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INTRACELLULAR RECORDINGS FROM MYENTERIC NEURONES IN THE HUMAN COLON

BY S. J. H. BROOKES, W. R. EWART AND D. L. WINGATE

From the Gastrointestinal Science Research Unit and the Department of Physiology, The London Hospital Medical College, 26 Ashfield Street, London E1 2AJ

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SUMMARY

1. Intracellular recordings were made from cells in the myenteric plexus of the human colon in freshly dissected tissue obtained from patients undergoing surgery for the removal of carcinomas or diverticular bowel.

2. Twenty-seven cells from ten preparations were classified as neurones and had overshooting action potentials, an average resting potential of -54 ± 9 mV, an average input impedance of $1{\cdot}05 \pm 0{\cdot}59 \times 10^8$ Ω and a variety of synaptic inputs.

3. Twenty-three (out of twenty-five neurones tested) received nicotinic fast excitatory synaptic inputs (fast e.p.s.p.s) that were blocked reversibly by hexamethonium and mimicked by acetylcholine. These nerve cells bore a close resemblance to S cells that have been characterized in the guinea-pig small-bowel myenteric plexus.

4. One cell had a long after-hyperpolarization following its impulses and was similar to AH cells in the guinea-pig small bowel.

5. Three neurones received inhibitory synaptic inputs, up to 15 mV in amplitude, lasting up to 10 s, associated with a decrease in input impedance and with a reversal potential between -80 and -90 mV.

6. Slow excitatory synaptic potentials were only detected in the single AH cell. The slow e.p.s.p. was associated with a depolarization of up to 12 mV, an increase in excitability and an increase in the input impedance of the neurone.

7. The proportion of S and AH cells differ considerably from that reported in the guinea-pig small-bowel preparation. Possible causes of the differences are discussed.

INTRODUCTION

Recordings of single neurones in the enteric nervous system have contributed greatly to our understanding of the physiology of the gut. Originally extracellular recording techniques were used (Wood, 1970, 1973; Ohkawa & Prosser, 1972) but these have been largely superseded by intracellular recordings since the pioneering studies of Nishi & North (1973) and Hirst, Holman & Spence (1974). These and many subsequent papers have shown that in the guinea-pig small intestine most myenteric neurones can be classed as either type 1/S cells or type 2/AH cells. AH cells are distinguished by the presence of a long after-hyperpolarization that lasts many

seconds after each of their impulses. S cells lack this after-potential but commonly have obvious fast excitatory synaptic inputs. Virtually all of the work on freshly dissected preparations of enteric nerve cells has been carried out in the myenteric plexus in the small bowel of the guinea-pig, although a number of studies have looked at submucous neurones from the same animal (Hirst & McKirdy, 1975; Nield, 1981; Surprenant, 1984*a*, *b*).

We have recently carried out what we believe to be the first comparative study, recording the activity of myenteric neurones in freshly dissected preparations of rat duodenum (Brookes, Ewart & Wingate, 1985, and in preparation). Our results indicate an over-all similarity to guinea-pig small-bowel myenteric neurones although a number of small but significant differences were also apparent. Our results also differ in some respects from those recently reported by Nishi & Willard (1985) and Willard & Nishi (1985*a*,*b*) on cultured neurones from the rat small-bowel myenteric plexus. This raises some doubts about the validity of extrapolating findings from cell culture to freshly dissected preparations and from one species to another.

From this work it became apparent that studies of human enteric neurones would be an essential step in being able to assess the relevance of animal models to man. The only studies to date of human enteric neurones using intracellular recording are those of Maruyama (1981) and Maruyama & Suzuki (1982) who looked at the action potential characteristics of cultured fetal myenteric neurones. Synaptic potentials were not recorded in these studies. Our aims, therefore, were first, to determine whether or not a viable preparation, suitable for intracellular recording, could be made from freshly dissected surgically excised tissue; and secondly to determine how human enteric nerve cells compared to neurones recorded in other preparations in terms of their neurophysiological profiles. Due to the availability of tissue we have been restricted to specimens of human distal colon, so direct comparison with published data on small-bowel neurones has to be attempted with caution. Nevertheless our results indicate that the neurophysiology of the myenteric plexus of the human colon is significantly different from that of the guinea-pig and rat small-bowel preparations, notably in the scarcity of AH cell activity and the paucity of slow e.p.s.p.s.

A preliminary report of this work has been presented to the Physiological Society (Brookes, Ewart & Wingate, 1986).

METHODS

With the co-operation of surgeons at the London Hospital specimens of distal colon were obtained from patients undergoing surgery for removal of colonic tumours or diverticular bowel. Immediately on removal from the patient, a small section of non-pathological tissue between the taenia coli was cut off and placed in Krebs solution chilled to 5 °C. The tissue was then pinned out, serosal side down and the mucosa and submucosa were cut off. The circular muscle layer was then peeled back to reveal the myenteric plexus and longitudinal muscle layers. About one-third of dissections were successful; in these the myenteric plexus was just visible under the dissecting microscope. This was considerably aided in some preparations by a creamy-white pigmentation of the nerve cell bodies. In these preparations the tissue was pinned out in a small organ bath lined with Sylgard (Dow-Corning) with a volume of 0·5–1·0 ml, and perfused at 2–3 ml/min with Krebs solution containing $1–5 \times 10^{-7}$ M-nicardipine, which prevented spontaneous muscle activity that would otherwise have dislodged the recording electrodes. Krebs solution contained NaCl (117 mM), KCl

(4·7 mM), $MgCl_2$ (1·2 mM), $CaCl_2$ (2·5 mM), $NaHCO_3$ (25 mM), NaH_2PO_4 (1·2 mM) and glucose (11 mM); pH 7·3, gassed with 95% O_2–5% CO_2, and was warmed to give a bath temperature of 36–38 °C. The preparation was viewed under epi-illuminated Nomarski optics at 75 times magnification.

Intracellular recordings were made using conventional fibre-filled glass micro-electrodes filled with 2 M-KCl with resistances of $6–10 \times 10^7$ Ω. Dye-filled electrodes were filled with 2% Lucifer Yellow with 200 mM-LiCl, and had resistances of $1·6–2·5 \times 10^8$ Ω. Signals were passed through an electrometer (Neuroprobe amplifier 1600, A and M Systems, U.S.A.) with internal bridge-balance circuitry, and simultaneously displayed on an oscilloscope and recorded on a Racal Store 4 tape-recorder for future analysis. Traces were printed on an $X–Y$ plotter (J. J. Lloyd) from images stored on a digital oscilloscope (Gould OS 4000). Synaptic events were evoked by focal stimulation of fibre tracts and ganglia with pairs of chloride-coated silver wires, insulated apart from their tips and driven from a constant-current isolation unit (Neurolog NL800). Acetylcholine was applied by pressure micro-ejection from one barrel of a multibarrelled pipette, and fixed-duration pulses of nitrogen (10–30 lbf/in²) were controlled by a solenoid-operated valve (Radio Spares, U.K.). Acetylcholine was used at a concentration of 10^{-3} M in the barrel and adjacent barrels were filled with Krebs solution to control for artifacts produced by fluid movement. Antagonists were applied in the perfusing Krebs solution. All drugs were obtained from Sigma Chemicals.

At the end of each day's experiment the tissue was routinely stained with methylene blue to reveal the myenteric plexus in order to determine that the recordings had in fact been made from a nerve ganglion.

RESULTS

To date we have recorded from thirty-three cells in ten preparations of human colonic myenteric plexus. Seven of these cells showed the lack of activity characteristic of identified glial cells in other enteric nerve cell preparations and were provisionally identified as glial cells. On penetration of a glial cell a steady resting potential of 55–80 mV was recorded and the average input impedance of these cells, measured with small constant-current hyperpolarizing pulses, was $3·5 \times 10^7$ Ω and generally remained constant for the duration of the recording (up to 60 min). Typically, the time constant of these cells was considerably shorter than for neurones; this was manifested in the rapid response to current pulses (see Fig. 1). Another typical feature of putative glial cells was their failure to show the marked rectification in response to depolarizing current pulses that was characteristic of most of the neurones recorded in the present study. Small pulses of depolarizing current evoked very similar amplitudes of change of membrane potential to similar hyperpolarizing current pulses in putative glial cells. Most neurones, on the other hand, had a smaller response to subthreshold depolarizing current pulses than to similar sizes of hyperpolarizing current. Glial cells also consistently failed to develop action potentials or any active membrane response to any sizes of depolarizing current steps. None showed a measurable potential or conductance change to acetylcholine (ACh) applied by pressure micro-injection. In both the rat and the guinea-pig small-bowel myenteric plexus preparations it has been reported that a subpopulation of supposed glial cells can develop neuronal activity after being recorded for long periods. This was not seen with any of the putative glia recorded in the present study.

On penetrating a neurone with a recording micro-electrode, a drop in resting potential of 30–50 mV was recorded that gradually stabilized over the next 2–5 min. This coincided with a gradual increase and subsequent stabilization of the input impedance and time constant of the cells. Only cells with steady resting potentials negative to −45 mV were used in the present study. The average resting potential

was -54 ± 9 mV, the range varying from -45 to -76 mV. The input impedance of cells, measured with small (less than 500 pA) hyperpolarizing pulses was $1{\cdot}05 \pm 0{\cdot}59 \times 10^8\ \Omega$ and varied from $0{\cdot}35 \times 10^8\ \Omega$ to $2{\cdot}85 \times 10^8\ \Omega$ in different cells. The current–voltage relationship of cells was generally linear for small hyperpolarizing current pulses but showed marked rectification for depolarizing current.

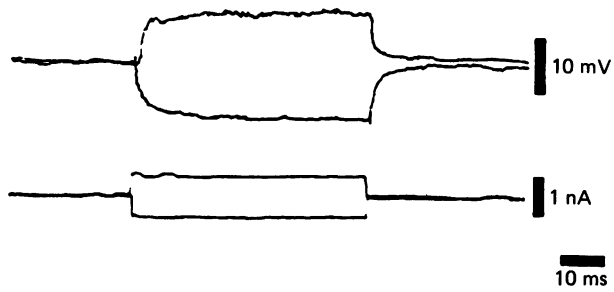

Fig. 1. Membrane potential of a presumed glial cell (upper traces) during the application of depolarizing and hyperpolarizing current pulses (lower traces). Putative glia typically showed a relatively small but fast potential change in response to injected current and little rectification of depolarizing current.

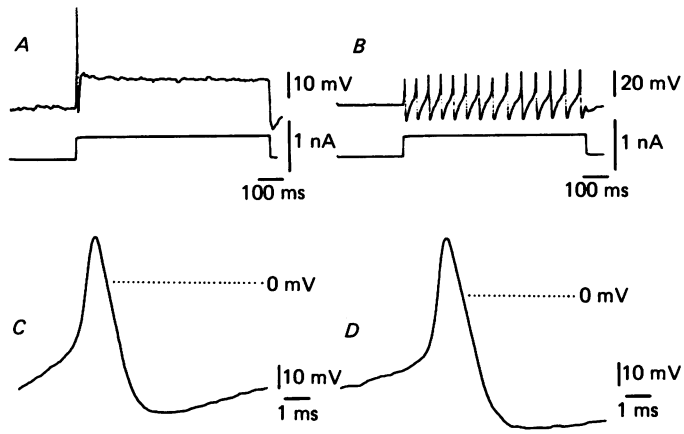

Fig. 2. Responses of neurones to intracellularly injected suprathreshold depolarizing current pulses. *A*, a neurone gave a single impulse (upper trace) in response to any amplitude of suprathreshold depolarizing current pulse (lower trace). The action potential is shown at a faster time base in *C*. *B*, a different neurone fired repeatedly in response to a similar suprathreshold depolarizing current pulse. One of the action potentials evoked in this way is shown on *D*. Action potentials are truncated in *A* and *B* due to the sampling frequency of the digital oscilloscope.

In response to suprathreshold depolarizing current pulses (generally 100–400 pA), neurones fired one or more action potentials that were up to 80 mV in amplitude and of short duration (less than 1·5 ms at half-peak amplitude). The majority of nerve cells fired only one impulse to any suprathreshold current pulse but about one-quarter of cells were capable of firing tonically (seven out of twenty-seven cells; see Fig. 2*A* and *B*). Action potentials were typically very rapid and lacked the 'shoulder' or inflexion on the falling phase that is often seen in AH cells in both the rat and the

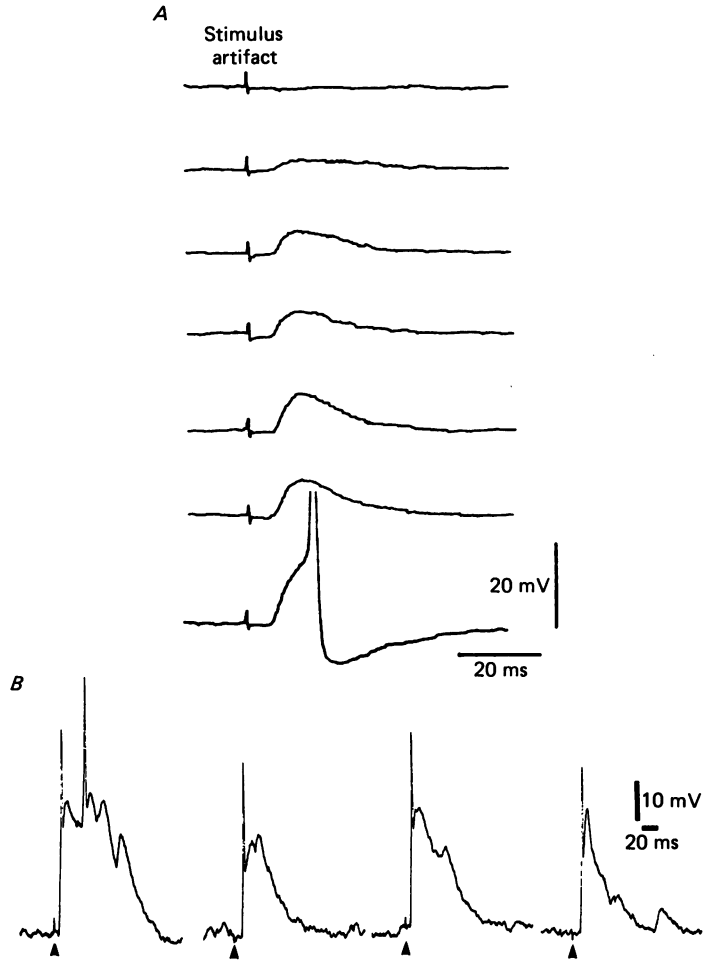

Fig. 3. *A*, an increasing series of stimulus currents (going down the page) evoked four different sizes of fast e.p.s.p. in this neurone, presumably as four different presynaptic fibres were successively recruited. The final summed fast e.p.s.p. was sufficient to initiate an action potential (lowest trace). *B*, four examples of a single stimulus pulse applied to an interganglionic connective close to the recording site (arrows) evoked a complex series of spatiotemporally summed fast e.p.s.p.s which reached threshold for action potential initiation. The considerable variability in the pattern of fast e.p.s.p.s evoked by identical stimuli and the very long latency of some of the synaptic events suggest that a number of polysynaptic pathways were excited.

guinea-pig. Impulses were followed by a short after-hyperpolarization lasting up to 500 ms and up to 14 mV below resting potential. Only one neurone showed the long after-hyperpolarization and the conductance changes following a single impulse that are typical of AH cells recorded from both the rat and the guinea-pig small-bowel myenteric plexus. However, following bursts of tonic firing, induced by long pulses of depolarizing current, another three cells showed an after-hyperpolarization (up to 8 mV in peak amplitude and lasting up to 10 s). The features of the spiking behaviour of the human colonic myenteric neurones reported in this study are comparable with

the characteristics of S cells described in other myenteric plexus neurone preparations. Two neurones showed spontaneous firing at a low frequency; both had small resting potentials (−48 and −52 mV) and had many spontaneous fast e.p.s.p.s which were revealed when impulses were blocked by hyperpolarizing the cell. It is possible that damage caused by the micro-electrode may also have contributed to the excitability of these neurones.

Focal stimulation of ganglia or interganglionic connectives evoked fast excitatory post-synaptic potentials (fast e.p.s.p.s) in twenty-three out of twenty-five cells tested. By varying the stimulus intensity, it was possible to evoke a number of different sizes of fast e.p.s.p. in most of the neurones (see Fig. 3*A*). This indicated that there was considerable convergence of input onto the recorded cells, and that as the stimulus current was increased more presynaptic fibres were excited. In different cells, from three to six different sizes of fast e.p.s.p.s could be evoked by increasing intensities of stimulus current applied to a single fibre tract. This is probably an underestimate of the number of presynaptic fibres that converge onto each neurone in the human colonic myenteric plexus, since it was difficult to distinguish very small changes in the amplitude of the evoked synaptic potential, and it was not usually possible to stimulate all of the many connectives entering each ganglion. Fast e.p.s.p.s were of relatively short duration, lasting from 15 to 50 ms and were up to 21 mV in peak amplitude. In a few cells quite complex spatiotemporal summation of fast excitatory synaptic inputs was seen (Fig. 3*B*). The rise times of e.p.s.p.s varied from 3 to 7 ms. Spontaneous fast e.p.s.p.s were seen in five of the neurones recorded to date; these showed similar time courses to the stimulus-evoked fast e.p.s.p.s and were up to 10 mV in amplitude. In two cells spontaneous fast e.p.s.p.s were seen to underlie the spontaneous firing. Following either single pulses or trains of stimuli, the frequency of spontaneous fast e.p.s.p.s was often increased for several seconds as reported by Furukawa, Taylor & Bywater (1986) in the mouse colon myenteric plexus.

When the cell was hyperpolarized by passing current tonically, fast e.p.s.p.s increased in amplitude (see Fig. 4); when cells were depolarized fast e.p.s.p.s became relatively smaller. This indicated that if these synaptic events have a true reversal potential, it must be positive to the normal resting potential of the cell. By extrapolating from plots of the size of the fast e.p.s.p. amplitude against membrane potential, the reversal potential was indirectly estimated to lie between −20 and +10 mV ($n = 7$). It was not possible to test this directly by depolarizing the cell with tonically applied current due to limitations in the current-passing capabilities of the micro-electrodes. When stimulated repeatedly at relatively high frequencies fast e.p.s.p.s often showed a marked depression after the first potential (see Fig. 5). That this was not caused by 'drop-out' or failure to stimulate some presynaptic fibres is suggested by the observation that it could be seen when a just-suprathreshold stimulus current was used (which was presumed to excite a single presynaptic axon).

Fast e.p.s.p.s were reversibly blocked by perfusing the preparation with 50–200 μM-hexamethonium (see Fig. 6); following wash-out of this nicotinic antagonist they recovered to their former amplitude. This indicated that fast e.p.s.p.s were probably mediated by the release of ACh acting on post-synaptic nicotinic receptors as has been shown in many other mammalian peripheral synapses. Atropine, phentolamine

and propranolol had no detectable effects on the amplitude of fast e.p.s.p.s. The potential changes associated with the fast e.p.s.p. could be mimicked by the application of ACh by local pressure micro-ejection onto the recorded neurone (see Fig. 7). ACh evoked a depolarization of rapid onset, up to 45 mV in amplitude in five out of five neurones, all of which received fast excitatory synaptic inputs. Associated

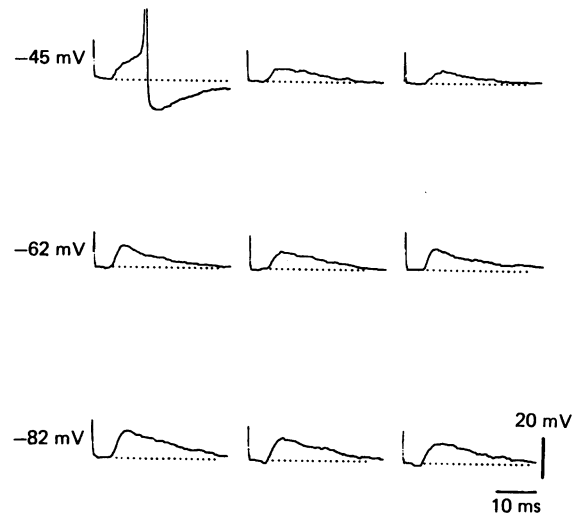

Fig. 4. Three fast e.p.s.p.s were evoked using an identical stimulus current at each of three different membrane potentials, maintained under current-clamp conditions. The normal resting potential of this neurone was −62 mV; at an apparent membrane potential of −82 mV (evoked by injecting 400 pA of hyperpolarizing current), fast e.p.s.p.s were clearly increased in size; at −45 mV (corresponding to 500 pA of depolarizing current) they were clearly reduced. At −45 mV an action potential was initiated by one of the fast e.p.s.p.s indicating that they can exceed the threshold for spike initiation.

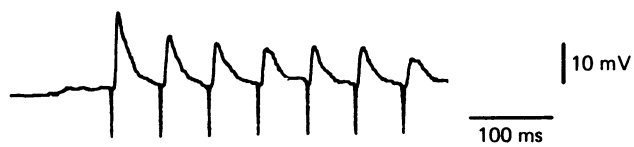

Fig. 5. With repeated stimulation fast e.p.s.p.s often showed a marked depression after the first event, as has been reported in the guinea-pig small bowel.

with this depolarization was a marked decrease in input impedance. The ACh-evoked depolarization had a considerably slower time course than the fast e.p.s.p., as has been reported for application by pressure micro-ejection in other studies (North & Surprenant, 1985). Both the depolarization and the increase in conductance were reversibly bocked by 100–200 μM-hexamethonium, but were unaffected by atropine (see Fig. 8). Like the fast e.p.s.p., the depolarization evoked by ACh increased in amplitude when the neurone was hyperpolarized and decreased when it was depolarized. The extrapolated reversal potential lay between −30 and +8 mV ($n = 5$). One cell had an additional slow response to ACh, showing a long duration de-

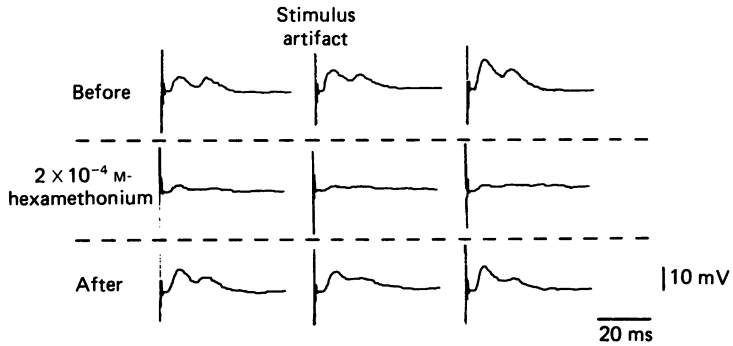

Fig. 6. Two fast e.p.s.p.s of slightly different latencies were evoked at constant intervals by stimulation of an interganglionic connective. After 2 min of perfusion with 2×10^{-4} M-hexamethonium, a specific nicotinic blocker, both fast e.p.s.p.s were blocked. After washing with fresh Krebs solution for 6 min (lowest set of traces) fast e.p.s.p.s returned, virtually to their former amplitude.

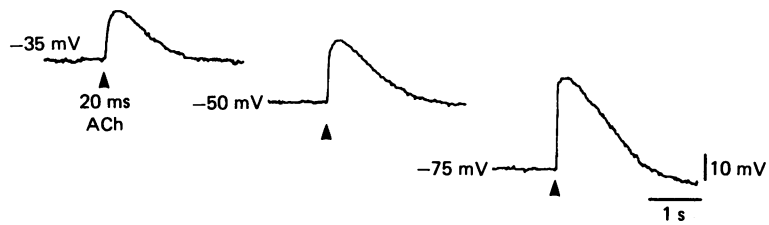

Fig. 7. Effects of ACh applied by pressure ejection onto a neurone. Short pulses of ACh (10^{-3} M, 20 ms duration, at the arrows) evoked rapid depolarizations that increased in amplitude when the cell was hyperpolarized, and decreased in amplitude when depolarizing current was passed. The membrane potentials shown were recorded whilst current was being injected on the assumption that the electrode resistance remained constant throughout the experiment. The time course of the ACh-induced depolarization was considerably slower than the fast e.p.s.p., as has been reported elsewhere for pressure micro-injection.

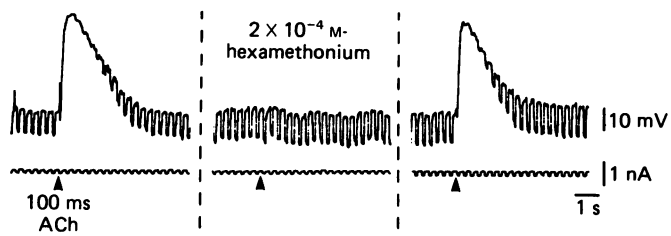

Fig. 8. The effect of hexamethonium on ACh-evoked depolarizations. A short pulse of ACh (arrows) evoked a rapid depolarization (left pair of traces) that was associated with a pronounced increase in membrane conductance, as shown by the reduction in the amplitude of electrotonic potentials evoked by constant-current hyperpolarizing pulses (lower trace of each pair). After 3 min of perfusion with 2×10^{-4} M-hexamethonium (middle traces) the response to ACh was blocked. After washing with fresh Krebs solution for 7 min (right pair of traces) the effects of ACh on membrane potential and conductance recovered.

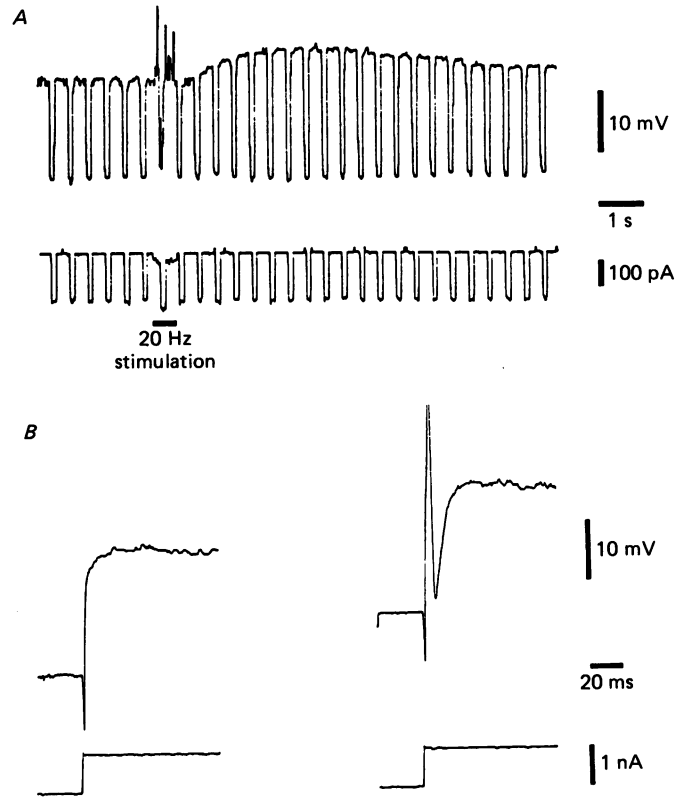

Fig. 9. *A*, repeated stimulation of an interganglionic fibre tract (filled bar) evoked in one cell a prolonged slow e.p.s.p. which was associated with a marked increase in input impedance, as revealed by the increase in amplitude of electronic potentials produced in response to small hyperpolarizing constant-current pulses (lower trace). *B*, effects of the slow e.p.s.p. on excitability. In the only cell seen to have a measurable slow e.p.s.p. (the same as that shown in *A*), depolarizing current pulses (0·9 nA) were ordinarily insufficient to evoke an action potential. However, during the slow e.p.s.p. evoked by repeated stimulation (right-hand traces) an identical current pulse consistently evoked a single impulse. This indicates that there was an increase in excitability during the slow e.p.s.p., as has been reported in guinea-pig small-bowel myenteric neurones.

polarization, of slow onset and recovery. This was associated with an increase in input impedance and was irreversibly abolished by 1×10^{-6} M-atropine.

Slow e.p.s.p.s, lasting many seconds, are commonly recorded in both guinea-pig and rat small-bowel myenteric neurones in response to repetitive stimulation. Only one cell out of twenty-five tested showed a slow excitatory synaptic potential; this cell also received fast e.p.s.p.s and had a long after-hyperpolarization after its impulses. During the slow e.p.s.p. the input impedance of the cell measured with small constant-current pulses increased (see Fig. 9*A*) and the cell became more excitable (see Fig. 9*B*).

Three neurones were seen to receive an inhibitory synaptic input (i.p.s.p.) that was associated with a hyperpolarization of the cell membrane of up to 15 mV lasting up

to 10 s, and a decrease in the input impedance (see Fig. 10). By tonically hyperpolarizing the cell with injected current, it was possible to reverse to i.p.s.p.; it appeared to have a reversal potential between −80 and −90 mV, close to the expected potassium equilibrium potential. All three cells with i.p.s.p.s also received fast e.p.s.p.s.

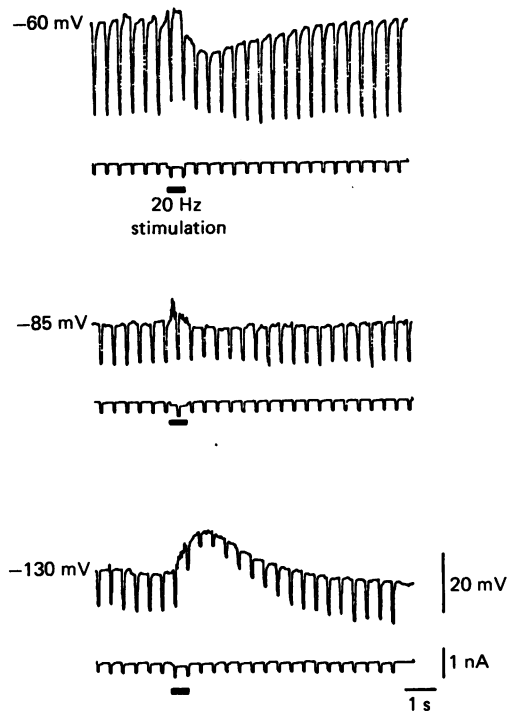

Fig. 10. In response to repeated stimulation at 20 Hz for 0·6 s (marked by the filled bar) an inhibitory synaptic potential was evoked. Constant-current hyperpolarizing pulses (the lower of each pair of traces) reveal the increase in conductance that was associated with the i.p.s.p. When the cell was strongly hyperpolarized (by passing 1·3 nA hyperpolarizing current; lowest pair of traces) the i.p.s.p. was seen to reverse. The reversal potential was between −85 and −95 mV (maintained by passing 0·6 nA hyperpolarizing current; middle pair of traces). Apparent membrane potentials were measured whilst current was being injected on the assumption that the electrode resistance remained reasonably constant.

In order to prove that our recordings were indeed of neuronal activity, we recorded the activity of a number of cells with micro-electrodes filled with a solution of the fluorescent dye Lucifer Yellow (Stewart, 1981). An example of this is shown in Fig. 11. After recording base-line activity and evoked synaptic potentials the two neurones in Fig. 11 were stained. The presence of a single axon (in one neurone orally directed and in the other aborally directed) proved the neuronal identity of the recorded cells. There is clearly a range of morphological types and physiological types of nerve cells in the human myenteric plexus; both of these cells received fast excitatory synaptic inputs and one cell also received an inhibitory synaptic input.

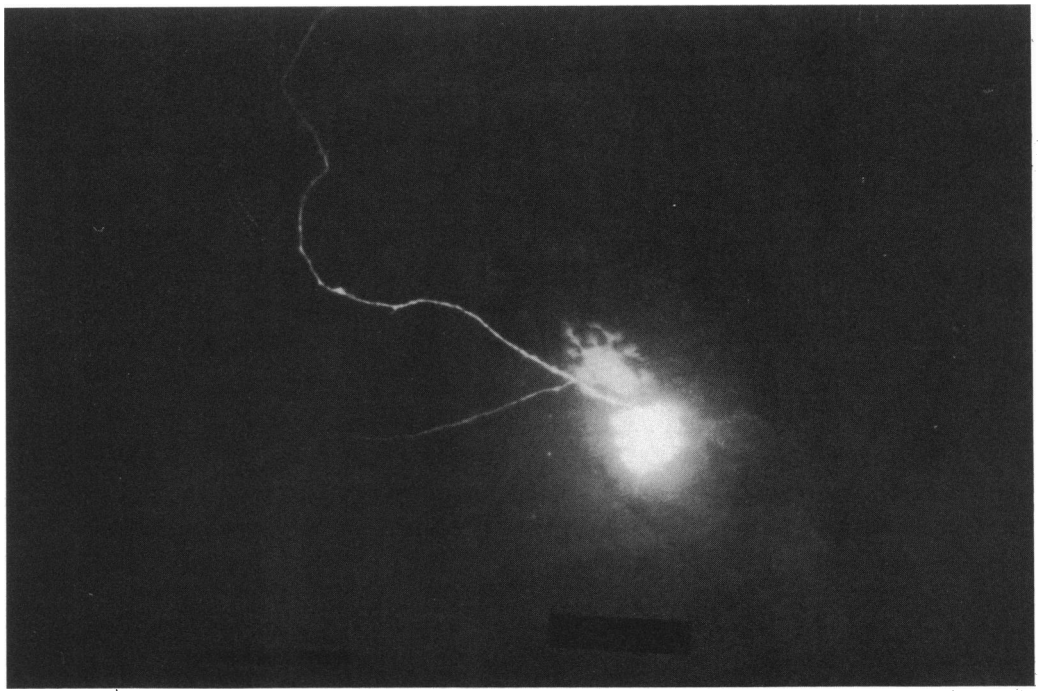

Fig. 11. Photomicrograph of two human colonic myenteric neurones intracellularly stained with the fluorescent dye Lucifer Yellow after recording their electrophysiological activity. Both cells received fast e.p.s.p.s; the lower cell body also received an i.p.s.p. Both somata give rise to a single axon, one leaving the ganglion in an oral direction, the other leaving aborally. One cell body is surrounded by short club-like processes, the other by short, thin, branching processes. This confirmed the neuronal identity of the source of our recordings (calibration: 100 μm).

DISCUSSION

The results presented in this report show that a viable preparation of human enteric neurones, suitable for intracellular recording, can be prepared from tissue removed from patients undergoing abdominal surgery. This is the first report of recordings of human neurones using these techniques and we hope that it will pave the way for a detailed study of human enteric neurones. The results indicate a number of differences in the electrophysiological characteristics of myenteric neurones in the human colon compared to the neural activity recorded in the guinea-pig small bowel which until recently has been the only source of enteric nerve cells studied in detail.

The presence of fast e.p.s.p.s in human colonic myenteric neurones is not surprising, neither is the observation that they are probably mediated by the release of ACh acting on post-synaptic nicotinic receptors. Similar synaptic potentials have been recorded from enteric neurones in the guinea-pig small-bowel myenteric plexus (Nishi & North, 1973; Hirst *et al.* 1974) and guinea-pig small-bowel submucous plexus (Hirst & McKirdy, 1975; Surprenant, 1984*a*, *b*), from rat small-bowel myenteric neurones (Brookes *et al.* 1985, and in preparation) and from cultured rat small-

bowel myenteric plexus nerve cells (Willard & Nishi, 1985*a*, *b*; Nishi & Willard, 1985) and in guinea-pig colonic myenteric neurones (Wade & Wood, 1985). What is rather surprising is the very high proportion of neurones that receive fast excitatory inputs; the proportion of S cells in this study is far higher than in any of the other studies cited above. The lack of long after-hyperpolarizations following impulses in all but one of the neurones recorded in the present study confirms the similarity of our recordings to S cells described elsewhere. It is interesting, however, that in a recent study of myenteric neurones in the mouse colon Furukawa *et al.* (1986) reported that 91% of neurones were S cells (eighty-two out of ninety), thus it may be that AH cell activity becomes less common in the more distal regions of the alimentary tract. It would not be surprising to find neurophysiological differences between the small intestine and the colon since the functions and gross patterns of activity of these parts of the gastrointestinal tract are very different (e.g. Sarna, Condon & Cowles, 1984). A dedicated study to compare the relative proportions of the two types of cells in different regions of the gut is needed to resolve this question. The added possibility of a genuine interspecific difference cannot be excluded at the present time. The presence of inhibitory synaptic inputs to three of the neurones is also interesting since these potentials are rather rare in guinea-pig ileum myenteric nerve cells (see Johnson, Katayama & North (1980)); our small sample size, however, makes it undesirable to speculate on the significance of this.

There are many possible reasons for the apparent scarcity of AH cells and slow e.p.s.p.s in our study, including the possibilities of interspecific variation and differences between the small and the large intestines as discussed above. A number of other explanations are also possible. It might be suggested that AH cells are common and that we have simply failed to record them, due perhaps to a sampling bias of our recording techniques. This seems unlikely for two reasons. First, in our hands in the guinea-pig and rat small-bowel preparations, AH cells are rather easier to impale and hold, perhaps because they are somewhat larger than S cells on average (North, 1982). Secondly, although only twenty-seven nerve cells were included in this study, about three times that number of cells were impaled and later rejected, mostly because of low resting potentials or the short duration of the recording. None of these short recordings showed AH-type activity. This makes it likely that AH-type activity is genuinely very rare in our preparations at the time of recording.

It is possible that the tissue used in this study was not physiologically normal, perhaps due to the disease state that led to surgical intervention. This does not appear to be very likely, since during removal of tumours it is customary to remove a margin of healthy tissue (in order to reduce the risk of local recurrence), and it was from this area of the excised segment that we obtained our specimens. Certainly the segments of colon that we studied were distant from the site of cancerous tissue and appeared in the dissecting microscope to be relatively normal. It should be pointed out that the patients from whom we obtained tissue were all over 60 years old; it has been shown that there are age-related changes in the innervation of the gut in humans (Koch, Go & Szurszewski, 1986) and it is possible, although unlikely, that this is why we failed to record significant numbers of AH cells and slow e.p.s.p.s. It seems improbable that a change as extreme as the loss of nearly all AH cell activity and slow e.p.s.p.s would not be associated with a profound gut dysfunction. Apart

from the local colonic lesion that necessitated surgical intervention, we are not aware that this was the case with any of the patients from whom we obtained specimens of tissue.

Another potential explanation lies in the treatment of the tissue prior to recording. Dissections of guinea-pig small bowel are generally carried out on tissue that has been quickly removed from freshly killed animals, while human tissue was obtained during long operations on anaesthetized patients. It is possible that the drugs administered during the premedication or to maintain anaesthesia could have had profound effects on peripheral neurones; however, in view of the long period (up to 5 h) of washing with fresh chilled oxygenated Krebs solution during the dissection it would seem probable that any residual pharmacological agents would have been removed before recording. Even so, the chance of prolonged effects cannot be entirely excluded. Another source of potential abnormality in the tissue is the actual surgical procedure. During the course of the operation the gut is handled extensively, and this nociceptive stimulation might be the cause of the abnormal range of neuronal activity recorded during the present study. During the removal of specimens of guinea-pig gut, handling is usually kept to a minimum. It is also possible then that the human tissue which we studied had already gone into a state of adynamic ileus and the possibility that the AH subpopulation of neurones is involved in this pathological state would be of great interest. The possibility that hypoxia was the cause of the lack of AH cells and slow e.p.s.p.s seems remote, since the period during which the human tissue was deprived of its circulation was comparable to the time taken to excise specimens in small-animal gut preparations.

These results convince us that it can no longer be assumed that what is true for the guinea-pig small bowel is necessarily directly applicable to other species, including man, and/or that there may be significant differences between various regions of gut. This preparation will allow us to explore some of the possibilities mentioned above and to study directly the physiology, pharmacology and morphology of the human enteric nervous system.

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