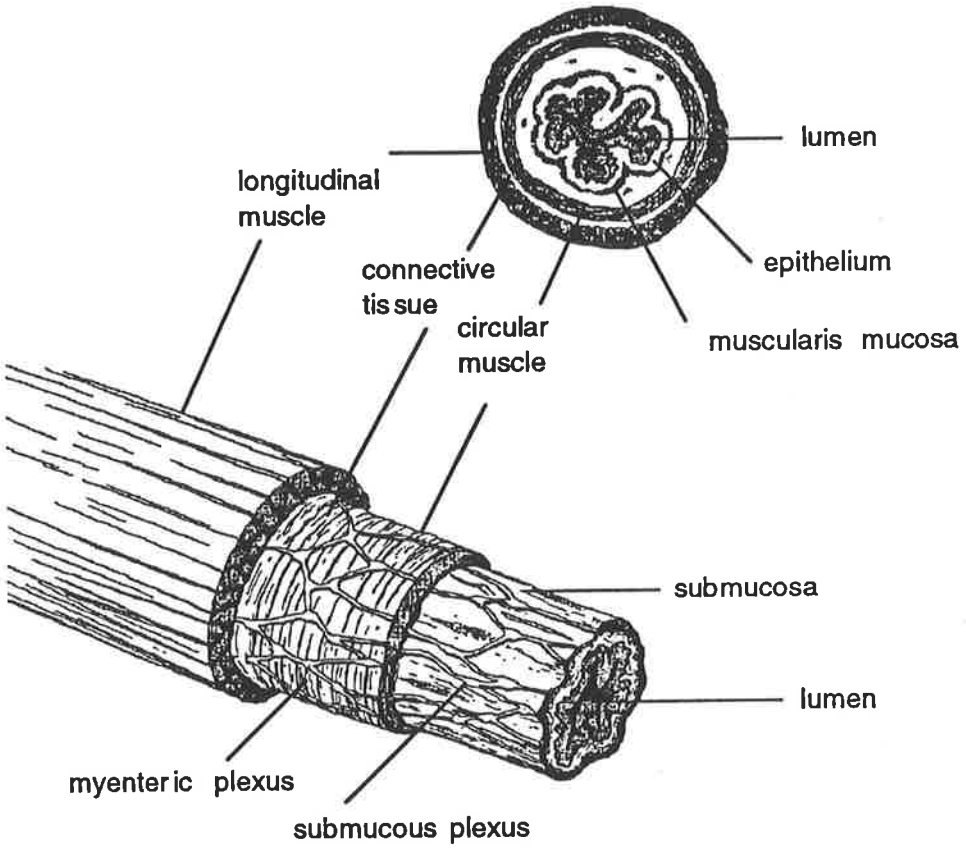

Figure 1.1

Structure of the small intestine showing the position of the myenteric and submucous plexuses (from Smout JPM and Akkermans LMA: Normal and Disturbed Motility of the Gastrointestinal Tract. UK, Wrightson Biomedical Publishing, 1992, p16; with permission).

1.4 NEURAL ANATOMY

1.4.1 Intrinsic innervation

Intrinsic nerves have a major role in the control of small intestinal motility as evidenced by the fact that extrinsically denervated canine small intestine is capable of mixing and propelling contents, though this is probably not as effective as in the normally innervated bowel (Quigley et al 1990). The enteric nervous system is known as the "little brain" of the gut and is arranged in overlapping segments which are replicated along the length of the intestine and serve to coordinate contractions at adjacent sites (Wood 1987).

The enteric nervous system consists of nerve cell bodies and their processes in the wall of the gut. There are two major plexuses: the myenteric plexus (Auerbach's) located between the longitudinal and circular muscle layers, and the submucous (Meissner's) plexus situated in the submucosa. Each plexus is composed of a three dimensional network of interconnected ganglia, each ganglion comprising between 10 and 100 neuronal cells (Furness & Costa 1987). The enteric nerves include motor neurones which innervate smooth muscle cells, interneurones which synapse on motor neurones within the myenteric plexus or connect the myenteric ganglia, and afferent neurones which carry sensory information from the gut wall and lumen to autonomic ganglia or the central nervous system (Gabella 1987).

The primary transmitter of excitatory motor neurones which innervate circular muscle is acetylcholine (Furness et al 1995). Both cholinergic and noncholinergic neurones also supply the mucosa and blood vessels of the small intestine. There is evidence that a number of transmitters have a substantial role in mediating neural inhibition, including nitric oxide, adenosine triphosphate (ATP) and vasoactive intestinal peptide (VIP) (Stark & Szurszewski 1992, Burnstock et al 1970, Allescher et al 1989, Furness et al 1995). Recent work has focused on the inhibitory role of nitric oxide (NO) in small intestinal smooth muscle (Sarna et al 1993, Stark et al 1993). Enteric interneurones form both ascending and descending pathways to ganglia along the length of the small

intestine. Small intestinal motor and epithelial function, as well as blood flow, are therefore controlled by an extensive motor, sensory and integrative neural network.

1.4.2 Extrinsic innervation

Although it is clear that the small intestine receives both sympathetic and parasympathetic neural inputs, the pathways responsible for both efferent and afferent central nervous system projections to the small intestine are poorly defined (Agostini et al 1957). The sympathetic supply is derived from the thoracic segments of the spinal cord; the cell bodies of the post ganglionic neurones that supply the small intestine are located in the coeliac and superior mesenteric ganglia. The sympathetic nerves travel in branches which accompany the arterial supply. The parasympathetic afferent supply is derived from the vagi, with cell bodies located in the dorsal motor nucleus of the vagus of the brain stem, and passes via the nerves of Latarjet to supply the pylorus and upper small intestine (Kennedy 1974).

It is clear that there are far more afferent than efferent fibers in the autonomic nerves of the gut (Grundy 1988). Approximately 90% of vagal fibers are unmyelinated sensory fibers which transmit information from mechanoreceptors and chemoreceptors in the upper gastrointestinal tract to the brain (Agostini et al 1957). The number of efferent fibres in relation to enteric neurones is therefore small and it is therefore likely that each efferent fibre modulates the function of a large number of enteric nerves extending over a considerable length of intestine.

1.5 INTERSTITIAL CELLS OF CAHAL

The origin of gastrointestinal electrical control was thought for many years to be primarily myogenic. However it has been suggested that the interstitial cells of Cahal may be responsible for electrical control activity of the small intestine (Thuneberg 1989). These are small cells, found in close relationship to networks of other interstitial cells, smooth muscle cells and neural structures. In the canine model, electrical control activity derives from the junction of the longitudinal and circular muscle, an area in

which there there are many interstitial cells of Cahal (Daniel & Allescher 1990). Furthermore, oscillations of electrical activity have been recorded from this area and removal of these cells abolishes electrical control activity in mammals (Hara et al 1986).

1.6 CONCLUSIONS

The gross anatomy and histology of the small intestine indicates that there are both myogenic and neural structures which allow regulation and organisation of motor patterns. The primary regulation of localised small intestinal motor function is provided by the enteric nervous system, with modulation by the central nervous system in response to local and distant sensory information. Furthermore, there are differences in the intestinal wall structure between the duodenum and ileum which are likely to be important in regional variations in motor patterns.

CHAPTER 2

MEASUREMENT OF SMALL INTESTINAL MOTILITY

2.1 INTRODUCTION

To fully understand the mechanics of small intestinal motor activity, the impact of individual contractions on transit needs to be evaluated. The effect of a small intestinal contraction on luminal flow is likely to be dependent on whether it results in lumen occlusion or not, as well as its temporal or spatial organisation. As there is no single technique which measures flow, intraluminal pressures, wall motion and electrical activity concurrently, most information is obtained by simultaneous use of a combination of techniques. In many cases only limited conclusions can be drawn from observations made in studies in which one or two of these factors has been measured. Furthermore, the methodology used in many earlier studies is now recognised to be suboptimal, and recordings were often made for insufficient time periods.

This chapter examines the available measurement techniques which can be used to investigate small intestinal motor activity (Table 2.1).

Table 2.1

Some of the available techniques for measurement of small intestinal motility in humans

1. Flow
   - radiography
   - scintigraphy
   - breath hydrogen test
   - impedance
   - intubation and aspiration of non absorbable markers
   - absorption of orally administered drugs

2. Wall motion
   - radiography
   - ultrasound

3. Intraluminal pressures
   - perfusion manometry
   - ambulatory manometry
   - barostat
   - radiotelemetry
   - electromyography

2.2 MEASUREMENT OF FLOW

A number of techniques have been used to assess the flow of chyme along the small intestine.

2.2.1 Radiography

The first descriptions of the movement of chyme through the small intestine were made by Cannon (1902) using fluoroscopy in cats . In these studies the impact of different patterns of small intestinal contractions on transit, and the effect of different nutrients on contractile patterns and flow were evaluated (Cannon 1904). The development of videofluoroscopy allowed a permanent record of flow to be obtained. In addition to the determination of flow patterns, fluoroscopy provides data on intestinal wall motion. Fluoroscopic imaging, however, has a number of deficiencies:

(i) Although passage of the head of the meal can be easily recognised, it is impossible to quantify the passage of the remainder of the meal along a segment of intestine.

(ii) The overlap of small intestinal loops within the abdomen hampers visualisation of individual regions of small intestine.

(iii) Despite image intensification techniques, there is a significant exposure to ionising radiation, which limits the use of the technique to short time periods.

(iv) Barium is a non-physiological substance which may cause mucosal irritation and alter small bowel motility and transit per se (Schonfeld et al 1995).

For these reasons, radiological techniques have limited applicability to the investigation of small intestinal motor activity in humans.

Laboratory based manometric techniques have been widely applied to research on small intestinal motility. The role of small intestinal manometry in clinical practice has not been clearly defined (Quigley 1992).

2.4.2 Ambulatory manometry

In an attempt to obtain prolonged small intestinal recording during normal daily activities, lightweight portable recording systems have been developed. Miniature electronic pressure transducers can be used in place of low compliance perfusion manometry (Kellow et al 1990, Husebye et al 1990). These transducers can easily be coupled to digital storage facilities to allow creation of a portable assembly. However, solid-state manometry has a number of limitations. Transducers are very fragile and expensive (Read & Sun 1992) and damage to a single transducer necessitates repair of the entire assembly. Sensors cannot be mounted less than 2 cm apart because this hampers detailed recognition of intestinal patterns. Finally placement of the assembly requires fluoroscopy. As a result of these limitations, ambulatory perfusion manometry has recently been developed but it is unclear as to how useful this technique will be in clinical practice (Samsom et al 1997). The potential advantage of ambulant recording is that the motor patterns detected may be more relevant, because the study conditions approximate a normal daily lifestyle more closely. These techniques are likely to be increasingly used in both research and clinical settings as technological improvement increases comfort and patient acceptability.

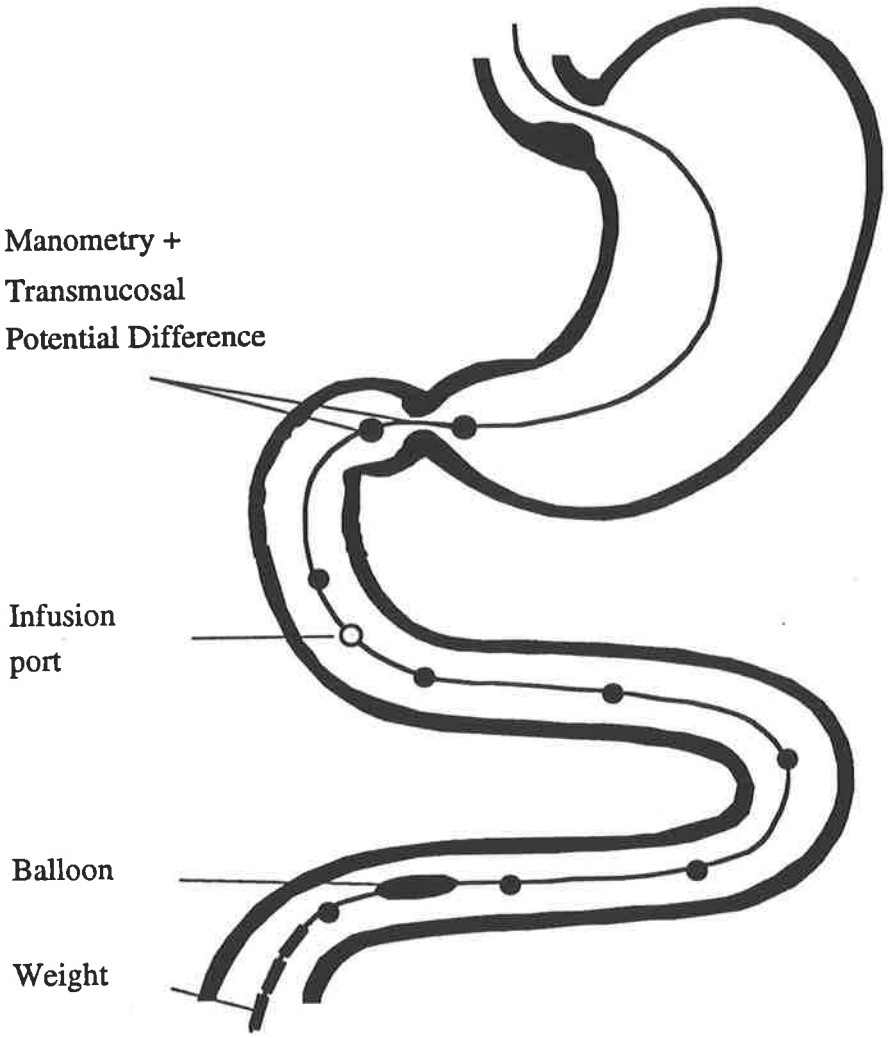

Figure 2.1

Diagram of the multilumen manometric catheter used in the small intestinal studies (Chapter 9 & 10). The two most proximal sideholes are 5cm apart in the antrum and duodenum respectively. The remainder of the sideholes are at 10 cm intervals and record pressures from the duodenum and proximal jejunum. An infusion port was located 55 cm from the catheter tip in the mid-duodenum. A 2.5 cm silicone balloon, positioned near the weighted tip, was inflated to facilitate passage of the assembly down the small intestine prior to starting the studies.

2.4.3 Barostat

A low compliance, thin-walled, balloon attached to a catheter and maintained at constant pressure can be used to detect changes in muscular tone or contraction in a hollow organ (Holtman et al 1996). The advantage of this so-called barostat technique is that visceral tone can be assessed and, in some cases reflex activity and sensation as well (Azpiroz & Malagelada 1985, Gregersen & Kassab 1996). This is not the case with conventional manometry which records only phasic contractions of the gut (see 2.4.1). The main disadvantage of the barostat technique is that tonic pressures recorded are from only one site, and contractions that occur proximal or distal to the balloon are not detected. The technique is also invasive and may disturb normal physiology of the gastrointestinal tract (Ropert et al 1993).

2.4.4 Radiotelemetry

Ingestible radiotelemetry capsules can be used to measure small intestinal pressures via radiotransmission to remote radio receivers (Dollinger et al 1975). This approach allows prolonged ambulatory recordings. Radiotelemetry has, however, significant disadvantages. The technique is technically challenging and the capsule detects pressure artefacts in addition to small intestinal pressures. As the radiopill may move during the study the exact recording site cannot be determined precisely. If the sensor is tethered, this increases the invasiveness of the procedure. The development of digital ambulatory manometric techniques has largely replaced radiotelemetry.

2.4.5 Electromyography (EMG)

The electrical activity underlying muscular contractions of the small intestine can be recorded by sensors implanted into the serosa of the intestinal wall. This technique can be used to assess the origin and spread of slow wave activity

(Lammers et al 1993). Accurate spacing of recording electrodes is critical to the accuracy of measurements. Electromyography has been used extensively in animals. Although a number of studies have used this technique post operatively in humans (Dauchel et al 1976) and animals (Bohm et al 1995) electromyographic studies have not been used widely in humans because of their invasive nature.

2.5 CONCLUSIONS

No single technique used to assess small intestinal motility has the capacity to evaluate all aspects of motor function. The combination of techniques which measure transit with an approach which assesses contraction force, such as manometry, provides a relatively comprehensive assessment. There are currently no methodologies which allow transit to be determined on a second by second basis in humans. The development of novel techniques is likely to provide insights into the mechanics of small intestinal function.

CHAPTER 3

PATTERNS OF SMALL INTESTINAL MOTILITY

3.1 INTRODUCTION

The overall organisation of small intestinal motility comprises two broad patterns of contractile activity: cyclical motor activity which occurs during the interdigestive or fasting state, and a so-called "fed" or irregular motor activity after nutrient ingestion. These patterns of motor activity have markedly different effects on the movement of small intestinal contents. Fasting motor patterns are the better characterised, in part because interdigestive motility is easier to categorise and quantify. However, it is likely that abnormalities of postprandial motor activity are clinically more important .

In this chapter the different patterns of small intestinal contractions and their impact on the movement of intraluminal contents are reviewed.

3.2 FASTING INTERDIGESTIVE MOTILITY

During fasting, the human small intestine undergoes cyclical motor activity, which is characterised by relatively long periods of motor quiescence or intermittent contractions interspersed with paroxysmal motor events. Szurszewski et al (1969) described electrical spike activity in the small intestine of the dog, which occurred at the maximal slow wave frequency, and migrated distally from the proximal duodenum to the ileocolonic junction: the migrating myoelectric complex (MMC). The MMC has subsequently been divided into three phases, according to differences in contractile frequency (Vantrappen et al 1977, Schemann & Ehrlein 1986) (figure 3.1a).

During phase I (motor quiescence) spike potentials and contractions are absent. In humans, phase I accounts for 30%-50% of the entire small intestinal MMC. Phase I is generally considered to be present when the contractile frequency is less than 2 per 10 minutes (Sarna 1985, Stam et al 1995). Phase II (which comprises 30%-50% of the entire MMC cycle) is a period of sporadic motor activity, with a contractile frequency between 2 and 10 per minute. Although bursts of contractions occur during phase II, these are not sustained for more than 1 minute and do not migrate for more than a few cm. During phase II the frequency of contractions increases gradually until phase III occurs. Phase III activity is characterised by an intense burst of regular motor activity which migrates aborally along the small intestine. Although phase III occupies less than 10% of the entire MMC cycle, it has received the most attention because it can be easily recognised. The propagation velocity of phase III decreases from approximately 4cm per minute in the jejunum to approximately 1cm per minute in the ileum (Kerlin et al 1982) and there is also a reduction in the frequency of contractions from 12 per minute in the jeunum to 6-9 per minute in the ileum. In contrast, there is an increase in duration of phase III from an average of about 9 minutes in the jejunum to about 14 minutes in the ileum. Approximately 50% of phase III activities commence in the oesophagus or stomach (Kellow et al 1986). In humans, unlike dogs phase III activity arising in the proximal jejunum migrates for a variable distance along the small intestine with less than 10% reaching the terminal ileum (Kellow et al 1986). It has been suggested that phase III is the "housekeeper" of the gut, responsible for the aboral transit of undigested food residues and cellular debris along the length of the bowel (Code 1979); this cleansing role may also be important in preventing bacterial overgrowth in the small intestine. Following phase III there is sometimes a transition period with sporadic activity prior to a return to motor quiescence which has been referred to as phase IV (Stam et al 1995).

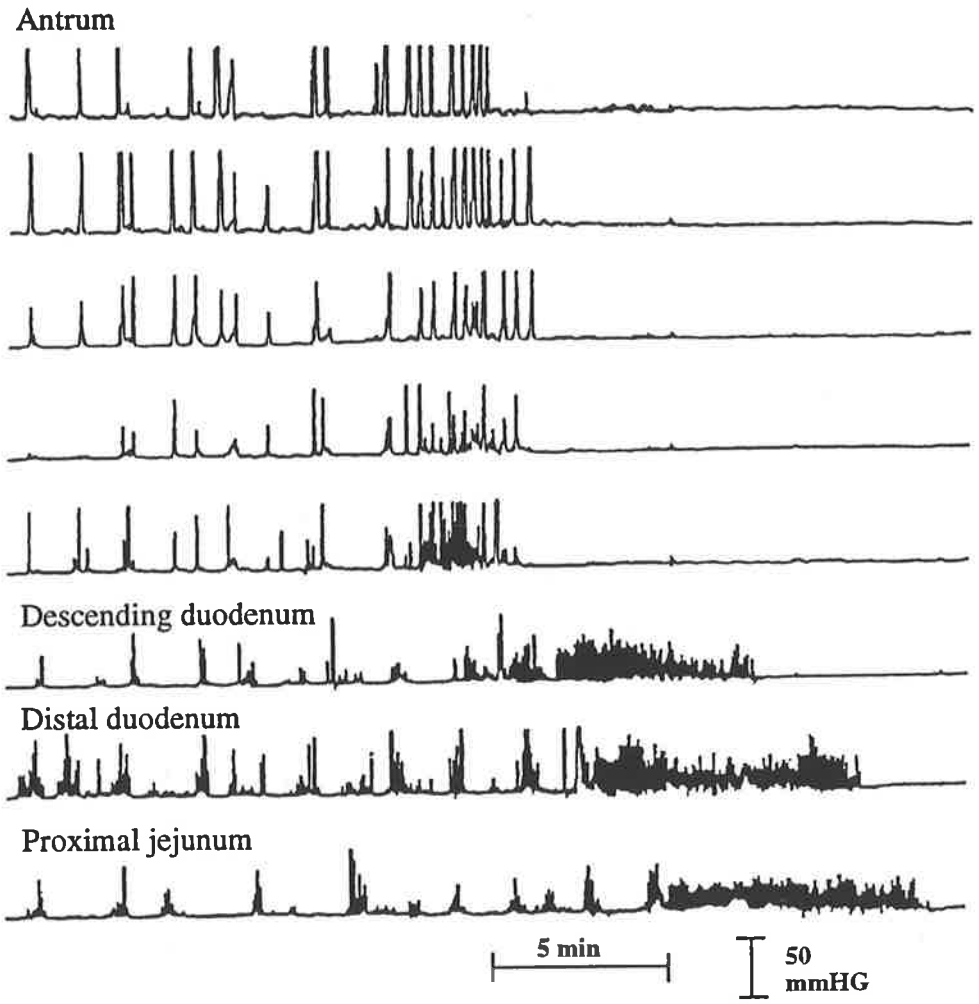

Figure 3.1a

Example of a manometric trace showing typical interdigestive motility from the stomach, duodenum and jejunum of a healthy human, including phase I, phase II and phase III (section 3.2) (reproduced from Malagelada J-R et al: Manometric Diagnosis of Gastrointestinal Motility Disorders. New York, Thieme Publishers, 1986. p45; with permission).

3.3 POSTPRANDIAL ACTIVITY

Ingestion of a meal and exposure of the small intestinal mucosa to nutrients alters motility, causing a change in the patterns of contractions and flow along the small intestine (Reinke et al 1967, Bueno et al 1975) (figure 3.1b). Both eating, and small intestinal nutrient infusion disrupts the MMC within 10-20 minutes, replacing the interdigestive motor activity with irregular contractions that persist for several hours (De Wever et al 1978, Schlang 1978).

A number of human postprandial motor patterns have been described, but there has been relatively little quantitative analysis. Initially following ingestion of a meal, clusters of contractions which propagate towards the ileum are observed, followed by an increase in segmental (non propagating) contractions which occur irregularly throughout the small intestine. These are interrupted intermittently by low amplitude propagated contractions that migrate for variable distances (Sarna et al 1989).

Schemann & Ehrlein (1986) used a combination of fluoroscopy and strain gauges to examine canine motor activity and the relationship between contractions and flow following infusion of a variety of nutrients. Non-nutrient, cellulose meals induced contractions which migrated the longest distance and were associated with the most rapid transit. Nutrients, such as fat or carbohydrate, induced stationary or segmental contractions interspersed with contractions which migrated for shorter distances. These latter contractions appeared to have a major impact on transit. In a recent study the effect of small intestinal nutrient-mediated feedback, on motility, transit and flow in the Troll minipig was examined (Hugh et al 1995). The delivery of higher nutrient loads to the mid-jejunum was associated with a decrease in the number and amplitude of jejunal contractions, a reduction in the length of migration of propagated contractions and an increase in

stationary contractions. These changes in motor activity were associated with a reduction in flow rate and an increase in intestinal absorption. In humans infusion of nutrient meals into the small intestine stimulates pressure waves which do not appear to propagate; the impact of individual contractions on flow is unknown (Sarna et al 1991).

It is recognised that there are regional differences in the response of the small intestine to luminal nutrients in humans. Ileal infusion of fat slows jejunal transit and reduces both total and propagating jejunal contractions (Holgate & Read 1985). A similar, but less pronounced effect is apparent during jejunal nutrient infusion (Riachi et al 1996). These effects may optimise the digestion and absorption of nutrients.

3.4 OTHER MOTOR PATTERNS

Although the overall motility of the intestine can be characterised broadly as either fasting or fed, a number of other contractile pressure wave patterns have also been described.

3.4.1 Migrating clustered contractions

Migrating clustered contractions have been described during both phase II of the MMC and postprandially in animals (Schemann & Ehrlein 1986, Fleckenstein & Oigaard 1978) and humans (Summers et al 1983). In animal studies Fleckenstein et al (1982) have referred to migrating clustered contractions as the "minute rhythm". Unlike the migrating motor complex, migrating clustered contractions do not have a regular frequency, are less than 1 minute in duration and have a velocity of up to 20 cm/sec. In the fasted state clustered contractions may migrate for up to 50 cm and have been shown to increase luminal flow (Kruis et al 1985). Postprandially, clustered contractions migrate aborally over short distances (2-4

cm). This motor pattern is associated with diarrhoeal states, intestinal obstruction and pseudoobstruction. Furthermore, abnormal occurrence of migrating clustered contractions has been reported in patients with partial mechanical obstruction (Summers et al 1983) and irritable bowel syndrome (Kellow et al 1987). In humans an increase in the number of migrating clustered contractions has been described during hyperglycaemia (Bjornsson et al 1994).

3.4.2. Retrograde propulsive contractions

In the dog retrograde propulsive contractions are frequently seen during vomiting (Lang et al 1986) and are believed to lead to rapid orad propulsion of intestinal contents. Retrograde propulsive contractions are recorded mainly in the mid or distal small intestine and are associated with proximal gastric relaxation and relaxation of the lower oesophageal sphincter (LOS). Individual contractions have an amplitude of about 2 times greater and a duration 2-4 times longer than that of contractions during an interdigestive migrating motor complex. Retrograde propulsive contractions migrate orally with a velocity of 8-10cm/sec up to a distance of 100cm.

3.4.3 Giant migrating contractions

Giant migrating contractions are isolated, high amplitude, contractions which occur mainly in the jejunum or ileum and migrate distally to the colon in the dog (Sarna et al 1987). This pattern of small intestinal motor activity has been characteristically observed in response to injury, in particular inflammation due to infection (Cowles & Sarna 1990), radiation enteritis (Otterson et al 1992) or noxious substances (Kruis et al 1985) in animals. Individual giant migrating contractions have an amplitude of 60 mmHg and a duration of 20-40 seconds (i.e. 1.5-2 times greater in amplitude and 4-6 times longer in duration than individual phasic contractions) and migrate distally at about 1cm/sec (Sarna 1987). They are

believed to cause rapid movement of small intestinal contents distally (Kruis et al 1985). Similar characteristics of giant migrating contractions have been described in humans. In irritable bowel syndrome patients, these contractions are associated with abdominal cramps and discomfort (Kellow et al 1987). In contrast to phase III activity giant migrating contractions usually propagate to the terminal ileum and often into the proximal colon (Quigley et al 1984).

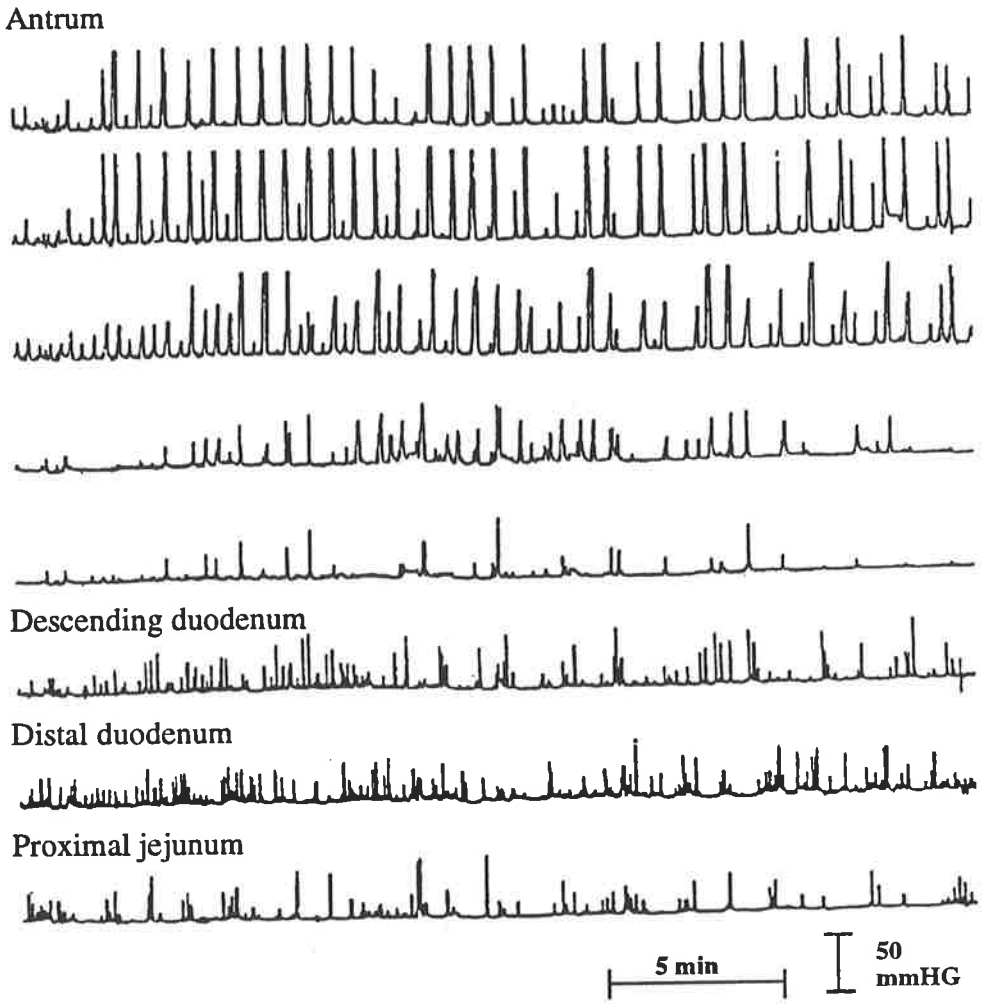

Figure 3.1b

Manometric tracing of healthy human postprandial motor activity, illustrating sporadic contractions in the antrum, duodenum and proximal jejunum (reproduced from Malagelada J-R et al: Manometric Diagnosis of Gastrointestinal Motility Disorders. New York, Thieme Publishers, 1986. p45; with permission).

3.5 DISORDERED SMALL INTESTINAL MOTILITY

Although disordered small intestinal motility has been proposed to underlie many of the symptoms associated with gastrointestinal symptoms such as distension, abdominal pain, nausea and vomiting, there is relatively little information about their temporal relationship to small intestinal motility. This situation is in part a reflection of the lack of consensus as to what constitutes normal motility. Disordered small intestinal motility may result from a number of diseases which cause motor or sensory dysfunction or a combination of these (Table 3.1). The best characterised of these are diabetes mellitus, progressive systemic sclerosis, myopathies and chronic pseudoobstruction. Attempts have been made to characterise motor dysfunctions as either myogenic and/or neurogenic on the basis of manometric studies (Malagelada & Stanghellini 1985).

3.5.1 Diabetes Mellitus

Gastrointestinal symptoms such as vomiting and diarrhoea occur frequently in patients with diabetes mellitus (Feldman and Schiller 1983, Schvarcz et al 1996). It is also clear that disordered small intestinal motility occurs frequently- probably in about 50% of unselected patients with diabetes (Kim et al 1991, Camilleri & Malagelada 1984, Jebbink et al 1993).

An early study in diabetic patients with diarrhoea reported an increase in duodenal pressure waves, both fasting and postprandially (Drewes 1971). More recent studies in type I diabetics, have also shown abnormalities in both fasted and fed small intestinal motor activity (Camilleri & Malagelada 1984, Samsom et al 1996). During fasting, the migrating motor complex cycle length is greater as a result of more prolonged phase II activity, whilst postprandially, early bursts of "phase III-like" activity are seen in the small intestine.

Camilleri & Malagelada (1984) have reported a reduction in the duodeno-jejunal phasic pressure activity as well as non-propagated long bursts of powerful contractions. Abnormal small intestinal transit (both slower and more rapid) has been reported in patients with diabetes mellitus (Scarpello et al 1976, Keshavarzian & Iber 1986). A recent study has reported that transit of a liquid meal in the distal small bowel may be more rapid than normal in type I diabetes (Rosa-e-Silva et al 1996).

The mechanisms responsible for disordered small intestinal motility in diabetes mellitus are unclear, but may be due to neuropathy, affecting enteric or extrinsic nerves, humoral changes or a combination of these (Horowitz & Fraser 1994). Gastrointestinal symptoms, and by inference disordered motility have been characteristically attributed to irreversible autonomic neuropathy (Rundles 1945). In other areas of the gastrointestinal tract, such as the oesophagus and stomach, the relationship between disordered motility, symptoms and autonomic nerve function has been poor (Horowitz et al 1991). Recent studies indicate that acute changes in the blood glucose concentration have a major effect on gastrointestinal motor function in the oesophagus (DeBoer et al 1992), stomach (Fraser et al 1991), colon (Sims et al 1995) and anorectum (Chey et al 1995). In previous studies of small intestinal motility in diabetes mellitus blood glucose concentrations were not stabilised or even monitored. It is, therefore, possible that some of the observed changes may have been due to effects of the blood glucose concentration.

3.5.2 Progressive systemic sclerosis

The hallmark of progressive systemic sclerosis is the replacement of smooth muscle with collagen (Scutellari et al 1990). The small intestine is frequently involved in progressive systemic sclerosis. Although myopathic factors

contribute to the motility problems in progressive systemic sclerosis, early changes are likely to reflect visceral neuropathy due to degenerative changes in the myenteric plexus (Rohrmann et al 1984, Greydanus & Camilleri 1989). In the late stages of progressive systemic sclerosis replacement of smooth muscle by collagen results in a reduction in small intestinal pressure waves. Small intestinal transit is delayed and symptoms characteristic of obstruction are common (Schuffler et al 1981). Bacterial overgrowth (Kaye et al 1995) occurs frequently, probably secondary to the loss of migrating motor complex activity and may contribute to malabsorption (Hendel 1994).

3.5.3 Irritable bowel syndrome (IBS)

Irritable bowel syndrome occurs commonly, and is characterised by abdominal pain, diarrhoea and/or constipation. The aetiology of this condition is unknown, but is likely to reflect both motor and sensory dysfunction. A number of disturbances in small intestinal motility have been described in the irritable bowel syndrome, in particular high amplitude contractions which are temporally associated with abdominal discomfort and pain (Kumar & Wingate 1985, Kellow & Phillips 1987, Kellow et al 1990). Because these latter contractions are absent during sleep (Kumar et al 1992, Gorard et al 1995), it has been proposed that irritable bowel syndrome is a result of abnormal central nervous system function rather than a disorder of gut function. When patients with irritable bowel syndrome are subjected to stress, there is a decrease in migrating motor complex activity. Stress appears to provoke small intestinal motor disturbances and consequently the symptoms of the disease (Wingate 1989). This issue remains unresolved. Furthermore, apparently identical motor patterns, unassociated with discomfort may sometimes be observed in healthy subjects (Quigley et al 1992).

3.5.4 Dystrophia myotonica

Myotonic dystrophy, an inherited disorder may be associated with a disordered motility of the entire gastrointestinal tract, most frequently the oesophagus (Eckardt et al 1986) and stomach (Kuiper 1971, Horowitz et al 1987). The small intestine is also affected (Lewis & Daniel 1981). This disease is characterised by collagen formation in smooth muscle cells of the gastrointestinal tract and a degenerative neuropathy of the myenteric plexus. In patients with dystrophia myotonica low amplitude jejunal contractions during the migrating motor complex and after feeding have been observed (Nowak et al 1984).

3.5.5 Chronic pseudo-obstruction

This term refers to functional obstruction to flow in the small intestine where there is no mechanical obstruction (Camilleri et al 1986). Symptoms include nausea, vomiting and abdominal pain which are sometimes severe enough to result in nutritional disturbances. Chronic pseudo-obstruction may be "primary" (disorder confined to the small intestine) or "secondary" as part of a systemic disease such as diabetes or progressive systemic sclerosis. Chronic pseudo-obstruction results from dysfunction of the neuromuscular control mechanism of the gut, which may be either myopathic (more commonly) or neuropathic in nature (Camilleri et al 1993). Manometric studies in the human small intestine show a variety of motor dysfunctions, which are likely to reflect, at least in part, differences in the underlying pathophysiology (Summers et al 1983). Reported abnormalities include a reduction in the frequency and amplitude of pressure waves, clustered migrating contractions, and a reduction or absence of phase III activity (Summers et al 1983). Abnormal propagation of interdigestive motor complexes, non propagating bursts of phasic pressure activity, and failure to convert to a fed pattern have also been reported (Camilleri et al 1986). It has not

been established which of these abnormalities contributes to a delay in small intestinal transit.

3.6 CONCLUSIONS

The differences in contractile activity of the small intestine during fasting and postprandially, have a major impact on flow. Although phase III of the interdigestive migrating motor complex is recognised to cause major shifts of material along the intestine other motor patterns are clearly important to flow.

Disturbances in small intestinal motor activity may be associated with severe gastrointestinal symptoms and compromise nutrition. The pathophysiology of disordered small intestinal motility is poorly defined and information about the mechanical correlates of abnormal small intestinal transit is also limited.

CHAPTER 4

REGULATION OF SMALL INTESTINAL MOTILITY

4.1 INTRODUCTION

Small intestinal motor activity is regulated by a complex system of control mechanisms arranged in a hierarchical fashion. The basic myogenic rhythm is modulated by both extrinsic and intrinsic neural pathways and circulating hormones. The triggering and maintenance of interdigestive and postprandial motor patterns involves a complex interaction between these different mechanisms. Pathways responsible for interdigestive or fasting motor activity are much better characterised than those regulating postprandial motility. This chapter focuses on the control mechanisms which underlie small intestinal motor activity.

4.2 INTRINSIC MUSCULAR CONTROL

4.2.1 Electrical Control Activity

The most basic control pathway underlying small intestinal motility is myogenic in nature. It has long been recognised that the resting membrane potentials of smooth muscle cells of the longitudinal layer of the small intestine undergo spontaneous depolarisations, which occur independently of the contractile state of the muscle (Alvarez & Mahoney 1922, Puestow 1932). These fluctuations in smooth muscle membrane potential are called slow waves or electrical control activity (ECA) (Szurszewski 1969 & 1987) because they are ultimately responsible for the occurrence and timing of smooth muscle contractions. Electrical control activity is believed to be generated by "pacemakers" along the small intestine which produce cyclical electrical changes at a frequency greater

than the surrounding areas. The exact location of pacemaker activity generation is uncertain, but the interstitial cells of Cahal have been proposed as a possible site (section 1.5). The frequency of the slow wave is highest in the duodenum and decreases with increasing distance from the pylorus; in humans the electrical control activity frequency is 10-12/min in the duodenum and 6-9/min in the ileum (Christensen et al 1966, Diamant & Bortoff 1969).

4.2.2 Modulation of electrical control activity

Although electrical control activity is intrinsic to smooth muscle, extrinsic factors modulate both the frequency and pattern of membrane potential fluctuations. In the cat, intraluminal nutrients have been shown to reduce the frequency of small intestinal electrical control activity, possibly via a vagal pathway (Melone 1986, Melone & Mei 1991). In addition, drugs such as erythromycin and opiates, may disrupt or even abolish electrical control activity in a dose-dependent fashion (Otterson & Sarna 1990, Sarna & Otterson 1990). Conditions such as radiation enteritis may also alter or even disrupt electrical control activity (Summers et al 1992). The mechanisms responsible for these changes are poorly defined.

4.2.3 Electrical Response Activity

Smooth muscle contraction is due to sudden depolarisation in membrane potential resulting in intracellular changes in calcium ion concentration. Contractions only occur when spikes (action potentials) are superimposed on the plateau of the slow waves (Duthie 1974). Such spike potentials do not occur with each electrical control activity, and are dependent on neural and hormonal inputs.

4.3 NEUROGENIC CONTROL PATHWAYS

Intestinal myogenic activity is extensively modulated by neural activity. Much of this modulation is provided by an intrinsic neural supply from the enteric nervous system, which is in turn regulated by inputs from the central nervous system.

4.3.1 Intrinsic neural control pathways

The enteric nervous system provides an intrinsic neural supply for many of the motor patterns of the gut. Local neural networks are responsible for contractile events such as peristalsis with aboral relaxation and oral contraction. These motor activities depend on neural networks which run both anally and orally. Hence transection and reanastomosis of the dog jejunum decreases the number of contractions which propagate distal to the transected site, resulting in slower intestinal transit (Johnson et al 1995). However, if microsurgery is performed to precisely oppose the neural endings propagation is restored, and small intestinal motility distal to the transection site is preserved after reanastomosis (Hart et al 1996).

A variety of neurotransmitters have been described which facilitate both excitatory and inhibitory functions. Many of these neurotransmitters are co-localised within the enteric nerves. Excitatory nerves contain substance P and acetycholine (Ach), whilst inhibitory neurones contain vasoactive intestinal polypeptide (VIP) and nitric oxide (NO). It has been suggested that the small intestine is under continuous tonic inhibition from nitric oxide-containing nerves and release from this may lead to phase III of the migrating motor complex (Gustaffson & Delbro 1993).

4.3.2 Extrinsic neural control pathways

The central nervous system (CNS) is believed to modulate small intestinal motor activity via the parasympathetic and sympathetic components of the autonomic nervous system (Gabella 1972). Parasympathetic neural pathways are carried by the vagus nerve. The majority of vagal fibers are afferent or sensory in nature, whilst efferent motor fibers make up <10% of vagal fibers (Agostini et al 1957). As a result one efferent fiber supplies many muscle fibers, and it is therefore not possible for the vagus to control motor activity such as peristalsis directly.

The sympathetic system supply to the small intestine is derived from the thoracolumbar portion of the spinal cord. The precise role of the sympathetic supply in the regulation of small intestinal motility is uncertain, although it is believed to have a mainly inhibitory effect (Ormsbee et al 1979, Telford & Szurszewski 1985).

The contribution of the extrinsic nervous supply to the small intestine has usually been investigated from studies performed after interruption of the vagus and/or the sympathetic nerves by surgical ablation or, in the case of the vagus, temporary cooling of the nerve.

Complete extrinsic denervation

In animals, autotransplantation, resulting in complete extrinsic denervation of the gut, does not appear to have a major impact on the occurrence and timing of fasting motility (Behrns et al 1995)- disruption to interdigestive small intestinal motor activity is transient and normal cyclical activity returns within 12 months (Quigley et al 1990). There is, however, loss of coordination of phase III activity in the denervated section of intestine, with the migrating motor complexes cycling independently in each small intestinal region without any temporal coordination. These findings suggest a modulatory role for the extrinsic nervous system in the

coordination of interdigestive motor activity. Autotransplantation does, however, disrupt the motor response to food, suggesting that the extrinsic nerves have a more important role in postprandial motility (Quigley et al 1990, Sarr & Kelly 1981).

Role of vagus nerve in small intestinal motility

The vagus appears to have only minor effects on the occurrence of the MMC although the proportions of the different phases in the fasting interdigestive motility are disturbed. A number of investigators have shown that cycling of the small intestinal MMC persists in the absence of vagal tone, both in dogs (Thor et al 1989) and in humans (Waterfall 1983). This appears to be true in both the acute situation produced by vagal cooling (Hall et al 1984) and more chronically following vagotomy (Marik & Code 1975).

Hall et al (1984) demonstrated that canine small intestinal phase III activity was independent of vagal tone, unlike phase I and II. Gleysteen et al (1985) reported that vagal cooling in the fasted dog caused only minor changes in either gastric or small intestinal migrating motor complexes. Vagal integrity appears to be more important for the initiation and maintenance of postprandial motility. A number of investigators have demonstrated in several animal models that conversion of fasting motor activity to a postprandial pattern is impaired after vagotomy (Marik & Code 1975, Ruckebusch & Bueno 1977, Greenwood & Read 1985, Chung & Diamant 1987).

Vagal function also appears important in the specialised motor activity associated with emesis. In dogs, the giant retrograde contractions which propel luminal contents aborally during vomiting appear to be cholinergically mediated by the vagus, as they are blocked by atropine (Lang et al 1986).

Effect of sympathetic neural activity on small intestinal motility

Sympathetic nervous inputs appear to have a limited role in the regulation of small intestinal motility. Interruption of the sympathetic nerves does not abolish initiation of phase III in the dog (Marlett & Code 1979). Sympathectomy does, however, lead to variability in cycle duration. Normal migrating complexes return to the duodenum within 2 weeks of sympathectomy. Ormsbee et al (1974) showed that in dogs, the sympathetic nervous system may have a role in the initiation of the small intestinal MMC. However, conversion of the fasted to fed pattern has been shown to be independent of sympathetic control pathways in the dog (Telford et al 1985). In humans, selective blockade of beta 1 adrenergic receptors increases the propulsive forces generated by contractions in the intestine leading to more rapid transit (Ahluwalia et al 1994).

4.4 HORMONAL CONTROL OF SMALL INTESTINAL MOTILITY

Intestinal smooth muscle and nerves are sensitive to a wide range of hormones and neurotransmitters. There is convincing evidence that hormonal influences are important in the regulation of both fasting and fed motor activity. To be recognised as physiologically important to a particular response, a hormone should meet certain criteria as stated by Grossman (1974): 1) The plasma concentration should rise in association with the response. 2) Exogenous infusion of the hormone at physiological doses should produce the response and 3) The physiological response should be blocked when an antagonist is administered. In establishing the potential role for many hormones a major limitation has been the lack of specific antagonists.

4.4.1 Motilin

The strongest evidence for hormonal involvement in the regulation of small intestinal motor activity is related to motilin, a 22-amino acid peptide localised in the enterochromaffin cells of the duodenum. The entire sequence is required for significant bioactivity. Motilin has been proposed as the major stimulus for phase III activity. Plasma concentrations of motilin fluctuate cyclically with the MMC with peak concentrations just before and during phase III of the stomach and duodenum (Vantrappen et al 1979). In humans, only gastric activity fronts are associated with plasma motilin peaks (Bormans et al 1987), suggesting that motilin is only involved in regulation of the gastric migrating motor complex. In the dog intravenous infusion of the hormone initiates premature migrating motor complexes during fasting, but has no effect on postprandial motor activity (Itoh et al 1976). Similar results have been reported in humans (Vantrappen et al 1979). Administration of motilin antiserum abolished phase III activity in the proximal small intestine of the rabbit (Lee et al 1983). Although administration of a specific motilin antagonist blocks motilide-induced in vitro, the effect of motilin antagonists in vivo are unknown (Peeters et al 1994). There is substantial evidence that the antibiotic, erythromycin, stimulates gastrointestinal motility by acting as a motilin agonist (Peeters et al 1989).

4.4.2 Insulin

The role of insulin in modulation of small intestinal motor activity is unclear because of the major effects of hypoglycaemia on vagal activity following insulin administration. During fasting there are conflicting reports about the effect of this hormone on small intestinal motility. In the dog (Prasad & Sarna 1986) insulin has been reported to stimulate MMC activity prior to any hypoglycaemic activity. However, in humans, although insulin-induced hypoglycaemia stimulates jejunal motor activity, this was not associated with phase III activity (Fellows et al 1987).

suggests that postprandial motor activity is mediated through both neural and hormonal mechanisms (Code & Marlett 1975).

4.5.1 Neurogenic

Neural modulation by the central nervous system of fed motor activity following meal ingestion has been suggested. Studies performed in animals relate to the central administration of neuropeptides, as well as interruption to extrinsic neural pathways.

Hall et al (1986) reported that interruption of vagal pathways prevented the initiation and maintenance of a postprandial pattern in the dog. Chung & Diamant (1987) however reported that vagal cooling did not prevent the occurrence of postprandial activity but resulted in a delay in onset of postprandial activity which was of short duration.

4.5.2 Hormonal

The role of hormones in the postprandial or fed pattern is poorly defined. A number of hormones released following meal ingestion appear to alter the migrating motor complex cycle. In addition, infusion at apparently physiological doses induces a motor pattern typical of the fed state, in fasted subjects. These hormones include gastrin (Marik & Code 1975, Weisbrodt et al 1974), insulin (Bueno & Ruckebusch 1976), cholecystokinin (Niederau & Karaus 1991), secretin (Mukhopadhyay et al 1977), glucagon (Gregersen et al 1988) and neurotensin (Thor et al 1982). There are however, no convincing studies demonstrating that any one hormone is responsible for initiation of postprandial motor activity. Modulation of the postprandial pattern by hormones may relate to a direct effect on smooth muscle, but is more likely a combination of actions via extrinsic and intrinsic nervous control.

Several neuropeptides may affect the pattern of gastrointestinal motility in animals through a central nervous system action (Tache et al 1990). Intracerebroventricular administration of neurotensin restores the migrating motor complex pattern in fed rats (Bueno et al 1985). Corticotropin releasing factor (CRF) (released in response to stress) suppresses the gastric MMC (Fargeas et al 1993).

4.6 EFFECTS OF BLOOD GLUCOSE CONCENTRATION ON SMALL INTESTINAL MOTILITY

4.6.1 Introduction

Gastrointestinal motor dysfunction occurs commonly in patients with diabetes mellitus and may involve a number of sites (Feldman et al 1983, Horowitz & Fraser 1994, Fraser et al 1990, Camilleri & Malagelada 1984, Schvarcz et al 1996). Abnormal motility has generally been attributed to irreversible autonomic neuropathy, but recent studies have shown that the blood glucose concentration is also important in the regulation of gastrointestinal motor function (De Boer et al 1992, Fraser et al 1991, Bjornsson et al 1994, Chey et al 1995). It has also been shown that more physiological levels of increases in blood sugar, such as those that occur postprandially also modulate gastrointestinal motility (Boeckxstaens et al 1996, Schvarcz et al 1997, Lingenfelser et al 1996).

4.6.2. Effects of blood glucose concentration on fasting small intestinal motility

The effects of blood glucose concentration on small intestinal motility are unclear. Initial reports suggested that hyperglycaemia (while suppressing antral phase III) had no effect on fasted duodenal phase III activity, (Barnett & Owyang 1988). However, acute hyperglycaemia has also been reported to stimulate duodenal phase III-like activity (Fraser et al 1991). It has also been documented

that more modest levels of hyperglycaemia (10mmol/l) reduce the migrating motor complex cycle length. Oster-Jorgensen et al (1992) showed that a blood glucose concentration of 10mmol/l is associated with a reduction in duodenal phase III activity (Oster-Jorgensen et al 1992). Subsequent studies have examined in greater detail the effects of increased blood glucose concentration on different components of the MMC in normal subjects. There appears to be a reduction in the number of propagated phase II contractions, but an increase in the number of clustered contractions during hyperglycaemia - non-propagating pressure wave sequences with a frequency of 10-12/min lasting approximately 30 seconds (Bjornsson et al 1994). Similar patterns have been described in patients with diabetes and it is unclear whether the latter observations may be attributable to high blood glucose concentrations, as these were not stabilised or monitored (Camilleri and Malagelada 1984).

There have been few studies on the effect of hypoglycaemia on small intestinal motor activity. Fellows et al (1987) and Fraser et al (1991) reported differential effects between the proximal and distal small intestine using low blood glucose concentrations. The effects of these motor changes on transit are unknown.

4.6.3 Effects of blood glucose concentration on postprandial small intestinal motility

Few studies have examined the effect of changes in blood glucose concentration on postprandial activity, which is more likely to be of importance in clinical practice.

A prolongation of duodeno-caecal transit of a non-nutrient meal has been reported in healthy humans during marked hyperglycaemia (blood glucose ~ 15mmol/l) (DeBoer et al 1993). The motor correlates of this slowing were not evaluated.

The effect of hyperglycaemia on postprandial small intestinal motor activity and transit are also unknown. No studies have examined the effect of hypoglycaemia on fed small intestinal motility and transit.

4.7 CONCLUSIONS

The changes in small intestinal motor patterns induced by meals are mediated by several interacting factors. The response to intraluminal contents, which act via intrinsic nerves and locally released chemicals, is modified by hormones and extrinsic nerves. Modulation of the postprandial pattern by hormones is likely to result primarily from actions via the extrinsic and intrinsic nerves rather than direct effects on smooth muscle. Hyperglycaemia has major effects on both fed and fasted small intestinal motor activity, which are likely to be important in patients with diabetes mellitus.

CHAPTER 5

ANATOMY OF THE ANORECTUM

5.1 INTRODUCTION

The anorectum forms the junction between the internal milieu of the gastrointestinal tract and the external environment. The major roles of this region are maintenance of faecal continence and control of defaecation; functions which require a complex series of interactions between visceral and somatic motor and sensory pathways under the control of local reflexes and the central nervous system. In this chapter the anatomical structures which underlie motor and sensory activity responsible for anorectal function are reviewed.

5.2 GROSS ANATOMY

The anorectum comprises two integrated anatomic structures: the rectum, and the anal canal, together with the internal and external anal sphincters and supporting structures (figure 5.1). The rectum forms the terminal part of the large intestine, extending from the rectosigmoid junction to the anal canal. It is approximately 12 cm in length with a proximal diameter of about 4 cm. Inferiorly, the lumen widens and forms the rectal ampulla, before narrowing rapidly as it approaches the anal canal (Farouk & Bartolo 1993). The passage of the rectum through the pelvis is marked by the occurrence of three curves. In addition there are three fixed rectal folds, the so-called valves of Houston formed over bands of connective tissue, which may play a role in retarding faecal flow (Abramson 1978). The anal canal is 3-4 cm long and descends as the continuation of the rectum. It begins immediately distal to the rectal ampulla and turns posteriorly at the pelvic floor to reach the anus. The transition between the rectum and the anal

canal is marked by the anorectal angle. The anorectal region has both visceral and somatic components, comprising the rectum and upper part of the anal canal, and the muscles of the external anal sphincter, the pelvic floor and the epithelium of the lower anal canal respectively (Kumar 1993).

The internal anal sphincter (IAS), formed by a thickening of the circular smooth muscle of the rectum, surrounds approximately 3cm of the anal canal. The muscle layer varies between 0.1 and 0.5 cm in thickness and extends from approximately 1cm below the junction of the epithelial lining of the rectum and the anus i.e the dentate line.

The external anal sphincter (EAS) is composed of striated skeletal muscle which surrounds the whole length of the anal canal (Kumar 1993). Although it is usual to divide the EAS into three components: subcutaneous, superficial and deep (Milligan & Morgan 1934), the sphincter muscle behaves functionally as one unit. The superficial components are attached to the perineal body and the coccyx, whilst the subcutaneous and deep layers form a ring around the anal canal.

The muscles of the pelvic floor comprise paired striated muscles known as the levator ani and puborectalis. The levator ani is a thin, broad muscle which runs between the pubis, the ischial spine and the obturator internus muscle. In the midline its fibers form a raphe with those from the opposite side. The levator ani is believed to act as a muscular support for the pelvic viscera when abdominal pressure increases during defaecation. The muscle fibers of the puborectalis overlap the deep part of the EAS. The puborectalis, which extends from the pubis, forms a U-shaped loop around the recto-anal junction, maintaining an anorectal angle of approximately 90°, except during defaecation when the muscle

relaxes and allows the angle to become less acute (Hajivassiliou et al 1996). Hence, the angulation between the axis of the rectum and that of the anal canal results from the tonic activity of the puborectalis portion of the levator ani muscles.

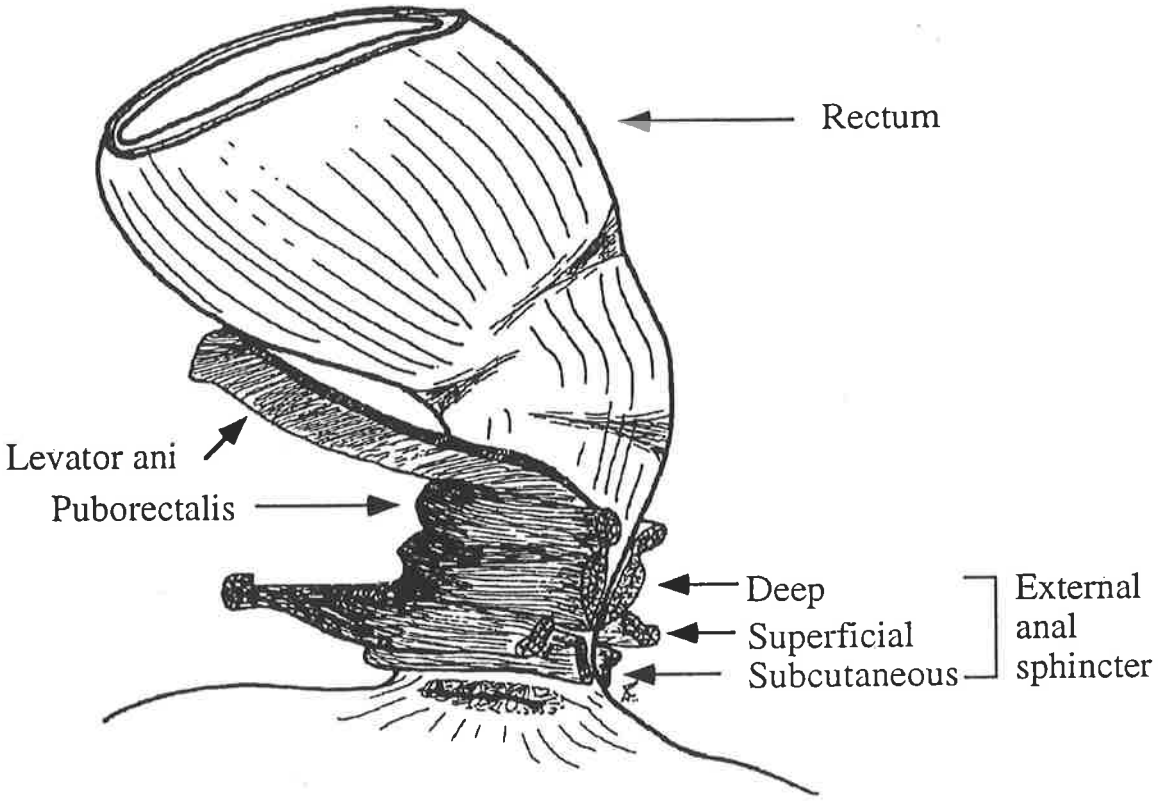

Figure 5.1

A diagram illustrating the rectum, external anal sphincter musculature and the levator ani (modified from Reeve DRE: Alimentary Sphincters and their Disorders. London, Macmillan Publishers, 1981, p18; with permission).

5.3 HISTOLOGY

Rectum

The wall of the rectum, in common with the rest of the gastrointestinal tract has four major layers: the mucosa, submucosa, muscularis propria and serosa (present only in the proximal two thirds of the rectum). The mucosa comprises mucous secreting columnar cells which occupy half of the anal canal, and abundant non-myelinated nerve fibers. The muscularis propria of the rectum is composed of outer longitudinal and inner circular layers of smooth muscle. The circular muscle of the rectum continues to become the internal anal sphincter (section 5.2). The longitudinal muscle layer joins with fibers from the pubococcyx passing through the EAS and inserting in the perineal skin. Between the two muscle layers lies the myenteric plexus. The myenteric ganglia become less prominent in the distal rectum and are absent at the level of the IAS (Aldridge &Campbell 1968).

Anal canal

The structure of the epithelial lining of the anal canal varies along its length; the upper part consists of simple columnar cells, with numerous tubular glands or crypts, while the lower part is lined by squamous epithelium, richly supplied with sensory fibers and sensitive to touch, pain, temperature and movement (Duthie & Gairns 1960). In approximately 50% of people a band of cuboidal epithelium at the junction of the upper and lower anal canal, known as the transitional zone, is present (Fenger 1987, Chattopadhyay et al 1993).

5.4 NEURAL ANATOMY

5.4.1 Intrinsic innervation

The neural supply of the anorectum is complex and includes input from motor and sensory components to the somatic and visceral structures (Stephens 1972).

The rectum has a well defined intrinsic (enteric) nervous system in continuity with the rest of the colon. The rectal enteric nervous system comprises myenteric and submucosal plexi whose ganglia are interconnected by numerous nerve fibers (Furness & Costa 1987). Rectal ganglia are smaller and fewer and the nerve bundles thinner than in the proximal colon. Neural fibers, which are mainly inhibitory in nature, run between the rectum and the IAS and are possibly responsible for relaxation of the IAS (Burleigh 1983).

5.4.2 Extrinsic innervation

Extrinsic innervation of the rectum and internal anal sphincter is via sympathetic and parasympathetic components of the autonomic nervous system. The external anal sphincter is innervated by the pudendal nerves which arise from the first four sacral segments.

Rectum

Branches of the parasympathetic nervous system arising from the sacral level of the spinal cord (S2-S4), leave by sacral ventral roots and run in the pelvic splanchnic nerves to synapse with post ganglionic intramural neurones to reach the rectum (Schuster 1968). Parasympathetic innervation to the anorectum is primarily excitatory.

The rectum also receives branches from the sympathetic system which arise from the lumbar level of the spinal cord with ganglion cells at L2 - L4 (Carlstedt et al 1988). Axons run in the lumbar splanchnic nerves, and synapse in the inferior mesenteric ganglion with postganglionic noradrenergic neurones whose axons reach the rectal ampulla via the hypogastric nerves (Baron et al 1985). The sympathetic innervation of the rectum may be excitatory or inhibitory.

Internal anal sphincter (IAS)

In keeping with the rest of the gastrointestinal tract, the internal anal sphincter is also innervated by the extrinsic autonomic nervous system, receiving sacral parasympathetic and thoracolumbar sympathetic innervation. However, the sympathetic nerves are excitatory and parasympathetic nerves inhibitory (Bouvier & Gonella 1981, Carlstedt et al 1991). Electrical stimulation of pelvic nerves relaxes the IAS in humans (Shepherd & Wright 1968); this inhibitory effect of the parasympathetic nervous system is due to the activation of cholinergic preganglionic neurones connected to non adrenergic, non-cholinergic (NANC) intramural inhibitory neurones.

The sympathetic neural supply to the IAS is excitatory. In the cat stimulation of the distal end of hypogastric nerves, lumbar colonic nerves, or second, third and fourth lumbar spinal nerves induces internal anal sphincter contraction (Carlstedt et al 1988). This excitatory effect of sympathetic innervation on the IAS has been demonstrated in other animal species (Costa & Furness 1973, Garrett et al 1974), as well as humans (Schuster 1968), and is caused by release of noradrenaline at postganglionic nerve endings acting directly on smooth muscle cells through noradrenergic alpha receptors (Bouvier & Gonella 1981).

External anal sphincter (EAS)

The EAS is supplied by the inferior rectal branch of the pudendal nerve and the perineal branch of the fourth sacral nerve (Gagnard et al 1986). Somatic nerves supply the voluntary muscles of the puborectalis; direct branches of the sacral plexus enter the muscle from its pelvic surface (Snooks & Swash 1986). The levator ani are supplied (on their pelvic surface), by the fourth sacral nerve, and (on their perineal aspect), by the inferior rectal, or perineal branches, of the pudendal nerves.

5.5 SENSORY

Rectum

While the sensation of rectal distension is transmitted via the autonomic nerves (Rogers 1992), the afferent pathway to the central nervous system is poorly defined. These fibers are responsible for the perception and discrimination of gaseous, liquid and solid rectal contents. Although the rectal mucosa comprises abundant non-myelinated nerve fibers, only one intra-epithelial receptor ending has been identified (Duthie &Gairns 1960). There is no sensation to touch or temperature in the rectum. Thus the most useful tests of rectal sensation involve balloon distension with either air or water (Read & Sun 1992). Both parasympathetic and sympathetic nervous pathways appear to be involved in communicating sensory information from the rectum in response to distension (MacDonagh et al 1992). The relative importance of these two pathways is dependent on the type of sensation e.g perception, wind, desire to defaecate, or pain. In the human oesophagus it has been suggested that the parasympathetic nerve fibres may be responsible for relay of perception, whereas the sympathetic nerve fibres are responsible for pain due to balloon distension (Weusten et al 1994). There are limited data about relay of sensory information from the rectum, although it has been suggested that sympathetic pathways play a role (MacDonagh et al 1992).

Anal canal

The anal canal is densely innervated with a variety of specialised sensory nerve endings: Krause end-bulbs respond to thermal stimuli, Golgi-Mazzoni bodies and Pacinian corpuscles respond to changes in tension and pressure and genital corpuscles respond to friction. As a consequence the mucosa of the upper anal canal is sensitive to heat, cold, touch and pain (Duthie & Bennett 1963). In addition, there are large diameter free nerve endings within the epithelium. The

pathway for anal canal sensation is via the inferior haemorrhoidal branches of the pudendal nerve to the sacral roots of S2, S3 and S4 (Gunterberg et al 1976). Anal sensation may be assessed quantitatively by the response to electrical stimulation (Rogers 1992).

The lower anal canal is composed of hairy perianal skin in which numerous sensory nerves reside. Sensation experienced in the lower anal canal is thought to be conveyed by the afferent fibers in the inferior rectal nerve, as sensation can be abolished by inferior rectal neve block (Gordon 1987).

5.5 CONCLUSIONS

The anorectal region has an extensive neural network which allows coordinated muscle function and central monitoring and control of rectal filling. The local and distal interactions are responsible for normal maintenance of continence and defaecation at the appropriate time.

CHAPTER 6

ANORECTAL MEASUREMENT TECHNIQUES

6.1 INTRODUCTION

In a number of previous studies of anorectal function only limited conclusions can be drawn because of suboptimal methodology (Schiller et al 1982, Rogers et al 1988, Wald & Tunuguntla 1984). Comprehensive assessment of anorectal function therefore requires measurement of both motor and sensory components, including the integrated measurement of rectal and anal sphincter motility, electrical activity of the internal and external sphincter, neural innervation of the anorectum and anal sphincter structure (Rao & Sun 1997). No single test provides complete information about all aspects of anorectal function and most tests examine only one specific function or component. Most information is therefore obtained from a combination of various techniques (Sun et al 1992). In this chapter techniques which are currently available for assessment of anorectal motor and sensory function are discussed.

6.2 STRUCTURAL ASSESSMENT

6.2.1 Anal ultrasound

Structural damage to anal sphincter muscles may occur during vaginal delivery, or as a result of some surgical procedures, with consequent impairment of anal sphincter function. Ultrasound is the only commonly available technique which allows assessment of sphincteric structural integrity (Bartram & Sultan 1995). Anorectal ultrasound is performed using a high frequency (7MHz), rotating transducer, covered by a plastic cone which is acoustically coupled to the anal canal by degassed distilled water (Farouk & Bartolo 1993). The technique is

quick and easy to perform, associated with minimal patient discomfort and requires no bowel preparation. The outer and inner edges of the internal and external anal sphincter can usually be delineated accurately. Anorectal ultrasound is indicated in patients with faecal incontinence or obstructed defaecation to assess the integrity and thickness of the internal anal sphincter, external anal sphincter, perineal body and to a lesser extent the puborectalis (Bartram & Sultan 1995). In addition, sequential changes in sphincteric structure after surgery or radiotherapy can be monitored. Anorectal ultrasound gives no information about motor or sensory mechanisms, and it is therefore best utilised in combination with other techniques (Yeoh et al 1996).

6.3 MEASUREMENT OF ANORECTAL PRESSURES

6.3.1 Water perfused manometry

Water perfused manometry is based on the principle of measuring the resistance to outflow of perfusate. When a lumen-occlusive contraction occurs, the force with which flow of water through the catheter is occluded is measured as pressure (Read & Sun 1992). Measurements of anorectal pressure are usually made using catheters of flexible silicone tubing, incorporating a terminal rectal balloon, and a number of sideholes to permit recordings from multiple sites in the anorectum (figure 6.1). While use of a catheter incorporating multiple, closely spaced sideholes facilitates interpretation of various parameters and allows evaluation of the pressure gradient between the rectum and the anal canal, the presence of multiple sideholes can also be a significant disadvantage because of artefact caused by excessive water in the anal canal. Furthermore, even when sideholes are placed closely, the highest point of pressure may not be detected.

The sleeve sensor, initially developed by Dent (1976) to measure lower oesophageal sphincter pressure, functions as a Starling resistor and records the

maximum pressure along its length (usually 4-5 cm). The sleeve sensor has been adapted for measurement of upper oesophageal sphincter (Wyman et al 1990), pyloric (Heddle et al 1988), ileocolonic (Quigley et al 1987) and anal pressures (Orkin et al 1991). The sleeve sensor, however, does not record pressure changes along the anal canal, nor discriminate between the pressure changes caused by the sphincter muscles. The anorectal catheter used by the author included a sleeve sensor as well as perfused sideholes to record maximum pressure and the pressure gradient in the anorectum.

Information relating to frequency, amplitude and duration of contractions can be recorded on a paper chart recorder or a computer for subsequent analysis. A number of parameters can be measured during manometric studies (Read & Sun 1992): the most common being the maximal and minimal resting anal pressures, maximum and plateau squeeze anal pressures, the rectoanal inhibitory reflex (i.e. the minimum rectal volume which induces anal relaxation), the contractile reflex (i.e. the contractile response of the external anal sphincter in response to rectal distension), threshold volume for perception of rectal sensation, rectal compliance (pressure-volume relationship curve) and both rectal and anal pressures during straining.

6.3.2 Ambulatory anorectal manometry

The requirement for prolonged anorectal motor function has been based on the difficulties in extrapolating observations derived from short term measurements of anorectal motility obtained in a laboratory to what is seen in normal daily activity. Because abnormal patterns of anorectal motility may occur randomly or intermittently, studies up to 24 hours in duration may be required for comprehensive pressure data to be obtained in a normal environment during normal daily activity (Kumar et al 1990). The ambulatory manometric technique

involves a lightweight portable recording system which incorporates a miniature catheter and computer for data storage. Even though prolonged recordings provide detailed descriptions of anorectal motility, there are a number of disadvantages to this technique which limit its sensitivity (section 2.4.2). Electrodes may be attached to the anal verge and used to measure skin impedance and hence episodes of incontinence. This technique known as impedance planimetry, is semi-quantitative and restricted mainly to ambulatory recordings (Dall et al 1993).

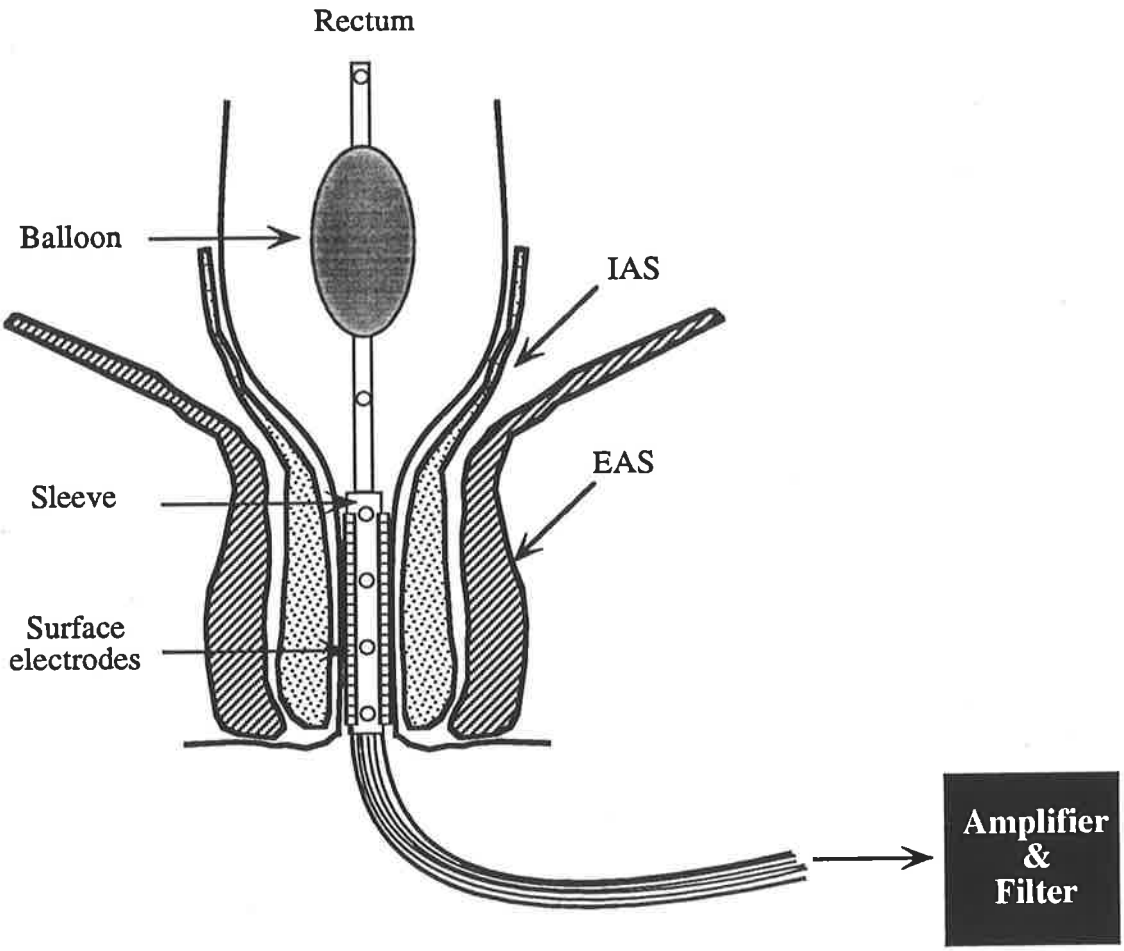

Figure 6.1

A schematic representation depicting anorectal anatomy and position of the perfused catheter used in the author's study (chapter 11). The sleeve straddles the anal canal and comprises 4 sideholes separated by 1cm. The lower and upper rectal sideholes are separated by a 5.5cm balloon used for rectal distension.

6.3.3 Microballoons

A modification of the perfused manometric system is the use of microballoons, which are filled with either air or water (Belliveau et al 1983). While this technique avoids the potential artefact caused by leakage of fluid in the anal canal by perfused catheters, microballoon catheters are not in widespread use, primarily because of the lower precision of recordings. In addition, larger balloon sizes may lead to distension of the anal canal and alter anorectal motility. The number of recording sites is also less when compared to perfused catheter systems.

6.3.4 Solid state transducers

Miniature pressure transducers incorporated into a catheter can be used to provide accurate readings of colorectal pressures (Crowell et al 1991). However, there are significant disadvantages to this approach. Apart from being fragile and expensive the number of solid state transducers which can be incorporated into the catheter is limited by the resulting diameter of the probe which may cause distension of the anus. Solid state tranducers may, however, be useful in ambulatory studies.

6.4 RECTAL SENSORY TESTING

Rectal sensation is important in the initiation of defaecation and maintenance of continence. Rectal sensory function is quantified by rectal distension with an air or water filled balloon, inflated at various rates, volumes and patterns. The rate and volume of inflation varies between different centres and includes ramp inflation (a slow continuous inflation), graded inflation (rapid intermittent balloon inflation at a rate of approximately 40ml/sec with volumes ranging between 10-250ml) (Sun et al 1990) and the use of a barostat (providing inflation of a thin walled bag attached to a catheter at either constant volume or pressure) (Bell et al

6.5.3 Saline continence test

This technique evaluates the capacity to maintain continence to liquid faeces, and is often performed at the end of standard anorectal manometry (Bartolo et al 1985). With the manometric assembly in position, an additional catheter is introduced into the rectum through which the rectum is slowly distended (60 ml/min) with prewarmed saline to a total volume of 1500ml (Haynes & Read 1982). Anorectal pressures, rectal sensation, the minimum volume of first leakage of infused saline and maximum retention volume can be determined. The saline continence test may detect subtle changes of anorectal function that are not apparent with other techniques (Penninckx et al 1995). This procedure is applied optimally to incontinent patients with leakage of liquid faeces, and can also be used as an indicator of improved anorectal function after treatment (Miner et al 1990).

6.6 NEUROPHYSIOLOGICAL ASSESSMENT

6.6.1 Electromyography

Electromyography (EMG) allows quantitative measures of electrical activity of the anal sphincters (Williams & Kumar 1994). The types of electrodes commonly used include surface (Orkin et al 1991), monopolar, bipolar (Basmajian & Stecko 1962) and concentric (Buchthal 1977). In combination with manometry, EMG not only facilitates interpretation of manometric recordings, but provides a better understanding of the relationship between motility and nerve function (Yeoh et al 1996). The author used electromyographic electrodes incorporated into the sleeve catheter , particulary as this design reduces patient discomfort (Chapter 11). For measurements of internal anal sphincter activity, a filter window of 0.15-3Hz frequency range is used, as opposed to 10-150Hz for external anal sphincter activity (Yeoh et al 1996). Slow waves, or regular oscillations of the internal anal sphincter, with an

approximate frequency of 10-20/minute and bursts (successive spikes) of the external anal sphincter can be readily identified. For example, as external anal sphincter activity increases in response to squeeze a quantitative assessment of change from baseline can be made. Similarly, internal anal sphincter activity can be assessed as a change in both amplitude and frequency of the slow waves. The technique has widespread application in combination with manometry but is rarely used on its own.

6.6.2 Pudendal nerve terminal motor latency

The integrity of the terminal portion of the pudendal nerve and the external anal sphincter is assessed by measurement of pudendal nerve terminal motor latency. If the pudendal nerve is damaged the time between stimulation and the myoelectric response of the sphincter is prolonged. Disposable electrodes mounted onto a gloved finger are inserted into the rectum allowing the pudendal nerve to be stimulated at 50V for 0.1 ms at 1 sec intervals (Neill & Swash 1980). The external anal sphincter response is measured using a separate pair of electrodes and recorded to EMG apparatus with standard external anal sphincter filter settings. A pudendal nerve latency of $\leq$ 2 ms is considered to be normal (Jost & Schimrigk 1994). It should be recognised that while this technique quantifies the degree of nerve damage, it only provides information about efferent nerve pathways.

6.6.3 Somatosensory evoked potentials

This technique involves stimulation of the rectum by an electrode mounted onto a rectal probe, with simultaneous recording of cerebral potentials from the skull using surface electrodes (Loening-Baucke et al 1991). The latency between stimulation of the rectum and the cerebral potential, as well as the amplitude of

the cerebral potential, can be determined to provide an assessment of afferent nerve function. At present, this technique remains primarily a research tool.

6.7 CONCLUSIONS

No single technique evaluates all aspects of anorectal function. Tests of structure, motor and sensory function and innervation provide complementary data concerning anorectal motility. In practice, a combination of selected techniques, guided by the patient's clinical condition, is usually employed to provide the most comprehensive assessment. In the work reported in this thesis, the author used perfused manometry, electromyography and assement of rectal sensation by balloon distension techniques to characterise the effects of changes in the blood glucose concentration on anorectal activity.

CHAPTER 7

ANORECTAL MOTILITY

7.1 INTRODUCTION

The function of the anorectum is to maintain faecal continence and, when appropriate, contribute to the controlled excretion of the waste products of digestion. This involves detection of rectal contents, discrimination of solid, liquid and gaseous components, retention of these, and finally, expulsion through the anal canal (Sun & Read 1989, Rasmussen 1994). This chapter discusses the patterns of anorectal motility which underlie both normal and disordered anorectal function.

7.2 RECTAL FUNCTION

The rectum was originally suggested to consist of two functional parts: an upper part responsible for storage of faecal material, and a lower part which was normally empty except during defaecation. Information on how rectal function correlates to motor activity is limited, as an understanding of colonic motor activity is also of integral importance. Studies of these two organs simultaneously is often cumbersome and difficult. To simulate arrival of a bowel motion in the rectum, balloon distension is commonly used. In response to rapid distension there is a transient increase in rectal pressure which gradually returns to a steady baseline. In response to slow distension there is also a transient increase in rectal pressure with minimal changes until a volume of approximately 300 ml is reached; this is known as the "accommodation response" (Duthie 1975). As the distending volume increases further the rectum fails to accommodate and increases in amplitude and duration of rectal contractions occur as a result of

increases in baseline pressures. This is usually associated with a desire to defaecate.

Three types of contractile activity in the human rectum have been described from short-term recordings: (i) simple non-propagating contractions occurring at a frequency of 5-10 cycles/min. (ii) slower static contractions at 3 cycles/min, with an amplitude of up to 100mmHg. (iii) slow propagated contractions occurring infrequently (Whitehead et al 1980, Scharli & Kiesewetter 1970).

Recent studies using prolonged manometry in ambulant human subjects, have further characterised rectal contractile activity. Periodic motor activity with strong bursts of sustained contractions have been described. These contractions have a frequency of 3/min, a maximum amplitude of about 50mmHg, and a duration of 3-10 mins. Cycles recur every 80-90 min during the day and every 50-60 min intervals at night. This periodic rectal activity is disrupted by food for approximately 150-180 mins and has been termed the "rectal motor complex" (RMC)(Kumar et al 1989). It's unlikely the rectal motor complex is related to phase III of the migrating motor complex in the small bowel. Even though the rectal motor complex occurs periodically, with a shorter cycle length during nocturnal sleep, there is no consistent relationship between the small bowel migrating motor complex and the rectal motor burst activity (Kumar et al 1990, Prior et al 1991). Furthermore the rectal motor complex does not propagate in either the orad or aborad direction (Kurakake et al 1993), and may function to keep the rectum empty by retropulsion of the stool into the colon.

7.3 ANAL SPHINCTER FUNCTION

7.3.1 Internal anal sphincter

The IAS is tonically contracted at rest and provides 85% of resting anal pressure (Holschneider 1976, Frenckner & Euler 1975). Regular fluctuations (5-25mmHg) in IAS pressure, termed slow waves, have a frequency of 10-20/min (Wankling et al 1968). Ultra slow waves (amplitude 30-100mmHg), with a frequency of less than 3/min are occasionally observed in normal subjects but are more frequently seen in patients with anal fissure or haemorrhoids (Gibbons & Read 1986, Read et al 1982). In addition spontaneous anal relaxations have been described in both health and disease. These are classified as a decrease in anal pressure of at least 20mmHg, with a duration of greater than 15 sec unassociated with any change in rectal pressure (Sun et al 1990)

Rectal balloon distension is also used to study IAS function. Rapid distension causes a decrease in anal pressure (Sun & Read 1989). At lower volumes (10-40ml), the IAS relaxes and then quickly returns to resting levels. As the distending volume increases (60-250ml), IAS relaxation increases in magnitude, with a duration equal to the time of inflation (Bannister et al 1989, Sun & Read 1989). When the balloon is deflated, anal canal pressure returns to baseline and often exceeds the values observed immediately before inflation.

7.3.2 External anal sphincter

Activity of the external anal sphincter contributes about 15%-20% of resting anal pressure (Frenckner & Euler 1975). During threats to continence the EAS can be voluntarily contracted to occlude the anus more firmly. External anal sphincter contraction is the final mechanism by which continence is maintained.

During rapid, intermittent balloon distension, EAS contractile activity initially increases and then returns to a steady state. With increasing balloon volumes

EAS contractile activity increases in amplitude and duration. The initial contractile response is thought to be mediated by spinal reflexes as it is observed in paraplegics (Parks et al 1962). The EAS response is also modulated by conscious mechanisms and is related to sensation. Therefore the response is absent if the subject does not perceive rectal distension and correlates with the duration of rectal sensation (Sun et al 1990).

7.4 RECTO-ANAL COORDINATION

The anal canal is surrounded by the internal and external anal sphincter muscles which produce a high pressure zone approximately 2-4 cm from the anal verge (Bennett & Duthie 1964) that prevents the escape of rectal contents except at the desired times. Contraction of the two sphincters normally keep the anal canal closed.

7.4.1 Normal defaecation

Defaecation has been described as an orderly series of events. Propagated colonic contractions propel faeces into the rectum, and if the stool size and weight are large enough the desire to defaecate may result (Read & Sun 1992). This sensation is usually associated with rectal contraction and IAS relaxation, which serve to introduce the stool into the proximal anal canal. Sensory mechanoreceptors in the rectum mediate awareness of rectal filling or distension. When the internal anal sphincter relaxes due to rectal filling, rectal contents come into contact with the sensitive anal mucosa and this facilitates discrimination of gas, liquid or solid rectal contents. This is known as the "sampling reflex" (Duthie 1975).

Under appropriate conditions, the subject adopts a sitting position, and contracts the diaphragm, abdominal muscles and levators. This is associated with external

anal sphincter relaxation. Faeces are then extruded as the anorectal angle becomes less acute.

7.4.2 Maintenance of continence

Continence to faeces is maintained by tonic contraction of the IAS under both resting conditions, during sleep and gradual rectal distension (Read & Sun 1992). Tonic activity of the EAS also facilitates continence. Contraction of the puborectalis makes the anorectal angle more acute, thereby allowing the rectum to retain faeces. Threats to continence like rectal distension, rectal contraction and increases in intraabdominal pressure may increase rectal pressure to greater than that produced by tonic contraction of the sphincter muscles. For example rectal distension and contraction cause a reflex relaxation of the IAS . Increases in intraabdominal pressure are usually of sufficient magnitude to overwhelm the resting sphincter pressure and may also induce sphincter relaxation.

Under threats of continence compensatory EAS contraction is the only means by which continence can be maintained. However, during sleep EAS activity is almost completely absent and does not increase in response to balloon distension (Whitehead et al 1982). In this case IAS contraction is the only mechanism by which continence can be maintained.

The relationship between contraction of the EAS and IAS in preservation of continence is complex and recording the function of both muscles simultaneously in humans is difficult. Both sphincter muscles function independently of one another and in a reciprocal fashion. For example, rectal distension and contraction causes IAS relaxation, but EAS contraction. In contrast, micturition is associated with IAS contraction and EAS relaxation (Salducci et al 1982).

7.5 DISORDERED ANORECTAL FUNCTION

Faecal incontinence and constipation can result from impairment of several components of the continence mechanisms (Table 7.1) (Bartolo & Sun 1997). To properly investigate the integrated function of each component, situations which pose a threat to continence must be reproduced and the techniques used should have little impact on the normal function of the organ. The most common causes of disordered anorectal function will be discussed.

Table 7.1

Some of the major causes of disordered anorectal motility

1. Faecal incontinence
   - diabetes mellitus
   - irritable bowel syndrome
   - impaired rectal sensation
   - anal sphincter trauma
   - spontaneous transient anal relaxation
   - impaired internal anal sphincter
   - pudendal neuropathy

2. Constipation
   - megarectum
   - haemorrhoids
   - Hirschsprung's disease
   - anismus

7.5.1 Faecal incontinence

Diabetes mellitus

Faecal incontinence is a problem in up to 20% of unselected patients with diabetes mellitus (Feldman & Schiller 1983, Schiller et al 1982, Wald & Tunuguntla 1984, Rogers et al 1988, Wald 1994). Multiple anorectal motor and sensory dysfunctions are present in diabetic patients with incontinence. Internal and external anal sphincter pressures are reduced (Schiller et al 1982, Rogers et al 1988), the internal anal sphincter is frequently unstable (Sun et al 1996) and rectal sensation may be impaired (Wald & Tunuguntla 1984, Caruana et al 1991) when compared to healthy subjects. Blood glucose concentrations were not monitored in any of these studies.

Acute changes in the blood glucose concentration has been shown to have a major, reversible, effect on motor and sensory function in a number of regions of the gastrointestinal tract (De Boer et al 1992, Barnett & Owyang 1988, Fraser et al 1990, Fraser et al 1991, DeBoer et al 1993, Sims et al 1995), both in patients with diabetes mellitus and in normal subjects. The impact of changes in blood glucose concentration on anorectal function is discussed in more detail in Chapter 8 (section 8.8).

Irritable bowel syndrome (IBS)

Patients with IBS are more sensitive to rectal distension than normal subjects (Sun & Read 1988, Prior et al 1990). Not only are symptoms perceived earlier, but patients with irritable bowel syndrome also have reduced rectal compliance (Sun & Read 1988). Low rectal volumes induce sustained IAS relaxation and repetitive rectal contractions with an associated weakness of the EAS. Approximately 25% of IBS patients suffer from faecal incontinence (Cann et al 1984).

Impaired rectal sensation

In a subgroup of these patients IAS relaxation induced by rectal distension or contraction, is not always associated with compensatory EAS contraction. Alternatively the EAS response is present but delayed (Sun et al 1990). The rectal volume which induces IAS relaxation is lower than that which induces rectal sensation and an increase in EAS activity. These patients have a grossly delayed sensory response.

Anal sphincter Trauma

Obstetric trauma resulting in anal tears is a common cause of faecal incontinence (Bek & Laurberg 1992). Patients often present with a problem many years after the last delivery. Radical haemorrhoidectomy and digital distension for the treatment of anal fissure may also lead to IAS damage. Measurement of IAS and EAS integrity using anal ultrasound facilitates differentiation between the two conditions. Manometrically the presentation of a weak sphincter is difficult to distinguish from those patients with pudendal neuropathy. In the first instance low tone of either the EAS alone or both sphincters is evident at rest and squeeze pressures are low. In the second case, low resting tone of the IAS is seen with no relaxation during rectal balloon distension. In contrast, squeeze pressures are normal. Surgery for sphincter repair usually has a good success rate if damage is restricted only to the EAS (Laurberg et al 1988).

Spontaneous transient anal relaxation

Approximately 20% of patients suffer from this phenomenon (Sun et al 1990) (section 7.3.1). Spontaneous transient internal anal sphincter relaxations are also seen in normal subjects, where, EAS contraction protects against incontinence. In incontinent patients, relaxations are of longer duration and of much greater magnitude. In addition, very few patients show a compensatory increase in EAS.

Anismus

This is characterised by a paradoxical contraction of the EAS during defaecation. In addition, the rectum of patients with anismus in chronic constipation is usually empty, suggesting a generalised disorder of defaecation (Preston & Lennard-Jones 1985). The underlying pathophysiology of this disorder is unknown. Defaecography, the balloon expulsion test and scintigraphic assessment of defaecation are all abnormal in patients with anismus (Pezim et al 1987a).

Other common anorectal and pelvic floor problems include rectal prolapse, solitary rectal ulcer syndrome, levator ani syndrome, anal fissure, imperforate anus, ileoanal pouch and pruritus ani.

7.6 CONCLUSIONS

As the aetiology of incontinence and constipation is often multfactorial in origin, evaluation of the underlying pathophysiology needs to be comprehensive. It is evident that for normal maintenance of continence and defaecation, coordinated function between the rectum and anal canal sphincters must occur (Bartolo & Sun 1997).

CHAPTER 8

REGULATION OF ANORECTAL MOTILITY

8.1 INTRODUCTION

Anorectal motor activity is modified by a number of control mechanisms. The basic myogenic control is regulated by intrinsic and extrinsic neural pathways as well as circulating hormones. This chapter will discuss the control mechanisms which underlie normal anorectal motor and sensory function and in addition, colonic electrical and motor activity. As one of the author's studies evaluated the effects of blood glucose concentration on anorectal motility, this issue is discussed in some detail in section 8.7.

8.2 INTRINSIC MUSCULAR CONTROL

8.2.1 Electrical control activity

The integration of a contraction is achieved by a slow electrical oscillation called the electrical slow wave. Initiation of a contraction is accomplished by a burst of much more rapid electrical oscillation or depolarisation, the spike bursts. Contractions only occur with those slow waves that carry a spike burst (Szurszewski 1987). Slow waves coordinate contractions and are also known as pacesetter potentials (PP) or the electrical control activity (ECA) (section 4.2.1).

Like other gastrointestinal smooth muscle, the human colon generates electrical signals that are related to muscle contraction. Reports of slow wave characteristics in the colon have been inconsistent, probably primarily because of variability in slow wave frequency, amplitude and coordination in time and space

in the different colonic segments and the two muscle layers (Sarna et al 1980, Chauve et al 1976).

The human colon displays "phase unlocking" (a spike does not correlate with a slow wave) in the proximal and distal segments, and does not have a frequency gradient as demonstrated in the small intestine. Hence, spikes do not always correlate with slow waves and propagate both orally and aborally in the colon (Sarna et al 1981).

Variable electrical control activity frequencies have been recognised, in particular lower (2-9 cycles per minute) and higher frequency (9-13 cycles per minute) rhythms. The proximal colon demonstrates ECA in the lower frequency range, whilst the transverse colon consists mainly of the high frequency component and is "phase locked" (1:1 ratio of spikes to slow waves) (Sarna et al 1980). Taylor et al (1975) and Snape et al (1976) observed a variety of ECA frequencies (6-12 cycles per minute as a dominant frequency and 2-4 cycles per minute as low frequency) in the human colon using a number of different recording electrodes. This discrepancy amongst authors may be due to different recording techniques, complex data analysis and different recording sites.

It appears that there are substantial differences in the characteristics of human colonic slow waves to those observed in the small intestine of the dog or cat (Taylor et al 1975, Huizinga et al 1985). Huizinga et al (1985) demonstrated in vitro, that electrical oscillatory activity of the human colon determines the spacing of the spikes and pattern of contractile activity. The circular muscle layer generates spontaneous electrical oscillatory activity with a wide frequency range of (4-60 cycles per minute) with or without superimposed spiking activity. The

longitudinal muscle displays slow electrical oscillations at frequencies betweem 24-36 cycles per minute.

In the cat, it has been shown that this electrical activity is generated in the circular muscle layer of the muscularis propria (Christensen et al 1969). Caprilli and Onori (1972) reported similar results with electrical slow waves detected in isolated strips of circular muscle of the cat colon but not in the longitudinal muscle.

8.2.2 Electrical response activity

Fioramonti et al (1985) described a variety of spike bursts in the colon of all mammalian species using an intraluminal probe. Weak and non-propagating contractions known as short spike bursts (1.5-4 sec duration) with a 10 cycle per minute rhythm were reported. These short spike bursts have also been described by Sarna et al (1981) and Schang & Devroede (1983) using an intraluminal tube, in the rectosigmoid area of healthy humans. Long spike bursts (~10 sec duration) with a 3 cycle per minute frequency which propagate over short distances (10-70 cm) in both directions and finally migrating long spike bursts, which migrate rapidly at 10cm/sec aborally have been described by Bueno et al (1980) in the human colon using a probe introduced rectally.

Reports of human rectal slow wave activity are inconsistent. Snape et al (1976) and Bueno et al (1980) have reported rhythms of 3 and 6 cycles per minute and 3 and 10 cycles per minute respectively using bipolar electrodes clipped to the mucosa of the rectum and rectosigmoid regions and a 1.5 m anal probe incorporating electrodes with concurrent electromyography. The rectosigmoid junction has also been characterised by short spike bursts appearing for long periods of 5-10 min followed by periods of quiescence for 10-20 min, and long

spike bursts (Bueno et al 1980). In fact, the rectosigmoid junction is thought to be a pacemaker site triggering the electrical control activity that paces the electrical response activity which initiates rectal contractile activity. Shafik (1994) demonstrated this rectal electrical activity in dogs. Electrical control activity was accompanied by electrical response activity, which had inconsistent frequencies and was associated with an increase in rectal pressure. Rectal distension led to an increase in both frequency and amplitude of electrical control activity and electrical response activity. Use of electrorectogram in normal subjects demonstrated similar results (Shafik 1995).

At rest the internal anal sphincter displays an electrical control activity of 6-20 cycles per minute, with the highest frequency occurring distally (i.e. closest to the anal verge). Tonic contraction of the IAS is responsible in part for maintenance of closure of the anal canal and contributes approximately 85% of resting anal pressure, with smaller contributions after sudden and constant rectal distension (40% and 65% respectively) in humans (Frenckner & Euler 1975). Bouvier & Gonella (1981) examined spontaneous EMG from the cat anal sphincter and showed that rhythmic slow variations of membrane potential in the circular muscle were accompanied by contractions.

At rest, the myogenic component of the EAS produces fluctuations and generates a pressure of approximately 7mmHg. Evidence of such tone has been obtained by recording EMG from electrodes applied to the skin over the EAS (Alva et al 1967). Using manometry and EMG in humans Duthie & Watts (1965) provided evidence that the EAS is a voluntary muscle that contributes to the tonic closure of the anal canal only when a mass is present in the rectum i.e. rectal distension. Frenckner & Euler (1975) recorded EMG from needle electrodes in the striated sphincter muscle of humans and confirmed that during rest there is a continuous

discharge of spiking, interpreted as evidence of tone. In addition they showed that the EAS contributes about 15-20% to resting tone, with larger contributions during rectal distension.

8.3 NEUROGENIC CONTROL PATHWAYS

Neural control is organised at three levels: enteric, autonomic and central. The enteric nervous system contains cholinergic and nonadrenergic noncholinergic (NANC) neurones. The autonomic nerves continuously monitor the anorectum and colon, providing a modulatory input when necessary. The central nervous system input coordinates motor activity of the colon, rectum and anal sphincters for orderly evacuation of faeces during defaecation.

8.3.1 Intrinsic neural control pathways

The intrinsic nerve supply to the colon is via the myenteric and submucosal plexi comprising uniform distribution of ganglion cells. Transmural electrical stimulation has demonstrated that the excitatory response involves both cholinergic and non-cholinergic transmitters (Grundy 1985, Taylor & Bywater 1988), whilst inhibitory innervation is via non-cholinergic non-adrenergic transmitters (Boeckxstaens et al 1993, Tam & Hillier 1992).

During rectal filling the classical response is IAS relaxation. In the cat this relaxation seems to be an enteric reflex, mediated via local nerve pathways in the circular muscle layer (Bouvier & Gonella 1981). Human studies have also shown the existence of this enteric neural reflex. Frenckner & Euler (1975) showed that the IAS response is intact irrespective of the spinal lesion. In addition, after posterior rhizotomy IAS relaxation is still evident in humans Sun et al (1995). However, damage to the enteric nerve supply affects the recto-anal reflex by interruption to the integrity of anorectal musculature. For example, in patients

with low anterior resection and coloanal anastomosis, anal relaxation in response to rectal distension is impaired (Sun et al 1994).

8.3.2 Non adrenergic non cholinergic (NANC) innervation

In addition to sympathetic and parasympathetic innervation to gastrointestinal smooth muscle, a subset of nerves which are non-adrenergic or non-cholinergic (NANC) have been identified.

In rat colonic circular muscle, nitric oxide plays an important role in NANC inhibition of smooth muscle relaxation (Serio et al 1995). In addition nitric oxide is involved in serotonin-induced relaxations of the guinea-pig colon (Briejer et al 1992). Similar results have been demonstrated in the human isolated colon using both circular (Boeckxstaens et al 1993) and longitudinal (Tam & Hillier 1992) muscle strips.

Inhibitory NANC neurotransmission in pig circular rectal muscle has been demonstrated in vitro (Stebbing et al 1995). In human rectal circular smooth muscle strips the nitric oxide synthase inhibitor N-omega-nitro-L-arginine, reduces the relaxant response to electrical field stimulation in a dose-dependent fashion (Stebbing et al 1996). The failure of nitric oxide synthase competitors to completely abolish the relaxant response suggests that additional neurotransmitters may be involved.

Bouvier & Grimaud (1984) demonstrated a NANC inhibition of relaxation in cat anal sphincter muscle. Further evidence for the role of nitric oxide as an inhibitory neurotransmitter is supplied by other animal studies (Rattan et al 1992, Chakder & Rattan 1993). In the opossum nitric oxide may also play a facilitatory role in the release of sympathetic neurotransmitters (Thatikunta et al 1993).

There is evidence in humans that nitric oxide is a NANC inhibitory neurotransmitter in the IAS (O'Kelly et al 1993). It is controversial however, as to whether other neurotransmitters, such as ATP (Burleigh et al 1979) and VIP (Biancani et al 1985, Chakder & Rattan 1995) also play a facilitatory role.

8.3.3 Extrinsic neural control pathways

Like other digestive smooth muscles, the colon, rectum and internal anal sphincter are innervated by the autonomic nervous sytem, which can be divided into sympathetic and parasympathetic components. To study the extrinsic innervation of the anorectum the autonomic nerve supply can be interrupted by selective anaesthesia or traumatic spinal transection.

The rectum is innervated by both afferent and efferent fibers of the parasympathetic and sympathetic nervous system. Parasympathetic innervation to the rectum is primarily excitatory (Carlstedt et al 1989). Sympathetic nerves have a dual effect on the rectum in the cat (Carlstedt et al 1988). In humans, stimulation of the hypogastric nerves causes variable responses in the rectum, with a weak contraction being most frequently observed (Carlstedt et al 1991). It is still unclear as to whether sympathetic innervation to the rectum is predominantly excitatory or inhibitory (Carlstedt et al 1988).

It is now well documented that the human IAS receives a sympathetic excitatory and a parasympathetic inhibitory innervation, as evidenced by loss of IAS tone after adrenoreceptor blockade (Guiterrez & Shah 1975). There is also evidence that presacral sympathetic (hypogastric) nerves cause relaxation of the IAS in humans (Shibamoto et al 1994). Frenckner &Ihre (1976) studied the effects of high and low spinal anaesthesia i.e. interruption to the parasympathetic and sympathetic innervation to the anorectum, and demonstrated unequivocally that

sympathetic innervation to the IAS is excitatory, whereas parasympathetic innervation is inhibitory. Their observations were subsequently confirmed by Rattan and Shah (1987) who demonstrated frequency-dependent IAS relaxation in response to electrical stimulation of the third and fourth sacral nerves in the opossum. By showing that anal resting pressure decreases with high spinal anaesthesia, these observations establish the classical theory of reciprocal innervation.

The striated external anal sphincter and puborectalis muscle are innervated only by somatic components from the 2nd, 3rd and 4th sacral roots, via branches of the pudendal nerves. In humans with traumatic transverse lesions of the spinal cord, the EAS response to rectal balloon distension is literally ablated (Frenckner 1975). The study of Frenckner & Ihre (1976) used high and low spinal anaesthesia. A tonic excitatory sympathetic discharge to the sphincter exists and accounts for some of the tone, but there is no tonic parasympathetic discharge. The EAS response appears to be regulated in part via a low centre of the spinal cord (Frenckner 1975), but it is not clear to what extent the reflex activity of the striated muscles is influenced by cerebral reflexes.

8.3.4 Spinal reflexes

Defaecation and preservation of faecal continence depends on the integrity of spinal reflexes controlling motor activity of the anorectum, and their modulation by conscious mechanisms (Parks et al 1962). The exact nature of the reflex mechanism is, incompletely understood. However, spinal reflexes controlling continence and defaecation can be eliminated by section of the sacral afferent nerve roots.

It is likely that the inhibitory influence on rectal activity during defaecation is conveyed via the sympathetic nervous system. Stimulation of the sympathetic

nerves is known to increase IAS tone and inhibit colorectal contraction in animals (Gonella et al 1987). Section of the posterior sacral roots eliminates the rectal contractile response to rectal distension, indicating a spinal reflex that is normally suppressed by descending pathways. Sun et al (1995) demonstrated that giant rectal contractions induced by rectal distension in spinal transection patients were eliminated after posterior rhizotomy.

After spinal transection IAS relaxation stimulated by rectal balloon distension is not abolished, indicating an enteric reflex. However, this reflex may be modulated by spinal pathways, because both the amplitude and duration of IAS relaxation are greater in these patients after posterior rhizotomy (Sun et al 1995).

It is now well accepted that external anal sphincter contraction is mediated by spinal reflexes, with extensive modulation by conscious mechanisms. Patients with traumatic spinal injuries lose conscious control of sphincter activity and discriminant rectal sensation during rectal balloon distension (MacDonagh et al 1992). This may explain why such patients experience uncontrollable reflex defaecation. Sun et al (1995) also reported that patients with complete spinal transection lose conscious control of the EAS. Because these patients are unable to achieve voluntary defaecation they must rely on spinal reflexes to control stool output.

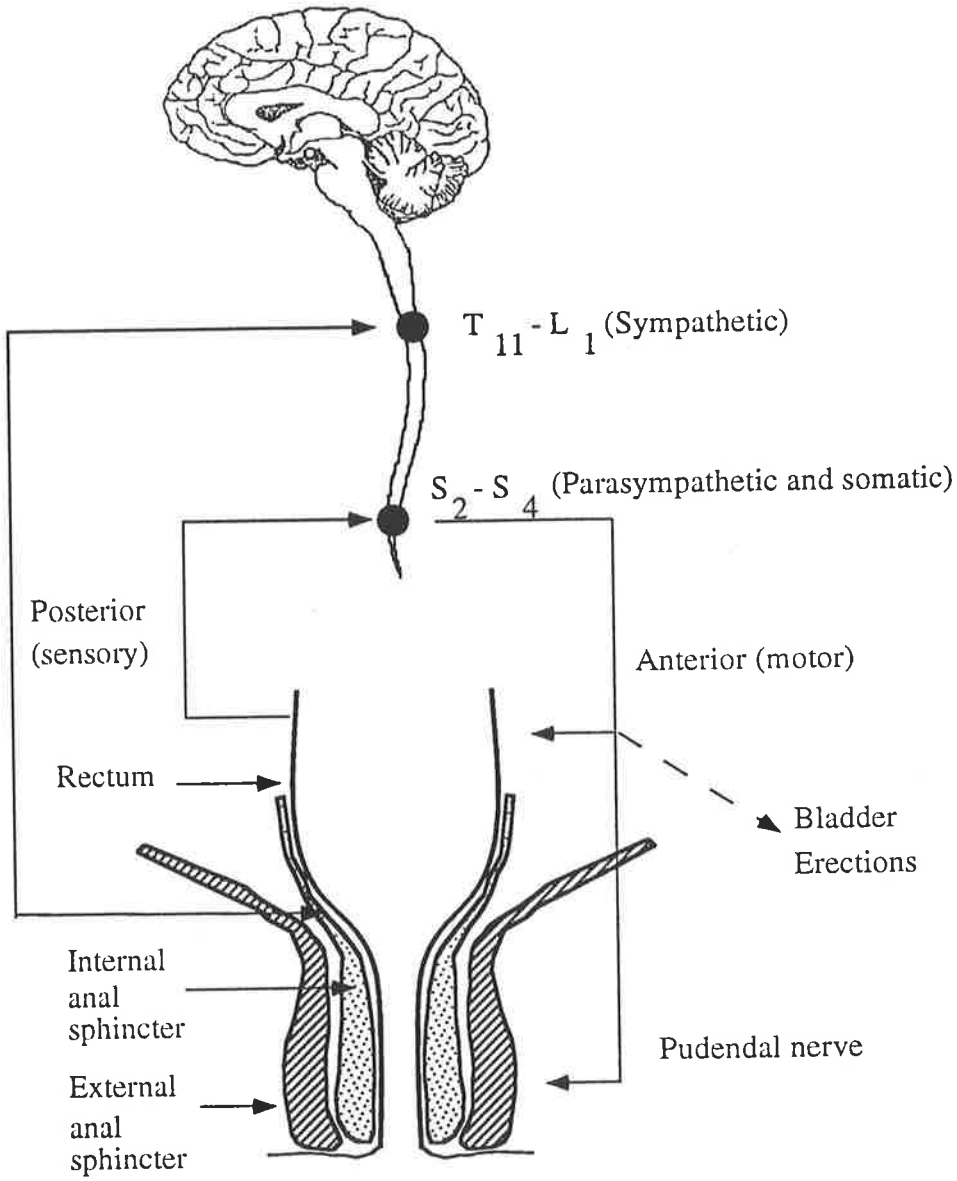

Figure 8.1

A simplified schematic of the innervation pathways (both motor and sensory) to the anorectal region.

8.4 SENSORY CONTROL

Little is known about the receptors and neural pathways contributing to the relay of sensory information. In contrast to the anal canal, which contains sensitive nerve endings responsible for fine discrimination, the rectum is thought to be insensitive to stimuli other than stretch (Rogers 1992). Most studies of rectal sensory function have employed rectal balloon distension. Subjective responses to balloon distension in different gastrointestinal tract regions have traditionally been used to estimate the sensitivity of afferent pathways mediating visceral perception (Ness & Gebhart 1990). However, the lack of standardized distension techniques and rigorous analysis to determine thresholds and stimulus-response characteristics of visceral afferents has limited greatly the ability of distension studies to implicate specific afferent pathways and mechanisms in the transmission of different types of rectal sensations.

In healthy humans sacral parasympathetic fibers (S2, S3, S4) running in the pudendal nerves mediate sensory information from pelvic organs, including the distal colon. They are the predominant pathways mediating physiological sensations from the rectum (Janig & Morrison 1986). Somatosensory anorectal afferents running in the pudendal nerve contribute to the transmissions of these physiological sensations (Loening-Baucke et al 1992, Todd 1964). In contrast, the role of splanchnic rectal afferents projecting to the lumbar spinal cord is poorly understood (Lembo et al 1994). Human studies have tried to elucidate these sensory control pathways, however small patient numbers and incomplete spinal lesions at different sites render results difficult to interpret.

MacDonagh et al (1992) reported that in patients with complete supraconal spinal lesions discriminant rectal sensation was abolished during rectal distension. In

patients with complete spinal transection after sacral posterior rhizotomy, discriminative rectal sensation is also lost (Sun et al 1995).

8.5 HORMONAL CONTROL OF ANORECTAL MOTILITY

Several peptides and chemicals are localised in the rectal and anal mucosal wall. In most cases their physiological role has not ben established, primarily because of the absence of specific antagonists.

8.5.1 Vasoactive intestinal polypeptide (VIP)

Vasoactive intestinal polypeptide is found in the human upper rectum and anal canal (Ferri et al 1988). The concentration is highest in the colon when compared with other regions of the gastrointestinal tract (Chayvialle et al 1980). VIP was originally implicated as the non-adrenergic non-cholinergic inhibitory transmitter in the gastrointestinal tract (Fahrenkrug et al 1978), especially the internal anal sphincter of the rabbit (Biancani et al 1985), and cat (Alumets et al 1978). Later studies in the opossum showed that VIP caused a dose-dependent fall in IAS pressure (Nurko & Rattan 1988). Human studies, however, fail to confirm this observation (Burleigh 1983), and there is increasing evidence that nitric oxide is the major neurotransmitter involved in IAS relaxation (O'Kelly et al 1993). In the opossum vasoactive intestinal polypeptide and nitric oxide synthase are present and frequently coexist in neurones of the IAS (Lynn et al 1985). These neurones may be an important source of inhibitory innervation mediating the rectoanal reflex-induced relaxation of the sphincter muscle. The exact site of nitric oxide release from the IAS in response to VIP is unknown.

8.5.2 Somatostatin

Somatostatin is found predominantly in the mucosa and submucosa of the human sigmoid colon (Ferri et al 1988). Lorentzen et al (1989) demonstrated that

intravenous administration of somatostain leads to a significant decrease in anal canal pressure in humans whereas vasoactive intestinal polypeptide and substance P had no effect. It has subsequently been reported that octreotide, a long-acting somatostatin agonist, reduces perception of rectal distension in both healthy volunteers and patients with irritable bowel syndrome, whereas afferent pathways involved in local reflexes and cutaneous perception are not affected (Hasler et al 1993). The concept that somatostatin plays a role in rectal sensation was supported by Chey et al (1995) who demonstrated that octreotide reduces perception of rectal electrical stimulation and suggested that this inhibition of perception was by spinal afferent inhibition.

8.5.3 Neuropeptide Y (NPY)

Neuropeptide Y is present in noradrenergic cell bodies of the inferior mesenteric ganglion, nerve endings on circular smooth muscle layers of the rectum and IAS, in the myenteric plexi and around blood vessels (Hellstrom et al 1989). Peak concentrations are found in the distal sigmoid colon, upper rectum and anal sphincter (Ferri et al 1988). Intravenous administration of NPY increases rectal tone and anal canal pressure in the cat (Hellstrom et al 1989). In addition NPY antagonises IAS relaxation caused by rectal distension in the opossum (Nurko & Rattan 1990).

8.5.4 Serotonin (5-HT)

5-HT positive nerve fibers are localised within the circular and longitudinal muscle layers of the rectum. Serotonin is associated with a dose-dependent relaxation of the IAS in rats (Goldberg et al 1986) and increases the threshold for perception of colo-rectal distension (Danzebrink & Gebgart 1991). Relaxation of the IAS is mediated neurally, as this response is abolished by hexamethonium in the rat (Fasth et al 1983). Serotonin induces both relaxation and contraction of

the fowl rectum, these responses being mediated through different pathways; the relaxation through non-adrenergic inhibitory neurones, and contraction via a direct myogenic action and indirect stimulation of non cholinergic excitatory neurones (Mishra & Raviprakash 1983).

Other peptides such as substance P and peptide YY and opiates coexist in the rectal and anal mucosa. However, evidence for these as direct regulatory peptides in anorectal function is at present lacking.

8.6 CENTRAL CONTROL OF ANORECTAL MOTILITY

Approximately 40% of patients with complete supraconal spinal cord lesions perceive a dull pelvic ache at maximum levels of rectal distension (MacDonagh 1992). Patients with thoracic cord lesions also perceive a dull pelvic sensation during rectal distension, even after rhizotomy (Sun et al 1995). The origin of this perception is unclear, although it could derive from afferent impulses conveyed to the brain along sympathetic nerves that enter the thoracic spinal cord above the level of the lesion.

8.7 EFFECTS OF BLOOD GLUCOSE CONCENTRATION ON ANORECTAL MOTILITY

Abnormal defaecation, as demonstrated by incontinence and constipation, occurs frequently in patients with diabetes mellitus (Schiller et al 1982, Wald & Tunuguntla 1984, Rogers et al 1988, Sun et al 1996). In an unselected group of outpatients with diabetes mellitus 20% had faecal incontinence (Schiller et al 1982). The anorectal and colonic dysfunctions in patients with diabetes are heterogeneous and poorly defined. Disordered anorectal motility in patients with diabetes has usually been attributed to irreversible (vagal) autonomic nerve dysfunction (Rundles 1945). However recent studies have demonstrated that

acute changes in blood glucose concentration have a major affect on gastrointestinal motor and sensory function (DeBoer et al 1992, Chey et al 1995, Fraser et al 1992, Bjornsson et al 1994). There is little information about the effects of changes in the blood glucose concentration on anorectal function. Sims et al (1995) reported that marked hyperglycaemia (blood glucose approximately 15mmol/l) blunted both the increase in colonic tone due to gastric distension (gastrocolonic) and the increase in proximal colonic tone due to colonic distension (colon-colon reflex). Chey et al (1995) studied the effects of hyperglycaemia (blood glucose approximately 15mmol/l) on anorectal motor and sensory function in normal subjects and reported an increase in the threshold for initial perception and urge to defaecate in response to rectal balloon distension, with no change in sphincter function. Apart from the degree of hyperglycaemia, the study design did not exclude a potential order effect. The study performed by the author (reported in Chapter 11) evaluated the effect of acute hyperglycaemia on anorectal motor and sensory function in normal subjects, including changes within the physiological range. Combined manometry and electromyography with rectal balloon distension were performed under both euglycaemic and hyperglycaemic conditions on three separate occasions.

8.8 CONCLUSIONS

The integrity of the anorectal musculature provides the mechanics for normal anorectal function. Several enteric, peripheral and central mechanisms contribute to the encoding and decoding of anorectal motor and sensory function. The integration of all these mechanisms is complex but nonetheless essential for orderly anorectal control and function.

CHAPTER 9

EFFECTS OF ACUTE HYPERGLYCAEMIA ON SMALL INTESTINAL MOTILITY AND TRANSIT

9.1 SUMMARY

The effects of hyperglycaemia on postprandial small intestinal motor activity are unclear. Duodenal and jejunal pressures and duodenocaecal transit were measured in eight healthy male volunteers during euglycaemia (blood glucose 4-6 mmol/l) and hyperglycaemia (blood glucose 12-15 mmol/l). Duodenal and jejunal pressures were recorded with a manometric assembly during intraduodenal infusion of 100ml nutrient liquid comprising 14% protein, 31.5% fat and 54.5% carbohydrate together with 15g lactulose. Duodenocaecal transit was determined by a breath hydrogen technique. The number of duodenal ($p<0.05$) and jejunal ($p<0.01$) pressure waves, excluding phase III episodes was reduced during hyperglycaemia compared to euglycaemia. Hyperglycaemia was associated with earlier onset of phase III activity (30 ± min vs 132 ± min; $p<0.05$). Duodeno-caecal transit was slower during hyperglycaemia when compared to euglycaemia (114 ±17 min vs 49±6 min, $p<0.01$). Induced hyperglycaemia has major effects on postprandial small intestinal motility. The reduction in duodenal and jejunal motor activity is likely to explain the retardation of small intestinal transit during hyperglycaemia.

9.2 INTRODUCTION

Gastric and small intestinal contractions are responsible for the passage of ingested nutrients along the gastrointestinal tract and it is now well recognised that the rate of gastric emptying, is a major determinant of postprandial blood

glucose concentrations. Disordered gastrointestinal motility occurs frequently in patients with both insulin dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) and is frequently associated with gastrointestinal symptoms such as nausea, vomiting, abdominal pain, bloating, and diarrhoea (Feldman & Schiller 1983). In patients with IDDM abnormal gastric emptying or small intestinal transit may impair blood glucose control by leading to a mismatch between absorption of nutrients and the onset of exogenous insulin action (Horowitz & Fraser 1991). There is limited and conflicting information about both small intestinal motility and transit in patients with diabetes mellitus. For example both slow (Scarpello et al 1976) and accelerated small intestinal transit (Keshavarzian & Iber 1986) have been reported. These discrepancies are likely to be attributable, at least in part, to the fact that blood glucose concentrations were not stabilised or even monitored during these studies. Recent data indicate that the blood glucose concentration has a major impact on motor function in a number of regions of the gastrointestinal tract including the oesophagus (De Boer et al 1992), stomach (Barnett & Owyang 1988, Fraser et al 1991, Hasler et al 1995), gall bladder (De Boer et al 1993), and colon (Sims et al 1995) in healthy humans, and that hyperglycaemia slows gastric emptying in patients with diabetes (Fraser et al 1990).

In healthy humans, induction of hyperglycaemia by intravenous infusion of glucose has been reported to reduce fasting small intestinal motility (Bjornsson et al 1994). The effect of hyperglycaemia on postprandial small intestinal motility has received limited attention (Kim et al 1990). The aims of the current study were to determine the effects of hyperglycaemia on small intestinal motility and transit during and after infusion of a nutrient load in healthy volunteers.

9.3 MATERIALS AND METHODS

9.3.1 Subjects

8 healthy male volunteers (mean age 26, range 22-41 years, body weight 77 kg range 58-97) were studied. No subject had a history of gastrointestinal surgery or was taking medication. Smoking was prohibited on the day of each test. Written informed consent was obtained from each subject and the study protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

9.3.2 Manometric technique

A 200 cm manometric assembly (diameter 4.4 mm) was used in all studies. The catheter incorporated 10 manometric lumina, an infusion port, and a 2.5 cm diameter balloon which was located 5 cm from the weighted tip. The opening of the central feeding channel was 55 cm from the end. Sideholes located 60, 50 and 40 cm and 30, 20, 10, 0 cm from the catheter end recorded motility in the duodenum and proximal jejunum respectively. A single sidehole was located in the distal antrum. The two most proximal channels were positioned on either side of the pylorus using monitoring of transmucosal potential difference (TMPD) (Fraser et al 1992, Heddle et al 1988, Fone et al 1989).

Intraluminal pressures were recorded using a pneumohydraulic perfused manometric technique. Manometric lumina were perfused at a rate of 0.5 ml/minute. The TMPD channels were used to measure both intraluminal pressure and transmucosal potential difference and were perfused with degassed saline from separate reservoirs. All other channels were perfused with degassed distilled water from a common reservoir. As in previous studies (Fraser et al 1992, Heddle et al 1988, Fone et al 1989) the manometric assembly was considered to be positioned correctly when the antral TMPD was less than -20 mV, the duodenal TMPD was more positive than -15 mV, and the difference

between the two was at least 15 mV. Pressures were measured via transducers (Abbott Critical Care, Illinois, U.S.A.) using a purpose-designed computer program (MAD, Synectics, Sweden). Data were recorded on both a Macintosh Quadra 700 computer and a 12 channel chart recorder (Grass Model 7E Polygraph: Grass instruments Co, Quincy, Mass, U.S.A.), for subsequent semi-automated analysis.

9.3.3 Protocol

All subjects were studied during both euglycaemia and hyperglycaemia in paired studies performed in a single-blind cross-over fashion. Studies were performed approximately 7 days apart. After an overnight fast, the manometric assembly was introduced at approximately 0900h. Volunteers lay in the right lateral position until the tip of the catheter passed beyond the pylorus (usually during fasting gastric MMC activity). The balloon was then inflated with 7 ml of air to facilitate movement of the catheter along the intestine. When the two most proximal sideholes were positioned astride the pylorus the balloon was deflated.

Intravenous cannulae were placed in antecubital veins of each arm for blood sampling and intravenous infusion of either glucose or normal saline. Each study began during phase I of the interdigestive motor complex and lasted approximately 3 hours. During hyperglycaemia a modified glucose clamp technique was used to maintain the blood glucose concentration at about 14 mmol/l (Fraser et al 1991). An intravenous bolus of 150 ml of 25% dextrose was initially administered over 5 minutes, followed by a variable infusion of 25 % dextrose. Blood glucose concentrations were determined at least every 10 minutes using a portable blood glucose meter (Medisense Companion II glucometer, Medisense Inc., Waltman, MA, U.S.A.) and the accuracy of these measurements confirmed subsequently using a hexokinase technique. During

euglycaemic studies, an initial 100 ml bolus of 0.9 % saline was infused intravenously, followed by a constant intravenous infusion of 0.9 % saline at a rate of 100 ml/hr. Ten minutes after the intravenous bolus, each subject received an intraduodenal infusion of 100 ml (106 kcal) of a commercial feeding emulsion comprising 14% protein, 31.5% fat and 54.5% carbohydrate (Ensure, Ross Laboratories, Columbus, Ohio, U.S.A.), and 15g lactulose (Janssen-Cilag Pty Ltd, Sydney, Australia) at a rate of 5 ml/minute for 20 minutes. At baseline and subsequently at 15 min intervals end expiratory breath samples were taken for measurement of breath hydrogen (Read 1991).

9.4 DATA ANALYSIS

9.4.1 Pressure waves

Pressure waves with an amplitude greater than 10 mmHg and duration more than 1 sec were evaluated. The number of duodenal and jejunal pressure waves in each hour of the study, was counted manually from the computer stored data, excluding phase III activity. The latter was defined as a burst of pressure waves greater than 10/minute, with a duration of greater than 2 minutes which migrated more than 40 cm (4 sideholes). The time of onset, pressure wave frequency, length of propagation, propagation velocity and origin (antral or duodenal) of each phase III, as well as the total number of phase III episodes, were determined.

9.4.2 Measurement of small intestinal transit

End-expiratory breath samples were collected in impermeable plastic tubes for later analysis. Breath hydrogen concentrations were measured using a gas chromatograph (Series 350 Thermal Conductivity detector, Gow Mac Instrument Co, Bridgewater, N.J., U.S.A.) (Read 1991). The caecal arrival of lactulose (duodeno-caecal transit time) was defined as a sustained rise in hydrogen of > 5 ppm.

9.4.3 Statistical analysis

Data were evaluated using Student's t-test for paired data (2-tailed) and are presented as mean values and standard errors. Significance was accepted at the $p < 0.05$ level in all analyses.

9.5 RESULTS

All subjects tolerated the study well and there were no adverse events. The mean blood glucose concentrations during euglycaemic and hyperglycaemic experiments are shown in figure 9.1.

9.5.1 Small intestinal pressures

Manometric tracings were evaluable for 2755 (95 %) of 2880 mins of recording time. During euglycaemia, intraduodenal infusion of the nutrient led to replacement of the fasting pattern with an irregular "fed" pattern of pressure waves (figure 9.2a) which persisted until the next phase III activity. In contrast, during hyperglycaemia there was an early onset of phase III activity and the fed pattern was not seen (figure 9.2b).

9.5.2 Pressure Waves

The number of duodenal pressure waves was reduced during hyperglycaemia when compared to euglycaemia (23 ± 7/h vs 121 ± 33/h, $p<0.05$) during the first hour but not the second and third hours though there was a trend for a reduction in the third hour (24 ± 8/h vs 80 ± 27/h, $p = 0.07$, figure 9.3a). The number of jejunal pressure waves was reduced during hyperglycaemia when compared to euglycaemia throughout the 3 hour recording period (figure 9.3b).

9.5.3 Phase III

The onset of phase III following the intravenous bolus, occurred earlier during hyperglycaemia when compared to euglycaemia (30 ± 12 min vs 132 ± 20 min, $p< 0.01$) (figure 9.4). The time between the last phase III during baseline measurements and the first phase III after induction of hyperglycaemia or euglycaemia was 46 ± 12 min during hyperglycaemia compared to 177 ± 19 min during euglycaemia ($p<0.01$).

There was no significant difference in the duration of phase III activity between the two studies. However, propagation distance was reduced during hyperglycaemia when compared to euglycaemia (56 ± 4 cm vs 73± 1 cm $p<0.05$). There was a non-significant trend ($p=0.09$) for the propagation velocity to be slower during hyperglycaemia. During hyperglycaemia all phase III activity commenced in the duodenum.

9.5.4 Duodenal-caecal transit

Duodeno-caecal transit was prolonged in all subjects during hyperglycaemia when compared to euglycaemia (114 ± 17 min vs 49 ± 6 min, $p< 0.01$, figure 9.5).

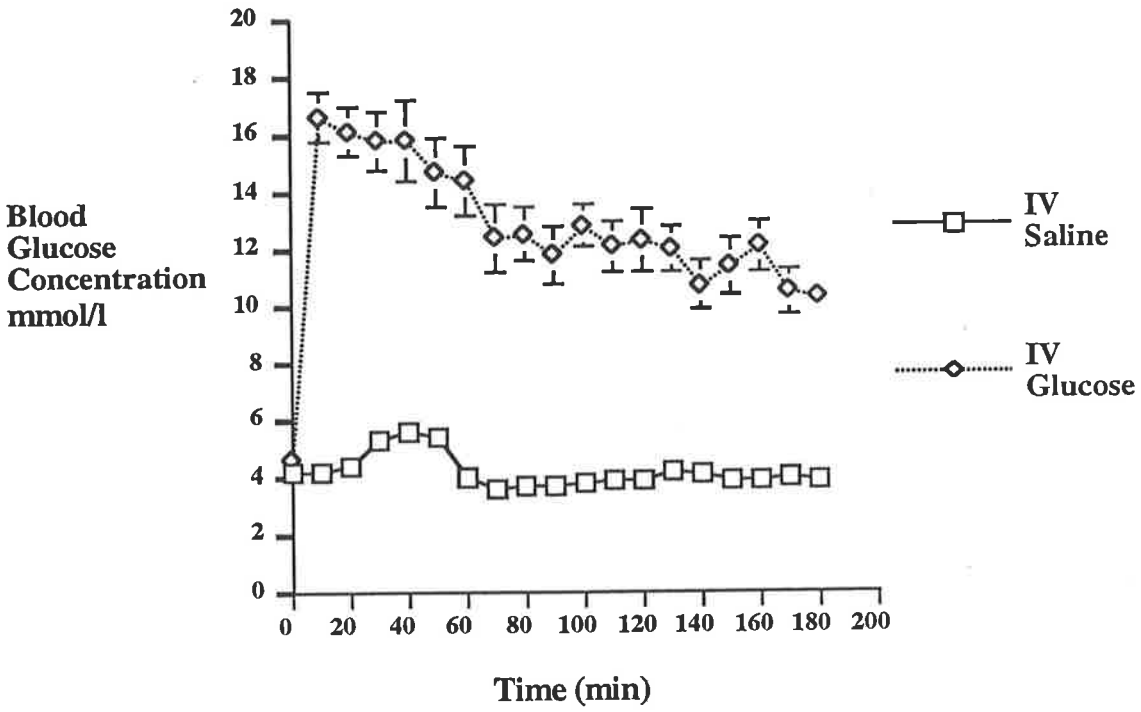

Figure 9.1

Blood glucose concentrations during euglycaemia and hyperglycaemia (mean±SEM).

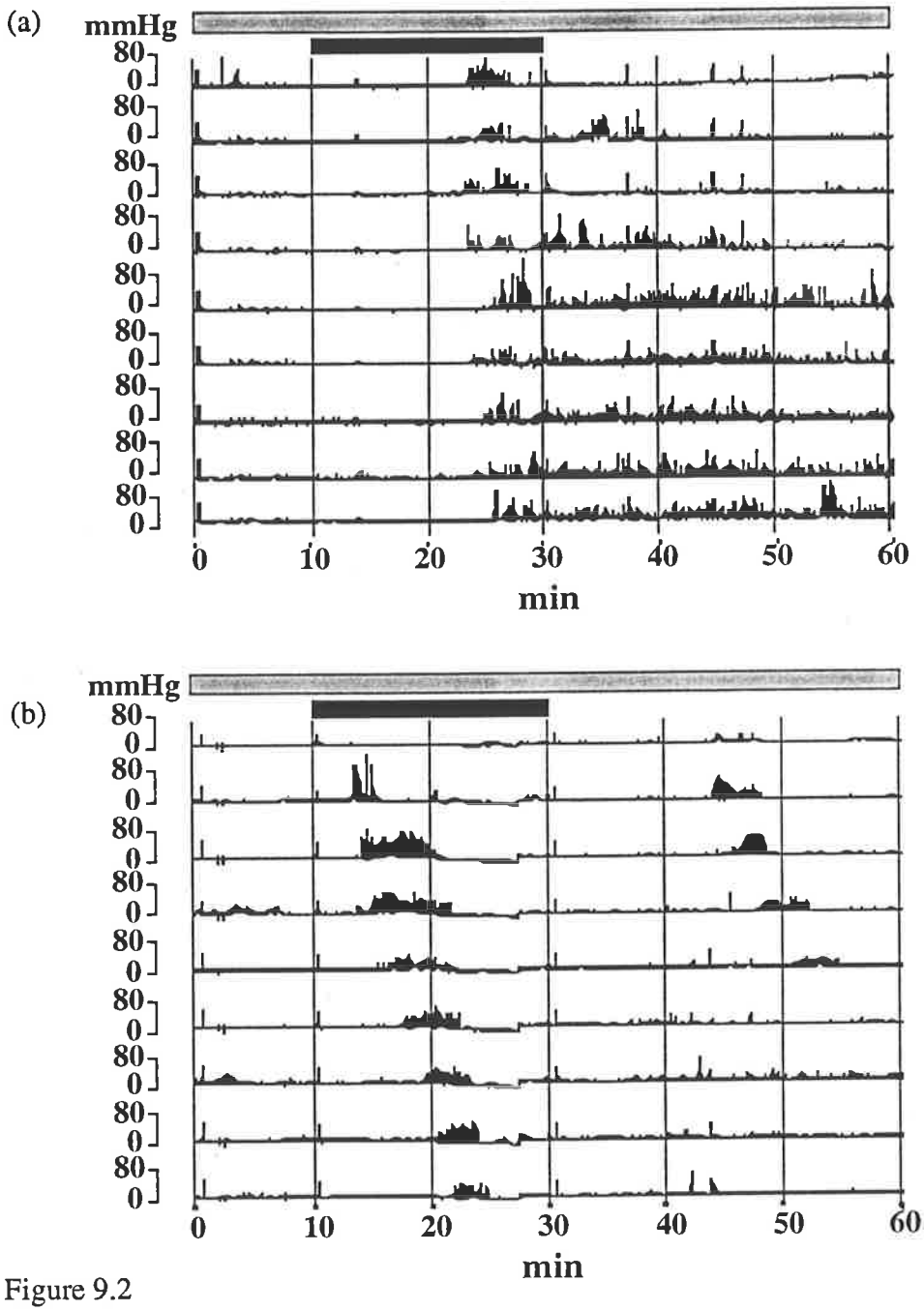

Figure 9.2

Example of a manometric recording during the first 60 min of (a) euglycaemia, and (b) hyperglycaemia. Recordings are arranged with the most proximal channel (antrum) at the top and the most distal (jejunal) at the bottom. The vertical axis shows the pressure scale in mmHg. Time 0 marks the start of intravenous infusion (dotted bar) and nutrient liquid was infused 10 minutes later (period of nutrient infusion shown by dark horizontal bar).

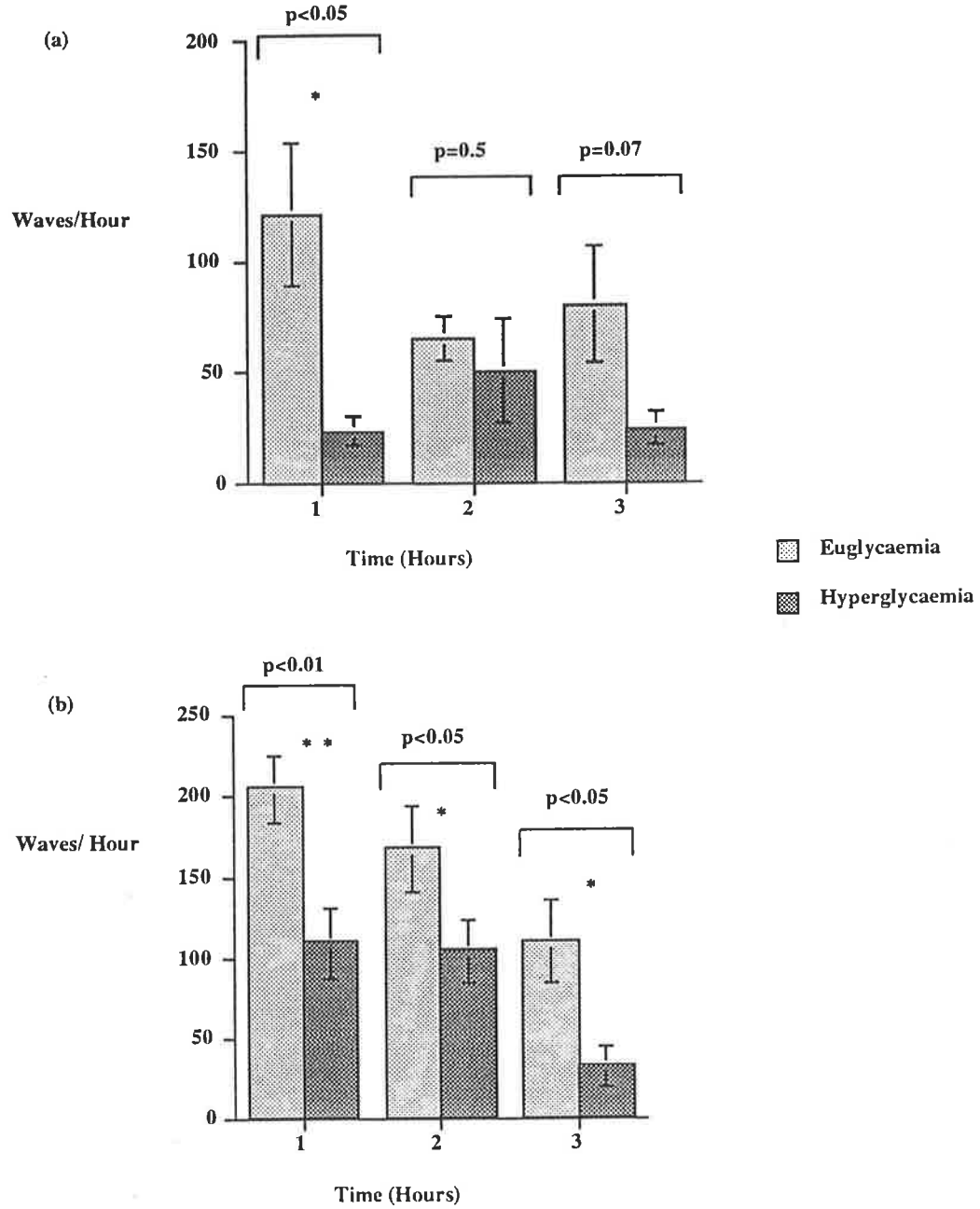

Figure 9.3

Number of (a) duodenal and (b) jejunal pressure waves (mean±SEM) during euglycaemia and hyperglycaemia for each hour of the study (* $p<0.05$, ** $p<0.01$, hyperglycaemia vs euglycaemia).

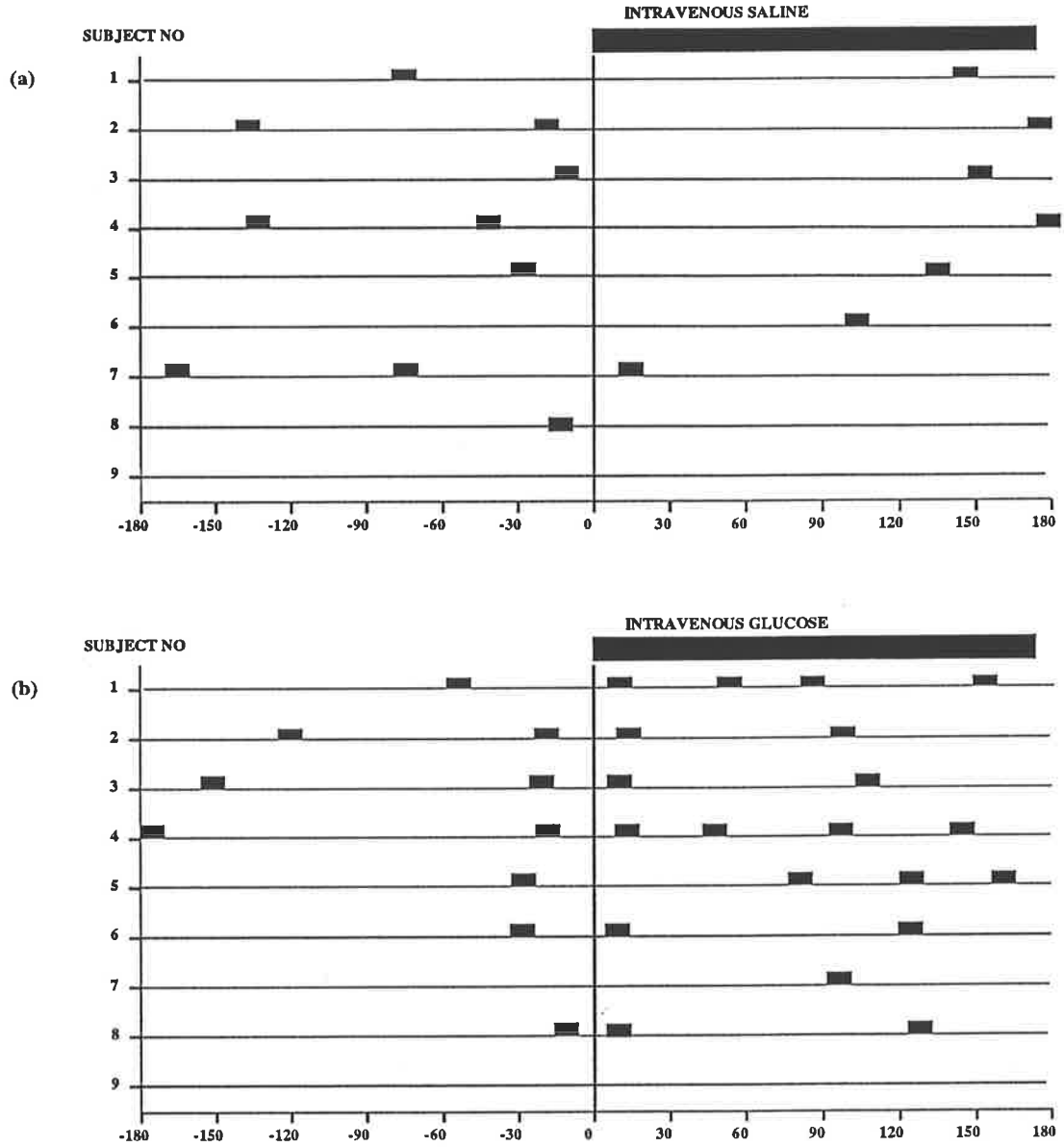

Figure 9.4

The number and timing of phase III motor activity during euglycaemia (a) and hyperglycaemia (b). In 6 of the 8 subjects, phase III activity occurred within 15 minutes of induction of hyperglycaemia.

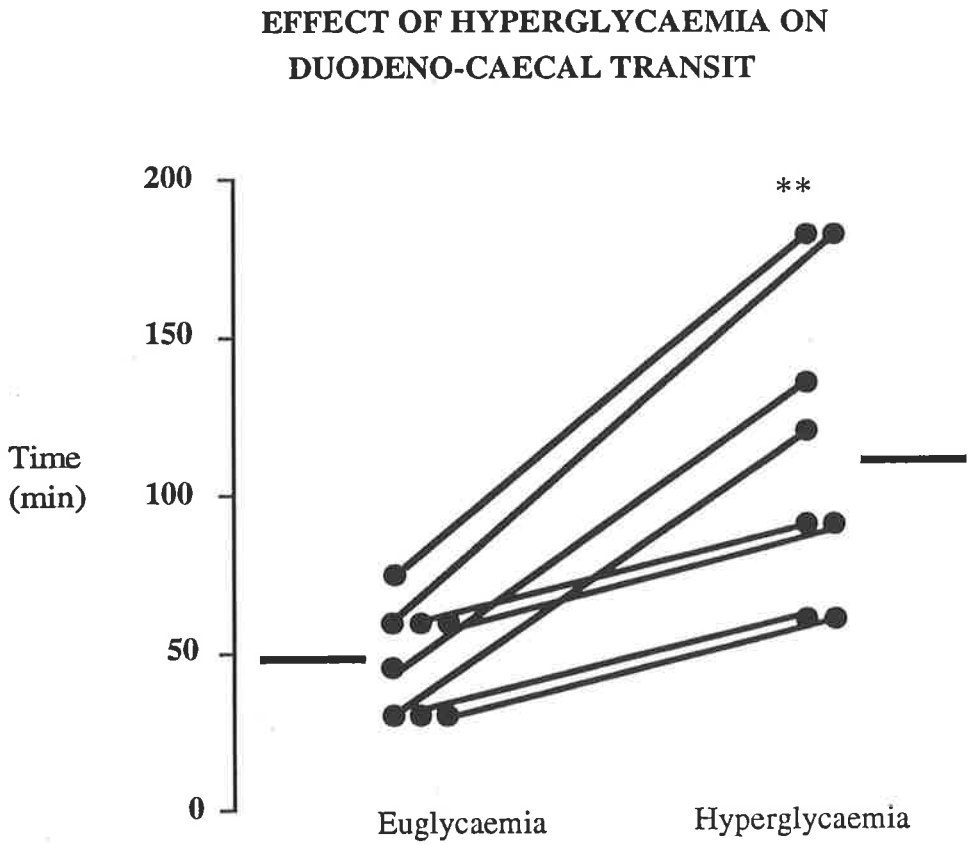

Figure 9.5

Individual values for duodeno-caecal transit during euglycaemia and hyperglycaemia. Mean transit times are shown as horizontal bars (** $p<0.001$ hyperglycaemia vs euglycaemia).

9.6 DISCUSSION

This study is the first to examine the effects of hyperglycaemia on the organisation of "postprandial" small intestinal motility. The data shows that acute hyperglycaemia has complex effects on "postprandial" small intestinal motor patterns in healthy human subjects with stimulation of premature phase III activity and an overall reduction in the number of non-phase III duodenal and jejeunal pressure waves. The study also confirms that hyperglycaemia retards duodeno-caecal transit time in normal subjects (De Boer et al 1993).

A previous study (Kim et al 1990) reported in abstract form, suggested hyperglycaemia (approximately 10 mmol/l) had no effect on the postprandial motility index in either the antrum, duodenum or jejunum in normal subjects. The patterns of motor activity were not described and an increase in phase III activity may be responsible for discrepancy with the findings of the current study. Their observations are also surprising in that a recent study demonstrated that antral motility is suppressed at a blood glucose concentration of 9.7 mmol/l (Hasler et al 1995). The observed overall reduction in non-phase III intestinal motor activity during hyperglycaemia is also consistent with previous studies during fasting where hyperglycaemia was associated with a reduction in the small intestinal motility index (Bjornsson et al 1994). Furthermore, the premature onset of phase III activity is in accord with previous studies in fasting humans. Bjornsson et al (1994) reported that acute hyperglycaemia reduced MMC cycle length. A previous study demonstrated that induction of hyperglycaemia in normal subjects is associated with bursts of duodenal pressure waves, but methodological limitations prevented full characterisation of the duodenal activity (Fraser et al 1991). The observations in the current study conflict with those of Barnett and Owyang who found no effect of hyperglycaemia on the frequency of occurrence of duodenal phase III activity (Barnett & Owyang et al 1988).

However, these investigators studied only a limited number of subjects and did not report on the timing of the phase III activity.

Phase III activity probably plays a major role in the aboral movement of indigestible solids, bacteria and cellular debris along the small intestine and an increase in phase III activity is associated with more rapid small intestinal transit (Sarna 1985). The slowing of intestinal transit despite stimulation of phase III activity was thus unexpected. However, during hyperglycaemia, the propagation distance of phase III activity was less than that seen during euglycaemia and hence luminal contents may not have moved as far distally. Previous studies also suggest that movement of luminal contents in the stomach and small intestine also occurs during phase II of the MMC (Oberle et al 1990) which is suppressed by hyperglycaemia (Bjornsson et al 1994). It is thus also possible that although phase III activity results in propulsion of chyme it may result in relatively slower transit when compared to normal postprandial contractile activity (Summers et al 1976).

The mechanisms responsible for the effects of hyperglycaemia on gastrointestinal motility are poorly defined. Possible sites of action which are not mutually exclusive include direct effects on the central nervous system, vagus nerve, myenteric plexus, smooth muscle and hormonal release (Horowitz & Dent 1991). Hyperglycaemia reduces vagal cholinergic activity, as evidenced by suppression of pancreatic polypeptide secretion (De Boer et al 1993). In addition, acute hyperglycaemia has major effects on the release of hormones such as insulin. Insulin has been reported to affect small intestinal motility in dogs, independent of its effect on blood glucose concentrations (Prasad & Sarna 1986) and insulin-induced hypoglycaemia in humans was associated with bursts of jejunal motor activity (Fellows et al 1987). However, recent data suggest that insulin release is

not a major mechanism for the effects of hyperglycaemia on gastrointestinal motility (Hasler et al 1995). In particular, hyperglycaemia affects gastrointestinal motility in patients with insulin-dependent diabetes mellitus, who have no endogenous insulin release (Fraser et al 1990). The stimulation of pyloric activity (Fraser et al 1991), and inhibition of antral (Barnett & Owyang 1988, Fraser et al 1991, Hasler et al 1995), fundic (Hebbard et al 1994) and gall bladder motility (De Boer et al 1993) also indicates that hyperglycaemia is unlikely to have a direct effect on smooth muscle. The factors which underlie the discrepant effects of hyperglycaemia on duodenal and jejunal motility, and the reduced propagation distance of phase III activity during hyperglycaemia are also unclear. These variations may reflect differences in sensitivity of the duodenum and jejunum to changes in the blood glucose concentration (Fellows et al 1987), or different control pathways.

Hyperglycaemia has been shown to affect motor function in every region of the gastrointestinal tract which has been examined (De Boer et al 1992, Barnett & Owyang 1988, Fraser et al 1991, Hasler et al 1995, De Boer et al 1993, Sims et al 1995, Fraser et al 1990, Bjornsson et al 1994, Hebbard et al 1994, Chey et al 1995). Although the majority of these studies have been conducted in healthy subjects, it is clear that the blood glucose concentration also has a major effect on gastrointestinal motility in patients with diabetes mellitus (Fraser et al 1990). There is relatively little information about the relationship between the blood glucose concentration and gastrointestinal motility. A dose-dependent reduction in fasting and postprandial antral motility has been reported with suppression occurring at blood glucose concentrations within the normal range (Barnett & Owyang 1988, Hasler et al 1995). These latter observations suggest that the blood glucose concentration may have a physiological role to modulate gastrointestinal motor function. While the current study involved healthy subjects

and evaluated the effects of marked hyperglycaemia, these observations support the concept that blood glucose concentrations should be monitored and, ideally, stabilised in studies of gastrointestinal motility in subjects with diabetes mellitus (Horowitz & Fraser 1994). In such patients, abnormalities of small intestinal motility may contribute to gastrointestinal symptoms (Camilleri & Malagelada 1984) and it is therefore possible that correction of hyperglycaemia may be of therapeutic benefit.

In conclusion, the candidate has shown that acute hyperglycaemia decreases duodenal and jejunal pressure waves, stimulates phase III activity and slows duodeno-caecal transit in normal subjects. The reduction in duodenal and jejunal pressure waves may contribute to slowing of small intestinal transit.

CHAPTER 10

EVIDENCE THAT NITRIC OXIDE (NO) MECHANISMS REGULATE SMALL INTESTINAL MOTILITY IN HUMANS

10.1 SUMMARY

Non-cholinergic non-adrenergic neural mechanisms involving nerves containing nitric oxide have been shown to modulate smooth muscle in the gastrointestinal tract and it has been suggested that release from tonic NO inhibition may be important in the regulation of cyclical fasting small intestinal motility. The aim of this study was to evaluate the role of NO mechanisms in the regulation of fasting small intestinal motor activity in humans. The candidate employed a specific nitric oxide synthase inhibitor, N^G- monomethyl-L-arginine (L-NMMA). In 7 healthy male volunteers, duodenal and jejunal pressures were measured for 4 h with a 9 lumen manometric catheter. Volunteers attended on four separate days on which they received an intravenous infusion (iv) of either saline or L-NMMA (0.5 mg/kg/h, 2 mg/kg/h or 4 mg/kg/h) in random order. IV infusions began during phase I of the interdigestive motor complex. The first episode of phase III activity occurred earlier after infusion of 2 mg/kg/h and 4 mg/kg/h L-NMMA when compared to 0.5 mg/kg/h L-NMMA and saline (mean and 95% confidence interval) 52(36-68) min (2 mg/kg/h) and 57(18-97) min (4mg/kg/h) vs 116 (69-193) and 145(64-226) min) respectively with a resultant MMC cycle length of (82(59-105) and 86(46-126) vs 132 (49-198) and 169(98-240) min. The total number of phase III activities was increased ($p<0.05$) by L-NMMA 4mg/kg/h (2(1-3)) but not 2mg/kg/h (1.5(1-2)) or 0.5 mg/kg/h (1.3(0.6-2)) when compared to saline (1.3(0.6-2)). L-NMMA had no effect on the duration, velocity, number of contractions per minute, length of migration or site

of origin of phase III of the MMC. The duration of phase I activity was less ($p<0.05$) during 4mg/kg/h when compared to saline (12(1-23) min vs 31(19-44) min). These observations indicate that NO mechanisms play a role in the regulation of fasting small intestinal motor activity in humans.

10.2 INTRODUCTION

During fasting, the upper gastrointestinal tract undergoes cyclical motor activity which usually commences in the oesophagus or stomach and migrates down the small intestine to the ileum; the interdigestive migrating motor complex (MMC) (Kellow et al 1986). The migrating motor complex is about 100-120 min in duration and comprises three phases: a period of quiescence (phase I), a period of irregular motor activity (phase II), and a burst of activity at maximal slow wave frequency which migrates aborally (phase III) (Wingate 1981). Phase III of the MMC is about 5 minutes in duration and has been called the "house-keeper" of the gut, as the contractions propel cellular debris, secretions, undigested food and bacteria distally along the small intestine (Code 1979). Absence of phase III activity is associated with bacterial overgrowth as a result of small intestinal stasis (Vantrappen et al 1977).

Although it is recognised that both small intestinal slow waves and contractile activity are dependent on neural influences, the mechanisms mediating MMC activity are poorly defined. Recent studies in animals suggest that nitric oxide (NO) is an important inhibitory neurotransmitter throughout the gastrointestinal tract (Bult et al 1990, Stark & Szurszewski 1992). The role of NO mechanisms is optimally evaluated with the use of inhibitors of NO synthase rather than agonists such as glyceryl trinitrate. In dogs inhibition of NO synthase by N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NNA) induces premature intestinal phase III activity and results in prolonged disruption of both

fed and fasted small intestinal motility (Rodriguez-Membrilla et al 1995, Sarna et al 1993). There is evidence that NO mechanisms are also important in gastrointestinal function in humans and previous studies have employed either agonists or in vivo scavengers of NO. The NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) has recently been used in humans as a supportive treatment for septic shock (Petros et al 1994). Using L-NMMA the candidate has now established that NO mechanisms are involved in the regulation of small intestinal motility in humans.

10.3 MATERIALS AND METHODS

10.3.1 Subjects

Studies were performed in 7 male volunteers, aged 21-39 years, body mass index (BMI) (22.5 - 28.4). No volunteer had a history of gastrointestinal disease, previous abdominal surgery or was taking medication. Smoking was prohibited from the evening before each study. Routine biochemical assessment of liver function was shown to be normal in all subjects prior to enrolment in the study. The experimental protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital and each subject gave written informed consent.

10.3.2 Manometric technique

Intraluminal pressures were recorded using a pneumohydraulic, perfused manometric technique, with a flow rate of 0.5 ml/min. The 200 cm manometric assembly (outer diameter 4.4 mm) incorporated 9 manometric lumina, and a 2.5 cm diameter balloon located 5cm from the weighted tip. The two most proximal sideholes were separated by 5 cm and the remainder were positioned at 10 cm intervals. The position of the two most proximal sideholes (antral and duodenal) relative to the pylorus was determined by mesurement of transmucosal potential difference (TMPD). The assembly was inserted or withdrawn to maintain the

antral TMPD equal to or less than -20mV and the duodenal TMPD equal to or more positive than -15mV, with a difference between the two of at least 15mV (Russo et al 1996). The TMPD channels measured both intraluminal pressure and transmucosal potential difference and were perfused with degassed saline from separate reservoirs. All other channels were perfused with degassed distilled water from a common reservoir. Pressures were measured via transducers (Abbott Critical Care, Illionois) using special manometric software (Dr C-H Malbert, MAD, Synectics, Sweden). Data were recorded onto a Macintosh Quadra 700 Computer and a 12 channel chart recorder (Grass Model 7E Polygraph: Grass Instruments Co, Quincy, Mass, U.S.A) for subsequent analysis.

10.3.3 Protocol

Subjects were studied for 4 hours on four occasions separated by at least 7 days, during intravenous infusion of saline and L-NMMA (Calbiochem, Sydney, Australia) in doses of 0.5, 2 and 4 mg/kg/h in single blind, randomised fashion (only 5 subjects received 0.5 mg/kg/h). At approximately 0900h, after an overnight fast, the manometric assembly was introduced via an anaesthetised nostril. Volunteers lay on their right side until the tip of the catheter passed the pylorus. The balloon was then inflated with 7ml of air to facilitate passage of the catheter along the intestine. When the two most proximal sideholes were positioned in the antrum and proximal duodenum respectively, the balloon was deflated.

An intravenous cannula was placed in the antecubital vein of the right arm for infusion of either saline or L-NMMA which commenced 10 minutes after completion of an episode of phase III activity. Saline was infused at an identical rate to the L-NMMA infusion (43 ml/min). Manometric measurements were performed for 4 hours. To minimise the possibility of an adverse reaction to L-

NMMA at the higher dose, intravenous infusions of L-NMMA commenced at 0.5mg/kg/h for 5 min followed by 1mg/kg/h for 5 min followed by either 2 or 4mg/kg/h. For the studies using 0.5 mg/kg/h and 2mg/kg/h of L-NMMA, a 2 hour infusion was followed by a 2 hour post infusion period. Because of the substantial cost of L-NMMA, it was only practical to use 4 mg/kg/h as a 1 hour infusion followed by 3 hours of post infusion recording. Blood pressure and heart rate were monitored every five minutes for the first 30 minutes and thereafter every 10 minutes for the duration of each study. Subjects were fed a light meal prior to leaving the laboratory. Screens for haemoglobin and liver enzyme abnormalities (gamma glutamyl transpeptidase, aspartate aminotransferase, alkaline phosphatase) were repeated 4 days after each infusion.

10.3.4 Analysis of small intestinal motility

The occurrence and timing of small intestinal phase III activity were analysed manually. Phase III was defined as a burst of pressure waves with a frequency greater than 10 per min, and a duration greater than 2 min, which migrated more than 40cm (i.e. four sideholes) (Russo et al 1996). The frequency, duration, velocity, length of pressure wave migration and origin (antral, duodenal or jejunum) of phase III were calculated. The time between the commencement of iv infusion and the next episode of phase III, and the duration of quiescence after this episode of phase III (phase I) were also determined.

10.3.5 Statistical Analysis

Data are represented as mean and 95% confidence intervals. Differences in motor activity, blood pressure and heart rate between the four experiments were evaluated using Mixed Mode ANOVA (SAS Version 6.11). Differences in the site of origin of phase III was assessed using a Chi squared test. A p value <0.05 was considered to be significant in all analyses.

10.4 RESULTS

The studies were well tolerated by all volunteers. L-NMMA had no significant effect on either blood pressure or heart rate when compared with intravenous saline infusion (data not shown). No subject reported dysphagia or chest pain following infusion of L-NMMA. There were no changes in either haemoglobin or liver function following L-NMMA infusion.

The lowest dose of L-NMMA (0.5 mg/kg/h) had no effect on small intestinal motility, whereas the two higher doses of L-NMMA induced premature phase III activity (figure 10.1). The time to onset of the first episode of phase III activity after the start of iv infusion was less ($p<0.05$) after both 2mg/kg/h (52(36-68)min) and 4mg/kg/h min (57(18-97)min) but not 0.5mg/kg/h (116 (69-193) min) L-NMMA when compared to saline (145(64-226) min) (figure 10.2a). In addition, L-NMMA reduced the MMC cycle length: 82(58-105) min (2mg/kg/h) and 86(46-105) min (4mg/kg/h) compared to 132 (49-198) (0.5mg/kg/h) and saline 169(98-240) min, ($p<0.05$ for each (figure 10.2b)). In all studies phase III activity originated in the antrum or duodenum and L-NMMA had no effect on the site of origin of phase III activity. The number of phase III activities was greater ($p<0.05$) during infusion of 4mg/kg/h L-NMMA (2(1-3)) but not 2 mg/kh/h, (1.5(1-2)) or 0.5mg/kg/h (1.3(1-2)), when compared to saline (1.3(0.6-2)). L-NMMA had no effect on the duration, velocity, or length of migration of phase III activity or the number of pressure waves per minute during phase III acitvity (Table 10.1). Following the first phase III activity after the IV infusion, the duration of phase I activity was reduced ($p<0.05$) during infusion of L-NMMA 4 mg/kg/h (12 (1-23)min) but not 2mg/kg/h (18 (2-39)min) or 0.5mg/kg/h (18 (3-31)min) L-NMMA when compared to saline (31 (19-44)min) (figure 10.2c).

Table 10.1

	Saline	0.5mg/kg/h L-NMMA	2mg/kg/h L-NMMA	4mg/kg/h L-NMMA
Contractions per min	11.4(11.1-12.7)	10.9 (10.7-11.2)	11.1(10.7-11.5)	11.3(10.7-11.8)
Velocity (cm/min)	7.7(5-10.4)	7.3 (5-8.6)	6.7(4.7-8.7)	5.5(1.8-9.1)
Duration (min)	6.0(4.0-8.0)	6.1(5.2-7.5)	5.7(4.3-7.1)	4.7(2.8-6.6)
Length of migration (cm)	64.3(62.8-65.8)	64.2(59.1-66.3)	57(26.5-87.5)	75(75-75)

Contractions per minute, velocity, duration and length of migration of phase III of the MMC during saline and both doses of L-NMMA. Data are mean and 95% confidence intervals.

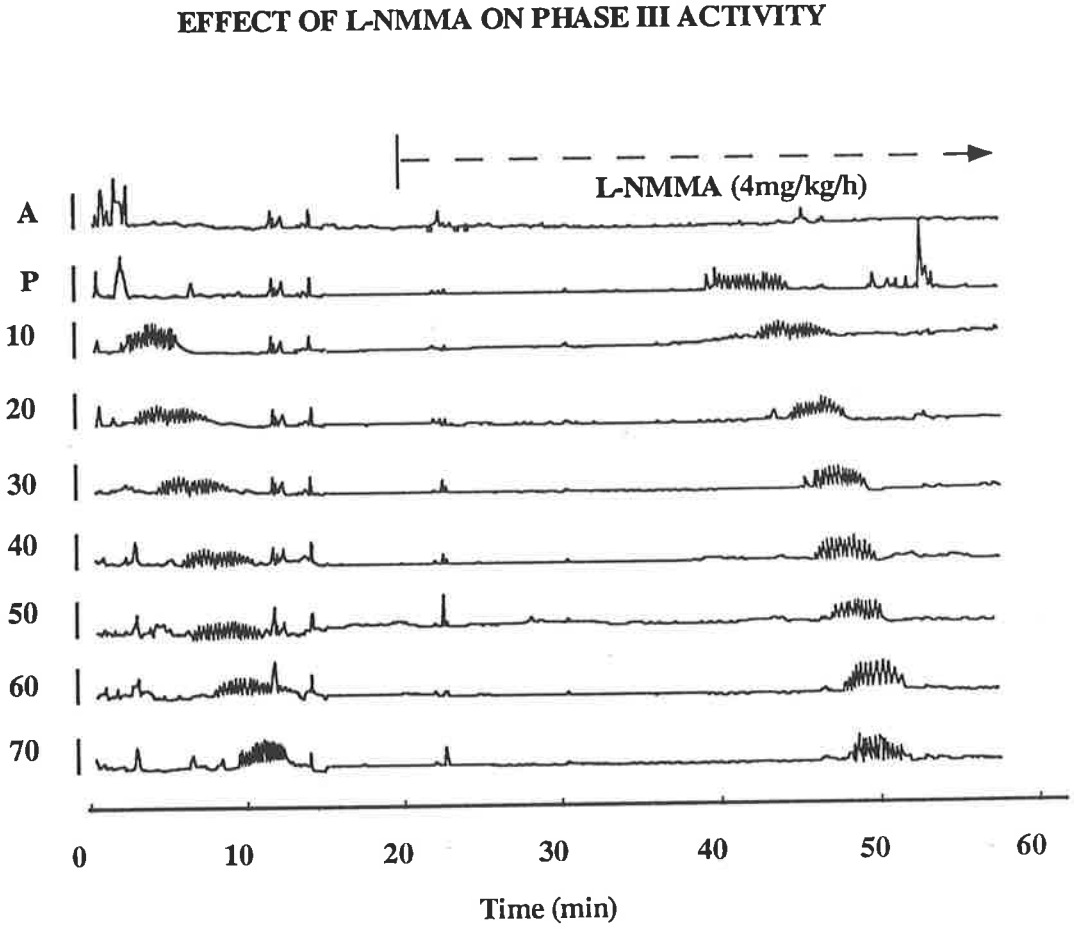

Figure 10.1

Example of a manometric recording showing an example of early stimulation of small intestinal phase III by 4mg/kg/h of L-NMMA. Sensor position is shown on the vertical axis (a=antrum, p=pylorus, numbers are distance in cm distal to the pylorus .

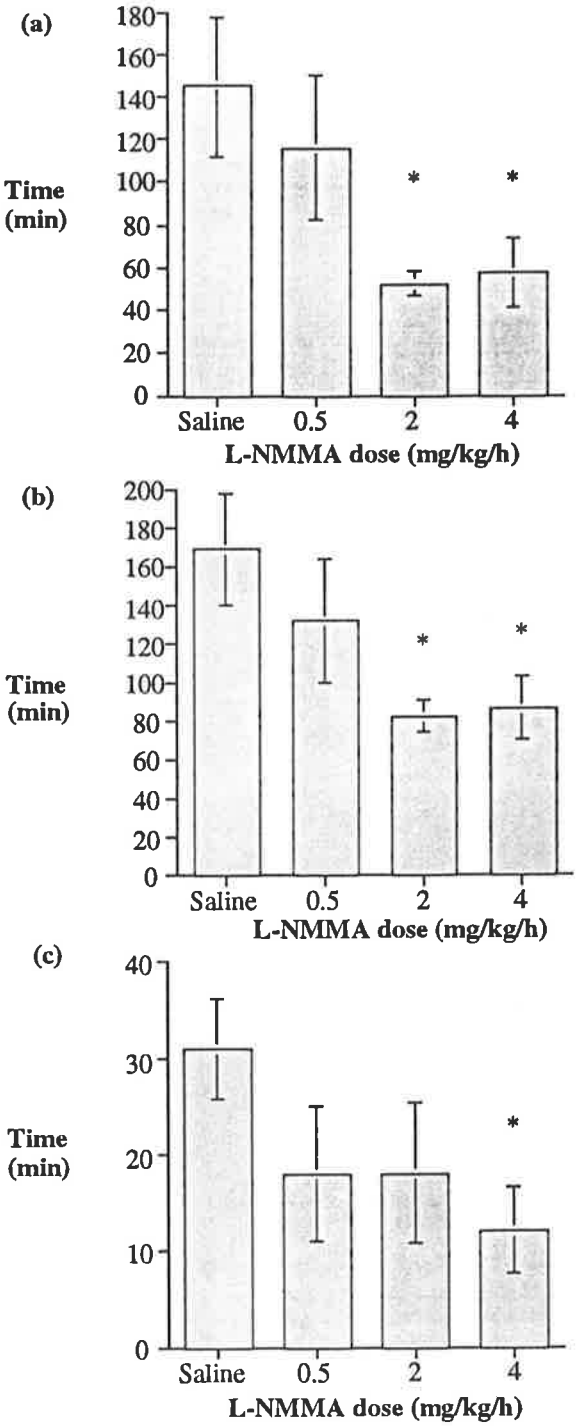

Figure 10.2

Effects of L-NMMA on time to phase III activity after the baseline MMC, cycle length and the duration of quiescence post-phase III (phase I). Values are mean and 95% confidence intervals.

10.5 DISCUSSION

The major observation in this study is that inhibition of endogenous NO synthase by L-NMMA stimulates early phase III activity in the human small intestine. This indicates that NO mechanisms play a role in the regulation of small intestinal motility in humans and is consistent with the concept that the small intestine is under tonic inhibition by NO and that release from NO is a mechanism for stimulation of phase III (Gustafsson & Delbro 1993).

The role of NO mechanisms is characteristically evaluated by reducing NO production by the use of inhibitors to block biosynthesis, or removing NO by a scavenging molecule such as haemoglobin (Murray et al 1995). The most direct approach to investigate the role of endogenous NO in humans is by reducing NO synthesis using specific antagonists. It has not previously been possible to conduct such experiments in humans because of concerns about the safety of NO synthase inhibitors such as N^G-nitro-L-arginine methyl ester and N^G-nitro-L-arginine. The novel NO synthase inhibitor L-NMMA has, however, recently been used in both cardiovascular research and in patients with septic shock with few adverse events (Hansen et al 1994, Petros et al 1994). Although NO is important in blood pressure regulation the candidate did not observe any effect on blood pressure or heart rate in this study in contrast to other studies which have investigated the effect of L-NMMA. However in these studies in healthy volunteers, higher doses of L-NMMA were infused over a shorter period of time (e.g. 3.6-6.7 mg/kg over 15 min (Hansen et al 1994) and only minor increases in blood pressure were reported. In contrast to a study of the role of NO mechanisms in oesophageal motility, in which NO was removed by recombinant human haemoglobin, we did not observe side effects such as chest pain or dysphagia (Murray et al 1995). The current study therefore suggests that L-NMMA is a useful and safe agent for the investigation of the role of NO

mechanisms in the gastrointestinal tract in both health and disease. Furthermore, as abnormalities of phase III activity are frequently associated with clinical problems such as bacterial overgrowth or diarrhoea, manipulation of small intestinal motility by NO synthase inhibitors may also have potential therapeutic applications.

The current study demonstrates a step wise dose-related alteration in small intestinal motility by L-NMMA: there was no change in motor patterns at the lowest (0.5mg/kg/h) dose, stimulation of phase III activity at an intermediate (2mg/kg/h) and highest (4mg/kg/h) doses, and a decrease in the period of motor quiescence at the highest dose. Dose-dependent effects with NO synthase inhibitors have not been previously described. In animals, more potent inhibitors such as L-NAME and L-NNA (the latter is approximately 10 times more potent than L-NMMA (Boeckxstaens et al 1990) have been administered in doses which are likely to completely block NO synthesis (Sarna et al 1993, Rodriguez-Membrilla et al 1995). In these studies, short term inhibition of NO synthesis by L-NNA and L-NAME was associated with prolonged disruption of both fed and fasting small intestinal motor activity (Sarna et al 1993, Rodriguez-Membrilla et al 1995). Thus in the dog, L-NNA infused at 3.75 mg/kg/h for 4 h induced premature phase III activity followed by prolonged disruption of the MMC cycle, probably reflecting motor disinhibition as a result of complete blockade of NO synthesis. Complete disruption of MMC activity by L-NNA or L-NAME infusion, was followed by a period when MMC cycle length was shortened significantly, probably as a result of partial inhibition during gradual recovery from the NO synthase antagonist. In the current study, L-NMMA in a dose of 4mg/kg/h produced an effect which resembled that of L-NNA and L-NAME in the dog during the recovery period. The relatively low dose of L-NMMA, and the

lack of effect on blood pressure or heart rate in our study are consistent with the concept of an incomplete blockage of NO responses.

The mechanisms underlying the initiation or triggering of phase III and the interdigestive motility pattern are unknown. The intestine contracts at maximal frequency during phase III, possibly reflecting a loss of inhibitory influences which could result from decreased endogenous synthesis of NO. Further support for this hypothesis is provided indirectly by reports of stimulation of phase III by opioids and somatostatin which also inhibit NO release. NO synthase is found thoughout the gastrointestinal tract and also within the central nervous system and the site of action of intravenous L-NMMA in the current study is therefore unclear. However, central inhibition of NO synthase is associated with inhibition of gastric and duodenal phase III activity (Ohta et al 1997), and it therefore appears likely that the effects of intravenous administration of L-NMMA result from a peripheral action, most probably within the enteric nervous system.

In conclusion, transient inhibition of NO synthesis by L-NMMA is associated with stimulation of phase III activity and a reduction in quiescence following this premature activity. L-NMMA is a useful and safe agent for the investigation of the role of NO mechanisms in the human gastrointestinal tract. In the future, manipulation of NO synthase activity may be of therapeutic value.

CHAPTER 11

ACUTE HYPERGLYCAEMIA AFFECTS ANORECTAL MOTOR AND SENSORY FUNCTION IN NORMAL SUBJECTS

11.1 SUMMARY

The pathogenesis of anorectal dysfunction, which occurs frequently in patients with diabetes mellitus, is poorly defined. Recent studies indicate that changes in the blood glucose concentration have a major reversible effect on gastrointestinal motor function. The aim of this study was to determine the effects of physiological changes in blood glucose and hyperglycaemia on anorectal motor and sensory function in normal subjects. In 8 normal subjects measurements of anorectal motility and sensation were performed on separate days while blood glucose concentrations were stabilised at 4mmol/l, 8mmol/l and 12mmol/l. Anorectal motor and sensory function was measured using a sleeve/sidehole catheter incorporating a balloon and electromyography (EMG). The number of spontaneous anal relaxations was greater at 12mmol/l than at 8mmol/l and 4mmol/l ($p<0.05$ for both). Anal squeeze pressures were less at a blood glucose of 12mmol/l when compared to 8mmol/l and 4mmol/l ($p<0.05$ for both). During rectal distension, residual anal pressures were not significantly different between the three blood glucose concentrations. Rectal compliance was greater ($p<0.05$) at a blood glucose of 12mmol/l when compared to 4mmol/l. The threshold volume for initial perception of rectal distension was less at 12 mmol/l when compared to 4mmol/l (40(20-100)ml vs 10(10-150)ml, $p<0.05$). An acute elevation of the blood glucose to 12 mmol/l inhibits internal and external anal sphincter function and increases rectal sensitivity in normal subjects. In contrast,

physiological changes in blood glucose do not have a significant effect on anorectal motor and sensory function.

11.2 INTRODUCTION

Anorectal dysfunction leading to faecal incontinence occurs in up to 20% of unselected patients with diabetes mellitus (Feldman & Schiller 1983, Schiller et al 1982, Wald & Tunuguntla 1984, Rogers et al 1988, Wald 1994). In diabetic patients with incontinence there are usually multiple anorectal motor and sensory dysfunctions. Internal and external anal sphincter pressures are reduced (Schiller et al 1982, Rogers et al 1988), the internal anal sphincter is frequently unstable (Sun et al 1996) and rectal sensation may be impaired (Wald & Tunuguntla 1984, Caruana et al 1991) when compared to healthy subjects. In considering the aetiology of faecal incontinence, it has not been established which is the most important defect. Furthermore, the mechanisms underlying disordered anorectal motor and sensory function in patients with diabetes mellitus are poorly defined.

Recent studies indicate that the blood glucose concentration may have a major, reversible, effect on motor and sensory function in a number of regions of the gastrointestinal tract, both in patients with diabetes mellitus and in normal subjects (De Boer et al 1992, Fraser et al 1990, Barnett & Owyang 1988, Fraser et al 1991, Schvarcz et al 1993, Hebbard et al 1996, Hebbard et al 1996, Hasler et al 1995, Bjornsson et al 1994, Russo et al 1996, De Boer et al 1993, Sims et al 1995). Acute changes in the blood glucose concentration affect motility in the oesophagus (De Boer et al 1992), stomach (Fraser et al 1990, Barnett & Owyang 1988, Fraser et al 1991, Schvarcz et al 1993, Hebbard et al 1996, Hebbard et al 1996, Hasler et al 1995), pylorus (Fraser et al 1991), small intestine (Bjornsson et al 1994, Russo et al 1996), gall bladder (De Boer et al 1993), colon (Sims et al 1995) and anorectum (Chey et al 1995). For example, gastric emptying is slowed

by hyperglycaemia (De Boer et al 1992) and accelerated during hypoglycaemia (Schvarcz et al 1993). Furthermore, changes in the blood glucose concentration within the normal physiological range may affect gastrointestinal motor function (Barnett & Owyang 1988, Hasler et al 1995, Boeckxstaens et al 1996, Groop et al 1989, Sun et al 1989). In previous studies of anorectal function in diabetic patients blood glucose concentrations were apparently not monitored (Feldman & Schiller 1983, Schiller et al 1982; Wald & Tunuguntla 1984, Rogers et al 1988, Sun et al 1996, Caruana et al 1991), and it is therefore possible that the dysfunctions observed in these patients may, at least in part, have been due to hyperglycaemia per se. Chey et al (Chey et al 1995) recently evaluated the effect of marked hyperglycaemia (~15mmol/l) on anorectal function in normal subjects and reported that both the perception of rectal distension and the rectoanal inhibitory reflex were blunted during hyperglycaemia. These authors did not observe any effect of hyperglycaemia on external or internal anal sphincter function and accordingly suggested that hyperglycaemia did not influence the efferent neural innervation to the anal sphincters (Chey et al 1995). In their study (Chey et al 1995) anal sphincter pressures were evaluated with multiport manometry, rather than a sleeve sensor, which is probably the optimal technique to evaluate external anal sphincter function (Orkin et al 1991). Furthermore, anal sphincter electrical activity was not evaluated. No studies have evaluated the effects of physiological changes in blood glucose on anorectal motility.

The candidate therefore measured anorectal motor and sensory function in normal subjects using a sleeve-sidehole catheter and electromyography (EMG), while blood glucose levels were maintained at 4mmol/l, 8mmol/l and 12mmol/l.

11.3 MATERIALS AND METHODS:

11.3.1 Subjects

8 healthy male volunteers (mean age 31 years, range 18-45), were studied. No subject had a history of gastrointestinal or urinary symptoms, gastrointestinal surgery or was taking medication. Smoking, caffeine and alcohol intake were prohibited on the day of each test. Written, informed consent was obtained from each subject and the study protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

11.3.2 Manometric technique

The manometric assembly (outer diameter 4.4 mm) incorporated 8 sideholes, a sleeve sensor and a 5.5cm silicone rubber balloon. Sideholes were located at 1, 2, 3, 4, 8 and 20 cm and the balloon was located between 10.5 and 16 cm from the anal verge. The sleeve (4 cm long) was positioned in the anal canal with one end just above the anal verge (Orkin et al 1991). All channels, except the balloon channel, were perfused with degassed distilled water at a rate of 0.5 ml/min by a pneumohydraulic pump. The channel used to measure intraballoon pressure was water-filled, but non-perfused. Balloon distensions with air were performed via a channel located in the centre of the manometric assembly. Two EMG electrodes, incorporated into the back of the sleeve, were used to record myoelectrical activity of both the internal and external anal sphincters. A ground electrode was placed on the subject's right buttock. For measurements of internal anal sphincter activity, a 0.15-3Hz frequency range was used, as opposed to 10-500 Hz for external anal sphincter activity (Orkin et al 1991). Data were recorded using a 12 channel chart recorder (Grass Model E Polygraph: Grass instruments Co, Quincy, Mass, USA) for manual analysis.

11.3.3 Protocol

In each subject measurements of anorectal motility and sensation were performed on separate days while blood glucose concentrations were maintained at 4mmol/l, 8mmol/l and 12mmol/l, in single-blind fashion, using a glucose clamp technique (DeFronzo et al 1979). The order of the three studies was randomised. Each study was separated by approximately 1 week and performed at the same time of the day.

Each subject fasted for at least 7 hours and was encouraged to empty their bowel before they arrived in the department. Digital examination was performed to ensure that the rectum was empty. The manometric assembly was introduced along the posterior wall of the anorectum and the subject lay in the left lateral position with the hips flexed at 90° for the duration of the measurements. Two intravenous cannulae were inserted. One was placed in an antecubital vein of one arm, for intravenous infusion of either 25% glucose or normal saline. The other was placed in a vein of the contralateral arm for blood sampling. This hand was kept heated, using an electric pad, to arterialise the venous blood. A bolus (adjusted to body weight) of either glucose or saline was given initially, followed by a variable infusion rate, to maintain the desired blood glucose concentration (Fraser et al 1991, DeFronzo et al 1979). Venous blood glucose concentrations were monitored at least every 5 minutes using a portable blood glucose meter (Medisense Companion II glucometer, Medisense Inc, Waltman, MA). The accuracy of these measurements was confirmed subsequently using a hexokinase technique.

Blood glucose concentrations were stabilised at the desired level for 60 minutes before measurements of anorectal motility were commenced. Anorectal pressures and sphincter electrical activities were initially recorded for 20 minutes under

resting conditions. The subject was then instructed to contract the anal sphincter maximally (squeeze) three times, with one minute between each effort. After a further 10 minutes the rectal balloon was serially inflated with 10, 20, 40, 60, 100 and 150ml of air (Sun et al 1990). Each inflation was maintained for one minute, and there was a one minute recovery period before the next inflation. The subject was asked to record the occurrence of rectal sensation using an event marker, indicating the onset and duration of the sensation. At the end of each rectal distension the subject was asked to report the nature of the sensation i.e. perception of the balloon, gas (wind), desire to defaecate, discomfort and pain (Sun et al 1990). The distension was terminated at a volume of 150ml, or if the subject felt discomfort or pain. After a further five minutes each subject was asked to blow up a party balloon (Ansell International, Glen Waverly, Vic, Australia) and thus increase the intra-abdominal pressure by forced expiration against a resistance. This was repeated three times, separated by one minute. Immediately after this time the intravenous infusion was stopped. Anorectal pressures and blood glucose concentrations were monitored for a further 30 minutes. On the completion of each study (~ 150min) the subject was given a light meal to prevent hypoglycaemia.

11.4 DATA ANALYSIS

Manometric recordings were analysed manually (without prior knowledge as to whether the study was conducted at 4, 8, or 12mmol/l) for the following parameters (Sun et al 1989, Sun et al 1990, Read & Sun 1992, Sun et al 1990):

(i) spontaneous anal relaxations, defined as sustained (>15 sec duration, >20 mm Hg decrease in pressure) reductions in anal pressure, unrelated to straining or any change in rectal pressure (Sun et al 1990). The number, minimum pressure and duration of each relaxation was recorded for the 20 minute periods at baseline (i.e. 60 minutes after the establishment of the desired blood glucose concentration)

and between 10 and 30 mintues after cessation of the intravenous glucose infusion (i.e. the last 20 minutes of the study).

(ii) the electrical activity of the internal anal sphincter, quantified by counting the number of slow waves before and during each spontaneous anal relaxation.

(iii) minimum basal sleeve pressure, defined as the lowest mean pressure at the end of respiration sustained for at least 2 minutes during the 20 minute baseline period.

(iv) the squeeze plateau pressure, defined as the visual mean of the pressure profile measured using the sleeve sensor (Yeoh et al 1996).

(v) the plateau anal pressure when the subject was blowing up a party balloon, measured using the sleeve sensor.

(vi) the minimum residual anal pressure during rectal balloon distension, defined as the minimum pressure recorded by the sleeve during each rectal distension.

(vii) the pressure-volume relationship for the balloon (Read & Sun 1992). In this calculation, the pressures in the balloon were corrected for by the pressure elicited by the balloon upon serial inflation in the atmosphere.

11.4.1 Statistical analysis

Data were evaluated using analysis of variance for repeated measures (ANOVA), the Wilcoxon signed rank test and Friedman's non-parametric test for repeated measures. Data are represented as mean ± SEM and, when not distributed normally, as median and range. A p value of <0.05 was considered to be significant.

11.5 RESULTS

The study protocol was well tolerated by all subjects. Mean blood glucose concentrations closely approximated the desired range (figure 11.1). In all experiments the blood glucose concentration had returned to the euglycaemic range (4-6 mmol/l) within 10 minutes after cessation of the intravenous infusion.

During the 20 minute baseline period, the number of spontaneous anal relaxations was greater at blood glucose of 12mmol/l when compared to both 8mmol/l and 4mmol/l (Table 1, $p<0.05$ for both). Spontaneous relaxations were not associated with external sphincter EMG activity, but rather a decrease in internal anal sphincter EMG activity, as demonstrated by a decrease in slow wave frequency (12(8-20)/min vs 7(4-11)/min, $p<0.05$). Both the minimum pressure and duration of these spontaneous anal relaxations were not significantly different between the three blood glucose concentrations. Leakage of perfusate did not occur in any subject. In the experiment conducted at a blood glucose of 12 mmol/l the number of spontaneous anal relaxations during the 20 minute baseline period was greater than the number between 10-30 minutes after cessation of the intravenous glucose infusion (7(3-12)/min vs 0(0-2)/min, $p<0.05$). In the latter period (i.e. 10-30 min) there was no difference ($p>0.05$) in the number of spontaneous relaxations between the three experiments (0(0-4)/min vs 0(0-2)/min and 0(0-2)/min respectively for 4,8 and 12mmol/l). There was no difference in the minimum basal pressures between the three blood glucose concentrations (Table 11.1). The mean squeeze pressure in the distal anal canal was lower at a blood glucose concentration of 12mmol/l when compared to 8mmol/l and 4mmol/l (Table 1, $p<0.05$ for both). Anal pressures recorded while blowing up a balloon were also less ($p<0.05$) at a blood glucose of 12mmol/l when compared to 4mmol/l (Table 1).

During rectal distension, the anal pressure decreased ($p<0.05$) in response to balloon distension, but there was no significant difference between the three blood glucose concentrations (Table 11.2). Intraballoon pressure increased ($p<0.05$) with increasing rectal distending volume. The magnitude of the increase in intraballoon pressure was less ($p<0.05$) at 12mmol/l when compared to a blood glucose concentration of 4mmol/l (figure 11.2).

Rectal balloon distension was perceived by all subjects. The threshold volume for initial sensation was less ($p<0.05$) at a blood glucose of 12mmol/l when compared to 4mmol/l (figure 11.3). The threshold volume for desire to defaecate was not significantly different between the three blood glucose concentrations (80(40-150) ml vs 70 (40-150)ml and 60 (10-150) ml, respectively for 4mmol/l, 8mmol/l and 12mmol/l.

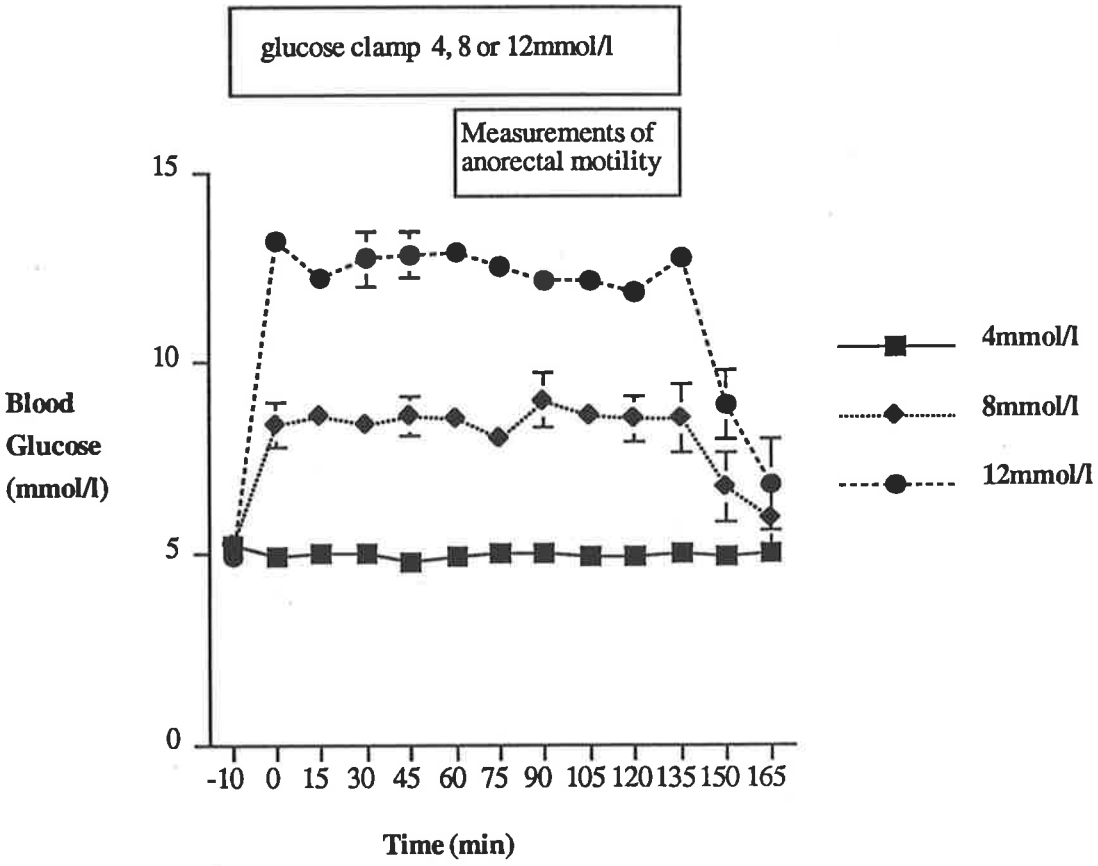

Figure 11.1

Blood glucose concentrations (mean±SEM) during the three experiments (4mmol/l, 8mmol/l and 12mmol/l).

Table 11.1:

Parameters of anorectal motility at blood glucose concentrations of 4mmol/l, 8mmol/l and 12mmol/l

	4mmol/l	8mmol/l	12mmol/l
Spontaneous anal relaxations			
Number (min^{-1})	2(0-5)	3(0-6)	7(3-12)*†
Lowest pressure (mmHg)	38±4.5	29±2.4	27±2.4
Duration (sec)	32±3.0	37±3.9	34±2.6
Minimum basal pressure (mmHg)	54±4.1	52±3.9	44±5.9
Squeeze pressure (mmHg)	162±10.5	164±8.0	140±12.1*†
Anal pressure while blowing up a balloon (mmHg)	117±13.1	102±9.7	88±11.2*

Mean± SEM (except for number of relaxations), * $p<0.05$, 12mmol/l cf 4mmol/l; †$p<0.05$, 12mmol/l cf 8mmol/l.

Table 11.2:

Anorectal motility during rectal distension at blood glucose concentrations of 4mmol/l, 8mmol/l and 12mmol/l

	10ml	20ml	40ml	60ml	100ml	150ml
Residual anal pressure						
4mmol/l	41±6.5	30±3.0	35±4.2	40±5.6	31±4.1	30±3.8
8mmol/l	41±4.7	35±3.5	30±3.7	38±2.6	36±2.6	34±3.2
12mmol/l	30±7.1	22±5.8	22±7.0	27±9.1	24±8.4	21±7.6

Mean ± SEM

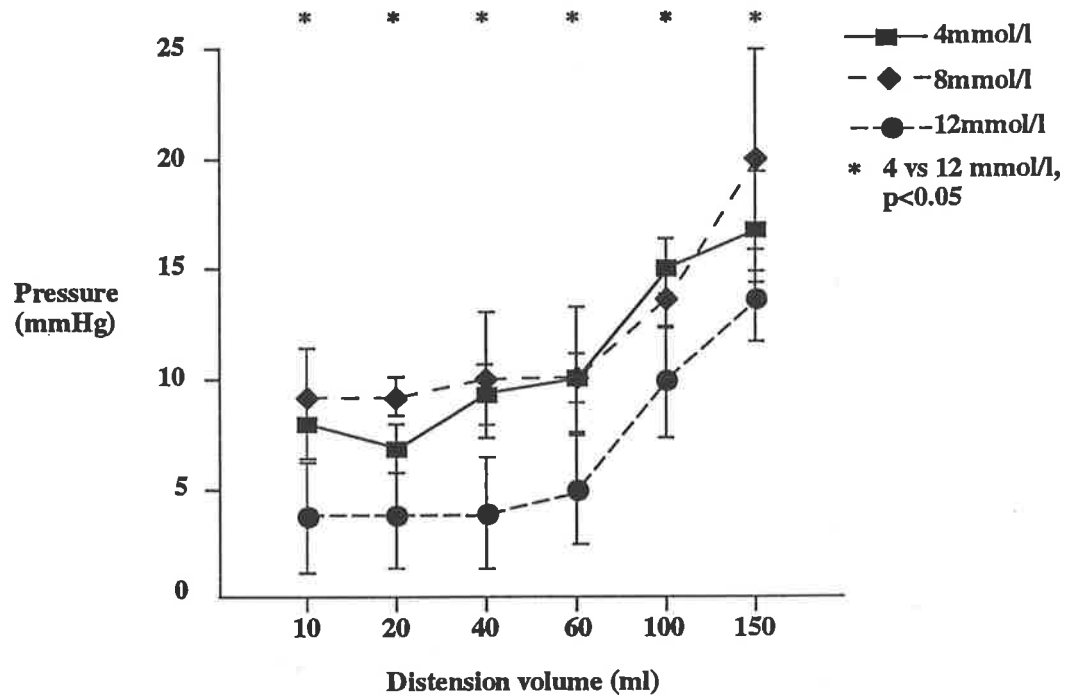

Figure 11.2

Relationship between intraballoon pressure and volume during rectal balloon distension at blood glucose concentrations of 4mmol/l, 8mmol/l and 12mmol/l. Data are mean ±SEM.

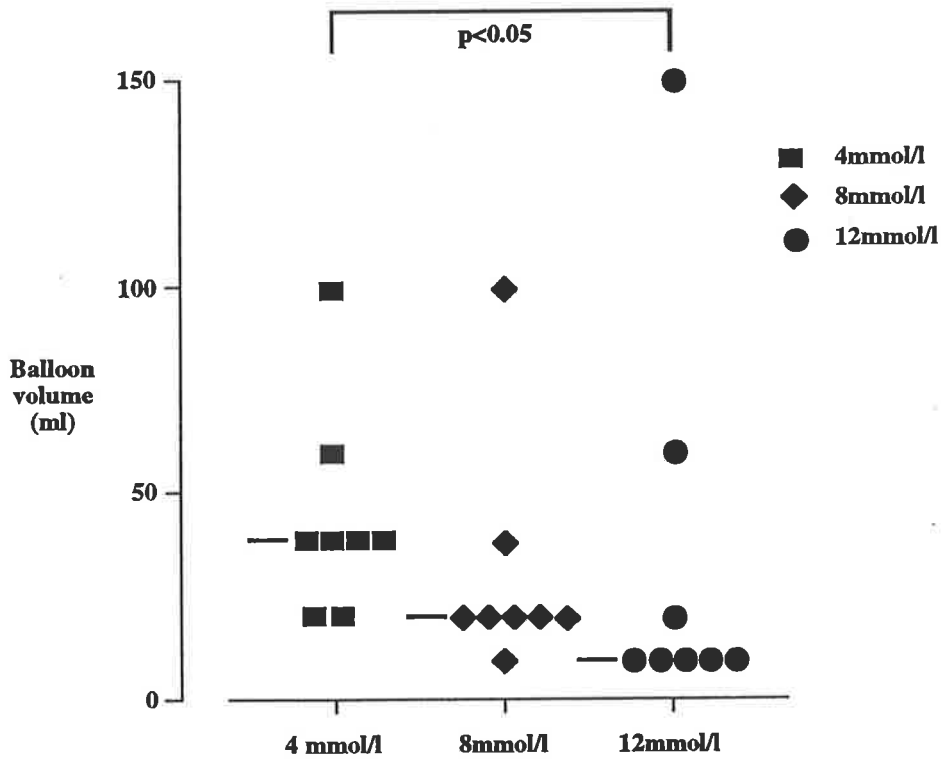

Figure 11.3

Volume for first perception of sensation during rectal balloon distension at blood glucose concentrations of 4mmol/l, 8mmol/l and 12mmol/l. The median value is shown by the horizontal line.

11.6 DISCUSSION

This study demonstrates for the first time that acute hyperglycaemia affects internal and external anal sphincter function, as well as rectal sensation and compliance in normal subjects. At a blood glucose concentration of 12mmol/l the number of spontaneous anal relaxations was greater, anorectal pressures generated by squeeze and blowing up a balloon were reduced and both rectal compliance and sensitivity to rectal balloon distension increased when compared to euglycaemia. In contrast, there was no significant effect of changes in blood glucose within the normal postprandial range on anorectal motility or sensation.

The observed increase in the number of spontaneous transient anal relaxations during hyperglycaemia is indicative of instability of the internal anal sphincter (Sun et al 1990). It has been reported that this phenomenon occurs frequently in diabetic patients with faecal incontinence, and is associated with leakage (Sun et al 1996). In normal subjects the candidate did not observe leakage of perfusate during internal anal sphincter relaxations because the residual anal pressure was higher than the rectal pressure, associated with compensatory external anal sphincter contraction. However, in diabetic patients the residual anal pressure is lower and compensatory external anal sphincter contraction impaired (Sun et al 1996). Hence, spontaneous transient anal relaxations are likely to contribute to incontinence. It is not surprising that in diabetic patients faecal incontinence occurs frequently during sleep, when there is no conscious control of anal sphincter function (Read & Sun 1992). The observed increase in rectal compliance during hyperglycaemia is also indicative of smooth muscle inhibition, consistent with the demonstration that acute hyperglycaemia increases proximal gastric compliance in normal subjects (Hebbard et al 1996, Hebbard et al 1996).

Although the minimum basal anal pressure was not affected by the blood glucose concentration, pressures generated by squeeze and in response to blowing up a balloon were reduced by hyperglycaemia, indicating that hyperglycaemia leads to a reduction in the strength of the external anal sphincter, which would favour incontinence. These findings conflict with Chey et al (Chey et al 1995) who found that hyperglycaemia (~15mmol/l) had no effect on maximal squeeze pressure. While there is no clear explanation to account for this discrepancy, there was a modest difference in blood glucose concentrations between the two studies. Furthermore, the sleeve sensor employed in the current study records the highest pressure along its length, while a simple multiport catheter may fail to record a narrow, high pressure zone (Orkin et al 1991).

The observation that the perception of rectal distension was increased during hyperglycaemia also conflicts with Chey et al (Chey et al 1995), who reported that hyperglycaemia increased the threshold for initial perception and urge to defaecate. In their study the balloon inflation rate was slower, and a larger diameter (9cm) balloon was used. Moreover, measurements of anorectal motility and sensation during hyperglycaemia were compared to "basal" measurements obtained before the induction of hyperglycaemia or euglycaemia hyperinsulinaemia (Chey et al 1995), thereby introducing the potential for an order effect. To support this approach the authors point out that there is a substantial day-to-day variability in measures of anorectal motility in normal subjects, which may lead to reduced sensitivity with a paired experimental design (Chey et al 1995). The rate and frequency of rectal balloon distensions were both relatively high in our study to mimic the rapid arrival of bowel contents, as opposed to evaluation of rectal sensation with a barostat technique which may allow more consistent and potentially unbiased distension (Read & Sun 1992). However, currently available barostats do not have the capacity for fast, low

volume rectal distensions. Although subjects were unaware of the timing, or volume, of rectal balloon distensions, the order of distensions was not randomised, introducing the potential for response bias. Despite this limitation, the observation of increased gut sensitivity during hyperglycaemia during the current study is consistent with other studies. For example, the perception of a number of sensations induced by distension of the proximal stomach (Hebbard et al 1996, Hebbard et al 1996), oesophagus (Boeckxstaens et al 1996) or duodenum (Lingenfelser et al 1996) and small intestinal nutrient infusion (Hebbard et al 1997) is greater during hyperglycaemia than euglycaemia. It is possible that the increase in rectal compliance during hyperglycaemia could lead to the observed increase in rectal sensitivity by increasing the exposure of the in parallel tension receptors in the rectal wall (Sun et al 1990, Read & Sun 1992). It remains to be determined whether the effect of hyperglycaemia on rectal sensitivity is also evident in patients with diabetes mellitus, particularly those patients with neuropathy. In particular, in previous studies the threshold for conscious rectal sensation has been reported to be elevated in diabetic patients who had faecal incontinence and evidence of neuropathy, but blood glucose concentrations were not monitored in these studies (Wald & Tunuguntla 1984, Caruana et al 1991).

The candidate was unable to demonstrate any effect of physiological changes in the blood glucose concentration on anal sphincter function or sensation. An elevation of blood glucose to 8mmol/l has been shown to affect motility in the oesophagus (Boeckxstaens et al 1996), stomach (Hasler et al 1995), and gallbladder (De Boer et al 1993). While it is possible that a threshold for an effect of hyperglycaemia varies in different regions of the gastrointestinal tract, it is also possible, as discussed previously, that subtle changes were not detected with the current study design.

The mechanisms mediating the effects of hyperglycaemia on gastrointestinal motility and sensation are poorly defined but both neural (central, spinal and peripheral) and hormonal mechanisms are likely to be responsible. A direct effect on smooth muscle is unlikely as both smooth muscle stimulation (Fraser et al 1991) and inhibition (Barnett & Owyang 1988, Hasler et al 1995) occur during hyperglycaemia. Hyperglycaemia (~15 mmol/l) suppresses parasympathetic tone in normal subjects (Chapter 12). Secondary hyperinsulinaemia is unlikely to be primarily responsible for the effects of hyperglycaemia on gastrointestinal motor and sensory function (Hasler et al 1995, Sims et al 1995, Chey et al 1995). In particular, in other regions of the gut the effects of hyperglycaemia are evident in patients with diabetes mellitus who have no endogenous insulin secretion (Fraser et al 1990). The inhibition of external anal sphincter activity (skeletal muscle) during hyperglycaemia may be secondary to impaired nerve conduction (Greene 1986).

While the effects of changes in the blood glucose concentration on anorectal motor function have not yet been evaluated in diabetic patients with and without faecal incontinence, it should be recognised that the observed effects of modifications in the blood glucose concentration on anorectal function in normal subjects, mimic a number of the abnormalities reported in diabetic patients with faecal incontinence (Sun et al 1996). This suggests that optimisation of blood glucose control may be an important component of treatment.

CHAPTER 12

HYPERGLYCAEMIA AFFECTS CARDIOVASCULAR AUTONOMIC NERVE FUNCTION IN NORMAL SUBJECTS

12.1 SUMMARY

The aim of this study was to evaluate the effect of acute hyperglycaemia on autonomic nerve function in normal subjects. Six healthy volunteers aged 19-32 yr underwent paired studies during euglycaemia (blood glucose 5.1 ± 0.04 mmol/l) and hyperglycaemia (blood glucose 15.7 ± 0.48 mmol/l) induced by intravenous infusion of glucose and maintained for 150 minutes. The order of the two studies was randomised. In each experiment supine heart rate, the heart rate variation with respiration, the ratio of maximum to minimum R-R interval after standing ("30:15" ratio), the systolic blood pressure response to standing and the diastolic blood pressure response to sustained handgrip were measured. Data were analysed using repeated measures analysis of variance. The supine heart rate was greater ($p=0.04$) and the "30:15" ratio less ($p=0.03$) during hyperglycaemia when compared to euglycaemia. Hyperglycaemia had no significant effect on any of the other cardiovascular reflex tests. These observations indicate that acute hyperglycaemia affects autonomic nerve function in healthy humans.

12.2 INTRODUCTION

Peripheral and autonomic neuropathy occur frequently in patients with diabetes mellitus and the risk of these complications is reduced by optimal glycemic control (The Diabetes Control and Complications Trial Research Group). Autonomic nerve function is usually evaluated using non-invasive cardiovascular

reflex tests (Ewing & Clarke 1982). Recent studies indicate that short-term changes in the blood glucose concentration may influence autonomic nerve function. For example, in patients with insulin dependent diabetes mellitus (IDDM) gastric emptying is slower during hyperglycaemia when compared with euglycaemia (Horowitz & Fraser 1994), and accelerated during hypoglycemia (Schvarcz et al 1993). In normal subjects the secretion of pancreatic polypeptide is suppressed by acute hyperglycaemia, consistent with a reduction in vagal activity (Schwartz 1983). In patients with IDDM acute hyperglycaemia slows peripheral nerve conduction velocity (Sindrup et al 1989). The effects of acute hyperglycaemia on autonomic nerve function have not been previously evaluated. The effects of hyperglycaemia on cardiovascular autonomic function were therefore assessed in normal subjects.

12.3 MATERIALS AND METHODS

12.3.1 Subjects

Six healthy volunteers (5 female, 1 male) mean age 24 years (range 19-32) and mean body mass index 21.6 (range 18.9-26.4) were studied. In each subject, studies were performed during euglycaemia and hyperglycaemia in a single-blind, randomised order. The two studies were separated by at least one week.

12.3.2 Evaluation of autonomic nerve function

Autonomic nerve function was evaluated using the following standardised cardiovascular reflex tests (Ewing & Clarke 1982): (a) Heart rate variation with respiration (test of parasympathetic function). Each subject was instructed to breathe in over five seconds and out over five seconds, over a one minute period. The difference between maximum and minimum heart rate was calculated (Ewing & Clarke 1982); (b) Heart rate response to standing "30:15" ratio (test of parasympathetic function). Each subject was asked to stand quickly from a

supine position. The ratio of the maximum R-R interval, occurring at or around the 30th beat, and the minimum R-R interval, occurring at or around the 15th beat following standing, was calculated (Ewing & Clarke 1982); (c) Systolic blood pressure response to standing (test of sympathetic function). Systolic blood pressure was measured at baseline and at one and two minutes after standing from a supine position (Ewing & Clarke 1982), using a semi-automated non-invasive blood pressure monitor (Critikon Dinamap 8100, Johnson and Johnson): (d) Blood pressure response to sustained handgrip (test of sympathetic function). A handgrip dynamometer was constructed using a standard blood pressure cuff and mercury sphygmomanometer. Each subject was initially asked to exert maximum handgrip force to determine the grip pressure which represented maximum exertion and subsequently asked to maintain 30% of maximum handgrip for five minutes, while blood pressure was recorded at one minute intervals (Ewing & Clarke 1982). Because two subjects were able to maintain handgrip for only four of the five minutes, when comparing the effect of euglycaemia and hyperglycaemia readings obtained at 0, 1, 2, 3, and 4 minutes were analysed.

12.3.3 Protocol

Each study commenced at 0900 hours following an overnight fast and abstinence from alcohol, caffeine, smoking and heavy exercise for at least 24 hours. With the subject recumbent, an intravenous cannula was sited in an antecubital vein of the left arm for blood sampling. A second cannula was placed in the right forearm for intravenous infusions. In the study performed during hyperglycaemia, 25% glucose was given as an initial 150 ml bolus, followed by an infusion at a variable rate to maintain the blood glucose concentration at approximately 15 mmol/l for the subsequent 150 minutes. In the study performed during euglycaemia 0.9% sodium chloride was given as an initial 150 ml bolus, followed by an infusion at a rate of 100 ml/hr for 150 minutes. The resting supine

heart rate, the heart rate variation with respiration, the heart rate response to standing and the systolic blood pressure response to standing were measured immediately before commencement of the infusions (baseline) and subsequently every fifteen minutes for two hours. The resting supine heart rate was calculated as the mean of 30 R-R intervals recorded while subjects were supine prior to standing for measurement of the "30:15" ratio. The diastolic blood pressure response to sustained handgrip was evaluated twice between 120 and 150 minutes. Venous blood samples were taken at baseline and subsequently at least every 10 minutes for 150 minutes. Blood glucose concentrations were initially measured using a portable blood glucose meter (Medisense Companion 2 glucometer, Medisense Inc., Waltman, MA) and the accuracy of these measurements confirmed subsequently using a hexokinase technique.

12.3.4 Statistical analysis

Data were evaluated using a repeated measures analysis of variance with contrasts (to compare specific time points) and linear regression analysis and are shown as mean values ± SEM. A p value of <0.05 was considered significant.

12.4 RESULTS

The studies were well tolerated by all subjects and no subject reported a need to urinate. The mean blood glucose was 5.1 ± 0.04 mmol/l during saline infusion and 15.7 ± 0.48 mmol/l in the studies performed during hyperglycaemia. 406 ± 4 ml was infused in the studies performed during euglycaemia and 693 ± 42 ml in the studies performed during hyperglycaemia ($p<0.01$).

12.4.1 Heart rate

Supine heart rate did not change significantly during saline infusion but increased during hyperglycaemia ($p=0.02$). The heart rate was greater ($p=0.04$) during

hyperglycaemia when compared to euglycaemia (figure 12.1). During hyperglycaemia the change in heart rate from baseline did not correlate significantly with the volume of fluid infused.

12.4.2 Parasympathetic function

The "30:15" ratio did not change significantly during saline infusion, but decreased ($p=0.003$) during hyperglycaemia. The "30:15" ratio was less ($p=0.03$) during hyperglycaemia when compared to euglycaemia (figure 12.2a). The change in the "30:15" ratio reflected an increase ($p=0.02$) in heart rate at or around beat 15 associated with a greater increase ($p=0.001$) in heart rate at or around beat 30 during hyperglycaemia when compared to euglycaemia (figure 12.2b). The heart rate variation with deep breathing did not change during either euglycaemia ($p=0.44$) or hyperglycaemia ($p=0.98$) and there was no difference between the two studies (data not shown).

12.4.3 Sympathetic function

There was no significant difference ($p=0.49$) between euglycaemia and hyperglycaemia in the systolic blood pressure response to standing (data not shown). Submaximal handgrip resulted in a progressive increase ($p<0.01$) in diastolic blood pressure, but there was no difference in the response between euglycaemia and hyperglycaemia (figure 12.3).

1985) suggesting that the more rapid heart rate during hyperglycaemia observed in the current study is unlikely to be the consequence of secondary hyperinsulinemia. This conclusion is supported by studies indicating that insulin is not responsible for the effects of hyperglycaemia on gastrointestinal motility (Horowitz & Fraser 1994, Hasler et al 1995). The increase in resting heart rate during hyperglycaemia is also unlikely to reflect an increase in plasma catecholamine concentrations (Rowe et al 1981).

While the volume of glucose given in studies performed during hyperglycaemia was greater than the amount of saline infused during euglycaemia, because of the inter-individual variation in the amount of intravenous glucose required to maintain hyperglycaemia, the rate of infusion of both saline and glucose were below the threshold at which volume expansion has been reported to affect heart rate responses (Vatner & Zimpfer 1981, Muir et al 1975). It is also possible, but unlikely, that a relative increase in plasma osmolality during hyperglycaemia may have resulted in altered nerve function.

In view of the current observations the effects of hyperglycaemia on cardiovascular autonomic nerve function in patients with diabetes mellitus require evaluation. It will also be appropriate to determine whether changes in blood glucose concentration within the physiological range affect autonomic nerve function, as suggested by recent studies (Hasler et al 1995). The potential effects of the blood glucose concentration may need to be taken into account when autonomic nerve function is evaluated in patients with diabetes.

APPENDIX

The material in this thesis formed the basis for the publications listed below.

Original Articles

Russo A, Fraser R, Horowitz M. The effect of acute hyperglycaemia on small intestinal motility in normal subjects. Diabetologia 1996;39: 984-989.

Russo A, Fraser R, Adachi K, Boeckxstaens G, Horowitz M. Evidence that nitric oxide (NO) mechanisms regulate small intestinal motility in humans (submitted to the Lancet).

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