A FUNCTIONAL ANALYSIS OF GASTROINTESTINAL MOTILITY IN THE GUINEA PIG AND HUMAN

A thesis submitted in total fulfilment of the requirements of the degree of doctor of philosophy

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5. SPONTANEOUS AND ELECTRICALLY EVOKED CONTRACTIONS IN SMALL SEGMENTS OF HUMAN COLON.

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5.1 Introduction

The enteric nervous system is important for many functions of the gastrointestinal tract in mammals. Laboratory animals have long been used to study the enteric neural circuitry in the gut wall. However, investigations of the functional enteric neural pathways in the human gastrointestinal tract have been limited. Most in vitro studies of contractility in isolated specimens of human gut have been conducted on narrow strips of muscle, usually 1-5 mm along the length of the colon and approximately 8-15mm in parallel with the circular muscle. Electrical field stimulation, pharmacological agents and physiological stimuli, can be used to demonstrate the contribution of neurons in either locally generated or spontaneous contractions (Bennett and Stockley, 1975, Keef et al., 1993, Huizinga et al., 1985). Restricted availability of larger specimens of human intestine has precluded detailed analysis. In this study, I examined whether differences in cross sectional area affected spontaneous activity of preparations.

The projections of motor neurons along the length of the gastrointestinal tract are polarised such that orally projecting motor neurons are typically excitatory and anally projecting motor neurons are inhibitory (Brookes et al., 1991). Human enteric excitatory motor neurons innervating the circular muscle layer project up to 10mm orally in the colon (Wattchow et al., 1995). Retrograde labelling combined with immunhistochemistry in the human colon have demonstrated that orally projecting excitatory motor neurons are immunoreactive for excitatory markers such as choline

acetyltransferase (ChAT) and tachykinin (TK). Aborally projecting inhibitory motor neurons are immunoreactive for inhibitory markers such as nitric oxide synthase (NOS) and vasoactive intestinal peptide (VIP) and project up to 16mm (Wattchow et al., 1997, Porter et al., 2002a). Interneuronal pathways are up to 68 mm in length (the maximum size of preparations studied): ascending interneurons are reactive for ChAT and TK; descending interneurons are either immunoreactive for ChAT, NOS or VIP (Wattchow et al., 1997, Porter et al., 2002a). However, there is little functional evidence correlating these anatomically defined pathways with the physiology of specimens of human gut tissue.

This study aimed to determine whether the size of preparations relative to known projections of either neuronal pathways modified contractility of specimens of human colon *in vitro*. I also tested whether the activation of cholinergic neurons in ascending pathways could modify patterns of contraction in isolated specimens.

5.2 Materials and Methods

5.2.1 Tissue preparation

Specimens of human colon were obtained, with prior written informed consent from patients undergoing surgery for removal of colon cancer in the Flinders Medical Centre (approved by the Flinders Clinical Research Ethics Committee now the Southern Adelaide Clinical Research Ethics Committee). Immediately following removal of the specimen an uninvolved segment was isolated and placed in oxygenated Krebs solution, at room temperature. Krebs solution contained mM: NaCl 118; KCl 4.70, $NaH_2PO_4 2H_2O$ 1; $NaHCO_3$ 25; $MgCl_2 6H_2O$ 1.2; D-Glucose 11; $CaCl_2.2H_2O$ 2.5; bubbled with 95% O_2 and 5% CO_2. Preparations were cut open along the longitudinal axis. Fat, mesentery, mucosa and most of the submucosa were removed by sharp dissection leaving the longitudinal and circular muscle layers, and the myenteric plexus intact.

5.2.2 Variations in contractility for preparations differing in cross sectional area.

Preparations of various rectangular dimensions were set up: (circumferential axis x longitudinal axis) 10x2mm, 20x4mm, 40x8mm and 80x15mm. Arrays of hooks were attached at either end of each specimen: one was connected to a force transducer via a cotton ligature, the other was fixed in position allowing circumferential contractions to be recorded (See Fig 5.1A). Each segment was placed into a 10ml water bath containing warmed Krebs solution (~36^{O}C) bubbled with 95% oxygen, 5% carbon dioxide. Tension recordings were digitised at 200Hz and stored via an A/D interface (MacLab 8SP, AD Instruments, Sydney, Australia) using Chart 6 software (ADInstruments, Australia).

5.2.3 Frequencies of contractions

Blinded to test condition and tissue size, I analysed patterns of contractile activity during analysis of contraction frequency. Contractions could be separated based on frequency, duration and amplitude. Three types of contraction could be distinguished:

Slow phasic contractions: occurred at frequencies of <1 cycle per minute. 600s epochs of data were used to analyse these contractions.

Intermediate contractions: occurred at 2-6 cycles per minute. 120s epochs of data were used to analyse these contractions.

High frequency contractions: of the shortest duration occurred at frequencies between 10-25 cycles per minute. 60s epochs of data were used to analyse these contractions.

Dominant frequencies were obtained from power spectra generated from fast Fourier transforms (FFT) of 600s epochs of data which were comprised of 12000 points. The FFT software was written in Matlab® (The MathWorks, Natick, Massachusetts, USA).

5.2.4 Ascending excitatory neurons and colonic contractility.

In a separate set of studies, preparations were cut into a "T" shape where the cross bar of the T was the oral end of the preparation. Maximal circumferential length was ~80mm and maximal longitudinal length was ~20mm. Preparations were pinned in

Sylgard-lined chamber (Dow Corning, Midland, MI, USA) with the circular muscle layer facing uppermost. An array of hooks attached along the longitudinal axis of the oral segment was connected to a force transducer to measure circumferential contractions. The aboral segment was pharmacologically isolated by a barrier of inert silicon grease with a glass coverslip. Both chambers were superfused with Krebs solution at 36^{O}C. Preparations were left to equilibrate for 1-2 hours before control data was collected.

Preparations were stimulated with electrical stimuli of for 5s of 80V, 0.4ms duration, at 10Hz via a Grass SD9 stimulator (See Fig 5.1B). The relationship between electrical stimulation and contractions was investigated quantitatively. The delay between an electrical stimulus and the next slow phasic contraction was measured. This was compared to the delay between a randomly selected point and the next spontaneous contraction. Five arbitrarily selected points were chosen by the investigator while unable to see the recording. At least three spontaneous slow phasic contractions were allowed to occur before the next bout of electrical stimulation, to minimise long-term interactions.

5.2.5 Drugs used

1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP) was prepared in Krebs solution at 1mM prior to experiments. Hexamethonium and tetrodotoxin were stored in aqueous solution (10^{-2}M) and diluted in Krebs solution to the required concentration immediately before use. All drugs were purchased from Sigma Aldrich (Castle Hill,

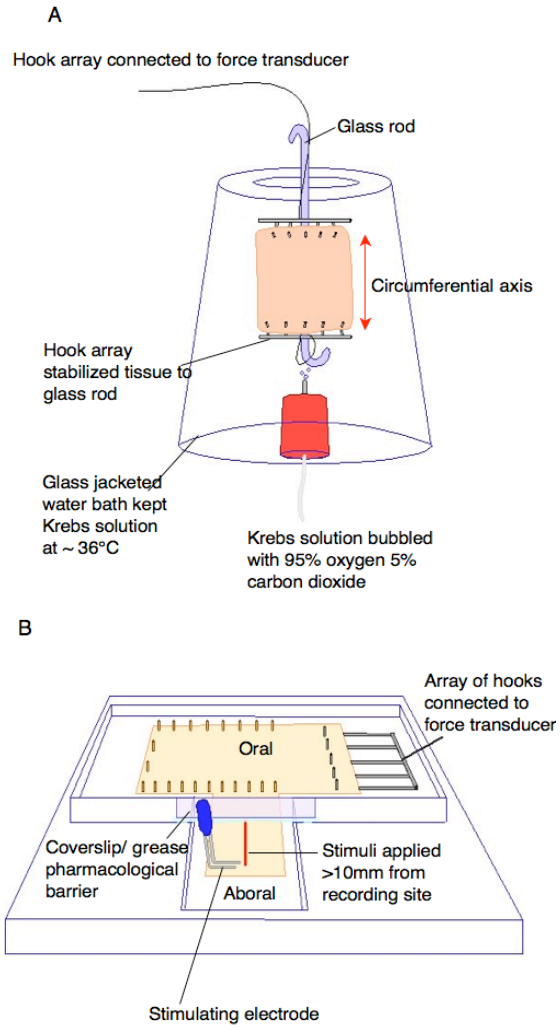

Figure 5.1 Experimental setups: A) study on preparations of varying cross sectional area B) study on ascending excitatory neurons and colonic contractility.

Australia). Antagonists were superfused for 30 minute before analysis was performed

5.2.6 Data analysis

Results are expressed as means ± standard deviation. The use of *"n"* refers to the number of patients. Statistical analysis was performed by: student's two-tailed *t*- test for paired samples using Microsoft Excel 2004; and either ANOVA or MANOVA using GraphPad Prism 4, software. One-way ANOVAs were used to analyse whether preparation size affected the frequency of slow events. MANOVA analysis was used to compare the frequency of slow events in either control or in the presence of KCl across all sizes of specimens. MANOVA analysis was used to compare the frequency of slow events across preparations of all sizes in control versus TTX. One-way ANOVA was used to compared the intervals between spontaneous contractions before and after electrical stimulation, with intervals between the evoked contraction and the next spontaneous contraction. One-way ANOVA and MANOVA were followed by Bonferroni post tests.

5.3 Results

4 of 12 patients were administered Celecoxib, a cycloxygenase inhibitor, prior to surgery to limit the occurrence of post-operative ileus (Wattchow et al., 2009). Unpublished data from a separate series of experiments compared the contractility of tissue obtained from patients with or without Celecoxib and revealed no significant differences in the mean frequency or amplitude of slow phasic or intermediary contractions.

5.3.1 The frequencies of contractions in muscle strips of varying size.

Tissue from 4 patients was compared: *n*=2 transverse colon; and *n*=2 descending colon. Previous studies have reported no significant differences in the contractile patterns between regions of colon (Choe et al., 2010, Rae et al., 1998). High,

intermediate and slow phasic contractions could be recorded in all tissues over the course of the recordings (Fig 5.2).

Power spectra analysis demonstrated that in general dominant frequencies clustered at 3 frequency ranges: 0-1, 2-6 and 10-25 cycles per minute. This corresponded with the frequencies calculated for slow phasic contractions, intermediate contractions and high frequency contractions identified visually. Calculated values ranged from 0.0-1.3, 1.5-4.0 and 7.0-25.0 cycles per minute. The presence or absence of visible slow phasic contractions corresponded with dominant frequencies from FFT between 0-1 cycles per minute in 13/16 recordings (81.25%). Visible intermediate contractions corresponded with dominant frequencies by FFT between 2-6 cycles per minute in 13/16 recordings (81.25%). Visible high frequency contractions corresponded with dominant frequencies by FFT between 10-25 cycles per minute 9/16 recordings (56.25%). Overall, there was a good correlation between visually identified rhythmic contractions and events calculated objectively by FFT, although this dropped away at the highest frequency.

5.3.2 Slow phasic contractions

The frequency of slow phasic contractions as determined by manual counting was not significantly different in preparations of different size in control. For specimens of all sizes, the overall mean frequency of slow phasic contractions was 0.4±0.3 cycles per minute (n=4, table 5.1). Contraction frequency was not affected by depolarising the membrane potential with increasing extracellular concentration of KCl (10, 15 and 20mM table 5.2, n=3). Slow phasic contractions occurred at slightly greater intervals in the presence of TTX compared to control specimens although this was not significant (n=4, P>0.05). The overall mean frequency of contractions reduced from 0.6±0.5 cycles per minute to 0.4±0.3 cycles per minute in TTX (1μM, n=4, P>0.05, table 5.5).

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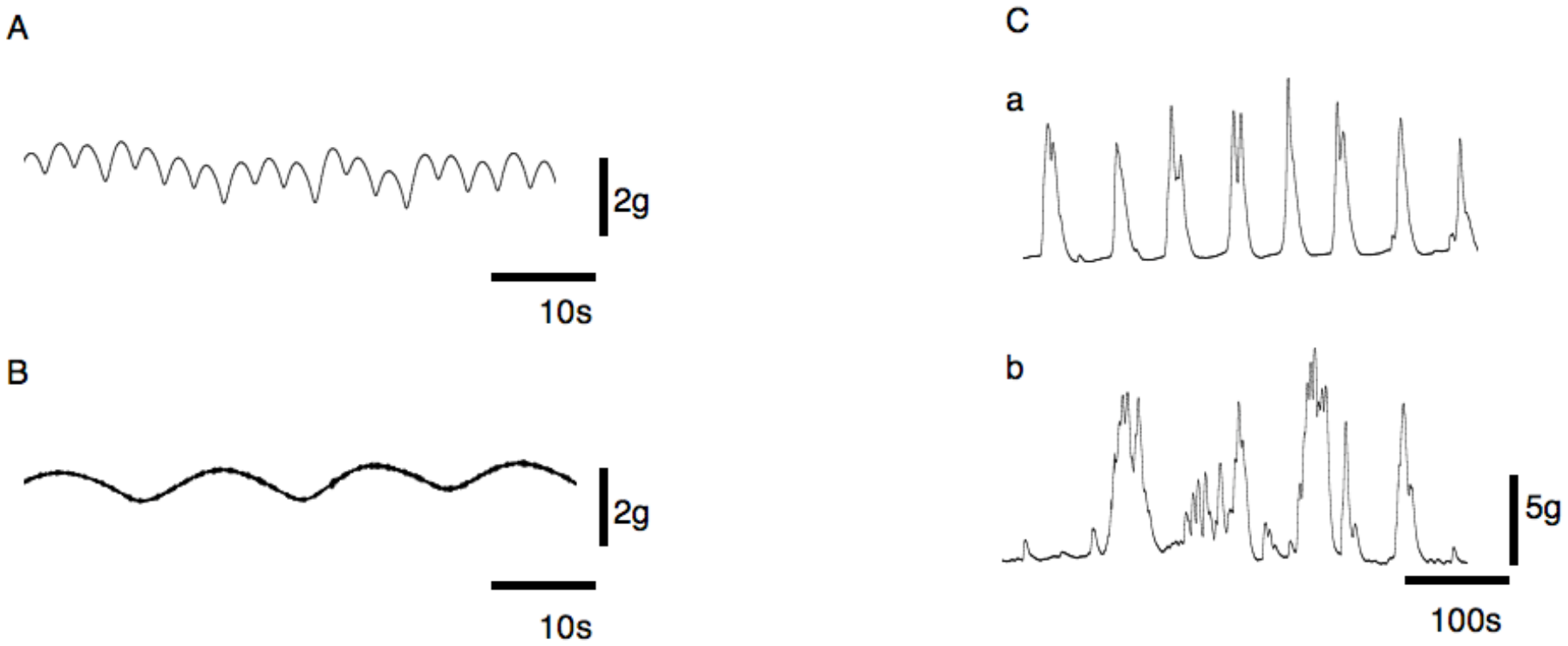


Figure 5.2 Preparations of human colon displayed various spontaneous motor patterns including: A) high B) intermediate frequency contractions and Ca) slow phasic contractions at either regular or b) irregular rhythms.

Table 5.1 The frequency of contractions from colon specimens varying in size. Frequencies were determined by visual analysis and confirmed with FFT. Values are mean± SD, *n*=4

	Size of tissue (mmxmm)			
	10x2	**20x4**	**40x8**	**80x15**
Slow phasic ± SD cycles per minute	0.5±0.3	0.6±0.5	0.4±0.2	0.5±0.2
Intermediate ±SD cycles per minute	3.3±0.3	1.7±0.3	2.9±1.4	2.0±0.5
High frequency ±SD cycles per minute	15.0±2.0	17.6±4.5	12.0±7.0	11.7±1.5

Table 5.2 The mean frequencies of slow phasic contractions do not significantly vary with increasing extracellular KCl concentrations. (P>0.05, n=3).

	Size of tissue (mmxmm)			
	10x2	**20x4**	**40x8**	**80x15**
control cycles per minute	0.3±0.3	0.6±0.7	0.3±0.3	0.3±0.2
10mM KCl cycles per minute	0.4±0.4	0.8±0.7	1.3±0.3	0.9±0.5
15mM KCl cycles per minute	0.3±0.5	0.2±0.2	0.4±0.3	0.7±0.6
20mM KCl cycles per minute	0.1±0.1	0.2±0.3	1.8±0.5	1.1±1.0

Table 5.3 The mean frequencies of intermediate contractions with increasing extracellular concentrations of KCl (*n*=3)

	Size of tissue (mmxmm)			
	10x2	**20x4**	**40x8**	**80x15**
control cycles per minute	3.5±0.0	1.8±0.4	3.2±1.6	1.8±0.4
10mM KCl cycles per minute	1.5±0.7	1.5±1.3	4.5±0.5	0.8±0.3
15mM KCl cycles per minute	1.3±1.0	3.0±2.8	4.0±2.6	3.2±0.8
20mM KCl cycles per minute	3.0±2.8	3.0±2.1	4.2±1.3	3.3±0.4

Table 5.4 The mean values for high frequency contractions with increasing extracellular concentrations of KCl. (*n*=3).

	Size of tissue (mmxmm)			
	10x2	**20x4**	**40x8**	**80x15**
control cycles per minute	14.0±1.4	20.0±2.8	4.2±4.0	11.7±1.5
10mM KCl cycles per minute	17.5±6.4	20.5±2.1	13.5±3.5	8.0±1.4
15mM KCl cycles per minute	13.7±3.8	18.0±0.0	10.0±0.0	9.0±0.0
20mM KCl cycles per minute	17.0±0.0	16.0±4.2	6.0±0.0	13.0±0.0

Table 5.5 The mean frequencies of contractions in control Krebs solution and with addition of TTX (1μM, *n*=4).

	Size of tissue (mmxmm)							
	10x2		**20x4**		**40x8**		**80x15**	
	Control	**TTX**	**Control**	**TTX**	**Control**	**TTX**	**Control**	**TTX**
Slow phasic ± SD cycles per minute	0.5±0.3	0.4±0.2	0.6±0.5	0.5±0.5	0.4±0.2	0.4±0.3	0.5±0.2	0.5±0.4
Intermediate ±SD cycles per minute	3.3±0.3	4.0±3.5	1.7±0.3	2.5±1.4	2.9±1.4	2.7±2.1	2.0±0.5	2.3±0.6
High frequency ±SD cycles per minute	15.0±2.0	10.3±9.0	17.6±4.5	16.8±4.3	12.0±7.0	13.0±6.1	11.7±1.5	17.7±4.0

The frequency of slow phasic contractions had a decreasing trend in TTX compared to control Krebs solution ($P>0.05$, $n=4$).

5.3.3 Intermediate contractions

The frequency of intermediate contractions did not significantly differ between specimens of different size. Across all specimens, the frequency of contractions averaged 2.5±1.0 cycles per minute (See table 1, *n*=4, table 5.1). Modifying the extracellular concentration of KCl (10, 15, 20mM) did not affect the frequency of contractions (table 5.3). TTX also had no affect on the frequency of contractions (control: 2.8±1.2 versus TTX: 2.5±1.7 cycles per minute, *n*=4, table 5.5)

5.3.4 High frequency contractions

The rate of high frequency contractions was not significantly different for specimens of different sizes. The mean frequency of these contractions averaged 13.2±5.4 cycles per minute (*n*=4, table 5.1). Increasing KCl concentration in the extracellular solution did not significantly modify the frequency of contractions (*n*=3, table 5.4). Addition of TTX also had no significant affect on the frequency of contraction (control: 16.4±11.3 versus 15.8±4.2 cycles per minute, *n*=4, table 5.5).

5.3.5 Spontaneous activity in 'T' segments of colon

Specimens were taken from 8 healthy, unobstructed segments of colon from the following regions: ascending colon (*n*=1), splenic flexure (*n*=1); descending colon (*n*=5), sigmoid colon (*n*=1). Evidence from the previous study suggested the frequency of slow phasic contractions may be modulated by neural input, therefore, slow phasic contractions were the sole focus of this study.

Slow phasic contractions occurred at a frequency of 0.62±0.31 cycles per minute (mean interval between contractions: 124.1±68.4s, *n*=6). Addition of the nicotinic receptor antagonist, hexamethonium (1μM) increased mean intervals between contractions to 278.1±138.3s ($P<0.005$, *n*=8, Fig 5.3B), however the mean amplitude of slow phasic contractions was not significantly affected (control: 6.0±3.5g versus hexamethonium: 6.5±4.1g, *n*=8, Fig 5.3C). Sequential addition of 1μM TTX did not significantly affect either the amplitude of contractions (5.3±2.9g, *n*=6), or the time interval between contractions (428.6±282.3, *n*=6).

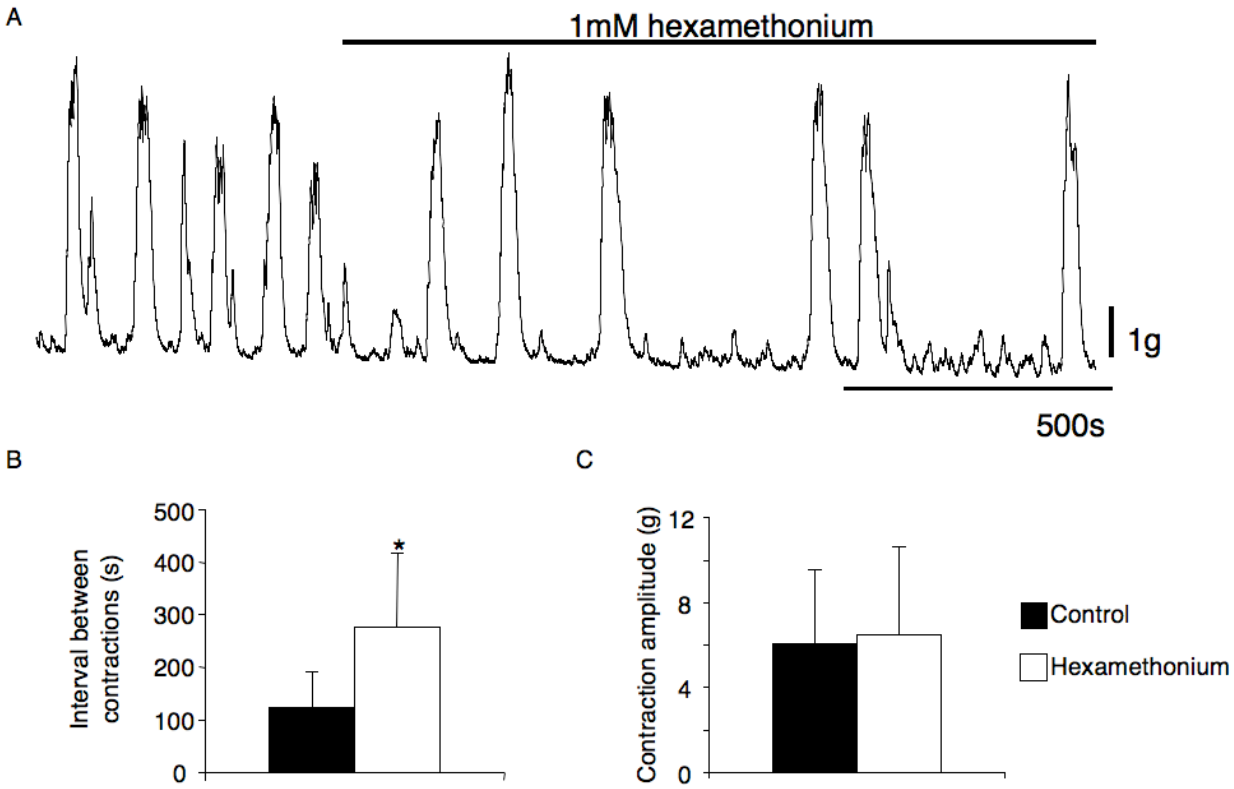


Figure 5.3 The frequency of slow phasic contractions was reduced in the presence of 1mM hexamethonium. A) An example of contractions in control solution and with addition of 1mM hexamethonium (top black bar). B) Time interval between contractions significantly increased in the presence of hexamethonium (* P<0.005, n=8). C) The amplitude of slow events were unaffected by hexamethonium (n=8).

5.3.6 Stimulation of ascending interneuronal pathways and its effects on slow phasic contractions

I applied transmural electrical nerve stimulation to the aboral segement of colon (see figure 5.1B). The time between the electrical stimulus and the peak of the following contraction was significantly smaller than the time between a randomly selected point and the peak of the following slow phasic contraction (10.8±2.3s vs 152.0±52.1s, $P<0.005$, $n=6$, Fig 5.4A) indicating that electrical stimulation caused a contraction. The amplitude of evoked contractions did not significantly differ from spontaneous slow phasic contractions (4.7±4.2g versus 6.1±3.5g, $n=6$, Fig 5.4B). Electrical stimulation re-set intervals between spontaneous contractions. The interval between a pair of spontaneous contractions preceding the electrical stimulus (200.6±109.8s, first spontaneous interval, Fig 5.5) was significantly shorter than the interval between the spontaneous contraction preceding the stimulus and the spontaneous contraction that followed the evoked contraction (329.8±188.1s, second spontaneous interval, $P<0.05$, $n=4$, Fig 5.5). The interval between the pair of contractions preceding the stimulus was not significantly different to interval between the evoked contraction and the next spontaneous contraction (241.1±127.5s, evoked interval, Fig 5.5). Electrical stimulation of the aboral segment, after it had been separated from the oral segment by cutting did not generate a premature contraction since the latency between stimulation and the peak of the following contraction was not significantly different from a random point and the next contraction peak (212.2±118.4s versus 135.7±21.8s, $n=1$, 3 repeats of stimulation).

5.3.7 Inhibiting nicotinic pathways: effects on premature contractions.

To test the role of nicotinic pathways in stimulus-evoked contractions, I applied hexamethonium to both chambers of the organ bath. Electrically evoked advancement of contractions persisted in the presence of hexamethonium (Fig 5.6A). The latency between the electrical stimulus and the peak of the following contraction was significantly shorter than that between a random point and the next contraction peak (11.8±4.9 versus 206.8±138.8s, $P<0.05$). The time to the following contraction in hexamethonium (11.8±4.9s) was very similar to that in control Krebs solution (10.8±2.3s) suggesting

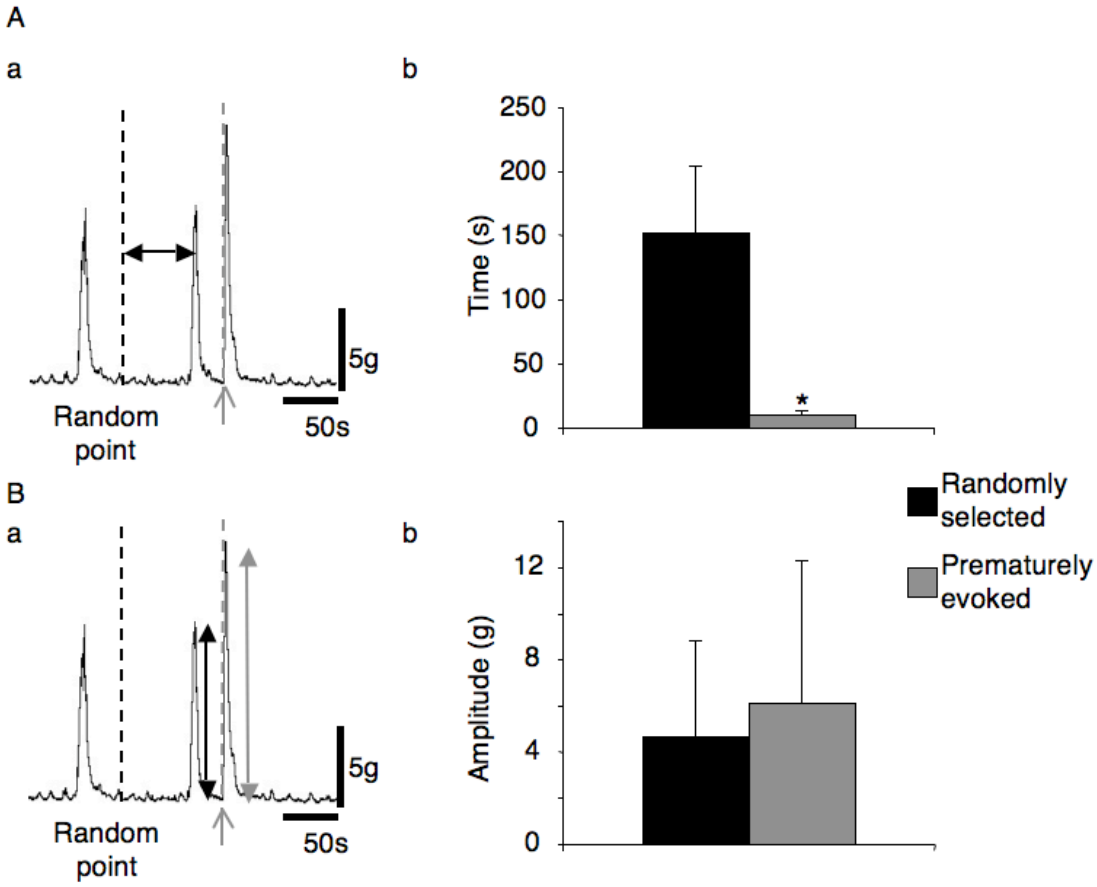
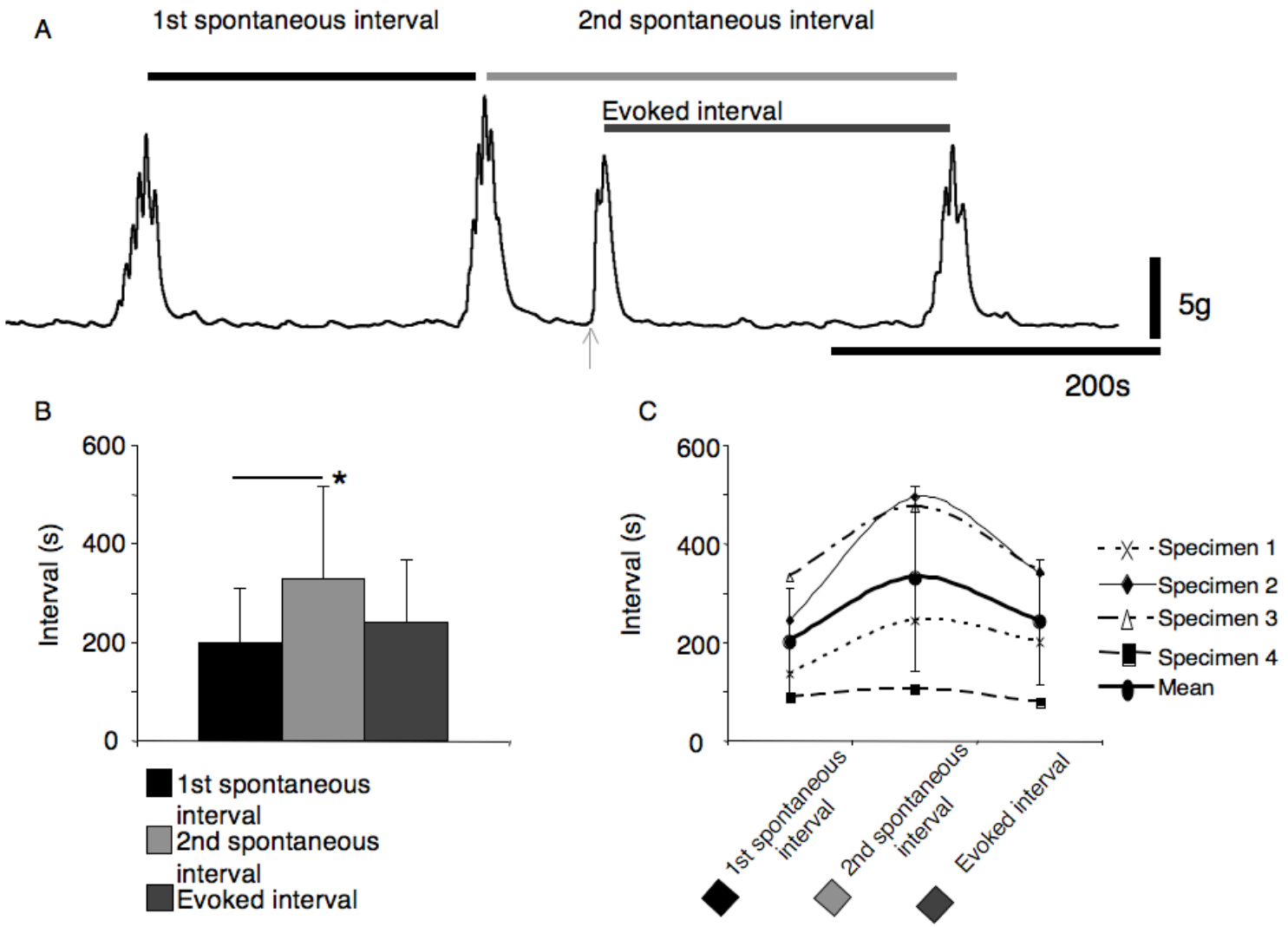


Figure 5.4 Aboral electrical stimulation resulted in a premature contraction. Aa) The time between the electrical stimulus (grey open arrow) and the peak of the next contraction was compared to the time from a randomly selected point and the peak of the following slow phasic contractions. Ab) The time of the contraction following an electrical stimulus was shorter than that following a randomly selected point (* $P<0.005$, $n=6$). Ba) The amplitude of the contraction following an electrical stimulus (grey open arrow) was compared to the amplitude of a slow phasic contraction following a randomly selected point. Bb) There was no significant difference in the amplitude of contractions.


A
1st spontaneous interval
2nd spontaneous interval
Evoked interval
5g
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400
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Interval (s)
*
1st spontaneous interval
2nd spontaneous interval
Evoked interval
C
600
400
200
0
Interval (s)
Specimen 1
Specimen 2
Specimen 3
Specimen 4
Mean
1st spontaneous interval
2nd spontaneous interval
Evoked interval

Figure 5.5 The interval between slow phasic contractions was reset by the evoked contraction. A) The interval between slow phasic contraction preceeding the electrical stimulus (1st spontaneous interval) was compared to the interval between the next pair of spontaneous contractions (2nd spontaneous interval) and the interval between the evoked contraction and the next spontaneous contraction. B) The 2nd spontaneous interval, interrupted by the electrical stimulus, was significantly longer than the 1st spontaneous interval (P<0.05, n=4.) C) Individual values for time intervals from each specimen.

that hexamethonium actually had little impact on the effects of electrical stimulation. The amplitudes of evoked contractions were not significantly affected by the addition of hexamethonium (4.0±3.8g versus 4.3±5.1g, *n*=5, Fig 5.6B). Electrically evoked contractions did not cause a premature event in the presence of TTX. The latency between stimulus and contraction was not significantly different between the latency from a random point to the following contraction peak (171.6±137.7s versus 207.9±84.4s, *n*=4). Since premature contractions persisted in hexamethonium, I tested whether the start of the premature contraction was delayed by inhibiting nicotinic pathways. The time from stimulus application to 10% amplitude of the contraction was compared in control with hexamethonium. Application of hexamethonium did not significantly affect this latency (4.7±1.2s to 3.8±.9s Fig 5.6C).

Application of DMPP (1mM) to the pharmacologically isolated aboral segment appeared to evoke a contraction 26.3±4.7s later (Fig 5.7A). This was slightly shorter than the latency between a random point on the trace and the peak of the following contraction (74.6±37.5s) however this did not reach significance (*P*=0.07, *n*=4, Fig 5.7B).

5.4 Discussion

Three sets of spontaneous contractions were distinguished based on their frequency: slow phasic, intermediate and high frequency contractions. Power spectra analysis confirmed the existence of these 3 dominant frequencies. High frequency contractions are likely to correspond with myenteric potential oscillations (MPOs) and have been associated with ICC-MY (Smith et al., 1987a, Rae et al., 1998). Intermediate contractions occurred at a slow wave frequency and are likely to be generated by ICC-SMP (Rae et al., 1998). It is interesting to note that these membrane oscillations are large enough to generate contractions in the smooth

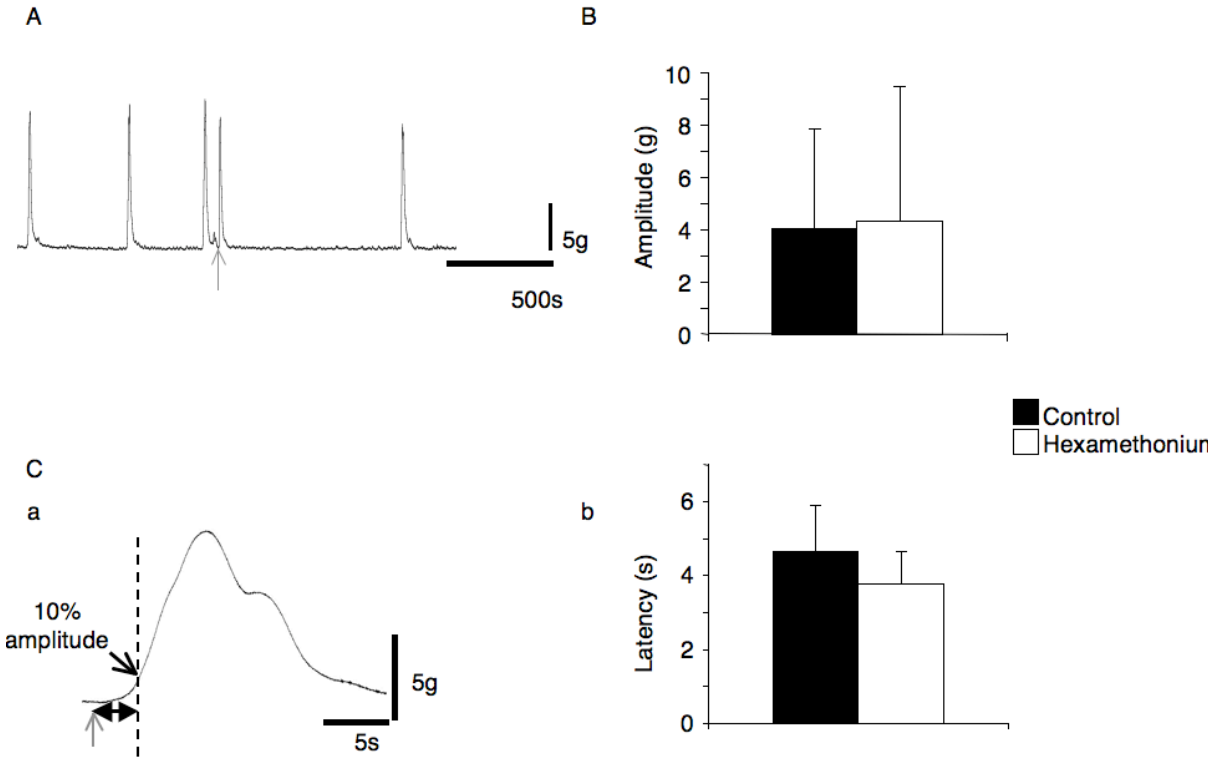

Figure 5.6 Contractions evoked by electrical stimulation persist in the presence of hexamethonium. A) Arrows indicate point of aboral electrical stimulation. Stimuli resulted in a premature contraction in the presence of hexamethonium. B) The amplitude of premature contractions were not significantly different from control and in hexamthonium (*n*=5). Ca) The time from the application of the stimulus (open arrow) and 10% amplitude of the contraction (dotted line) was compared. Cb) This was not significantly reduced in hexamethonium (*n*=4).

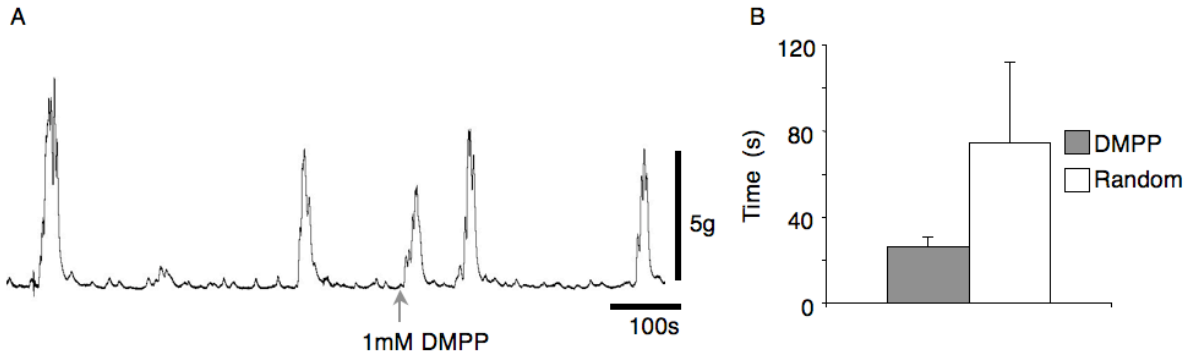

Figure 5.7 Direct activation of ascending nicotinic pathways can generate an orad contraction. A) Application of 1mM DMPP (closed arrow) to the pharmacologically isolated aboral segment. B) The time between the peak of the contraction following DMPP application (grey) was shorter than the time of a contraction following a random point (white) however this was not significant.

muscle cells. Since their frequency was not modulated by inhibiting neural activity with TTX I did not continue to study these contractions in the later part of this study.

5.4.1 Spontaneous slow phasic contractions

The ‘slow phasic contractions’ that I have described are similar to ‘phasic contractions’ or ‘slow contractions’ reported previously (Huizinga and Waterfall, 1988b, Huizinga et al., 1985). These papers used suction electrodes to identify the electrical correlates of phasic or slow contractions. These typically involved bursts of spikes superimposed on top of slow waves (Huizinga et al., 1988, Huizinga et al., 1985). Intracellular recordings from circular smooth muscle cells near the myenteric border have demonstrated slow waves oscillating at around 1cycle per minute with superimposed action potentials(Rae et al., 1998). In human, canine and pig colonic muscle cells, Huizinga and colleagues demonstrated that these contractions and spike bursts persist in the presence of TTX (Huizinga et al., 1988, Huizinga et al., 1985) and our results, using mechanical recordings, support this observation. In the rat mid colon, TTX inhibited similar contractions occurring at a similar frequency but with addition of carbachol these contractions resumed at similar frequency. It seems that neural input can modulate the frequency of these spike bursts, however, the mechanisms that generate them are unclear (Huizinga et al., 2011). It is interesting to note that in this study the amplitudes of slow phasic contractions did not significantly differ from contractions evoked by electrical stimulation and that these evoked contractions reset the interval between spontaneous slow phasic contractions. This suggests that there is a pattern generator in the human colon oscillating at less than 1 cycle per minute that can be activated by neural input but is not required for its activation.

Slow phasic contractions persisted at a reduced frequency in the presence of hexamethonium, the nicotinic receptor antagonist. Therefore slow phasic contractions differ from colonic migrating motor complexes (CMMCs) which are blocked by nicotinic antagonists in the mouse colon (Spencer et al., 1995, Fida et al., 1997). Sequential addition of TTX maintained the decrease in frequency but the

amplitude of contractions was unaffected. The effects of hexamethonium demonstrate the involvement of acetylcholine acting via nicotinic receptors, in regulating the frequency of slow phasic contractions. Application of carbachol and acetylcholine increase the frequency of the spikes and contractions in human colonic smooth muscle (Huizinga et al., 1986, Huizinga and Waterfall, 1988b, Huizinga et al., 1985). Depolarising the membrane potential of smooth muscle cells by increasing the concentration of extracellular K^+ did not have consistent effects on the occurrence of slow phasic contractions. Put together these results suggest that slow phasic contractions are not simply a product of slow waves interacting with myenteric potential oscillations (MPOs). Rather they appear to be an all-or nothing activity that occurs periodically and which may be triggered prematurely by activation of neural pathways. It would appear that their normal frequency is partially determined by ongoing nicotinic cholinergic neuronal activity although reducing the dimension of preparations did not reduce this. In turn this suggests that slow phasic contractions maybe modulated by very short local pathways in preparations.

5.4.2 Ascending excitatory pathways in the human colon

I found that electrically stimulating intrinsic neurons in an aboral segment of human colon caused a premature contraction orally. Electrical stimulation of the aboral segment isolated by cutting did not evoke a premature contraction. This suggests stimulation in the intact preparation must be due to activation of interneuronal pathways characterised previously in retrograde labelling experiments (Wattchow et al., 1995). Electrical stimuli were applied > 10mm from the recording site; this is further away than the longest ascending motorneurons in circular muscle layer of the human colon (Wattchow et al., 1995). Therefore, stimulation either directly activated ascending neural pathways, or antidromically activated descending interneurons. A comparable study was conducted by Spencer and colleagues who reported in 4 of 6 specimens electrical stimulation of intact hemi-colons *in vitro* generated a premature contraction at the site of stimulation which then propagated orally (Spencer et al., 2012). It has been reported in flat sheet preparations of human ileum (6x3cm) that stretching an aboral segment stimulated a contraction orally (Grider, 1989)

confirming the existence of functional ascending excitatory enteric neural pathways in human colon.

DMPP may have evoked a premature contraction orally, and this supports the idea that functional ascending excitatory neural pathways exist in the human colon. However, electrically evoked contractions persisted in the presence of hexamethonium. These results demonstrate that while nicotinic receptors have a role in ascending neural pathways, nicotinic transmission is not a requirement for ascending neuro-neuronal transmission. While a role for multiple neurotransmitters should be considered in this pathway, the involvement of tachykinins or muscarinic receptors seem likely options. The involvement of tachykinins in neuro-neuronal transmission has been demonstrated in the human ileum (Grider, 1989) but not in the colon. In the human colon, there is evidence for tachykinins in neuro-muscular transmission since application of exogenous substance P and neurokin A results in contraction of smooth muscle which persists with TTX (Aulí et al., 2008, Cao, 2000). NKA, the preferred transmitter for NK2 receptors, may mediate some of the electrically evoked contraction of human colonic smooth muscle cells since electrically evoked contractions persisted in the presence of NK1 antagonists but were inhibited by NK2 antagonists (Cao, 2006). In combined retrograde labelling and immunohistochemistry experiments conducted on human colon, 9% of circular muscle motor neurons were immunoreactive for tachykinins and all of these projected orally (Wattchow et al., 1997). For retrogradely labelled myenteric neurons, 23% of orally projecting interneurons were immunoreactive for TK (Wattchow et al., 1997).

5.4.3 The importance in studying different sized preparations.

While studying small muscle strips can offer some insight into the mechanisms underlying colonic motility, the contractions recorded *in vitro* do not correspond with what is recorded *in vivo.* Colonic manometry can be used to measure pressure generated by contractions as they occur *in vivo.* In a study of normal humans, Dinning and colleagues noted the fastest anterograde propagating sequences

occuring at 65 cycles per 24 hours, and retrograde sequences at 17 cycles per 24 hours (Dinning et al., 2009). These propagating sequences have been associated with flow of content in the colon (Dinning et al., 2008). While lack of sensitivity of the recording device may account for manometry studies missing some contractions recorded in muscle strips, muscle strip preparations do not reveal an activity pattern occurring with the same frequencies as propagating sequences. In transducer recordings from intact hemi-colons, contractions occurring at 4 minute intervals propagated along the colon, (Spencer et al., 2012) Neither *in vivo* manometry recording, nor *in vitro* force transducer recordings from muscle strips show this 4 minute pattern of contractions. The marked differences in behaviour observed between recordings from *in vivo* humans, and intact colon preparations point to fundamental differences between the specimens or the recording methods and resolutions. In the intact human there exists an array of extrinsic neural influences and perhaps blood-borne factors which are absent in the *in vitro* bath experiments.

No significant differences in the frequency of spontaneous contractions were observed between different sized preparations (10x2 to 80x150mm) in this study. However, it is interesting to note that previous experiments on the cat (Christensen et al., 1974) and mouse (Lyster et al., 1993) demonstrated that the myoelectric complexes associated with CMMCs are lost when intact tube preparations were cut down into rings segments less than 15 mm long. It is possible that the range of sizes of specimens tested in our experiments may not have been large enough to demonstrate any differences.

5.5 Conclusion

Results from this study demonstrate that a neural pathways with ascending trajectories can be used to generate premature contractions in specimens of human colon. While these pathways involve cholinergic transmission, other neurotransmitters appear to play important roles. Slow phasic contractions, occurring at a frequency slower than slow waves, persist in the presence of TTX but at a reduced frequency. This demonstrates that although they are mediated

myogenically, their frequency can be modulated neurally. The frequency of spontaneous contractions in specimens of varying cross sectional area was unchanged. In order to associate the recordings made in human *in vivo* experiments to mechanistic experiments conducted *in vitro* perhaps even larger specimens may need to be tested.